SCOTTISH HOSPITALS INQUIRY

Bundle of documents for Oral hearings commencing from 19 August 2025 in relation to the Queen Elizabeth University Hospital and the Royal Hospital for Children, Glasgow

Bundle 44 - Volume 2
Expert Reports in Response to GGC Expert (HAD) Report and Associated Documentation

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Scottish Hospitals Inquiry

Joint Response of Professor Hawkey, Dr Drumright and Dr Agrawal to HAD Questionnaire 1

The Scottish Hospitals Inquiry has been provided with the report you entitled "*Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the QEUH & RHC*" dated 24 July 2024 "the Report")[1]. In order to understand your Report, the Inquiry Team need to ask you a series of questions about the work you carried out in preparing the report, your knowledge about the QEUH & RHC and the information you have been supplied with by NHS GGC following your instruction by the Board on 21 November 2022.

To answer the questions in this questionnaire please type your answer in the answer area marked [Type your answer here] below the question, you will note that your type comes up in a different font from that of the question – this is to allow your answer to be read with ease.

Please note that some questions are described as being for just Dr Drumright and some questions are for just Professor Hawkey and Dr Agrawal. If you cannot answer a question because you consider it outwith your expertise, please explain why and which of your colleagues should answer the question.

Please do not insert pictures or documents into your written answers. All our hearing bundles are on our website https://www.hospitalsinquiry.scot/. If you would like to refer to a document within our bundles which captures your answer to the question, then please refer to the relevant document in the format **(Bundle X, Document Y, Page Z)**. If you wish to refer to your own document, then describe the document in your statement, list all such documents at the end of the statement and provide us with a copy of that document in order that we can process the document in accordance with Inquiry protocols.

This document constitutes your Joint Response to HAD Questionnaire 1. Each question has been answered by the expert whose initials appear alongside it. Where no initials are provided, the response is to be taken as jointly given and agreed by all three experts: Professor Hawkey, Dr Drumright, and Dr Agrawal.

A. You confirm that the facts stated in this Joint Response are true to the best of your knowledge and belief. You also confirm that you agree with the contents of the HAD Report. Finally, you understand that this statement may be used as evidence before the Inquiry and may be published on the Inquiry's website. Instruction of Professor Hawkey and Dr Agrawal

1) The Inquiry has been provided with your letter of instruction from the Central

[1] Bundle 44 Vol 1, Document 1, page 5.

Legal Office on behalf of NHS GGC on 21 November 2022[2]. *These questions are for Professor Hawkey and Dr Agrawal*.

a) What discussions did you have with representatives of NHS GGC or their solicitors and counsel about the QUEH/RHC prior to the date of that letter?

Dr Samir Agrawal [SA]: See my answer to # 5. I cannot recall the details of any discussions between myself, Professor Hawkey and [redacted] (representing the CLO) prior to the instruction letter. However, I believe we discussed how to approach answering the question of whether patients at QEUH/RHC were at increased risk of infection because of contamination of the hospital's water/ventilation systems.

Professor Hawkey [PMH]: Dr Agrawal's response is in agreement with my recollection. I was first approached by Prof Alistair Leonard who asked if I was willing to prepare a report on the case notes review, when I was in discussion with [redacted] to include other aspects as outlined by SA.

b) At the foot of the second page of that letter it is stated that the purpose of your "instruction was to consider the question of risk, specifically whether there is objective/data that would support the proposition that since the QUEH & RHC opened in 2015 patients were exposed to a greater risk of infection than would be expected due to a widespread issue with the water and/or ventilation systems". Is this an accurate summary of the purpose of your instructions as you then understood it?

SA: No, this is a limited description and hence the points in the appendix which expand on the text in the body of the letter.

PMH: I agree the appendix must be referred to for complete understanding.

c) What role did you understand that the seven questions set out in the Appendix to the letter play in the scope, purpose or production of the Report?

SA: See #1b above. The Appendix was a guide, a framework, to help us address the remit we had been given.

[2] Bundle 44, Vol 1, Documents 3 & 4, pages 234 - 243.

PMH: I agree.

d) The author of the letter then records that they understood following discussions with you that "it should be possible to address this at high level" and then describes "the intended approach". Is the description of "the intended approach" in the letter an accurate summary of the approach that you had proposed?

SA: Yes, it is accurate, with the specific questions in the Appendix being part of the remit.

PMH: I agree

e) How did the data that you required to review in order to address the purpose of the instruction set out in the letter of 21 November 2022 come to be identified. How much of that data was identified by representatives of NHS GGC or their solicitors and counsel propose?

We assume the final word, "propose" is a typo. We requested the complete microbiological blood culture data available from the time QEUH/RHC opened relating to paediatric oncology patients and adult haemato-oncology patients, as well as data available from the sites housing these groups prior to the opening of QEUH/RHC. This information was supplied to us by NHS GGC.

f) The letter informs you that the *"next step would be for you and Dr Agrawal to meet in order to discuss and define the scope of the data that needs to be reviewed"*. When did you first meet or speak to Dr Agrawal about the QUEH/RHC, who else was present and what was the substance of that discussion?

SA: My records indicate we spoke on 08/12/2022 and 19/12/2022. No one else was present. We clarified our understanding of the timing, location and specific organisms involved of the suspected nosocomial infections; the relevant patient populations; and patient ward locations and movements to different locations in the hospital over time. From this we identified and refined the data that would be required and how this might be identified through hospital electronic records.

PMH: My records indicate my first meeting with [redacted] was on 4.11.22 I think it was just myself and we discussed the same topics as SA stated above. I suggested we would need an experienced haem/oncology physician. I think [redacted] suggested SA and I agreed. Thereafter we had a number of meetings together. At some time later it became clear we needed an expert statistician with experience in this area and SA suggested Dr Lydia Drumright [LD] who was included in the group.

g) Was there any particular division of labour in preparing the Report? Did you, Dr Agrawal or Dr Drumright take a particular lead or responsibility in preparing any particular part of the Report? Please set that out here.

SA: The report represents a joint effort from all three of us. Although some sections are principally the work of one or other of us, we all provided input to all areas. The executive summary is a good example of the need to combine our different areas of expertise to address the questions put to us. With these caveats, chapters 2-5 are the focus of PMH; chapter 7 – LD; chapters 6 and 8 – SA.

PMH: I agree.

B. Involvement of Dr Drumright

2) The Inquiry holds the letter of instruction to Dr Drumright date 8 February 2024[3].

a) *This question is for Professor Hawkey and Dr Agrawal*. How was it that Dr Drumright came to be involved in the production of the Report? What role did you play in that decision? What did you consider was the assistance that Dr Drumright would be able to provide to the production of the Report?

SA: It rapidly became clear to Professor Hawkey and myself that given the scale of data that would need to be analysed, it required an experienced statistician, who ideally had a background in infection/epidemiology. As I had previously worked with Dr Lydia Drumright [LD] on infection issues and I was aware of her background working with very large data-sets, I proposed her as an

[3] Bundle 44, Vol 1, Document 5, page 244.

independent person rather than seeking internal statistical support at NHS GGC.

PMH: I agree - see my comment above.

b) *This question is for Professor Hawkey and Dr Agrawal*. What specifics did you provide to Dr Drumright following her instruction on 8 February 2024? Please produce your correspondence with her.

SA: As far as I can recall, neither I nor Professor Hawkey gave Dr Drumright specific instructions. Once Dr Drumright was aware of the remit of the work and the questions we had been posed, we discussed together the data and the various statistical analyses to be performed.

PMH: I agree.

c) *This question is for Dr Drumright.* How was it that you came to be involved in the production of the Report? What role did Professor Hawkey and Dr Agrawal play in that decision? What was the assistance that you were thought to be able to provide to the production of the Report?

LD: Dr Agrawal is a longtime collaborator of mine, starting from when I was faculty at the University of Cambridge. We work together studying the natural history of and risk factors for healthcare associated infections among haemato-oncology patients. I am an epidemiologist, expert in statistical modelling, and health informatician. Dr Agrawal and Prof Hawkey identified the need for statistical and epidemiological expertise in their report and Dr Agrawal recommended me based on his knowledge of my expertise. He and Prof. Hawkey discussed this and agreed to recommend me. They sent my name to the CLO. I met with [redacted] from the CLO, the CLO vetted my credentials and expertise and invited me to serve as an expert consultant.

d) *This question is for Dr Drumright*. What specifics did Professor Hawkey and Dr Agrawal provide to you following you instruction on 8 February 2024? Please produce your correspondence with them?

LD: This is an oddly worded question and slightly unclear, my apologies if it is not answered correctly. No “instruction” was provided. Prof Hawkey, Dr

Agrawal, Dr Rebello and I met to discuss the objectives, the available data and reports, and how we could examine these to provide evidence for environmental microorganisms resulting in increased bacterial bloodstream infection cases among QEUH patients due to environmental contamination.

3) *This question is for Professor Hawkey, Dr Agrawal and Dr Drumright*. The letter of instruction to Dr Drumright of 8 February 2024 refers to "data from Dr Agrawal's hospital etc". Was the data from St Bartholomew's hospital referred to in paragraph 8.1.2 of the Report the only data from that hospital made available to you, Dr Drumright and Dr Agrawal? If other data from that hospital was available, what was it?

This data in section 8.1.2 of the report is in the published literature, as referenced in the report. It is not the data referred to in the letter of instruction to Dr Drumright of 8 February 2024. The data referred to in the letter of instruction to Dr Drumright was not, in fact, used (nor even looked at) for the Report.

The remaining questions are for Professor Hawkey, Dr Agrawal and Dr Drumright.

C. Other involvement with or about the QUEH/RHC

4) The copy of the Report provided to the Inquiry is identified as version 1.7. In what way did the report develop in draft from version 1.1 to version 1.7 and why were these changes made by you and your fellow authors? Please provide the Inquiry with a copy of each version 1.1 to 1.6.

Versioning of reports, manuscripts and other documents is a standard procedure in scientific inquiry that helps keep everyone working off the same document and prevents confusion. It is standard scientific behaviour to share early versions of analyses, literature reviews and interpretation of findings and then meet to discuss. This was the purpose of our document versioning. Please not that Inquiry team member, [redacted] confirmed by email on 28/03/2025 that we did not need to provide every version of the report.

5) Have you received any other instructions from representatives of NHS GGC or their solicitors and counsel to consider issues, to give advice, provide reports or to issue an opinion that are in any way related to the QUEH/RHC? If the answer is yes, please provide details including dates upon which such advice was given.

SA: Yes. I was asked to produce a report for Greater Glasgow Health Board (GGHB) with respect to an Improvement Notice (IN) that was served on them by the Health and Safety Executive (HSE) in December 2020. The IN alleged the GGHB, “failed to ensure, so far is reasonably practicable, that the ventilation system within Ward 4C [of QEUH] is suitable and sufficient to ensure that high risk patients who are vulnerable to infection are protected from exposure to potentially harmful airborne microbiological organisms”. The GGHB had appealed. My report addressed the issues of ventilation, the risk of infection in highly vulnerable patients, and the observed infection rates on 4C. My report (from May 2021) is already included in the evidence submitted to the SHI.

PMH: I was initially approached by Prof Leonard. No further instructions after contact with AR.

LD: No.

6) Have you produced any other reports, opinions or written advice for NHS GGC about issues that relate to the QUEH/RHC? What subjects were addressed, when were they instructed and when did you produce any such reports, opinions or written advice?

SA: See answer to (5).

PMH: No.

LD: No

D. Information considered by you

7) In the Report you list at Item 10 a total of 101 documents to which you and your colleagues have referred to within the report.

a) What criteria did you use in selecting journal articles for citation and then citing them at various points in the body of the report?

Literature review is a standard scientific process. It is a comprehensive, critical summary of existing research on a particular question. It involves systematically searching for, evaluating, and synthesizing scholarly

information, including journal articles, books, conference abstracts, grey literature, and other academic information to understand the current state of knowledge in the field. Normally, scientists start by using one or more search engines, such as PubMed, Google Scholar, Scopus, etc., to identify articles, books and abstracts to review. They will read these and continue to search cited documents in the references of these papers to expand their search. They will also search the grey literature, such as government white papers and other documents that may exist. It is an iterative process with the objective of identifying what is known and what gaps exist in knowledge regarding the topic of interest. This approach was used. Any abstract, manuscript, report, etc. that provided sufficient information on the subject was included and cited as a standard scientific approach.

b) For what reason do you cite journal articles that relate to particular factual circumstances that may, or may not, be comparable to the circumstances in the QUEH from 2015 to 2020?

SA: I am not clear as to what this question is alluding to? The scientific literature was used to provide evidence to support specific points, to provide context, to provide expected rates of infection etc. Of course, no two hospitals can have the same circumstances. However, without comparative data, it would be impossible to assess if the infection rates at QEUH represent an increase, a decrease, or the expected rates of infection in these at-risk populations.

PMH: I agree I carried out a standard literature search which was informed as we analysed the data.

LD: This question is unclear. The information provided in the chapters including literature review sets the background of our knowledge for the topic area. For example, if one is worried about the hospital environment increasing risk of bloodstream infection among patients, then information on which organisms can survive in the environment and subsequently colonize a patient, risk factors for developing bloodstream infections in different patient groups, previous observations of this occurring, etc. are relevant pieces of information to support a scientific inquiry into whether or not a given patient population in

a given hospital could have acquired microorganisms that were present in the environment, become colonized and subsequently ended up with a bloodstream infection. Without this background, there would be no biological plausibility in which to examine existing data or justify the role of the environment in risk of a bloodstream infection among patients.

c) Have you considered any other information in preparing the Report? If, yes please describe this information at the end of this questionnaire and the use you made of it.

SA: Not that I am aware of.

PMH: No.

LD: No

d) Is the report by Dr Chaput referenced at Item 13 on that list the report that can be found within the Inquiry's hearing bundle, Bundle 18, Volume 1, Document 2.

SA: Yes - as far as I can ascertain.

PMH: I agree.

LD: It appears to be the same report, however, given that we are no longer accessing the Teams site set up by NHS GGC, we cannot confirm that these two reports are identical.

e) Is the HPS report reference at Item 41 the report that can be found within the Inquiry's hearing bundle, Bundle 18, Volume 1, Document 11, Page 819.

SA: Yes - as far as I can ascertain.

PMH: I agree.

LD: It appears to be the same report, however, given that we are no longer accessing the Teams site set up by NHS GGC, we cannot confirm that these two reports are identical

f) Do you still have access to the Microsoft Teams site said (in the letter of instruction to Dr Drumright date 8 February 2024[4]) to contain "The relevant documentation and data")? What is contained on that site?

SA: We were all provided access by the CLO as a secure platform for documentation. I cannot know when I last accessed the site, but I have certainly not accessed this site after June 2024. The contents of the site are in the hands of the CLO.

PMH: I agree.

LD: During the period of time that we were working on our report we had access to this Teams site. No screen shot or other factual documentation confirming what was made available on this site is available, as the information provided were considered confidential information and properly handled as such. I have not tried to access this site since submission of the report in June 2024. As we have now signed the Novation Agreement, trying to access the site is now is likely to be a violation of the Novation agreement. Therefore, we cannot confirm what was on this site previously or currently or whether or not we can access the site. The NHS GGC (or CLO) is likely to be able to answer this question for you accurately.

E. The decision to consider the work of the Case Notes Review

8) The letter of instruction dated 21 November 2022 makes no reference to the Case Notes Review and the extent to which it might be of assistance to the Inquiry in determining whether the built environment at the QEUH and RHC posed an increased risk of infection, yet this appear to be a significant part of the scope of the report. When was it first proposed that such a consideration be part of the scope of the report? Who suggested that consideration and is it set out in any letter of instruction or email from NHS GGC or their solicitors that you can produce?

[4] Bundle 44, Vol 1, Document 5, page 244.

SA: I am not sure what this question is alluding to? All documentation provided to us were from the CLO on behalf of the NHS GGC, including the Case Notes Review (CNR). The documentation was added to over time and, with that, our approach to the remit we had been given also evolved. The CNR is an in-depth analysis of infections in the paediatric unit on a case by case basis. Each of us found, independently, significant causes for concern about the CNR's findings. Addressing this is fundamental to our report, as it highlights why our findings differ dramatically from those of the CNR.

PMH: I agree, for me as a microbiologist this was a critical analysis, although as mentioned in our report, the methodology of the CNR is not clear.

LD: The Case Note Review is indeed mentioned in the first paragraph of the instructions in the letter dated 21 November 2022, "All of these infections have been the subject of intensive investigations, both by NHS GCC, and by external bodies (such as HPS/ARHAI and the Case Note Review)". The purpose of the instruction as stated in the letter was "to consider the question of risk, specifically whether there is objective evidence/data that would support the proposition that since the QEUH & RHC opened in 2015 patients were exposed to a greater risk of infection than would be expected due to a widespread issue with the water and/or ventilations systems.". It is therefore logical to review and comment on documents mentioned, including the Case Note Review, as it pertains to addressing the purpose of the instruction. This is what was done and prior investigations that were made available were reviewed and commented on as appropriate. The Case Note Review was available when I started work on the report.

9) Chapter 3 of the report is entitled "Clinical significance and epidemiology of the most common groups of presumed environmental bacteria selected for inclusion by the CNR" (page 18).

a) What is the purpose of this chapter? Are you proposing that the CNR should have not considered certain bacteria in their work?

PMH: No, as the main author that is not my intention. The CNR authors have grouped a very diverse collection of species of Gram negative bacteria (GNB)

together as most likely to be derived from the environment. As an expert who has worked and researched on GNB since 1980, I could see this as an erroneous assumption. In order to inform the inquiry of the nature of these bacteria, and their variable likelihood of coming from the ward environment, I wrote this section.

SA: As a haemato-oncologist managing adults with haematological cancers, I was concerned at the assumption of the CNR of an environmental source for some of the infections. There appeared to be a lack of consideration of the patient's own gut and skin micro-organisms as the source of infections, although these sites represent the main sources for the vast majority of GNB infections.

LD: I agree – no additional comments.

b) Given the purpose and scope of the Case Notes Review as set out in Section 2.2 of the CNR Overview Report (Bundle 6, Document 38 at page 1000) how do you think the patient cohort used by the CNR been selected?

PMH: The overall cohort is as I understand it those patients with bacteremia caused by GNB in the time period of interest. What is not explained is the methodology used to, with varying degrees of certainty, categorise them as coming from the environment. For instance, the CNR highlights Stenotrophomonas maltophilia (SM) as one of the more common species causing bacteremia and attributes many of the infections to an environmental source. They have ignored the work of Aitken et al. 2021 which elegantly demonstrates that in their leukemic patients with a high rate of infection caused by SM, it was the use of carbapenem antibiotics causing oral colonisation and subsequent infection rather than discrete environmental sources. My own work [Denton et al 1998] with this bacterium in cystic fibrosis patients showed that exposure to water in the home is just as likely to result in colonisation with SM. One of the periods with increased isolation of SM coincided with an increase in the use of carbapenems (due to lack of availability of piperacillin-tazobactam experienced by many units). My own haem/oncology unit in Birmingham followed this path and soon had a

significant increase in SM infections. We restricted carbapenems and the problem resolved. There was a similar episode at that time in Glasgow, yet the CNR failed to consider this as the most likely cause.

SA: I agree.

LD: I agree.

F. The water and ventilation systems at the QEUH/RHC and the IPC response to potentially HAIs/HCAIs in the Schiehallion Unit

10) Specifically, have you been provided with or have considered any of the following documents or reports about the water or ventilation systems of the QEUH/RHC? If you have considered any of the following, please set out who supplied them to you, when you considered them, what relevance they had to the issues addressed in the Report and for what purpose they were supplied to you?

a) The DMA Canyon 2015 L8 Risk Assessment (Bundle 6, Document 29, Page 122)

This was not within the scope of our remit.

b) The DMA Canyon 2017 L8 Risk Assessment (Bundle 6, Document 30, Page 416)

This was not within the scope of our remit.

c) Minutes of the NHS GGC Water Technical Group on 13 April 2018 and 20 April 2018 (Bundle 10, Document 2, Page 9 and Document 3, Page 14)

This was not within the scope of our remit.

d) Notes of the Water Incident Debrief meeting on 15 May 2018 (Bundle 14, Volume 2, Page 211)

This was not within the scope of our remit.

e) Full Incident Management Team Report covering the IMTs from 2 March 2018 to 13 April 2018 dated 5 June 2018 (Bundle 27, Volume 5, Document 19, Page 46

This was not within the scope of our remit.

f) Legionella Control Authorising Engineer Audit – QUEH - 23 July 2018 (Bundle 18 Volume 2, Document112 at page 909)

This was not within the scope of our remit.

g) Report by Innovated Design Solutions, Feasibility Study Regarding Increasing Ventilation Air Change Rates within Ward 2B, 15 October 2018 (Bundle 6, Document 33, Page 656)

This was not within the scope of our remit.

h) Report by Innovated Design Solutions, Feasibility Study Regarding Increasing Ventilation Air Change Rates within Ward 2A, 24 October 2018 (Bundle 6, Document 34, Page 674)

This was not within the scope of our remit.

i) SBAR which was used to brief the Chair of NHS GGC, Professor Brown, on or about 13 November 2018 (Bundle 4, Document 32, Page 133)

This was not within the scope of our remit.

j) The NHS GGC Review of Issues Relating to the Hospital Water Systems' Risk Assessment (Bundle 8, Documents 34 to 40, Page 150)

This was not within the scope of our remit.

k) The HFS Water Management Issues Technical Review, March 2019 (Bundle 7, Document 4, Page 70)

This was not within the scope of our remit.

l) Legionella Control Authorising Engineer Audit – QUEH - 30 and 31 January 2020 (Bundle 18, Volume 2, Document 125, Page 1355)

This was not within the scope of our remit.

m) Legionella Control Authorising Engineer Audit – QUEH - 4 and 5 February 2021 (Bundle 18, Volume 2, Document 126, Page 1402)

This was not within the scope of our remit.

n) Legionella Control Authorising Engineer Audit – QUEH - 28 February and 1 March 2022 (Bundle 18, Volume 2, Document 124, Page 1335)

This was not within the scope of our remit.

o) Legionella Control Authorising Engineer Audit – QUEH - 11 January 2023 (Bundle 15, Document 45, Page 1226)

This was not within the scope of our remit.

p) Legionella Control Authorising Engineer Audit – QUEH - 11 January 2024 (Bundle 27, Volume 1, Document18 at page 252)

This was not within the scope of our remit.

11) Specifically, have you been provided with or have considered any of the following presentations by Dr Chaput about water testing results at the QEUH/RHC? If you have considered any of the following, please set out who supplied them to you, when you considered them and for what purpose?

a) Presentation by Dr Chaput, RHC Ward 2A/2B Water Test Results, 28 March 2023 (Bundle 18, Volume 2, Document 117, Page 1030).

Yes, we did consider this for our report to inform our understanding. This was supplied from the NHS GGC CLO.

b) Presentation by Dr Chaput, RHC Ward 2A/2B Water Test Results, 8 February 2022 (Bundle 18, Volume 2, Document 118, Page 1040).

Yes, we did consider this for our report to inform our understanding. This was supplied from the NHS GGC CLO.

c) Presentation by Dr Chaput, RHC Ward 2A/2B Water Test Results, 1 March 2023 (Bundle 18, Volume 2, Document 119, Page 1063).

Yes, we did consider this for our report to inform our understanding. This was supplied from the NHS GGC CLO.

d) Presentation by Dr Chaput, RHC Ward 2A/2B Water Test Results, 18 February 2022 (Bundle 18, Volume 2, Document 120, Page 1073).

Yes, we did consider this for our report to inform our understanding. This was supplied from the NHS GGC CLO

12) To what extent have you been provided with or have you become aware of details of the design, structure, specifications and management systems of the water

system of the QEUH/RHC as it stood at the following points of time and what were you told and when were you told it?

a) At handover on 26 January 2015?

This was not within the scope of our remit.

b) At the start of the 'Water Incident' on 1 March 2018?

This was not within the scope of our remit.

c) At the time the adult BMT patients returned to Ward 4B in the QUEH on 30 June 2018?

This was not within the scope of our remit.

d) At the time of the 'decant' from Wards 2A/2B RHC to Wards 6A/4B in the QEUH on 26 September 2018?

This was not within the scope of our remit.

e) At the point that Ward 6A was closed to new admissions in August 2019?

This was not within the scope of our remit.

13) To what extent have you been provided with or have you become aware of details of the design, structure, specifications and management systems of the ventilation system of the QEUH/RHC as it stood at the following points of time and what were you told?

a) At handover on 26 January 2015?

This was not within the scope of our remit.

b) At the start of 2018?

This was not within the scope of our remit.

c) At the time the adult BMT patients returned to Ward 4B in the QUEH on 30 June 2018?

This was not within the scope of our remit.

d) At the point that Ward 6A was closed to new admissions in August 2019?

This was not within the scope of our remit.

14) What knowledge do you have about the experience of NHS GGC haemato-oncology clinicians of potential HAIs and HCAIs in the Schiehallion Unit in Yorkhill and then RHC from 2005 to 2022?

We do not have any knowledge of the experience of clinicians in the Schiehallion Unit in Yorkhill and then RHC from 2005 to 2022.

15) What knowledge do you have about the experience of NHS GGC ICNs, ICDs and microbiologists of potential HAIs and HCAIs in the Schiehallion Unit in Yorkhill and then RHC from 2005 to 2022

We do not have any knowledge of the experience of ICNs, ICDs and microbiologists in the Schiehallion Unit in Yorkhill and then RHC from 2005 to 2022.

G. Contemporaneous Epidemiology

16) Specifically, have you been provided with or have considered any of the following documents or reports that consider the epidemiology of infections at the QEUH/RHC from 2015 to 2019? If you have considered any of the following, please set out who supplied them to you, when you considered them, what relevance you consider they have to your instructions and why they were provided to you?

a) The HPS Situational Assessment, Wards 2A/B, Royal Hospital for Children, NHS Greater Glasgow and Clyde (Bundle 7, Document 5, Page 194) and, in particular, Appendix 4 to that document (Bundle 7, Document 5, Page 205).

It was not in our instructions to review and critique the management of infection control.

b) The HPS SBAR: To support NHS GGC IMT Mycobacterium chelonae cases and the Incidence of gram-negative bacteraemia in the paediatric haemato-oncology, September 2019 (Bundle 3, Document 16, Page 127).

It was not in our instructions to review and critique the management of infection control.

c) Draft HPS Review of NHS GG&C Infection Outbreaks in the Paediatric

Haemato-oncology Data October 2019 (Bundle 7, Document 6, Page 214)

It was not in our instructions to review and critique the management of infection control.

d) HPS Review of NHS GG&C Infection Outbreaks in the Paediatric Haemato-oncology Data October 2019 - 29 November 2019 (Bundle 7, Document 7, Page 250).

It was not in our instructions to review and critique the management of infection control.

e) Presentation by Kathleen Harvey-Wood and Dr Christine Peters: Bacteraemia rates and Resistance Paediatric Haemato-oncology 2014- 2018, 30 August 2018 (Bundle 27 Volume 6, Document 9, page 107).

It was not in our instructions to review and critique the management of infection control.

f) Report by Dr Iain Kennedy: Descriptive Analysis of Trends in Bacteraemia Rates for Selected Gram-Negative Organisms, 1 October 2018 (Bundle 6, Document 27, page 95).

It was not in our instructions to review and critique the management of infection control.

g) Draft report by C Peters and K Harvey-Wood: Bacteraemia rates and resistance patterns in paediatric haematology/oncology patients 2014- 2018, 10 October 2018 (Bundle 19, Document 19, Page 143).

It was not in our instructions to review and critique the management of infection control.

h) Report by Dr Iain Kennedy: Descriptive analysis of trends in bacteraemia rates for selected gram-negative organisms, July 2019 (Bundle 6, Document 28, page 104)

It was not in our instructions to review and critique the management of infection control.

i) Presentation by Dr Iain Kennedy and Jennifer Rodgers: Paediatric Haemato-oncology RHC – Summary of Data, September 2019 - Presented at IMT

meeting of 20 September 2019 (Bundle 27, Volume 13, Document 13, Page 77)

It was not in our instructions to review and critique the management of infection control.

H. Water

17) The Inquiry has heard a large amount of evidence on the question of homogeneity or polymicrobial nature of biofilms in water in the context of WGS and to that it would assist if you can further justify your statement at the end of Section 5.3.2 that *"In our experience, when clusters of infection occur caused by potentially environmentally sourced bacteria in immune supressed patients, investigation with detailed typing very often reveals a homogeneous cluster of strains and helps tremendously in identifying the source and introducing control measures"* If you can referred to published studies that would be of great assistance.

There is an extensive literature, which will require a review to be supplied shortly by Prof Hawkey.

I. Ventilation

18) At page 51 you discuss the specifications required in HTM 03-01, Part A for "wards with neutropenic patients'. From your knowledge of the QEUH/RHC which wards in the adult and childrens' hospital can properly be described as "wards with neutropenic patients' in that context?

Wards 2A, 2B, 4B, 4C and 6A – ie, paediatric and adults wards principally caring for haemato-oncology patients.

J. Numbers of potentially significant infections amongst paediatric patients

19) Is a reader correct to understand that Tables 5A to 8 list all of the identified bacteraemia for the listed species/genera amongst all adult hospitals in NHS GGC or only in respect of patients in the care of the South, North or BMT sectors?

Data including South, North and BMT sectors were provided for analyses of adult patients. No data on adult patients was provided for other GGC sectors. See list of hospitals included in #27. Please note that Tables 5A through 8 list only bacteraemia attributed to microorganisms that were consider environmentally relevant.

20) Is a reader correct to understand that Tables 11A to 11F, 12, 13, 14 of the Report list all bacteraemia for each of the listed species/genera amongst paediatric patients between January 2005 to December 2022 and that is the broad equivalent of Tables 4.2 and 4.3 of the Case Notes Review Overview Report (Bundle 6, Document 38 at page 1028)?

"Broadly equivalent" is a very general term that is not normally used in epidemiology or scientific study. The data included in Tables 11A-11F, 12, 13, and 14 of our report include all data on bloodstream infections/bacteraemia downloaded from GGC databases for the Yorkhill and QEUH RCH between 2005 and 2022. The data contained in Tables 4.2 and 4.3 could very well be a subset of these data, but it is difficult to determine from the Case Note Review as there is not a clear indication of where the datasets came from. Additionally, it appears that there are more cases of certain organisms in the Case Note Review then in the longer time period including the Yorkhill hospital in addition to the QEUH RCH, for example with Pseudomonas putida, suggesting either a different set of data were used or a different case definition was used.

K. Background rates:

21) In respect of each of the following bacteria to what extent would you agree with the statement that "there is no background rate for these organisms" amongst the cohort of paediatric haemato-oncology patients treated by the Schiehallion Unit:

We are not clear what counsel means by "background rate".

a) Achromobacter xylosoxidans,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I cannot comment on it for all organisms in question. We did observe one case of bacteraemia attributed to Achromobacter xylosoxidans in May 2010 at Yorkhill in the Schiehallion Ward based on the data provided to us.

b) Acinetobacters Iwoffi,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I

cannot comment on it for all organisms in question. We did observe two cases of bacteraemia attributed to Acinetobacters Iwoffi in Feb 2005 and Sept 2006 2010 at Yorkhill in the Schiehallion Ward based on the data provided to us.

c) Burkholderia cepacia in general,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were numerous cases of bacteraemia attributed to Burkholderia cepacian in the Schiehallion Ward, Schiehallion DCU and Ward 5A between 2005 and 2014 at Yorkhill and two cases, in May 2017 and June 2018 in Ward 2A at QEUH.

d) Burkholderia cepacia (other than in Cystic Fibrosis patients),

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. As above, there were numerous cases. Additionally, no data on the cystic fibrosis status of the patients was provided, therefore I cannot provide numbers of cases stratified by cystic fibrosis.

e) Cedecea lapagei,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. This organism was not reported as being isolated in any paediatric blood sample in the data provided.

f) Chryseobacterium indologenes,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were 6 cases of bacteraemia attributed to Chryseobacterium indologenes, in Yorkhill, 4 in the Schiehallion Ward, 1 in Schiehallion DCU and 1 in Ward 5A between 2005 and 2014, as well as two cases, in May 2017 and June 2018 in Ward 2A at QEUH.

g) Commamonas testosterone,

This is not a bacterium that exists. Perhaps you mean Comamonas testosteroni? There were no bacteraemia cases of Comamonas among paediatric cases in our dataset. This is not the same, however, as stating that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients".

h) Cupriavidus gilardii,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Cupriavidus gilardii, over the time period of the data.

i) Cupriavidus pauculus,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I cannot comment on it for all organisms in question. There were 3 cases of bacteraemia attributed to Cupriavidus pauculus over the time period of the data. One occurred in Yorkhill in the Schiehallion DCU in Mar 2012, one in Ward 2B Day Surgery in QUEH in Sept 2017, and one in QEUH Ward 3C in Jan 2018.

j) Delftia acidovorans,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I cannot comment on it for all organisms in question. In the dataset provided there was 1 case of bacteraemia attributed to Delftia acidovorans in Yorkhill in the Schiehallion DCU in Feb 2013 and 4 cases in 4 different wards at QEUH between 2017 and 2020 occurring 4 or more months apart.

k) Elizabethkingia meningospetica,

The statement that "there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients" is unclear and imprecise, so I cannot comment on it for all organisms in question. Three cases of

bacteraemia attributed to Elizabethkingia meningospetica occurred in Yorkhill in 2014, one per month from May-July on the Schiehallion Ward and DCU. Three cases of bacteraemia attributed to Elizabethkingia meningospetica also occurred in QEUH over the time period of the data provided, one occurred in Sept of 2016 on Ward 1B Day Surgery and the other two occurred in Dec 2016 and Jan 2017 on Ward 2A.

l) Pantoea agglomerans,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. Three cases of bacteraemia attributed to Pantoea agglomerans occurred at Yorkhill, one each in Nov and Dec of 2005 on the Schiehallion Ward and OPs and one in Feb 2014 on the Schiehallion Ward.

m) Paracoccus sp,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Paracoccus spp. over the time period of the data.

n) Pseudomonas chlororaphis,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Pseudomonas chlororaphis over the time period of the data.

o) Pseudomonas fluorescens,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There was one case of

bacteraemia attributed to Pseudomonas fluorescens at Yorkhill in the Schiehallion Ward in Oct 2007.

p) Pseudomonas oryzihabitans,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Pseudomonas oryzihabitans over the time period of the data.

q) Pseudomonas putida,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were three cases of bacteraemia attributed to Pseudomonas putida in the Yorkhill Schiehallion Ward over the time period 2 in 2007 and 1 in 2013. There were 6 cases of bacteraemia attributed to Pseudomonas putida in the QEUH RCH occurring between 2015 and 2019 on Ward 2A and the Emergency Department.

r) Pseudoxanthomonas Mexicana,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Pseudomonas mexicana over the time period of the data.

s) Ralstonia picketii,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There was one case of bacteraemia attributed to Ralstonia pickettii in Yorkhill on the Schiehallion Ward in Oct 2007.

t) Rhizobium radiobacter,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were 8 cases of bacteraemia attributed to Rhizobium radiobacter at Yorkhill, all occurring on the Schiehallion Ward, with the exception of one occuring on Schiehallion DCU, from 2007-2014. One case of bacteraemia attributed to Rhizobium radiobacter occurred in QEUH RCH on Ward 2A in Nov 2016.

u) Serratia fonticola,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Serratia fonticola over the time period of the data.

v) Shewanella puterfaciens,

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were no cases of bacteraemia attributed to Shewanella puterfaciens over the time period of the data.

w) Sphingomonas species

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were three cases of bacteraemia attributed to Sphingomonas spp. in the data over this time period, two at Yorkhill and one at QEUH, all in different years on different wards.

x) Stenotrophomonas maltophilia

The statement that “there is no background rate of these organisms in the cohort of paediatric haemato-oncology patients” is unclear and imprecise, so I cannot comment on it for all organisms in question. There were numerous

bacteraemias attributed to Stenotrophomonas maltophilia at both Yorkhill and QEUH, many occurring on the Schiehallion Ward. This was not surprising as this is a more commonly observed bloodstream infection in vulnerable patient populations.

L. Assessment of Clustering

22) In the parts of the report that discuss a qualitative assessment of clustering what information did you have access to about the events that surround each identified bacteraemia beyond the information contained within the relevant table and the WGS work of Professor Evans, Professor Leonord and Dr Brown?

The remit of this report was to focus on existing data and information that could provide new evidence that infections in patients at QEUH could be attributed to environmental contamination. There was some interest in trying to determine if cases clustered. The presence of two or more patients on the same ward at the same with a bacteraemia with the same microorganism is not sufficient evidence that the two infections are related. Even detecting the same species of organism in a ward provides no evidence that there is a connection between the environment and a bloodstream infection in a patient. Sequencing information is the only way to determine if two microorganisms of the same species are genetically related, and therefore potentially transmitted between people or the environment and people. We therefore asked if there were sequencing data available to help with this inquiry. Other data, such as case notes, were not used as they could not add additional objective information to determine if a cluster existed.

23) Did you have access to IMT or PAG Minutes, reports to the IPC SMT, AICC, BICC or to HPS/ARHAI or the relevant medical records from which you could draw further information about the potential for an epidemiological association between particular bacteraemia?

We were not instructed to analyse the decisions of the infection control staff.

M. Rate of infections per 1,000 bed days

24) You appear to have reported Bed Days by sector for adult patients at Table 4 of the Report (page 66). You describe in section 7.2.7 being supplied with data on bed days from Yorkhill and RHC from January 2005 to December 2022. Where

in the Report is the equivalent table to Table 4 for the paediatric patient population? If it is not in the report, please supply it.

This will be supplied as discussed on the teleconference on 28 March 2025.

25) What is your understanding of the nature of the patient cohort in each of the 'South', 'North' and 'BMT' consultant sectors for which you have reported bed days in Table 4 (page 66)? It would be of assistance if you can identify which wards in which hospitals these groups were patients in and which consultants treated each group.

For all consultant sectors, BMT, North and South, these were predominantly haemato-oncology patients. For these patients with detected bacteraemia, 31 different consultants were on the record for ordering the blood cultures and patients were on 98 different wards when the cultures were ordered. Please see Tables 1 and 2 at the bottom of this questionnaire for the consultants whose names appear on the blood culture orders and which wards the patients were on at the time of the order.

26) For clarity, to what extent do Figures 4 to 8 show bacteraemia incidence only for adult haemato-oncology patients or all patients or some other group of patients?

It is our understanding that patients in the BMT, North, and South are haemato-oncology patients. Only patients from BMT, North and South are included in the data that we analysed and therefore, figures 4 to 8 reflect this patient population.

27) What are the "Other Hospitals" that produce the red lines in Figures 4 to 8? Which hospitals or which wards or services within those hospitals are encompassed within the category of "Other Hospitals"?

The other hospitals encompass BMT, North, and South sectors and include the following hospitals: Beatson West of Scotland Cancer Center, Gartnavel General Hospital, Glasgow Royal infirmary, Inverclyde Royal Hospital, Royal Alexandra Hospital, Stobhill Hospital, Vale of Leven Hospital, New Victory Hospital and West of Glasgow Ambulatory Care Hospital. Please see Table 2 for wards from which blood samples were sent and had a bacteraemia detected.

28) Does the category "QEUH" that produces the blue lines in Figures 4 to 8 encompass the whole of the QUEH and all patients there in or is it limited to a

particular subset of patients, wards of services within the QUEH? If the latter, please provide details.

The QEUH patients are only those from BMT and South sectors when they were located at QEUH as follows: the South sector became QEUH in May 2015 and remained part of QEUH through 2023 and BMT became part of QEUH for a period of approximately one and a half months across the months of June and July in 2015 and then from June 2018 onward.

29) In section 7.2.3 on page 70 you state in respect of certain microorganisms that *"none of these organisms appeared at unexpected incidence by consultant sector or hospital"*. What is the expected incidence of Achromobacter spp., Aeromonas spp., Brevundimonas spp., Burkholderia spp., Cellulosimicrobium spp., Chryseobacterium spp., and Chryseomonas spp. amongst these patients?

Haemato-oncology patients are a very vulnerable population who frequently experience immune compromised states and are considered at high risk for bloodstream infections and bacteraemia. While we often see more cases of bacteraemia in these patients attributed to Escherichia spp., Klebsiella spp., Pseudomonas spp., Enterobacter spp., Enterococcus spp., Staphylococcus spp., Stenotrophomonas spp., Streptococcus spp., and Acinetobacter spp. among others, it is not surprising to see isolation of less common species such as Achromobacter spp., Aeromonas spp., Brevundimonas spp., Burkholderia spp., Cellulosimicrobium spp., Chryseobacterium spp., and Chryseomonas spp. and others among these patients given how susceptible to bacteraemia these patients are. Among these less common species of bacteria that are grown in blood culture, we would not be surprised to see a few cases over a prolonged period of time across multiple hospitals or wards; which is what was observed in the dataset. What would be of concern is if we observed a sufficient number over a small period of time, or even 5-10 in one year in a given setting, such as a single ward, as we expect to see these microorganisms infrequently attributable to bacteraemias.

30) The Inquiry has heard evidence of number of attempts made in 2018 and 2019 to consider the epidemiology of infections amongst the paediatric haemato-oncology patients in the RHC. Whilst noting that your category "*micro-organism species of environmental concern"* as defined in Table 1 of the Report differs to

some extent from the category "*Environmental Including Enteric (ENT) Group"* in the October 2019 HPS Review of NHS GG&C Infection Outbreaks in the Paediatric Haemato-oncology Data October 2019 (Bundle 7, Document 6 at Page 219) it seems to be the case that in that report (see Figure 6 at Page 230) the 'Rate of infections per 1,000 bed days' ranges between zero and seven infections per 1,000 bed days and yet the rate of infections shown in Figure 22 of your Report appears to range zero to seventeen infections per 1,000 bed days over a comparable period. Can you assist the Inquiry in why there might be such a difference in the rate of infections between the two reports?

There could be numerous reasons for these differences such as different data sources for the bacteraemia/bloodstream infections (numerator) or bed days (denominator); differences in case definitions; or some property of the SPC charts, which are not clearly described in the HPS Review to name a few. It is possible to determine the exact reason with discussion between those who prepared the HPS Review and us and with additional information from the CLO and those who extracted data the HPS Review on exactly what databases numerators and denominators were extracted from and how.

31) Your report does not provide the tables setting out the BSI rates per 1,000 bed days used to construct the various figures and how these were calculated by reference to numbers of BSIs and the denominator used in each case. Please provide these to the Inquiry.

We are happy to provide these and will do so in the request bundle to come on 11 April 2025, as indicated in the email from Kirsten McMillan dated 28 March 2025.

32) At a number of places within the report you discuss "spikes" or "occasional spikes" in incidence of bacteraemia (see Pages 68, 69, 80 and 82) and a number of what appear to be spikes appear at both Yorkhill and the RHC in Figure 22. To what extent is it valid to consider whether such spikes were noted by treating clinicians or members of the IPC team and if so how would they be aware of such changes in the incidence of bacteraemia at the time?

Trends in data, such as these, are very easy to observe when plotting data after it has been collected. It is much more difficult to determine what is happening in real-time, even when data are being continuously plotted. There is a huge area of research focused on detection of data anomalies in real time and numerous

algorithms have been used to determine if an increase in the number of cases of a given condition are a signal for an anomaly. This type of work is carried out by people with specialist training in very specific and complex statistical modelling and is usually led by people with a PhD with expertise specifically in this area of research. It is unfair to presume that treating physicians would or should have this skillset, and the time required to collate, clean and analyse data would not be efficient use of a physician's time. It is unlikely that physicians would notice periodic increases in number of cases of bloodstream infection or bacteraemias, especially as patient confidentiality dictates that providers focus on results from the patients who they are treating and do not review notes of patients that they are not treating unless there is a clinical reason to do so. Hospitals could employ specialists with skills to model their data to identify increased numbers of cases, but in the NHS, it would only be recommended if there was a significant cost benefit. As bacteraemia/ bloodstream infections are not transmissible in an "outbreak" sense, there may be little gain for the cost of employing someone to do this job.

N. Whole Genome Sequencing ("WGS")

33) The Report discusses the use of WGS at Section 7.3 which is entitled "*What does the available sequency show?"* and in Table 15A-15C. At page 96 reference is made to *"Report on Enterobacter Related Infection by Professor T Evans & Whole Genome Sequencing Report by Prof Leanord*". There are further references to Professor Evans at pages 76, 95, 117, 118 and yet no reports or papers by Professor Evans or Professor Leonard are listed in Chapter 10. To what extent are these references to the following reports or are you referring to other reports or papers?

a) Report by Professor Alistair Leanord and Doctor Derek Brown titled "Application of Whole Genome Sequencing to Identify Relationships..." Dated 18 January 2023 (Bundle 6, Document 40, Page 1195).

PMH: I find searching your bundles very difficult. The reports listed below have the same titles as those supplied to me by CLO. If necessary, I can send the PDFs I worked from.

b) The Report on Cupriavidus Related infections by Professor T Evans dated March 2023 (Bundle 8, Document 44, Page 223).

PMH: I find searching your bundles very difficult. The reports listed below have the same titles as those supplied to me by CLO. If necessary, I can send the PDFs I worked from

c) The Report on Enterobacter related infections by professor T Evans dated March 2023 (Bundle 8, Document 45, Page 230).

PMH: I find searching your bundles very difficult. The reports listed below have the same titles as those supplied to me by CLO. If necessary, I can send the PDFs I worked from.

d) The Report on Stenotrophomonas Infections by Professor T Evans dated March 2023 (Bundle 8, Document 46, Page 239)

PMH: I find searching your bundles very difficult. The reports listed below have the same titles as those supplied to me by CLO. If necessary, I can send the PDFs I worked from.

34) Have you had access to any other reports or data describing results of WGS carried out to samples taken from patients or the environment at the QEUH or RHC after 26 January 2015? What reports or data are these?

PMH: none

O. Comparison between hospitals

35) Your Report refers to comparison to “other hospitals” and lists infections at some other NHS GGC hospitals in Tables 5 to 8. Which hospital are these and on what basis do you consider it appropriate to compare rates of infection in adult patients in those hospitals with rates of infection in adult patients in the QEUH?

Please see question #27 for hospitals included. The comparison was considered appropriate as the patient population was the same, they were all haemato-oncology patients.

36) At the end of section 7.2.2 you explain your intention to compare rates of infection in paediatric patients at Yorkhill with rates of infection in paediatric patients at the RHC. On what basis do you consider it appropriate to compare these two hospitals in this manner?Lydia ? discuss what they are after?

Yorkhill was the primary haemato-oncology service for children in the GGC until June 2015 when the entire service moved to QEUH RHC. As this is the same service, treating the same patient population and the GGC has no other paediatric haemato-oncology service, it is the only comparator if one wants to determine if paediatric haemato-oncology patients in the GGC are experiencing a greater number of infection due to the new RHC setting. Without a comparator or regular and consistent whole genome sequencing of both the environment and microbial isolates from bloodstream infection there is no evidence that a given hospital is creating an elevated environmental risk for patient with respect to bloodstream infection.

P. Aspergillus

37) Chapter 8 of the Report is an *'analysis of infection rates and data from QEUH & RHC'.* A few questions arise:

a) Can you assist in explaining the nature of the patient cohort in each of the 'Adult South', 'Adult North' and 'Adult BMT' services and the extent to which their vulnerability or otherwise to Aspergillus infection is sufficiently similar so as to make such a comparison between them appropriate? It would be of assistance if you can identify which wards in which hospitals these groups were patients in and which consultants treated each group.

Adult BMT, Adult South and Adult North refer to the services treating haemato-oncology patients. All adult patients having a haematopoietic stem cell transplant (HSCT), were managed within the Adult BMT service. Figure 23 in chapter 8 shows the time periods when the adult BMT service was located at QEUH - the green horizontal bars at the top of the figure - and outside of these time periods, the Adult BMT service was located at the Beatson West of Scotland Cancer Centre.

As discussed in 6.1.4 of our report, those at greatest risk of *Aspergillus* infection are "… the group of patients with haematological, and other, cancers undergoing intensive chemotherapy and/or HSCT." Such patients were cared for in Adult BMT, Adult South and Adult North sectors.

b) At section 8.1.4 you explain that the variation over time between the period before and after the move to the RHC "is expected" by reference to "the

repeated relocation on the QUEH campus". The Inquiry is only aware of re-location to the RHC in June 2015, decant to Wards 6A/4B in September 2018 and a short term decant to the CDC in January 2019. What evidence do you have to support the statement that *"repeated relocation on the QUEH campus, with associated disruption, would be a risk factor of invasive aspergillosis"* in each of 2015, 2016, 2017, 2018 or 2019?

SA: I apologise if the text is confusing. I was referring to the same relocations the Inquiry is aware of – I am also unaware of any others. The movement of patients from one location to another disturbs their environment, particularly with respect to airborne particulate matter (ie, fungal spores). The movement of beds, equipment, any remedial building works, will all lead to an increased risk of infection.

The "expected" variation in rates of *Aspergillus* infection does not relate to the relocations. It seems the text has been misunderstood:

"This variation over time is expected. The repeated relocation on the QEUH campus, with the associated disruption, would be a risk factor for increased rates of invasive aspergillosis. However, there are no marked differences in case numbers by location…"

So, while the relocations would lead to increased risk of *Aspergillus* infections, this was not actually observed clinically.

c) In your Report at page 125 "*Table 20: Paediatric and adult cases with evidence of infection with Aspergillus species over time on the QEUH/RHC sites"* appears to contain no row for Ward 2A RHC or any other ward in the RHC. Why is that? What should such a row or rows show?

SA: Table 20 shows data for the periods of co-location of the paediatric and adult services – both BMT and non BMT data – during the period of co-location of services between 26/09/2018 to March 2022 on QEUH 4B and 6A. Since they were never co-located on any wards in RHC, there is no data and hence no rows corresponding to these locations. However, Table 19 shows the distribution of paediatric cases with evidence of infection with Aspergillus species by year and location in RHC (as well as QEUH).

Additional Information Considered

38) If you answered yes to question 7(c) please set out the other information you considered and the use you made of it.

Not applicable.

Table 1: Consultants whose names appear on blood culture orders for data from BMT, South and North Sectors between January 2013 and August 2023 (in alphabetical order by first name)

1	Alison Gibson
2	Alison McCaig
3	Alistair Hart
4	Andrew D Clark
5	Anne Louise Latif
6	Anne Morrison
7	Anne Parker
8	Catherine Bagot
9	Catherine Ogilvie
10	David Irvine
11	David McLaughlin
12	Gail Loudon
13	I Grant McQuaker
14	I Novitzky-Basso
15	Ian MacDonald
16	J Ryan Rodgers
17	Jennifer Laird
18	Jennifer Travers
19	L McLeod-Kennedy
20	Louisa McIlwaine
21	M Glen Rainey
22	Mark Drummond
23	Mark Rafferty
24	Matthew Wilson
25	Michael Leach
26	Pamela McKay
27	R Campbell Tait
28	Richard Soutar
29	Susan McNeill
30	Susan Rhodes
31	Ted Fitzsimons

Table 2: Wards of patients when the blood culture was ordered for data from BMT, South and North Sectors between January 2013 and August 2023 (in alphabetical order by ward name)

Ward Name (as it appears in the dataset)
A&E QEUH
A&E RAH
A&E VIC
ARU2 Respiratory QEUH
ARU3 Gastroenterol. QEUH
Adhoc VIC
B8 Haem Day Unit Beatson
B8/9 Day Unit Beatson
B9 OP Beatson
B9 OncologyDay Un Beatson
Beatson OP WIG
Beatson Unknown Location
Bone Marrow Transplant QEUH
Brownlee Cent Gen Med GGH
CAU Beatson
CCU Coronary CareUnt QEUH
CRF Clin Researc Fac QEUH
Clinic 1 QEB GRI
Clinic A ACH VIC
Clinic B 1st Fl ACH STO
Clinic B ACH VIC
Clinic C STO ACH
Clinic G 2nd Fl ACH STO
Clinic H ACH VIC
Clinic P ACH VIC
Clinical Res Unit Beatson
Coronar Care Un Crit QEUH
CritCar Un4 HDU31-40 QEUH
CritCar Un5 HDU41-49 QEUH
CritCar Un6 HDU50-59 QEUH
CritCare Un1 HDU1-10 QEUH
CritCare Un2 HDU1120 QEUH
CritCre Un3 HDU21-30 QEUH
Day Medical Unit VIC
Gastro Day Surg Unit GRI
General OP QEUH
General OP West Glasg ACH
HDU C19 60-79 QEUH
HDU QEUH
Haem Onco Secreta Beatson
Haem Oncol MedRec Beatson
Haematol Day Bed Unit RAH
Haematology Day Unit QEUH
Haematology Day Unit VIC
Haematology Day Ward VOL
Haematology OP GGH

Haemophilia Unit GRI
ITU Ward WIG
Level 8 Ortho WIG
Lomond Ward 6 VOL
MDUA Medical Day Unit STO
MacMillan DCU Beatson
Marie Curie Hospice
Medical Day Unit QEUH
Oncol. Assess St Beatson
Outpatients OPD Beatson
SATA GP Expects GRI
Stem Cell Laboratory GGH
Surgical ITU QEUH
Unknown Location GRI
Unknown Location SGH
Unknown Location WIG
Ward 11 GRI
Ward 18 RAH
Ward 2 RAH
Ward 24 SGH
Ward 2A GGH
Ward 2A QEUH
Ward 3 VOL
Ward 4 Lightburn Hosp
Ward 4A Ren. HighAcu QEUH
Ward 4B Haematology
Ward 4B1 BMT QEUH
Ward 4B2 QEUH
Ward 4C1H Haem QEUH
Ward 4CH Haem QEUH
Ward 5A Beatson High Acui
Ward 5B GGH
Ward 5B QEUH
Ward 5D QEUH
Ward 5D QEUH
Ward 6D QEUH
Ward 8B QEUH
Ward 8C QEUH
Ward 9B QEUH
Ward B L&Islands Hos
Ward B VIC
Ward B1 Beatson
Ward B2 Beatson
Ward B2A Beatson
Ward B3 Beatson
Ward B4 Beatson
Ward B5 Beatson
Ward B6 Beatson
Ward B7 Beatson
Ward B8 Beatson
Ward B9 Beatson
Ward I L&Islands Hosp

Inventory of Documents Provided by Dr Lydia Drumright and Dr Samir Agrawal in support of the GGC Expert (HAD) Report

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Dr Lydia Drumright

Doc No.	Document Name	Comments	Date From DD/MM/YYYY	Date to DD/MM/YYYY	Patient/Staff Identifiable Data
1	A52792290 - Responses to Data Query sent 11042024_v20250504	Included in this Bundle.		11/04/2025	No
2	A52792289 – Paeds Microorganism Detections Used	Not included in this Bundle as it contains patient identifiable information.			Yes
3	A52792293 – Figure 1 Data	Included in this Bundle.	2013	2022	No
4	A52792295 – Figure 2 Data	Included in this Bundle	2013	2023	No
5	A52792294 – Figure 4 Data	Included in this Bundle.	2013	2023	No
6	A52792291 – Paeds BSI Count Bed Days Rates	Included in this Bundle.	2005	2022	No
7	A52792292 – Paeds Bed Days	Included in this Bundle.	2013	2023	No
8	A52792296 – Paeds Micro Showing Deduplicated and 14-day drop	Not included in this Bundle as it contains patient identifiable information.			Yes
9	A52792299 – Adult Bed Days	Included in this Bundle.	2013	2023	No

10	A52792297 – Adult BSI Count Bed days rates sector	Included in this Bundle.	2013	2023	No
11	A52792301 – Adult BSI Count Bed Days Rates QEUH	Included in this Bundle.	2013	2023	No
12	A52792298 – Organisms Isolated Adult Paeds Bacteraemia Data GGC	Not included in this Bundle as it contains patient identifiable information.			Yes
13	A52792300 – Adult Microorganism Detections Used	Not included in this Bundle as it contains patient identifiable information.			Yes
14	A52792302 – Adult Micro showing Deduplicated and 14-day drop	Not included in this Bundle as it contains patient identifiable information.			Yes
15	A53003943 – Table 19	Included in this Bundle.	2005	2022	No

Dr Samir Agrawal

16	A53044379 – Cases over time Protected Updated	Included in this Bundle.		19/06/2025	Yes (with redactions)
17	A53044380 - GGC Expert (HAD) Report – Chapter 8 – Dr Samir Agrawal's Calculations re Invasive Aspergillosis	Included in this Bundle.			No

SHI Source Identification and Supporting Workings Request Dated April 11, 2025

Requests in bold, followed by responses.

For the following pages, could you please confirm the exact spreadsheet or data source used (including the period the dataset covers), and provide the working spreadsheets, tables, and any relevant methodological details:

(1) Page 60 - Data mentioned in relation to QEUH and comparable hospitals.

Tables 1 and 2 are simple summaries of the organisms that were reported in the blood culture isolate data from two data files provided by the CLO entitled: "Final Combined GGC Paeds Onc CHI only 01.01.05 - 31.12.22" and "Adult HaemOnc 2013-31Aug23 - matched GMC". These included specimens from haemato-oncology adult patients from Beatson Cancer Center (96.5% of non-QEUH), Glasgow Royal Infirmary, Queen Elizabeth University Hospital (QEUH), Royal Alexandra Hospital, Stobhill Hospital, Vale of Leven Hospital, Victoria Infirmary, Gartnavel General Hospital between 2013 and 2023 and haemato-oncology paediatric patients from Yorkhill and Queen Elizabeth University Hospital between 2005 and 2022. Table 1 includes only microorganisms of environmental concern including potential sources of infection, this information comes from the literature including expert review by Prof Peter Hawkey. Table 2 includes all microorganisms isolated in suspected bacteraemia cases among both adult and paediatric patients as described.

A combined file of these organisms with date and differentiation between adult vs paediatric patients has been provided in an Excel file as requested labelled:
"Organisms_Isolated_Adult_Paeds_Bacteraemia_Data_GGC"

(2) Page 62 –

(a) Organism detection data differentiated by patient ID.

I have provided two separate files, one for adult and one for paediatric patients as follows:

- ADULT: "Adult_microorganism_detections_used"
- PAEDIATRIC: "Paeds_microorganism_detections_used"

(b) Data used to calculate Bed Days per month (including the period that it covers).

Please note that these data were not calculated. Bed days were provided by month and year by the CLO for both adult, by sector, and paediatric, by hospital, patients. Please see Excel spreadsheets labelled:

- ADULT: "Adult_beddays"
- PAEDIATRIC: "Paeds_beddays"

(c) Working spreadsheet(s) that demonstrate the process used to define a new case (14-day rule), monthly case counts, environmental organism classification, and calculation of incidence rates per 1,000 bed days.

This request is unclear. People with proper statistical training do not use spreadsheets to clean data. Consistent with modern gold standard statistical practice, I use Stata, R or similar analytical package to aggregate and analyze data. Cases were based on microorganism species identified in every individual patient. As patients with organisms detected in blood culture may be monitored frequently, it is not uncommon to observe multiple blood cultures very close together, therefore we decided that an incident case in the same individual with the same microorganism required a ≥15 day gap between detections. If that patient had the same microorganism detected within ≤14 days, that was considered the same bacteraemia episode. These types of automated calculations are straightforward using a statistical package such as Stata, R, SAS and others, anyone trained in statistical analysis should know how to do this. I have provided the following spreadsheets to show all microorganism detections and those that were kept for analysis and those that were not because they either were duplicate entries or were ≤14 days apparat.

- ADULT: “Adult_micro_showing_deduplicated_and14_day_drop”
- PAEDIATRIC: “Paeds_micro_showing_deduplicated_and14_day_drop”

Once individual incident cases were identified, monthly cases were aggregated based on calendar year and month. The number of cases per month was divided by bed days (provided by the CLO as described in 2b) and then multiplied by 1000 to get bed days per 1000 patients. Spread sheets by month and year of cases, including bed days and incidence per 1000 have been provided stratified by section (adult) or hospital (paediatric) and all vs organisms of environmental concern in the following files:

- ADULT: “Adult_bsi_count_beddays_rates_sector” & “Adult_bsi_count_beddays_rates_qeuh”
- PAEDIATRIC: “Paeds_bsi_count_beddays_rates”

These can easily be checked by aggregating data in the spreadsheets labelled “Adult_microorganism_detections_used” and “Paeds_microorganism_ detections_used”

As stated in the report and again in this response, organisms of environmental concern are not identified through calculation, these are based on biological knowledge and expertise and can be confirmed by thorough literature search and review of biology texts.

(3) Page 64 -

(a) Historical (2005 – May 2015) vs. current paediatric hospital data (June 2015 – 2022) - (Yorkhill vs. RHC).

The data are in the Excel spreadsheet labelled: “Paeds_bsi_count_beddays_rates”

All dates are contained within these data. “Historical data” (Yorkhill) are those data dated Jan 2005- May 2015 and label with “Yorkhill” under hospital, “current data” (QEUH) are those dated June 2015-Dec 2022 and labelled with QEUH-RHC under hospital.

Please provide additional information if this is not what you are looking for.

(b) Data used in Figures 1 and 2. (ADULT)

Data for these figures are in the following Excel files:

- Figure 1: “Figure 1 data”
- Figure 2: “Figure 2 data”

(4) Page 68 – Data used in Figure 4, please also clarify if it is fair to assume that references to ‘other hospitals’ prior to page 69, do indeed refer, and always refer to the combined data from Beatson and SGH?

In the dataset provided by the CLO, there was data on blood cultures from 11 different GGC hospitals including Queen Elizabeth University Hospital (QEUH) corresponding to consultant sectors: BMT, North and South. Among the hospitals classified as “other’, i.e. not QEUH, 96.5% of blood cultures came from the Beatson Cancer Center while the remainder came from Glasgow Royal Infirmary, Lightburn Hospital, Lorn and Island Hospital, Matie Curie Huntershill, Royal Alexandra Hospital, Stobhill Hospital, Vale of Leven Hospital, Victoria Infirmary, and Gartnavel General Hospital; of which, organisms grew from patients located at Beatson Cancer Center, Glasgow Royal Infirmary, Royal Alexandra Hospital, Stobhill Hospital, Vale of Leven Hospital, Victoria Infirmary, and Gartnavel General Hospital.

Data for Figure 4 can be found in the Excel file labelled: “Figure 4 data”

(5) Page 65 –If available, please also provide the tables and workings for Figures 4–22.

I am not sure what “workings” refer to, but the data tables are provided at the end of this document and the raw data have been provided based on the other questions in this document, as well as by the CLO as stated in [redacted] email dated 22 April 2025.

(6) Pages 64–69: Data source(s) for references to “other hospitals in NHS GGC” and related figures.

See question number 4. I was provided with two datasets for adult haemato-oncology patients. One with all blood-related samples sent to microbiology for growth and results including dates, unique IDs, consultant sectors, wards and hospitals and the other containing bed days by month and year for the sectors, BMT, North and South for the time periods outlined in the report and above. As I understood it, these data were extracted from the GGC NHS database systems.

Terminology Clarifications

(7) Page 60: Please provide an explanation of what the 'ticks' and 'crosses' along the Adult and Paediatric columns in Table 1 and 2, in order to clarify the intent.

A check mark specifies that that species or organism was detected and an "X" indicates that it was not in that patient group.

(8) Pages 64–69: Please confirm whether "other hospitals in NHS GGC" consistently refers to Beatson and SGH across the report.

See number 4.

Figure Labels and Consistency

(9) Page 65: Could you confirm whether Figures 1 through 22 represent incidence rates (cases per activity/time point) as opposed to incidence (cases per time point)?

Incidence rates per month per 1,000 bed days.

(10) As many figures currently lack data labels, the provision of numerical tables would significantly aid in replicating and verifying your findings.

See question number 5.

(11) If the data you have drawn upon appears in the documents contained within Bundle 44 Vol 1, please refer to them as Bundle X and Page Y. If the sources are different or are not clearly identifiable within that bundle, we would be grateful if you could supply the specific spreadsheet(s), workings, and documentation as requested.

Data sources in the bundles are not clear.

The CLO, confirmed by [redacted] in an email dated 22 April 2025, that the council has received the same datasets that I was provided with. I have shared those data in this request.

If you could please share with me (1) raw data files used in Bundle 44 vol1 and (2) the code used for analyses, so that I can double check that we are indeed working with the same data. I will also be better able to answer queries about any differences. Please note if the code are in R, Stata or Python, I can take code in that format. If code are in SAS, SPSS or another statistical package, please provide code in a document file as I can read it, but do not have a software license for these.

Data Tables for Figures 4-22

Per request data tables for all figures have been provided below as supplemental tables to the report.

Supplemental Table 1: Data table for Figure 1 providing incidence rates per 1,000 bed days of all bacteraemia by month and year by consultant sector (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
BMT												
2014			1.79	30.30	21.91	32.32	29.29	50.42	32.59	31.8	23.5	35.52
2015	42.79	56.18	29.72	46.91	25.70	27.59	50.44	49.35	49.91	36.98	26.36	51.80
2016	28.63	59.96	32.42	36.40	16.63	47.17	52.40	55.03	47.23	58.09	40.15	50.00
2017	67.98	52.51	82.71	69.28	75.47	58.95	50.55	30.15	78.89	53.42	84.53	71.83
2018	50.35	69.65	60.66	57.80	43.36	106.45	40.64	47.53	73.49	88.69	59.06	84.24
2019	43.42	73.46	51.44	91.45	52.40	45.30	39.28	66.67	60.44	43.61	71.22	44.39
2020	37.42	61.13	125.60	96.26	74.47	25.64	30.63	44.66	53.65	46.23	85.87	62.31
2021	36.45	37.70	47.35	37.30	40.73	54.33	26.69	59.38	43.05	31.60	25.54	80.65
2022	28.57	46.15	33.58	41.67	23.04	45.98	25.27	50.26	59.78	67.83	50.08	111.39
2023	43.83	60.09	84.94	87.91	57.53	296.30						
NORTH												
2014				8.27	9.77	4.04	7.77	10.59	10.87	6.15	12.77	7.97
2015	7.44	27.44	7.65	23.88	6.59	4.53	13.70	21.90	26.09	20.55	13.09	11.71
2016	22.95	8.28	17.95	3.29	27.54	12.38	34.81	20.53	22.60	31.55	25.53	35.50
2017	16.72	18.37	23.20	12.09	21.03	9.75	12.82	14.20	20.79	28.63	43.59	8.28
2018	31.18	36.59	19.08	36.36	14.26	39.79	9.58	21.35	7.38	17.41	18.96	12.74
2019	18.31	28.09	4.53	17.20	16.59	59.23	26.74	7.25	22.29	14.35	15.41	18.69
2020	22.17	21.86	28.37	28.00	28.04	9.71	26.58	62.18	6.11	24.17	31.25	26.83
2021	29.51	2.83	13.97	32.39	33.54	16.48	26.14	26.72	22.03	7.21	16.72	11.39
2022	24.24	16.23	17.04	20.55	23.26	25.35	29.02	42.80	38.66	19.74	4.44	28.69
2023	12.47	22.56	42.44	13.27	34.13	16.22						
SOUTH												
2013	10.49	24.24	16.95	3.52	12.54	11.11	21.40	63.16	8.28	24.51	24.59	9.76
2014	33.33	31.25	15.75	22.37	54.65	29.92	26.09	21.92	11.24	32.71	13.27	14.97
2015	4.51	4.93	24.34	10.36	26.32	42.17	7.78	11.24	37.04	38.22	18.52	24.27
2016	36.65	41.38	23.53	12.20	3.53	45.69	18.52	11.45	200.00	20.94	35.93	21.90
2017	72.29	46.30	26.42	94.83	73.47	0	31.01	32.65	29.07	17.60	35.18	51.61
2018	42.45	44.44	36.70	28.90	60.61	27.32	30.30	37.82	30.30	30,30	39.84	54.80
2019	69.77	0	62.11	37.27	27.21	15.00	5.58	80.00	52.91	52.63	147.06	18.75
2020	31.11	56.60	34.04	62.50	61.86	62.94	10.64	8.77	57.97	25.21	54.35	21.90
2021	52.29	98.04	43.29	7.41	96.77	13.51	73.17	44.12	16.22	20.33	12.45	96.55
2022	0	54.69	23.26	33.82	15.06	91.67	30.62	19.14	57.69	8.07	59.32	8.48
2023	52.63	14.93	27.21	25.64	0	88.24						

<u>Supplemental Table 2:</u> Data table for Figure 2 providing incidence rates per 1,000 bed days of all bacteraemia by month and year by QEUH vs other hospitals in NHS GGC (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	10.49	24.24	16.95	3.52	12.54	11.11	21.40	63.16	8.28	24.51	24.59	9.76
2014	33.33	31.25	6.16	20.99	24.19	20.72	21.02	28.18	19.70	21.30	18.21	23.52
2015	18.69	29.36	21.30	31.08	16.27	4.53	13.70	35.18	38.96	29.44	21.03	32.15
2016	25.59	33.68	26.81	24.21	22.04	30.19	46.75	41.48	36.86	47.56	34.70	43.70
2017	47.68	36.25	53.37	44.50	48.12	33.40	33.14	22.94	49.77	42.74	67.65	37.95
2018	43.30	53.43	40.26	48.67	31.73	39.79	9.58	21.35	7.38	17.41	18.96	12.74
2019	18.31	28.09	4.53	17.20	16.59	59.23	26.74	7.25	22.29	14.35	15.41	18.69
2020	22.17	21.86	28.37	28.00	28.04	9.71	26.58	62.18	6.12	24.17	31.25	26.83
2021	29.51	2.83	13.97	32.39	33.54	16.48	26.14	26.72	22.03	7.21	16.72	11.39
2022	24.24	16.23	17.04	20.55	23.26	25.35	29.02	42.80	38.66	19.74	4.44	28.69
2023	12.47	22.56	42.44	13.27	34.13	16.22						
QEUH												
2015					26.32	30.30	35.06	11.24	37.04	38.22	18.52	24.27
2016	36.65	41.38	23.53	12.20	3.53	45.69	18.52	11.45	200.00	20.94	35.93	21.90
2017	72.29	46.30	26.42	94.83	73.47	0	31.01	32.65	29.07	17.60	35.18	51.61
2018	42.45	44.44	36.70	28.90	60.61	77.08	39.34	44.59	64.58	68.92	52.70	75.88
2019	50.41	51.84	54.10	72.0	42.55	32.85	23.50	69.42	57.87	45.83	83.95	33.42
2020	35.41	60.48	76.92	93.60	70.18	31.67	27.24	38.16	54.21	42.67	78.57	50.22
2021	40.54	43.24	46.11	31.52	50.41	43.16	31.99	55.66	35.92	29.34	21.33	85.71
2022	22.58	48.97	30.63	38.80	20.35	55.86	26.07	41.99	59.52	55.08	51.56	73.44
2023	44.44	54.41	72.18	75.18	43.42	246.48						

**Please note the red line in Figure 2 should be labelled "Other hospitals" not "North"*

<u>Supplemental Table 3:</u> Data table for Figure 3 providing incidence rates per 1,000 bed days of all bacteraemia by month and year by Consultant Sector with adjusted denominators based on average of other years for those denominators presented in red in Table 4 (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
BMT												
2014			1.79	30.30	21.91	32.32	29.29	50.42	32.59	31.78	23.56	35.52
2015	42.79	58.99	29.72	46.91	25.70	27.5	50.44	49.35	49.91	35.44	26.36	51.80
2016	28.63	59.96	32.42	36.40	16.63	47.17	52.40	55.03	47.23	58.09	40.15	50.00
2017	67.98	52.51	82.71	69.28	75.47	58.95	50.55	30.15	78.89	53.42	84.53	71.83
2018	52.08	67.16	60.66	57.80	43.36	106.45	40.64	47.53	73.49	88.69	59.06	84.24
2019	43.42	73.46	51.44	88.50	52.40	45.30	39.28	66.67	60.44	43.61	71.22	44.39
2020	37.42	61.13	125.60	96.26	74.47	25.64	30.63	44.66	53.65	46.23	85.37	62.31
2021	36.45	37.70	47.35	37.30	38.70	54.33	26.69	59.38	43.05	31.60	25.54	80.65
2022	28.57	46.15	33.58	41.67	23.04	45.98	25.27	50.26	59.78	67.83	50.08	111.39
2023	43.83	60.09	84.94	87.91	57.53	66.39						
North												
2014				8.27	9.77	4.04	7.77	10.59	10.87	6.15	12.77	7.97
2015	7.44	27.44	7.65	23.88	6.59	4.53	13.70	21.90	26.09	20.55	13.09	11.71
2016	22.95	8.28	17.95	3.29	27.54	12.38	34.81	20.53	22.60	31.55	25.53	35.50
2017	16.72	18.37	23.20	12.09	21.03	9.75	12.82	14.20	20.79	28.63	43.59	8.28
2018	31.18	36.59	19.08	36.36	14.26	39.79	9.58	21.35	7.38	17.41	18.96	12.74
2019	18.31	28.09	4.53	17.20	16.59	59.23	26.74	7.25	22.29	14.35	15.41	18.69
2020	22.17	21.86	28.37	28.00	28.04	9.71	26.58	62.18	6.11	24.17	31.25	26.83
2021	29.51	2.83	13.97	32.39	33.54	16.48	26.14	26.72	22.03	7.21	16.72	11.39
2022	24.24	16.23	17.04	20.55	23.26	25.35	29.02	42.80	38.66	19.74	4.44	28.69
2023	12.47	22.56	42.44	13.27	34.13	16.22						
South												
2013	10.49	24.24	16.95	3.52	12.54	11.11	21.40	63.16	8.28	24.51	24.59	9.76
2014	33.33	31.25	15.75	22.37	54.65	29.92	26.09	21.92	11.24	32.71	13.27	14.97
2015	4.51	4.93	24.34	10.36	26.32	42.17	7.78	11.24	37.04	38.22	18.52	24.27
2016	36.65	41.38	23.53	12.20	3.53	45.69	18.52	11.45	41.10	20.94	35.93	21.90
2017	72.29	46.30	26.42	94.83	73.47	0	31.01	32.65	29.07	17.60	35.18	51.61
2018	42.45	44.44	36.70	28.90	60.61	27.32	30.30	37.82	30.30	30.30	39.84	54.80
2019	69.77	0	62.11	37.27	27.21	15.00	5.58	80.00	52.91	52.63	51.28	18.75
2020	31.11	56.60	34.04	4.81	61.86	62.94	10.64	8.77	18.27	25.21	54.35	21.90
2021	52.29	35.72	43.29	7.41	96.77	13.51	73.17	44.12	16.22	20.33	12.45	96.55
2022	0	54.69	23.26	33.82	15.06	91.67	30.61	19.14	13.70	8.07	59.32	8.48
2023	10.26	7.14	27.21	25.64	0	17.34						

<u>Supplemental Table 4</u>: Data table for Figure 4 providing incidence rates per 1,000 bed days of all bacteraemia by month and year by QEUH vs Other Hospitals with adjusted denominators based on average of other years for those denominators presented in red in Table 4 (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	10.49	24.24	16.95	3.52	12.54	11.11	21.40	63.16	8.28	24.51	24.59	9.76
2014	33.33	31.25	6.16	20.99	24.19	20.72	21.02	28.18	19.70	21.30	18.21	23.52
2015	18.69	29.36	21.30	31.08	16.27	4.53	13.70	35.18	38.96	29.44	21.03	32.15
2016	25.59	33.68	26.81	24.21	22.04	30.19	46.75	41.48	36.86	47.56	34.70	43.70
2017	47.68	36.25	53.37	44.50	48.12	33.40	33.14	22.94	49.77	42.74	67.65	37.95
2018	43.30	53.43	40.26	48.67	31.73	39.79	9.58	21.35	7.38	17.41	18.96	12.74
2019	18.31	28.09	4.53	17.20	16.59	59.23	26.74	7.25	22.29	14.35	15.41	18.69
2020	22.17	21.86	28.37	28.00	28.04	9.71	26.58	62.18	6.12	24.17	31.25	26.83
2021	29.51	2.83	13.97	32.39	33.54	16.48	26.14	26.72	22.03	7.21	16.72	11.39
2022	24.24	16.23	17.04	20.55	23.26	25.35	29.02	42.80	38.66	19.74	4.44	28.69
2023	12.47	22.56	42.44	13.27	34.13	16.22						
QEUH												
2015					26.32	30.30	35.06	11.24	37.07	38.22	18.52	24.27
2016	36.65	41.38	23.53	12.20	3.53	45.69	18.52	11.45	41.10	20.94	35.93	21.90
2017	72.29	46.30	26.42	94.83	73.47	0	31.01	32.65	29.07	17.60	35.18	51.61
2018	42.45	44.44	36.70	28.90	60.61	77.08	39.34	44.59	64.58	68.92	52.70	75.88
2019	50.41	51.84	54.10	72.00	42.55	32.85	23.50	69.42	57.87	45.83	63.91	33.42
2020	35.41	60.48	76.92	48.10	70.18	31.67	27.74	38.16	42.34	42.67	78.57	50.22
2021	40.54	37.27	46.11	31.52	50.41	43.16	31.99	55.66	35.92	29.34	21.33	85.71
2022	22.58	48.97	30.63	38.80	20.35	55.86	26.07	41.99	42.59	55.08	51.56	73.44
2023	34.43	47.86	72.18	75.17	43.42	53.44						

<u>Supplemental Table 5</u>: Data table for Figure 5 on incidence rates per 1,000 bed days of bacteraemia from organisms of environmental significance by QEUH vs other NHS GGC hospitals (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	3.50	6.06	0	0	0	5.56	7.78	0	2.07	0	4.10	0
2014	9.52	0	1.23	3.23	1.08	5.58	3.66	5.33	2.46	5.11	2.60	2.61
2015	1.50	6.42	0	4.29	1.09	4.53	0	1.26	5.00	2.76	1.05	4.59
2016	4.09	6.32	6.95	0	4.20	9.66	3.05	3.46	2.38	5.01	2.57	3.86
2017	5.30	3.75	3.81	3.93	3.52	2.02	2.37	3.67	8.10	2.85	6.34	0.88
2018	3.02	2.74	0	2.21	2.44	531	0	7.12	3.69	7.46	4.74	0
2019	4.58	0	2.26	2.45	4.74	10.45	2.67	0	0	0	3.85	6.23
2020	2.46	2.73	0	0	4.67	0	3.32	0	0	6.04	6.95	4.88
2021	3.28	0	5.59	10.12	3.05	5.50	3.27	7.63	4.41	2.40	3.35	1.90
2022	2.02	3.25	0	2.28	3.88	2.82	5.28	23.35	2.58	2.19	2.22	8.20
2023	2.49	0	10.61	2.21	0	5.41						
QEUH												
2015					0	4.49	4.21	0	0	6.37	0	4.85
2016	5.24	6.90	5.88	0	0	0	0	3.82	22.22	5.24	11.98	7.30
2017	12.05	0	3.77	8.62	8.16	0	0	4.08	5.81	8.80	5.03	19.36
2018	0	0	9.17	0	0	6.09	5.08	6.37	10.42	5.87	1.32	0
2019	8.23	5.02	4.64	4.00	1.33	2.05	1.74	4.96	5.43	7.05	0	2.67
2020	2.83	5.38	9.05	0	7.02	3.39	1.54	1.59	1.87	5.69	9.52	2.18
2021	6.76	1.80	0	2.87	6.50	1.23	0	7.18	2.87	1.63	1.33	10.99
2022	0	7.73	5.33	1.76	1.02	1.80	0	7.63	7.14	5.16	1.36	4.69
2023	1.85	3.75	1.50	3.50	2.55	7.04						

<u>Supplemental Table 6:</u> Data table for Figure 6 on incidence rates per 1,000 bed days of bacteraemia from organisms of environmental significance by QEUH vs other NHS GGC hospitals with adjusted denominators from Table 4 of the report (rounded to the nearest hundredths place; greyed out cells indicate no data available, last 2 months of 2023 appeared to have incomplete data and therefore not included)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	3.50	6.06	0	0	0	5.56	7.78	0	2.07	0	4.10	0
2014	9.52	0	1.23	3.23	1.08	5.58	3.66	5.33	2.46	5.11	2.60	2.61
2015	1.50	6.42	0	4.29	1.09	4.53	0	1.26	5.00	2.76	1.05	4.59
2016	4.09	6.32	6.95	0	4.20	9.66	3.05	3.46	2.38	5.01	2.57	3.86
2017	5.30	3.75	3.81	3.93	3.52	2.02	2.37	3.67	8.10	2.85	6.34	0.88
2018	3.02	2.74	0	2.21	2.44	531	0	7.12	3.69	7.46	4.74	0
2019	4.58	0	2.26	2.45	4.74	10.45	2.67	0	0	0	3.85	6.23
2020	2.46	2.73	0	0	4.67	0	3.32	0	0	6.04	6.95	4.88
2021	3.28	0	5.59	10.12	3.05	5.50	3.27	7.63	4.41	2.40	3.35	1.90
2022	2.02	3.25	0	2.28	3.88	2.82	5.28	23.35	2.58	2.19	2.22	8.20
2023	2.49	0	10.61	2.21	0	5.41						
QEUH												
2015					0	4.49	4.21	0	0	6.37	0	4.85
2016	5.24	6.90	5.88	0	0	0	0	3.82	4.57	5.24	11.98	7.30
2017	12.05	0	3.77	8.62	8.16	0	0	4.08	5.81	8.80	5.03	19.35
2018	0	0	9.17	0	0	6.09	5.08	6.37	10.42	5.87	1.32	0
2019	8.23	5.02	4.64	4.00	1.33	2.05	1.74	4.96	4.53	7.05	0	2.67
2020	2.83	5.38	9.05	0	7.02	3.39	1.54	1.59	1.46	5.69	9.52	2.18
2021	6.76	1.55	0	2.87	6.50	1.23	0	7.18	2.87	1.63	1.33	10.99
2022	0	7.73	5.33	1.76	1.02	1.80	0	7.63	5.11	5.16	1.36	4.69
2023	1.43	3.30	1.50	3.50	2.55	1.53						

<u>Supplemental Table 7:</u> Data table for Figure 7 showing incidence rates per 1,000 of clusters (unlikely, possible, probable) of bacteraemia of environmental significance by hospital and date

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	0	6.06	0	0	0	5.56	5.84	0	0	0	4.10	0
2014	0	0	3.70	0	1.01	1.59	0.91	3.05	1.64	3.41	0.65	0.87
2015	1.50	3.67	0	0	0	4.53	0	2.51	2.00	1.84	1.05	2.30
2016	2.05	2.11	4.97	0	4.20	2.42	2.03	2.30	1.19	6.26	1.29	1.29
2017	1.33	1.25	2.54	2.62	0	2.02	2.37	3.67	3.47	0.95	4.23	0.88
2018	2.01	0	0	2.21	2.44	2.65	0	1.78	3.69	4.98	0	0
2019	4.58	0	0	0	2.37	3.48	0	0	0	0	1.93	3.11
2020	2.46	0	0	0	0	0	3.32	0	0	0	3.47	0
2021	3.28	0	5.59	4.05	0	2.75	0	3.82	0	0	0	0
2022	2.02	3.25	0	0	0	2.82	2.64	19.46	2.58	0	2.22	8.20
2023	2.49	0	0	0	0	0						
QEUH												
2015					0	1.12	0	0	0	0	0	0
2016	0	6.90	0	0	0	0	0	0	22.22	5.24	0	7.30
2017	0	0	0	0	8.16	0	0	0	5.81	2.93	5.03	6.45
2018	0	0	9.17	0	0	6.09	1.27	6.37	6.25	2.93	0	0
2019	6.17	3.34	3.09	4.00	0	0	0.87	3.31	1.81	2.35	0	1.34
2020	1.42	0	4.53	0	0	2.26	0	0	1.87	2.85	2.38	0
2021	3.38	1.80	0	2.87	6.50	1.23	1.39	3.59	1.44	0.82	0	4.40
2022	0	2.58	2.66	1.76	1.02	1.80	0	2.55	0	1.72	0	4.69
2023	0	1.88	1.50	1.75	0	0						

<u>Supplemental Table 8:</u> Data table for Figure 8 showing incidence rates per 1,000 of clusters (unlikely, possible, probable) of bacteraemia of environmental significance by hospital and date using adjusted denominators based on report Table 4

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	0	6.06	0	0	0	5.56	5.84	0	0	0	4.10	0
2014	0	0	3.70	0	1.01	1.59	0.91	3.05	1.64	3.41	0.65	0.87
2015	1.50	3.67	0	0	0	4.53	0	2.51	2.00	1.84	1.05	2.30
2016	2.05	2.11	4.97	0	4.20	2.42	2.03	2.30	1.19	6.26	1.29	1.29
2017	1.33	1.25	2.54	2.62	0	2.02	2.37	3.67	3.47	0.95	4.23	0.88
2018	2.01	0	0	2.21	2.44	2.65	0	1.78	3.69	4.98	0	0
2019	4.58	0	0	0	2.37	3.48	0	0	0	0	1.93	3.11
2020	2.46	0	0	0	0	0	3.32	0	0	0	3.47	0
2021	3.28	0	5.59	4.05	0	2.75	0	3.82	0	0	0	0
2022	2.02	3.25	0	0	0	2.82	2.64	19.46	2.58	0	2.22	8.20
2023	2.49	0	0	0	0	0						
QEUH												
2015					0	1.12	0	0	0	0	0	0
2016	0	6.90	0	0	0	0	0	0	4.57	5.24	0	7.30
2017	0	0	0	0	8.16	0	0	0	5.81	2.93	5.03	6.45
2018	0	0	9.17	0	0	6.09	1.27	6.37	6.25	2.93	0	0
2019	6.17	3.34	3.09	4.00	0	0	0.87	3.31	1.81	2.35	0	1.34
2020	1.42	0	4.53	0	0	2.26	0	0	1.46	2.85	2.38	0
2021	3.38	1.55	0	2.87	6.50	1.23	1.39	3.59	1.44	0.82	0	4.40
2022	0	2.58	2.66	1.76	1.02	1.80	0	2.55	0	1.72	0	4.69
2023	0	1.65	1.50	1.75	0	0						

<u>Supplemental Table 9:</u> Data table for Figure 9 showing BSI incidence rates among adult haemato-oncology patients in the GGC by hospital and whether or not the organism isolated in culture is of environmental significance or not

	Other Hospitals		QEUH	
Month	**Environmental**	**Non-environmental**	**Environmental**	**Non-environmental**
		2013		
Jan	3.50	6.99		
Feb	6.06	18.18		
Mar	0	16.95		
Apr	0	3.52		
May	0	12.54		
Jun	5.56	5.56		
Jul	7.78	13.62		
Aug	0	63.16		
Sep	2.07	6.21		
Oct	0	24.51		
Nov	4.10	20.49		
Dec	0	9.76		
		2014		
Jan	9.52	23.81		
Feb	0	31.25		
Mar	1.23	4.93		
Apr	3.23	17.76		
May	1.08	23.19		
Jun	5.58	15.14		
Jul	3.66	17.37		
Aug	5.33	22.85		
Sep	2.46	17.24		
Oct	5.11	16.18		
Nov	2.60	15.61		
Dec	2.61	20.91		
		2015		
Jan	1.50	17.19		
Feb	6.42	22.94		
Mar	00	21.29		
Apr	4.29	26.80		
May	1.09	15.18	0	26.32
Jun	4.35	0	4.49	25.81
Jul	0	13.70	4.21	30.86
Aug	1.26	33.92	0	11.24
Sep	5.00	33.97	0	37.04
Oct	2.76	26.68	6.37	31.85
Nov	1.05	19.98	0	18.52
Dec	4.59	27.55	4.85	19.42
		2016		
Jan	4.09	21.49	5.24	31.41
Feb	6.32	27.37	6.90	34.48
Mar	6.95	19.86	5.88	17.65
Apr	0	24.21	0	12.20
May	4.20	17.84	0	3.53
Jun	9.66	20.53	0	45.69
Jul	3.05	43.70	0	18.52
Aug	3.46	38.02	3.82	7.63
Sep	2.38	34.48	4.57	177.79

Oct	5.01	42.55	5.24	15.71
Nov	2.57	32.13	11.98	23.95
Dec	3.86	39.85	7.30	14.60
		2017		
Jan	5.30	42.38	12.05	60.24
Feb	3.75	32.50	0	46.30
Mar	3.81	49.56	3.77	22.64
Apr	3.93	40.58	8.62	86.21
May	3.52	44.60	8.16	65.31
Jun	2.02	31.38	0	0
Jul	2.37	30.77	0	31.01
Aug	3.67	19.27	4.08	28.57
Sep	8.10	41.67	5.81	23.26
Oct	2.85	39.89	8.80	8.80
Nov	6.34	61.31	5.03	30.15
Dec	0.88	37.07	19.36	32.26
		2018		
Jan	3.02	40.28	0	42.45
Feb	2.74	50.69	0	44.44
Mar	0	40.26	9.17	27.52
Apr	2.21	46.46	0	28.90
May	2.44	29.29	0	60.61
Jun	5.31	34.48	6.09	70.99
Jul	0	9.58	5.08	34.26
Aug	7.12	14.24	6.37	38.22
Sep	3.69	3.69	10.42	54.17
Oct	7.45	9.95	5.87	63.05
Nov	4.74	14.22	1.32	51.38
Dec	0	12.74	0	75.88
		2019		
Jan	4.58	13.73	8.23	42.18
Feb	0	28.09	5.02	46.82
Mar	2.26	2.26	4.64	49.46
Apr	2.45	14.74	4.00	68.00
May	4.74	11.85	1.33	41.22
Jun	10.45	48.78	2.05	30.80
Jul	2.67	24.06	1.74	21.76
Aug	0	7.25	4.95	64.46
Sep	0	22.29	5.43	52.44
Oct	0	14.35	7.05	38.78
Nov	3.85	11.56	0	83.95
Dec	6.23	12.46	2.67	30.75
		2020		
Jan	2.46	19.70	2.83	32.58
Feb	2.73	19.13	5.38	55.11
Mar	0	28.37	9.05	67.87
Apr	0	28.00	0	93.60
May	4.67	23.37	7.02	63.16
Jun	0	9.71	3.39	28.28
Jul	3.32	23.26	1.54	26.19
Aug	0	62.18	1.59	36.57
Sep	0	6.12	1.46	52.34
Oct	6.04	18.13	5.69	36.98
Nov	6.95	24.31	9.52	69.05
Dec	4.88	21.95	2.18	48.04
		2021		

Jan	3.28	26.23	6.76	33.78
Feb	0	2.83	1.55	41.44
Mar	5.59	8.38	0	46.11
Apr	10.12	22.27	2.87	28.65
May	3.05	30.49	6.50	43.90
Jun	5.50	10.99	1.23	41.92
Jul	3.27	22.88	0	31.99
Aug	7.63	19.08	7.18	48.47
Sep	4.41	17.62	2.87	33.05
Oct	2.40	4.81	1.63	27.71
Nov	3.35	13.38	1.33	20.00
Dec	1.90	9.49	10.99	74.73
		2022		
Jan	2.02	22.22	0	22.58
Feb	3.25	12.99	7.73	41.24
Mar	0	17.04	5.33	25.30
Apr	2.28	18.27	1.76	37.04
May	3.88	19.38	1.02	19.33
Jun	2.82	22.54	1.80	54.05
Jul	5.28	23.75	0	26.07
Aug	23.35	19.46	7.63	34.35
Sep	2.58	36.08	5.11	52.38
Oct	2.19	17.54	5.16	49.10
Nov	2.22	2.22	1.36	50.20
Dec	8.20	20.49	4.69	68.75
		2023		
Jan	2.49	9.98	1.43	42.59
Feb	0	22.56	3.30	50.66
Mar	10.61	31.83	1.50	70.68
Apr	2.21	11.06	3.50	71.68
May	0	34.13	2.55	40.87
Jun	5.41	10.81	1.53	239.44

Supplemental Table 10: Data table for Figure 9 showing BSI incidence rates among adult haemato-oncology patients in the GGC by hospital and whether or not the organism isolated in culture is of environmental significance or not using adjusted bed days as the denominator (see report Table 4)

	Other Hospitals		QEUH	
Month	**Environmental**	**Non-environmental**	**Environmental**	**Non-environmental**
		2013		
Jan	3.50	6.99		
Feb	6.06	18.18		
Mar	0	16.95		
Apr	0	3.52		
May	0	12.54		
Jun	5.56	5.56		
Jul	7.78	13.62		
Aug	0	63.16		
Sep	2.07	6.21		
Oct	0	24.51		
Nov	4.10	20.49		
Dec	0	9.76		
		2014		

Jan	9.52	23.81		
Feb	0	31.25		
Mar	1.23	4.93		
Apr	3.23	17.76		
May	1.08	23.19		
Jun	5.58	15.14		
Jul	3.66	17.37		
Aug	5.33	22.85		
Sep	2.46	17.24		
Oct	5.11	16.18		
Nov	2.60	15.61		
Dec	2.61	20.91		
		2015		
Jan	1.50	17.19		
Feb	6.42	22.94		
Mar	00	21.29		
Apr	4.29	26.80		
May	1.09	15.18	0	26.32
Jun	4.35	0	4.49	25.81
Jul	0	13.70	4.21	30.86
Aug	1.26	33.92	0	11.24
Sep	5.00	33.97	0	37.04
Oct	2.76	26.68	6.37	31.85
Nov	1.05	19.98	0	18.52
Dec	4.59	27.55	4.85	19.42
		2016		
Jan	4.09	21.49	5.24	31.41
Feb	6.32	27.37	6.90	34.48
Mar	6.95	19.86	5.88	17.65
Apr	0	24.21	0	12.20
May	4.20	17.84	0	3.53
Jun	9.66	20.53	0	45.69
Jul	3.05	43.70	0	18.52
Aug	3.46	38.02	3.82	7.63
Sep	2.38	34.48	22.22	36.53
Oct	5.01	42.55	5.24	15.71
Nov	2.57	32.13	11.98	23.95
Dec	3.86	39.85	7.30	14.60
		2017		
Jan	5.30	42.38	12.05	60.24
Feb	3.75	32.50	0	46.30
Mar	3.81	49.56	3.77	22.64
Apr	3.93	40.58	8.62	86.21
May	3.52	44.60	8.16	65.31
Jun	2.02	31.38	0	0
Jul	2.37	30.77	0	31.01
Aug	3.67	19.27	4.08	28.57
Sep	8.10	41.67	5.81	23.26
Oct	2.85	39.89	8.80	8.80
Nov	6.34	61.31	5.03	30.15
Dec	0.88	37.07	19.36	32.26
		2018		
Jan	3.02	40.28	0	42.45
Feb	2.74	50.69	0	44.44
Mar	0	40.26	9.17	27.52
Apr	2.21	46.46	0	28.90

May	2.44	29.29	0	60.61
Jun	5.31	34.48	6.09	70.99
Jul	0	9.58	5.08	34.26
Aug	7.12	14.24	6.37	38.22
Sep	3.69	3.69	10.42	54.17
Oct	7.45	9.95	5.87	63.05
Nov	4.74	14.22	1.32	51.38
Dec	0	12.74	0	75.88
		2019		
Jan	4.58	13.73	8.23	42.18
Feb	0	28.09	5.02	46.82
Mar	2.26	2.26	4.64	49.46
Apr	2.45	14.74	4.00	68.00
May	4.74	11.85	1.33	41.22
Jun	10.45	48.78	2.05	30.80
Jul	2.67	24.06	1.74	21.76
Aug	0	7.25	4.95	64.46
Sep	0	22.29	5.43	52.44
Oct	0	14.35	7.05	38.78
Nov	3.85	11.56	0	63.91
Dec	6.23	12.46	2.67	30.75
		2020		
Jan	2.46	19.70	2.83	32.58
Feb	2.73	19.13	5.38	55.11
Mar	0	28.37	9.05	67.87
Apr	0	28.00	0	48.10
May	4.67	23.37	7.02	63.16
Jun	0	9.71	3.39	28.28
Jul	3.32	23.26	1.54	26.19
Aug	0	62.18	1.59	36.57
Sep	0	6.12	1.87	40.88
Oct	6.04	18.13	5.69	36.98
Nov	6.95	24.31	9.52	69.05
Dec	4.88	21.95	2.18	48.04
		2021		
Jan	3.28	26.23	6.76	33.78
Feb	0	2.83	1.80	35.71
Mar	5.59	8.38	0	46.11
Apr	10.12	22.27	2.87	28.65
May	3.05	30.49	6.50	43.90
Jun	5.50	10.99	1.23	41.92
Jul	3.27	22.88	0	31.99
Aug	7.63	19.08	7.18	48.47
Sep	4.41	17.62	2.87	33.05
Oct	2.40	4.81	1.63	27.71
Nov	3.35	13.38	1.33	20.00
Dec	1.90	9.49	10.99	74.73
		2022		
Jan	2.02	22.22	0	22.58
Feb	3.25	12.99	7.73	41.24
Mar	0	17.04	5.33	25.30
Apr	2.28	18.27	1.76	37.04
May	3.88	19.38	1.02	19.33
Jun	2.82	22.54	1.80	54.05
Jul	5.28	23.75	0	26.07
Aug	23.35	19.46	7.63	34.35

Sep	2.58	36.08	7.14	37.48
Oct	2.19	17.54	5.16	49.91
Nov	2.22	2.22	1.36	50.20
Dec	8.20	20.49	4.69	68.75
2023				
Jan	2.49	9.98	1.85	33.00
Feb	0	22.56	3.75	44.56
Mar	10.61	31.83	1.50	70.68
Apr	2.21	11.06	3.50	71.68
May	0	34.13	2.55	40.87
Jun	5.41	10.81	7.04	51.91

Supplemental Table 11: Data table for Figure 11 showing BSI incidence rates attributable to non-environmentally significant microorganisms among adult haemato-oncology patients in the GGC by hospital (*Note trend lines are fitted statistically and cannot be tabulated)*

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	6.99	18.18	16.95	3.52	12.54	5.56	13.62	63.16	6.21	24.51	20.49	9.76
2014	23.81	31.25	4.93	17.76	23.19	15.14	17.37	22.85	17.24	16.18	15.61	20.91
2015	17.19	22.94	21.29	26.80	15.18	0	13.70	33.92	33.97	26.68	19.98	27.55
2016	21.49	27.37	19.86	24.21	17.84	20.53	43.70	38.02	34.48	42.55	32.13	39.85
2017	142.38	32.50	49.56	40.58	44.60	31.38	30.77	19.27	41.67	39.89	61.31	37.07
2018	40.28	50.69	40.26	46.46	29.29	34.48	9.58	14.24	3.69	9.95	14.22	12.74
2019	13.73	28.09	2.26	14.74	11.85	48.78	24.06	7.25	22.29	14.35	11.56	12.46
2020	19.70	19.13	28.37	28.00	23.37	9.71	23.26	62.18	6.12	18.13	24.31	21.95
2021	26.23	2.83	8.38	22.27	30.49	10.99	22.88	19.08	17.62	4.81	13.38	9.49
2022	22.22	12.99	17.04	18.27	19.38	22.54	23.75	19.49	36.08	17.54	2.22	20.49
2023	9.98	22.56	31.83	11.06	34.13	10.81						
QEUH												
2015					26.32	25.81	30.86	11.24	37.04	31.85	18.52	19.42
2016	31.41	34.48	17.65	12.20	3.53	45.69	18.52	7.63	36.53	15.71	23.95	14.60
2017	60.24	46.30	22.64	86.21	65.31	0	31.01	28.57	23.26	8.80	30.15	32.26
2018	42.45	44.44	27.52	28.90	60.61	70.99	34.26	38.22	54.17	63.05	51.38	75.88
2019	42.18	46.82	49.46	68.00	41.22	30.80	21.76	64.46	52.44	38.78	83.95	30.75
2020	32.58	55.11	67.87	93.60	63.16	28.28	26.19	36.57	52.34	36.98	69.05	48.04
2021	33.78	41.44	46.11	28.65	43.90	41.92	31.99	48.47	33.05	27.71	20.00	74.73
2022	22.58	41.24	25.30	37.04	19.33	54.05	26.07	34.35	52.38	49.10	50.20	68.75
2023	42.59	50.66	70.68	71.68	40.87	239.44						

Supplemental Table 12: Data table for Figure 12 showing BSI incidence rates attributable to non-environmentally significant microorganisms among adult haemato-oncology patients in the GGC by hospital with adjusted denominators (*Note trend lines are fitted statistically and cannot be tabulated)*

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Other Hospitals (96% of Isolates from Beatson)												
2013	6.99	18.18	16.95	3.52	12.54	5.56	13.62	63.16	6.21	24.51	20.49	9.76
2014	23.81	31.25	4.93	17.76	23.19	15.14	17.37	22.85	17.24	16.18	15.61	20.91
2015	17.19	22.94	21.29	26.80	15.18	0	13.70	33.92	33.97	26.68	19.98	27.55
2016	21.49	27.37	19.86	24.21	17.84	20.53	43.70	38.02	34.48	42.55	32.13	39.85
2017	142.38	32.50	49.56	40.58	44.60	31.38	30.77	19.27	41.67	39.89	61.31	37.07
2018	40.28	50.69	40.26	46.46	29.29	34.48	9.58	14.24	3.69	9.95	14.22	12.74
2019	13.73	28.09	2.26	14.74	11.85	48.78	24.06	7.25	22.29	14.35	11.56	12.46
2020	19.70	19.13	28.37	28.00	23.37	9.71	23.26	62.18	6.12	18.13	24.31	21.95
2021	26.23	2.83	8.38	22.27	30.49	10.99	22.88	19.08	17.62	4.81	13.38	9.49
2022	22.22	12.99	17.04	18.27	19.38	22.54	23.75	19.49	36.08	17.54	2.22	20.49
2023	9.98	22.56	31.83	11.06	34.13	10.81						
QEUH												
2015					26.32	25.81	30.86	11.24	37.04	31.85	18.52	19.42
2016	31.41	34.48	17.65	12.20	3.53	45.69	18.52	7.63	177.79	15.71	23.95	14.60
2017	60.24	46.30	22.64	86.21	65.31	0	31.01	28.57	23.26	8.80	30.15	32.26
2018	42.45	44.44	27.52	28.90	60.61	70.99	34.26	38.22	54.17	63.05	51.38	75.88
2019	42.18	46.82	49.46	68.00	41.22	30.80	21.76	64.46	52.44	38.78	63.91	30.75
2020	32.58	55.11	67.87	48.10	63.16	28.28	26.19	36.57	40.88	36.98	69.05	48.04
2021	33.78	35.71	46.11	28.65	43.90	41.92	31.99	48.47	33.05	27.71	20.00	74.73
2022	22.58	41.24	25.30	37.04	19.33	54.05	26.07	34.35	37.48	49.10	50.20	68.75
2023	33.00	44.56	70.68	71.68	40.87	51.91						

Supplemental Table 13: See Supplemental Table 5, trend lines are fitted statistically and cannot be tabulated

Supplemental Table 14: See Supplemental Table 6, trend lines are fitted statistically and cannot be tabulated

Supplemental Table 15: Data tables for Figure 15 showing incidence rates of environmentally relevant bacteraemia within BMT patients only over time (blue font indicates when BMT was in QEUH and black font indicates when BMT was in North)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2014			1.79	6.99	1.99	5.70	6.28	10.50	6.11	8.48	3.93	5.33
2015	4.51	16.85	0	4.94	2.14	5.52	4.39	2.60	5.55	1.54	0	4.51
2016	6.61	12.85	9.72	0	0	16.51	2.99	5.69	2.05	8.30	1.91	6.82
2017	8.77	4.77	5.01	6.93	4.72	4.21	4.40	5.03	11.60	1.67	7.19	1.89
2018	3.47	2.49	0	3.85	2.71	6.45	5.81	7.31	13.12	8.87	0	0
2019	7.00	7.11	6.17	5.90	2.18	3.48	3.27	6.25	8.24	4.67	0	2.34
2020	2.08	6.27	14.49	0	5.32	4.05	1.80	1.94	0	1.71	9.15	0
2021	0	1.98	0	3.55	4.07	1.70	0	2.38	3.91	1.02	0	6.45
2022	0	3.85	1.87	0	1.54	0	0	8.67	8.15	6.57	1.62	7.43
2023	1.99	4.29	0	4.40	3.38	9.26						

Supplemental Table 16: Data tables for Figure 16 showing incidence rates of environmentally relevant bacteraemia within BMT patients only over time with corrected denominators (blue font indicates when BMT was in QEUH and black font indicates when BMT was in North)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2014			1.79	6.99	1.99	5.70	6.28	10.50	6.11	8.48	3.93	5.33
2015	4.51	16.85	0	4.94	2.14	5.52	4.39	2.60	5.55	1.54	0	4.51
2016	6.61	12.85	9.72	0	0	16.51	2.99	5.69	2.05	8.30	1.91	6.82
2017	8.77	4.77	5.01	6.93	4.72	4.21	4.40	5.03	11.60	1.67	7.19	1.89
2018	3.47	2.49	0	3.85	2.71	6.45	5.81	7.31	13.12	8.87	0	0
2019	7.00	7.11	6.17	5.90	2.18	3.48	3.27	6.25	8.24	4.67	0	2.34
2020	2.08	6.27	14.49	0	5.32	4.05	1.80	1.94	0	1.71	9.15	0
2021	0	1.98	0	3.55	4.07	1.70	0	2.38	3.91	1.02	0	6.45
2022	0	3.85	1.87	0	1.54	0	0	8.67	8.15	6.57	1.62	7.43
2023	1.99	4.29	0	4.40	3.38	2.08						

Supplemental Table 17: Data table for Figure 17 showing incidence rates of all cases of bacteraemia among paediatric patients over the study period (black font indicates Yorkhill, blue font indicates QEUH-RHC)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005	16.18	32.46	16.37	9.60	17.71	27.83	24.05	22.86	21.36	16.28	41.75	34.99
2006	21.04	10.14	16.37	26.87	14.49	29.82	28.06	26.67	21.36	25.32	24.67	14.73
2007	21.04	14.20	16.37	19.19	27.38	11.93	26.05	19.05	21.36	19.89	15.18	16.58
2008	11.77	22.78	12.59	17.09	50.13	17.43	26.08	35.38	15.48	17.43	26.08	35.38
2009	22.11	5.25	13.77	19.72	13.72	27.12	9.00	22.27	28.21	21.20	30.11	33.97
2010	9.90	7.91	18.71	21.28	13.91	21.32	30.23	7.86	7.08	30.43	9.10	13.81
2011	14.63	18.25	17.54	23.37	26.69	22.96	8.83	12.53	11.88	15.31	18.79	14.93
2012	8.96	17.67	9.43	10.20	8.05	23.14	20.33	44.87	14.76	12.01	8.57	7.51
2013	11.52	9.79	14.29	22.17	10.87	27.17	16.39	20.17	42.02	5.62	3.93	9.75
2014	11.26	11.94	33.85	17.20	17.88	29.01	16.67	6.73	34.48	17.01	9.63	25.54
2015	12.33	20.64	20.95	15.23	20.17	20.53	18.14	11.27	10.40	20.76	10.11	13.97
2016	25.29	10.91	30.22	13.55	12.49	14.57	39.08	15.93	18.32	21.03	18.21	23.44
2017	24.22	36.73	37.04	28.48	28.64	37.98	15.20	30.79	20.54	25.64	32.90	27.11
2018	15.15	13.33	46.82	28.17	23.56	24.72	6.51	19.10	26.03	9.55	13.01	29.82
2019	12.69	12.17	25.00	8.27	17.01	20.55	12.09	10.42	22.17	15.53	8.98	1.61
2020	7.98	18.87	6.96	15.63	7.22	12.00	6.21	17.91	13.70	6.67	10.55	10.53
2021	2.56	3.17	3.59	6.76	7.98	11.46	11.39	5.63	11.74	12.31	10.64	2.86
2022	10.26	0	0	2.19	3.24	11.39	4.59	2.63	2.43	4.39	13.67	4.59

Supplemental Table 18: Data table for Figure 18 showing incidence rates of bacteraemia attributed to organisms of potential environmental concern among paediatric patients over the study period (black font indicates Yorkhill, blue font indicates QEUH-RHC)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005	3.24	8.11	1.49	1.92	8.05	13.92	10.02	11.43	0	5.43	17.08	12.89
2006	8.09	2.03	4.46	5.76	3.22	17.89	10.02	1.91	5.83	1.81	1.90	1.84
2007	3.24	0	2.98	3.84	8.05	3.98	10.02	9.52	7.77	7.23	0	1.84
2008	3.21	4.56	1.05	3.42	15.83	12.06	0	22.35	3.10	13.08	0	0
2009	3.16	0	5.01	1.41	3.43	3.39	2.57	13.36	10.97	4.99	4.30	8.49
2010	3.96	1.98	4.16	3.87	5.56	0	7.56	4.89	1.77	12.17	0	2.76
2011	1.63	3.65	1.75	7.01	7.12	2.55	2.21	0	3.96	5.10	0	2.49
2012	1.49	8.83	3.15	2.04	2.01	7.71	4.07	19.23	5.54	0	0	1.88
2013	0	3.26	0	6.65	0	5.44	8.20	14.41	5.60	0	0	1.95
2014	1.88	2.99	7.81	2.15	1.79	5.80	8.33	1.35	17.24	3.78	1.93	5.89
2015	1.37	2.29	1.91	1.69	2.88	0	0	0	0	1.89	0	2.00
2016	0	0	2.75	0	1.25	5.47	3.55	7.08	3.66	9.35	3.41	7.81
2017	4.61	5.01	3.09	5.09	5.97	4.75	7.60	8.10	6.42	6.41	9.87	6.02
2018	2.53	1.91	16.85	11.74	13.09	13.31	2.17	6.95	10.85	4.09	1.45	4.59
2019	7.61	1.74	9.09	1.65	3.78	10.27	5.18	3.47	7.39	3.11	4.49	0
2020	0	0	2.32	5.21	0	4.00	2.07	0	6.85	3.33	2.64	0
2021	0	0	0	2.52	2.00	2.87	9.49	0	5.87	3.08	0	0
2022	5.13	0	0	0	0	4.56	1.53	0	2.43	2.93	0	0

<u>Supplemental Table 19:</u> Data table for Figure 19 showing incidence rates of bacteraemia cases attributable to environemntally relevant microorganisms that may cluster edpidemiologically among haemato-oncology paediatric patients in GGC period (black font indicates Yorkhill, blue font indicates QEUH-RHC) ***Pending – currently reviewing clustering data***

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005												
2006												
2007												
2008												
2009												
2010												
2011												
2012												
2013												
2014												
2015												
2016												
2017												
2018												
2019												
2020												
2021												
2022												

<u>Supplemental Table 20</u>: Data Table for Figure 20 providing incidence rates per 1,000 bed days per month (rounded to the hundredths place) and year by organisms detected in bacteraemia/bloodstream infection assessments of environmental relevance ("Environmental") and all others ("Other")

	Yorkhill		QEUH-RHC	
Month	**Environmental**	**Other**	**Environmental**	**Other**
	Year: 2005		**Year 2015**	
Jan	3.24	12.95		
Feb	8.11	24.34		
Mar	1.49	14.88		
Apr	1.92	7.68		
May	8.05	9.66		
Jun	13.92	13.92	0	20.53
Jul	10.02	14.03	0	18.14
Aug	11.43	11.43	0	11.27
Sep	0	21.36	0	10.40
Oct	5.43	10.85	1.89	18.87
Nov	17.08	24.67	0	10.11
Dec	12.89	22.10	2.00	11.98
	Year: 2006		**Year 2016**	
Jan	8.09	12.95	0	27.59
Feb	2.03	8.11	0	10.91
Mar	4.46	11.91	2.75	27.47
Apr	5.76	21.11	0	13.55
May	3.22	11.27	1.25	11.24
Jun	17.89	11.93	5.47	9.11
Jul	10.02	18.04	3.55	35.52
Aug	1.91	24.76	7.08	8.85
Sep	5.83	15.53	3.66	14.65
Oct	1.81	23.51	9.35	11.68
Nov	1.90	22.77	3.41	14.79
Dec	1.84	12.89	7.81	15.63
	Year: 2007		**Year 2017**	
Jan	3.24	17.80	4.61	19.61
Feb	0	14.20	5.01	31.72
Mar	2.98	13.39	3.09	33.95
Apr	3.84	15.36	5.09	23.40
May	8.05	19.32	5.97	22.67
Jun	3.98	7.95	4.75	33.23
Jul	10.02	16.03	7.60	7.60
Aug	9.52	9.52	8.10	22.69
Sep	7.77	13.59	6.42	14.12
Oct	7.23	12.66	6.41	19.23
Nov	0	15.18	9.87	23.03
Dec	1.84	14.73	6.02	21.08

	Year: 2008		Year 2018	
Jan	3.21	8.57	2.53	12.63
Feb	4.56	18.22	1.91	11.43
Mar	1.05	11.54	16.85	29.96
Apr	3.42	13.68	11.74	16.43
May	15.83	34.30	13.09	10.47
Jun	12.06	5.36	13.31	11.41
Jul	0	26.08	2.17	4.34
Aug	22.35	13.04	6.95	12.15
Sep	3.10	12.38	10.85	15.18
Oct	13.08	16.82	4.09	5.46
Nov	0	10.33	1.45	11.56
Dec	0	6.14	4.59	25.23
	Year: 2009		**Year 2019**	
Jan	3.16	18.96	7.61	5.08
Feb	0	5.30	1.74	10.43
Mar	5.01	8.76	9.09	15.91
Apr	1.41	18.31	1.65	6.61
May	3.43	10.29	3.78	13.23
Jun	3.39	23.73	10.27	10.27
Jul	2.57	6.43	5.18	6.91
Aug	13.36	8.91	3.47	6.95
Sep	10.97	17.24	7.39	14.78
Oct	4.99	16.21	3.11	12.42
Nov	4.30	25.81	4.49	4.49
Dec	8.49	25.48	0	1.61
	Year: 2010		**Year 2020**	
Jan	3.96	5.94	0	7.98
Feb	1.98	5.93	0	18.87
Mar	4.16	14.55	2.32	4.64
Apr	3.87	17.41	5.21	10.42
May	5.56	8.35	0	7.22
Jun	0	21.32	4.00	8.00
Jul	7.56	22.67	2.07	4.14
Aug	4.89	3.37	0	17.91
Sep	1.77	5.30	6.85	6.85
Oct	12.17	18.26	3.33	3.33
Nov	0	9.10	2.64	7.92
Dec	2.76	11.05	0	10.53
	Year: 2011		**Year 2021**	
Jan	1.63	13.01	0	2.56
Feb	3.65	14.60	0	3.17
Mar	1.75	15.79	0	3.59
Apr	7.01	16.36	2.52	4.51
May	7.12	19.57	2.00	5.99
Jun	2.55	20.41	2.87	8.60

Jul	2.21	6.62	9.49	1.90
Aug	0	12.53	0	5.63
Sep	3.96	7.92	5.87	5.87
Oct	5.10	10.20	3.08	9.23
Nov	0	18.79	0	10.64
Dec	2.49	12.44	0	2.86
	Year: 2012		**Year 2022**	
Jan	1.49	7.46	5.13	5.13
Feb	8.83	8.83	0	0
Mar	3.15	6.29	0	0
Apr	2.04	8.16	0	2.19
May	2.01	6.04	0	3.24
Jun	7.71	15.42	4.56	6.83
Jul	4.07	16.26	1.53	3.06
Aug	19.23	25.64	0	2.63
Sep	5.54	9.23	2.43	0
Oct	0	12.01	2.93	1.46
Nov	0	8.57	0	13.67
Dec	1.88	5.63	0	4.59
	Year: 2013			
Jan	0	11.52		
Feb	3.26	6.53		
Mar	0	14.29		
Apr	6.65	15.52		
May	0	10.87		
Jun	5.44	21.74		
Jul	8.20	8.20		
Aug	14.41	5.76		
Sep	5.60	36.42		
Oct	0	5.62		
Nov	0	3.93		
Dec	1.95	7.80		
	Year: 2014			
Jan	1.88	9.38		
Feb	2.99	8.96		
Mar	7.81	26.04		
Apr	2.15	15.05		
May	1.79	16.07		
Jun	5.80	23.21		
Jul	8.33	8.33		
Aug	1.35	5.38		
Sep	17.24	17.24		
Oct	3.78	13.23		
Nov	1.93	7.71		
Dec	5.89	19.65		
	Year: 2015			

Jan	1.37	10.96		
Feb	2.29	18.35		
Mar	1.91	19.05		
Apr	1.69	15.54		
May	2.88	17.29		

<u>Supplemental Table 21</u>: Data Table for Figure 21 providing incidence rates per 1,000 bed days per month (rounded to the hundredths place) and year by organisms with no environmental relevance detected in bacteraemia/bloodstream infection assessments (font in black for Yorkhill attributed cases and in blue for QEUH-RHC attributed cases)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005	12.95	24.34	14.88	7.68	9.66	13.92	14.03	11.43	21.36	10.85	24.67	22.10
2006	12.95	8.11	11.91	21.11	11.27	11.93	18.04	24.76	15.53	23.51	22.77	12.89
2007	17.80	14.20	13.39	15.36	19.32	7.95	16.03	9.52	13.59	12.66	15.18	14.73
2008	8.57	18.22	11.54	13.68	34.30	5.36	26.08	13.04	12.38	16.82	10.33	6.14
2009	18.96	5.30	8.76	18.31	10.29	23.73	6.43	8.91	17.24	16.21	25.81	25.48
2010	5.94	5.93	14.55	17.41	8.35	21.32	22.67	3.37	5.30	18.26	9.10	11.05
2011	13.01	14.60	15.79	16.36	19.57	20.41	6.62	12.53	7.92	10.20	18.79	12.44
2012	7.46	8.83	6.29	8.16	6.04	15.42	16.26	25.64	9.23	12.01	8.57	5.63
2013	11.52	6.53	14.29	15.52	10.87	21.74	8.20	5.76	36.42	5.62	3.93	7.80
2014	9.38	8.96	26.04	15.05	16.07	23.21	8.33	5.38	17.24	13.23	7.71	19.65
2015	10.96	18.35	19.05	15.54	19.65	20.53	18.14	11.27	10.40	18.87	10.11	11.98
2016	27.59	10.91	27.47	13.55	11.24	9.11	35.52	8.85	14.65	11.68	14.79	15.63
2017	19.61	31.72	33.95	23.40	22.67	33.23	7.60	22.69	14.12	19.23	23.03	21.08
2018	12.63	11.43	29.96	16.43	10.47	11.41	4.34	12.15	15.18	5.46	11.56	25.23
2019	5.08	10.43	15.91	6.61	13.23	10.27	6.91	6.95	14.78	12.42	4.49	1.61
2020	7.98	18.87	4.64	10.42	7.22	8.00	4.14	17.91	6.85	3.33	7.92	10.53
2021	2.56	3.17	3.59	4.51	5.99	8.60	1.90	5.63	5.87	9.23	10.64	2.86
2022	5.13	0	0	2.19	3.24	6.83	3.06	2.63	0	1.46	13.67	4.59

Supplemental Table 22: Data Table for Figure 22 providing incidence rates per 1,000 bed days per month (rounded to the hundredths place) and year by organisms with environmental relevance detected in bacteraemia/bloodstream infection assessments

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005	3.24	8.11	1.49	1.92	8.05	13.92	10.02	11.43	0	5.43	17.08	12.89
2006	8.09	2.03	4.46	5.76	3.22	17.89	10.02	1.91	5.83	1.81	1.90	1.84
2007	3.24	0	2.98	3.84	8.05	3.98	10.02	9.52	7.77	7.23	0	1.84
2008	3.21	4.56	1.05	3.42	15.83	12.06	0	22.35	3.10	13.08	0	0
2009	3.16	0	5.01	1.41	3.43	3.39	2.57	13.36	10.97	4.99	4.30	8.49
2010	3.96	1.98	4.16	3.87	5.56	0	7.56	4.89	1.77	12.17	0	2.76
2011	1.63	3.65	1.75	7.01	7.12	2.55	2.21	0	3.96	5.10	0	2.49
2012	1.49	8.83	3.15	2.04	2.01	7.71	4.07	19.23	5.54	0	0	1.88
2013	0	3.26	0	6.65	0	5.44	8.20	14.41	5.60	0	0	1.95
2014	1.88	2.99	7.81	2.15	1.79	5.80	8.33	1.35	17.24	3.78	1.93	5.89
2015	1.37	2.29	1.91	1.69	2.88	0	0	0	0	1.89	0	2.00
2016	0	0	2.75	0	1.25	5.47	3.55	7.08	3.66	9.35	3.41	7.81
2017	4.61	5.01	3.09	5.09	5.97	4.75	7.60	8.10	6.42	6.41	9.87	6.02
2018	2.53	1.91	16.85	11.74	13.09	13.31	2.17	6.95	10.85	4.09	1.45	4.59
2019	7.61	1.74	9.09	1.65	3.78	10.27	5.18	3.47	7.39	3.11	4.49	0
2020	0	0	2.32	5.21	0	4.00	2.07	0	6.85	3.33	2.64	0
2021	0	0	0	2.52	2.00	2.87	9.49	0	5.87	3.08	0	0
2022	5.13	0	0	0	0	4.56	1.53	0	2.43	2.93	0	0

year	month	beddays_r	beddays_s	beddays_t	bsi_bmt (c	bsi_north (	bsi_south	bsi incider	bsi incider	bsi incidence rate_south
2013	1	443	286	505			3			10.48951
2013	2	316	165	225			4			24.24242
2013	3	339	177	332			3			16.94915
2013	4	489	284	441			1			3.521127
2013	5	389	319	364			4			12.53918
2013	6	396	180	355			2			11.11111
2013	7	460	514	405			11			21.40078
2013	8	450	95	398			6			63.15789
2013	9	300	483	399			4			8.281573
2013	10	379	204	552			5			24.5098
2013	11	383	244	584			6			24.59016
2013	12	461	205	452			2			9.756098
2014	1	385	210	457			7			33.33333
2014	2	398	96	405			3			31.25
2014	3	277	254	558	1		4	1.792115		15.74803
2014	4	363	447	429	13	3	10	30.30303	8.264462	22.37136
2014	5	307	183	502	11	3	10	21.91235	9.771987	54.64481
2014	6	495	234	526	17	2	7	32.31939	4.040404	29.91453
2014	7	386	230	478	14	3	6	29.2887	7.772021	26.08696
2014	8	472	365	476	24	5	8	50.42017	10.59322	21.91781
2014	9	460	267	491	16	5	3	32.58656	10.86957	11.23596
2014	10	488	214	472	15	3	7	31.77966	6.147541	32.71028
2014	11	548	226	764	18	7	3	23.56021	12.77372	13.27434
2014	12	251	334	563	20	2	5	35.52398	7.968128	14.97006
2015	1	672	222	444	19	5	1	42.79279	7.440476	4.504505
2015	2	328	406	356	21	9	2	58.98877	27.43902	4.926108
2015	3	392	452	471	14	3	11	29.72399	7.653061	24.33628
2015	4	335	193	405	19	8	2	46.91358	23.8806	10.36269
2015	5	455	228	467	12	3	6	25.69593	6.593407	26.31579
2015	6	221	166	725	20	1	7	27.58621	4.524887	42.16867
2015	7	292	257	456	23	4	2	50.43859	13.69863	7.782101
2015	8	411	178	385	19	9	2	49.35065	21.89781	11.23596
2015	9	460	189	541	27	12	7	49.90758	26.08696	37.03704
2015	10	438	157	649	23	9	6	35.43914	20.54795	38.21656
2015	11	382	216	569	15	5	4	26.36204	13.08901	18.51852
2015	12	427	206	444	23	5	5	51.8018	11.7096	24.27184
2016	1	523	191	454	13	12	7	28.63436	22.94455	36.64922

2016	2	483	145	467	28	4	6	59.95717	8.281573	41.37931
2016	3	390	170	617	20	7	4	32.41491	17.94872	23.52941
2016	4	304	246	522	19	1	3	36.39847	3.289474	12.19512
2016	5	472	283	481	8	13	1	16.63202	27.54237	3.533569
2016	6	404	197	424	20	5	9	47.16981	12.37624	45.68528
2016	7	316	162	668	35	11	3	52.39521	34.81013	18.51852
2016	8	341	262	527	29	7	3	55.02846	20.52786	11.45038
2016	9	354	45	487	23	8	9	47.22792	22.59887	200
2016	10	317	191	482	28	10	4	58.09129	31.54574	20.94241
2016	11	255	167	523	21	6	6	40.15297	23.52941	35.92814
2016	12	338	137	440	22	12	3	50	35.50296	21.89781
2017	1	299	83	456	31	5	6	67.98245	16.72241	72.28915
2017	2	381	108	419	22	7	5	52.50597	18.3727	46.2963
2017	3	388	265	399	33	9	7	82.70676	23.19588	26.41509
2017	4	331	116	433	30	4	11	69.28407	12.08459	94.82758
2017	5	428	245	424	32	9	18	75.47169	21.02804	73.46939
2017	6	513	12	475	28	5	0	58.94737	9.746589	0
2017	7	390	129	455	23	5	4	50.54945	12.82051	31.00775
2017	8	493	245	597	18	7	8	30.15075	14.19878	32.65306
2017	9	433	172	431	34	9	5	78.88631	20.78522	29.06977
2017	10	454	341	599	32	13	6	53.42237	28.63436	17.59531
2017	11	390	199	556	47	17	7	84.53237	43.58974	35.17588
2017	12	604	155	529	38	5	8	71.83365	8.278146	51.6129
2018	1	417	212	576	30	13	9	52.08333	31.17506	42.45283
2018	2	328	135	402	27	12	6	67.16418	36.58537	44.44444
2018	3	524	109	544	33	10	4	60.66177	19.08397	36.69725
2018	4	385	173	519	30	14	5	57.80347	36.36364	28.90173
2018	5	491	99	738	32	7	6	43.36044	14.25662	60.60606
2018	6	377	183	310	33	15	5	106.4516	39.7878	27.3224
2018	7	522	99	689	28	5	3	40.63861	9.578544	30.30303
2018	8	562	238	547	26	12	9	47.53199	21.35231	37.81512
2018	9	271	99	381	28	2	3	73.49081	7.380074	30.30303
2018	10	402	231	451	40	7	7	88.6918	17.41294	30.30303
2018	11	422	251	508	30	8	10	59.05512	18.95735	39.84064
2018	12	314	146	368	31	4	8	84.23913	12.73885	54.79452
2019	1	437	258	714	31	8	18	43.41737	18.30664	69.76744
2019	2	356	176	422	31	10	0	73.45972	28.08989	0
2019	3	442	161	486	25	2	10	51.44033	4.524887	62.1118

2019	4	407	161	339	30	7	6	88.49557	17.19902	37.26708
2019	5	422	294	458	24	7	8	52.40174	16.58768	27.21088
2019	6	287	200	287	13	17	3	45.29617	59.23345	15
2019	7	374	538	611	24	10	3	39.27987	26.73797	5.576208
2019	8	552	125	480	32	4	10	66.66666	7.246377	80
2019	9	314	189	364	22	7	10	60.43956	22.29299	52.91005
2019	10	418	209	642	28	6	11	43.61371	14.35407	52.63158
2019	11	519	68	337	24	8	10	71.21661	15.41426	147.0588
2019	12	321	320	428	19	6	6	44.39252	18.69159	18.75
2020	1	406	225	481	18	9	7	37.42204	22.16749	31.11111
2020	2	366	106	638	39	8	6	61.12852	21.85792	56.60378
2020	3	282	235	207	26	8	8	125.6039	28.36879	34.04255
2020	4	250	16	187	18	7	1	96.25668	28	62.5
2020	5	214	97	188	14	6	6	74.46809	28.03738	61.85567
2020	6	206	143	741	19	2	9	25.64103	9.708738	62.93706
2020	7	301	94	555	17	8	1	30.63063	26.57807	10.6383
2020	8	193	114	515	23	12	1	44.66019	62.17617	8.77193
2020	9	327	69	466	25	2	4	53.64807	6.116208	57.97102
2020	10	331	119	584	27	8	3	46.23288	24.16918	25.21008
2020	11	288	92	328	28	9	5	85.36585	31.25	54.34783
2020	12	410	137	321	20	11	3	62.3053	26.82927	21.89781
2021	1	305	153	439	16	9	8	36.44647	29.5082	52.28758
2021	2	353	51	504	19	1	5	37.69841	2.832861	98.03922
2021	3	358	231	528	25	5	10	47.34848	13.96648	43.29004
2021	4	494	135	563	21	16	1	37.30018	32.38866	7.407407
2021	5	328	124	491	19	11	12	38.69654	33.53659	96.77419
2021	6	364	222	589	32	6	3	54.32937	16.48352	13.51351
2021	7	306	82	637	17	8	6	26.6876	26.14379	73.17073
2021	8	262	136	421	25	7	6	59.38242	26.71756	44.11765
2021	9	454	185	511	22	10	3	43.05284	22.02643	16.21622
2021	10	416	246	981	31	3	5	31.60041	7.211538	20.3252
2021	11	299	241	509	13	5	3	25.54028	16.72241	12.44813
2021	12	527	145	310	25	6	14	80.64516	11.3852	96.55173
2022	1	495	130	490	14	12	0	28.57143	24.24242	0
2022	2	308	128	260	12	5	7	46.15385	16.23377	54.6875
2022	3	587	215	536	18	10	5	33.58209	17.03577	23.25581
2022	4	438	207	360	15	9	7	41.66667	20.54795	33.81643
2022	5	258	332	651	15	6	5	23.04148	23.25581	15.06024

2022	6	355	120	435	20	9	11	45.97701	25.35211	91.66666
2022	7	379	98	554	14	11	3	25.27076	29.02375	30.61225
2022	8	257	209	577	29	11	4	50.25996	42.80156	19.13876
2022	9	388	52	368	22	15	3	59.78261	38.65979	57.69231
2022	10	456	124	457	31	9	1	67.83369	19.73684	8.064516
2022	11	451	118	619	31	2	7	50.08078	4.43459	59.32203
2022	12	244	236	404	45	7	2	111.3861	28.68852	8.474576
2023	1	401	38	502	22	5	2	43.8247	12.46883	52.63158
2023	2	266	67	466	28	6	1	60.08584	22.55639	14.92537
2023	3	377	147	518	44	16	4	84.94209	42.44032	27.21088
2023	4	452	117	455	40	6	3	87.91209	13.27434	25.64103
2023	5	293	192	591	34	10	0	57.52961	34.12969	0
2023	6	185	34	108	32	3	3	296.2963	16.21622	88.23529

year	month	beddays_r	beddays_s	beddays_b	was BMT Q	Was North	Was South	bsi_bmt (c	bsi_north (	bsi_south	beddays_q	bsi_qeuh_(	bsi incider	beddays o	bsi count c	bsi incidence rate other hospitals
2013	1	443	286	505	no	no	no			3				286	3	10.48951
2013	2	316	165	225	no	no	no			4				165	4	24.24242
2013	3	339	177	332	no	no	no			3				177	3	16.94915
2013	4	489	284	441	no	no	no			1				284	1	3.521127
2013	5	389	319	364	no	no	no			4				319	4	12.53918
2013	6	396	180	355	no	no	no			2				180	2	11.11111
2013	7	460	514	405	no	no	no			11				514	11	21.40078
2013	8	450	95	398	no	no	no			6				95	6	63.15789
2013	9	300	483	399	no	no	no			4				483	4	8.281573
2013	10	379	204	552	no	no	no			5				204	5	24.5098
2013	11	383	244	584	no	no	no			6				244	6	24.59016
2013	12	461	205	452	no	no	no			2				205	2	9.756098
2014	1	385	210	457	no	no	no			7				210	7	33.33333
2014	2	398	96	405	no	no	no			3				96	3	31.25
2014	3	277	254	558	no	no	no	1		4				812	5	6.157636
2014	4	363	447	429	no	no	no	13	3	10				1239	26	20.98466
2014	5	307	183	502	no	no	no	11	3	10				992	24	24.19355
2014	6	495	234	526	no	no	no	17	2	7				1255	26	20.71713
2014	7	386	230	478	no	no	no	14	3	6				1094	23	21.02377
2014	8	472	365	476	no	no	no	24	5	8				1313	37	28.17974
2014	9	460	267	491	no	no	no	16	5	3				1218	24	19.70443
2014	10	488	214	472	no	no	no	15	3	7				1174	25	21.29472
2014	11	548	226	764	no	no	no	18	7	3				1538	28	18.20546
2014	12	251	334	563	no	no	no	20	2	5				1148	27	23.51916
2015	1	672	222	444	no	no	no	19	5	1				1338	25	18.6846
2015	2	328	406	356	no	no	no	21	9	2				1090	32	29.3578
2015	3	392	452	471	no	no	no	14	3	11				1315	28	21.29278
2015	4	335	193	405	no	no	no	19	8	2				933	29	31.08253
2015	5	455	228	467	no	no	yes	12	3	6	228	6	26.31579	922	15	16.26898
2015	6	221	166	725	yes	no	yes	20	1	7	891	27	30.30303	221	1	4.524887
2015	7	292	257	456	yes	no	yes	23	4	2	713	25	35.06311	292	4	13.69863
2015	8	411	178	385	no	no	yes	19	9	2	178	2	11.23596	796	28	35.17588
2015	9	460	189	541	no	no	yes	27	12	7	189	7	37.03704	1001	39	38.96104
2015	10	438	157	649	no	no	yes	23	9	6	157	6	38.21656	1087	32	29.43882
2015	11	382	216	569	no	no	yes	15	5	4	216	4	18.51852	951	20	21.03049
2015	12	427	206	444	no	no	yes	23	5	5	206	5	24.27184	871	28	32.14696
2016	1	523	191	454	no	no	yes	13	12	7	191	7	36.64922	977	25	25.58854
2016	2	483	145	467	no	no	yes	28	4	6	145	6	41.37931	950	32	33.68421
2016	3	390	170	617	no	no	yes	20	7	4	170	4	23.52941	1007	27	26.81231
2016	4	304	246	522	no	no	yes	19	1	3	246	3	12.19512	826	20	24.21308
2016	5	472	283	481	no	no	yes	8	13	1	283	1	3.533569	953	21	22.03568
2016	6	404	197	424	no	no	yes	20	5	9	197	9	45.68528	828	25	30.19324
2016	7	316	162	668	no	no	yes	35	11	3	162	3	18.51852	984	46	46.74797
2016	8	341	262	527	no	no	yes	29	7	3	262	3	11.45038	868	36	41.47466

2016	9	354	45	487	no	no	yes	23	8	9	45	9	200	841	31	36.86088
2016	10	317	191	482	no	no	yes	28	10	4	191	4	20.94241	799	38	47.55945
2016	11	255	167	523	no	no	yes	21	6	6	167	6	35.92814	778	27	34.70437
2016	12	338	137	440	no	no	yes	22	12	3	137	3	21.89781	778	34	43.7018
2017	1	299	83	456	no	no	yes	31	5	6	83	6	72.28915	755	36	47.68212
2017	2	381	108	419	no	no	yes	22	7	5	108	5	46.2963	800	29	36.25
2017	3	388	265	399	no	no	yes	33	9	7	265	7	26.41509	787	42	53.36722
2017	4	331	116	433	no	no	yes	30	4	11	116	11	94.82758	764	34	44.50262
2017	5	428	245	424	no	no	yes	32	9	18	245	18	73.46939	852	41	48.12207
2017	6	513	12	475	no	no	yes	28	5	0	12	0	0	988	33	33.40081
2017	7	390	129	455	no	no	yes	23	5	4	129	4	31.00775	845	28	33.13609
2017	8	493	245	597	no	no	yes	18	7	8	245	8	32.65306	1090	25	22.93578
2017	9	433	172	431	no	no	yes	34	9	5	172	5	29.06977	864	43	49.76852
2017	10	454	341	599	no	no	yes	32	13	6	341	6	17.59531	1053	45	42.73504
2017	11	390	199	556	no	no	yes	47	17	7	199	7	35.17588	946	64	67.65327
2017	12	604	155	529	no	no	yes	38	5	8	155	8	51.6129	1133	43	37.95234
2018	1	417	212	576	no	no	yes	30	13	9	212	9	42.45283	993	43	43.30312
2018	2	328	135	402	no	no	yes	27	12	6	135	6	44.44444	730	39	53.42466
2018	3	524	109	544	no	no	yes	33	10	4	109	4	36.69725	1068	43	40.26217
2018	4	385	173	519	no	no	yes	30	14	5	173	5	28.90173	904	44	48.67257
2018	5	491	99	738	no	no	yes	32	7	6	99	6	60.60606	1229	39	31.73312
2018	6	377	183	310	yes	no	yes	33	15	5	493	38	77.07911	377	15	39.7878
2018	7	522	99	689	yes	no	yes	28	5	3	788	31	39.3401	522	5	9.578544
2018	8	562	238	547	yes	no	yes	26	12	9	785	35	44.58599	562	12	21.35231
2018	9	271	99	381	yes	no	yes	28	2	3	480	31	64.58334	271	2	7.380074
2018	10	402	231	451	yes	no	yes	40	7	7	682	47	68.91496	402	7	17.41294
2018	11	422	251	508	yes	no	yes	30	8	10	759	40	52.70092	422	8	18.95735
2018	12	314	146	368	yes	no	yes	31	4	8	514	39	75.87549	314	4	12.73885
2019	1	437	258	714	yes	no	yes	31	8	18	972	49	50.41152	437	8	18.30664
2019	2	356	176	422	yes	no	yes	31	10	0	598	31	51.83947	356	10	28.08989
2019	3	442	161	486	yes	no	yes	25	2	10	647	35	54.09583	442	2	4.524887
2019	4	407	161	339	yes	no	yes	30	7	6	500	36	72	407	7	17.19902
2019	5	422	294	458	yes	no	yes	24	7	8	752	32	42.55319	422	7	16.58768
2019	6	287	200	287	yes	no	yes	13	17	3	487	16	32.85421	287	17	59.23345
2019	7	374	538	611	yes	no	yes	24	10	3	1149	27	23.4987	374	10	26.73797
2019	8	552	125	480	yes	no	yes	32	4	10	605	42	69.42149	552	4	7.246377
2019	9	314	189	364	yes	no	yes	22	7	10	553	32	57.86618	314	7	22.29299
2019	10	418	209	642	yes	no	yes	28	6	11	851	39	45.82844	418	6	14.35407
2019	11	519	68	337	yes	no	yes	24	8	10	405	34	83.95061	519	8	15.41426
2019	12	321	320	428	yes	no	yes	19	6	6	748	25	33.42246	321	6	18.69159
2020	1	406	225	481	yes	no	yes	18	9	7	706	25	35.41077	406	9	22.16749
2020	2	366	106	638	yes	no	yes	39	8	6	744	45	60.48387	366	8	21.85792
2020	3	282	235	207	yes	no	yes	26	8	8	442	34	76.92308	282	8	28.36879
2020	4	250	16	187	yes	no	yes	18	7	1	203	19	93.59606	250	7	28
2020	5	214	97	188	yes	no	yes	14	6	6	285	20	70.17544	214	6	28.03738

2020	6	206	143	741	yes	no	yes	19	2	9	884	28	31.67421	206	2	9.708738
2020	7	301	94	555	yes	no	yes	17	8	1	649	18	27.73498	301	8	26.57807
2020	8	193	114	515	yes	no	yes	23	12	1	629	24	38.1558	193	12	62.17617
2020	9	327	69	466	yes	no	yes	25	2	4	535	29	54.20561	327	2	6.116208
2020	10	331	119	584	yes	no	yes	27	8	3	703	30	42.67425	331	8	24.16918
2020	11	288	92	328	yes	no	yes	28	9	5	420	33	78.57143	288	9	31.25
2020	12	410	137	321	yes	no	yes	20	11	3	458	23	50.21834	410	11	26.82927
2021	1	305	153	439	yes	no	yes	16	9	8	592	24	40.54054	305	9	29.5082
2021	2	353	51	504	yes	no	yes	19	1	5	555	24	43.24324	353	1	2.832861
2021	3	358	231	528	yes	no	yes	25	5	10	759	35	46.11331	358	5	13.96648
2021	4	494	135	563	yes	no	yes	21	16	1	698	22	31.51863	494	16	32.38866
2021	5	328	124	491	yes	no	yes	19	11	12	615	31	50.40651	328	11	33.53659
2021	6	364	222	589	yes	no	yes	32	6	3	811	35	43.1566	364	6	16.48352
2021	7	306	82	637	yes	no	yes	17	8	6	719	23	31.98887	306	8	26.14379
2021	8	262	136	421	yes	no	yes	25	7	6	557	31	55.6553	262	7	26.71756
2021	9	454	185	511	yes	no	yes	22	10	3	696	25	35.91954	454	10	22.02643
2021	10	416	246	981	yes	no	yes	31	3	5	1227	36	29.33985	416	3	7.211538
2021	11	299	241	509	yes	no	yes	13	5	3	750	16	21.33333	299	5	16.72241
2021	12	527	145	310	yes	no	yes	25	6	14	455	39	85.71429	527	6	11.3852
2022	1	495	130	490	yes	no	yes	14	12	0	620	14	22.58064	495	12	24.24242
2022	2	308	128	260	yes	no	yes	12	5	7	388	19	48.96907	308	5	16.23377
2022	3	587	215	536	yes	no	yes	18	10	5	751	23	30.62583	587	10	17.03577
2022	4	438	207	360	yes	no	yes	15	9	7	567	22	38.8007	438	9	20.54795
2022	5	258	332	651	yes	no	yes	15	6	5	983	20	20.34588	258	6	23.25581
2022	6	355	120	435	yes	no	yes	20	9	11	555	31	55.85585	355	9	25.35211
2022	7	379	98	554	yes	no	yes	14	11	3	652	17	26.07362	379	11	29.02375
2022	8	257	209	577	yes	no	yes	29	11	4	786	33	41.98473	257	11	42.80156
2022	9	388	52	368	yes	no	yes	22	15	3	420	25	59.52381	388	15	38.65979
2022	10	456	124	457	yes	no	yes	31	9	1	581	32	55.07745	456	9	19.73684
2022	11	451	118	619	yes	no	yes	31	2	7	737	38	51.56038	451	2	4.43459
2022	12	244	236	404	yes	no	yes	45	7	2	640	47	73.4375	244	7	28.68852
2023	1	401	38	502	yes	no	yes	22	5	2	540	24	44.44444	401	5	12.46883
2023	2	266	67	466	yes	no	yes	28	6	1	533	29	54.409	266	6	22.55639
2023	3	377	147	518	yes	no	yes	44	16	4	665	48	72.18045	377	16	42.44032
2023	4	452	117	455	yes	no	yes	40	6	3	572	43	75.17483	452	6	13.27434
2023	5	293	192	591	yes	no	yes	34	10	0	783	34	43.42273	293	10	34.12969
2023	6	185	34	108	yes	no	yes	32	3	3	142	35	246.4789	185	3	16.21622

year	month	beddays_north	beddays_south	beddays_south_adjust	beddays_bmt	beddays_bmt_adjusted	was BMT Q	Was North	Was South	bsi_bmt (c	bsi_north (	bsi_south	qeuh_bsi_	beddays_c	ther_hosp	other_hos	qeuh_bed	qeuh_bsi_incidence_rate_adjusted
2013	1	443	286	286	505	505	0	0	0			3		286	3	10.48951		
2013	2	316	165	165	225	225	0	0	0			4		165	4	24.24242		
2013	3	339	177	177	332	332	0	0	0			3		177	3	16.94915		
2013	4	489	284	284	441	441	0	0	0			1		284	1	3.521127		
2013	5	389	319	319	364	364	0	0	0			4		319	4	12.53918		
2013	6	396	180	180	355	355	0	0	0			2		180	2	11.11111		
2013	7	460	514	514	405	405	0	0	0			11		514	11	21.40078		
2013	8	450	95	95	398	398	0	0	0			6		95	6	63.15789		
2013	9	300	483	483	399	399	0	0	0			4		483	4	8.281573		
2013	10	379	204	204	552	552	0	0	0			5		204	5	24.5098		
2013	11	383	244	244	584	584	0	0	0			6		244	6	24.59016		
2013	12	461	205	205	452	452	0	0	0			2		205	2	9.756098		
2014	1	385	210	210	457	457	0	0	0			7		210	7	33.33333		
2014	2	398	96	96	405	405	0	0	0			3		96	3	31.25		
2014	3	277	254	254	558	558	0	0	0	1		4		812	5	6.157636		
2014	4	363	447	447	429	429	0	0	0	13	3	10		1239	26	20.98466		
2014	5	307	183	183	502	502	0	0	0	11	3	10		992	24	24.19355		
2014	6	495	234	234	526	526	0	0	0	17	2	7		1255	26	20.71713		
2014	7	386	230	230	478	478	0	0	0	14	3	6		1094	23	21.02377		
2014	8	472	365	365	476	476	0	0	0	24	5	8		1313	37	28.17974		
2014	9	460	267	267	491	491	0	0	0	16	5	3		1218	24	19.70443		
2014	10	488	214	214	472	472	0	0	0	15	3	7		1174	25	21.29472		
2014	11	548	226	226	764	764	0	0	0	18	7	3		1538	28	18.20546		
2014	12	251	334	334	563	563	0	0	0	20	2	5		1148	27	23.51916		
2015	1	672	222	222	444	444	0	0	0	19	5	1		1338	25	18.6846		
2015	2	328	406	406	356	356	0	0	0	21	9	2		1090	32	29.3578		
2015	3	392	452	452	471	471	0	0	0	14	3	11		1315	28	21.29278		
2015	4	335	193	193	405	405	0	0	0	19	8	2		933	29	31.08253		
2015	5	455	228	228	467	467	0	0	1	12	3	6	6	922	15	16.26898	228	26.31579
2015	6	221	166	166	725	725	1	0	1	20	1	7	27	221	1	4.524887	891	30.30303
2015	7	292	257	257	456	456	1	0	1	23	4	2	25	292	4	13.69863	713	35.06311
2015	8	411	178	178	385	385	0	0	1	19	9	2	2	796	28	35.17588	178	11.23596
2015	9	460	189	189	541	541	0	0	1	27	12	7	7	1001	39	38.96104	189	37.03704
2015	10	438	157	157	649	649	0	0	1	23	9	6	6	1087	32	29.43882	157	38.21656
2015	11	382	216	216	569	569	0	0	1	15	5	4	4	951	20	21.03049	216	18.51852
2015	12	427	206	206	444	444	0	0	1	23	5	5	5	871	28	32.14696	206	24.27184
2016	1	523	191	191	454	454	0	0	1	13	12	7	7	977	25	25.58854	191	36.64922
2016	2	483	145	145	467	467	0	0	1	28	4	6	6	950	32	33.68421	145	41.37931
2016	3	390	170	170	617	617	0	0	1	20	7	4	4	1007	27	26.81231	170	23.52941
2016	4	304	246	246	522	522	0	0	1	19	1	3	3	826	20	24.21308	246	12.19512
2016	5	472	283	283	481	481	0	0	1	8	13	1	1	953	21	22.03568	283	3.533569
2016	6	404	197	197	424	424	0	0	1	20	5	9	9	828	25	30.19324	197	45.68528
2016	7	316	162	162	668	668	0	0	1	35	11	3	3	984	46	46.74797	162	18.51852
2016	8	341	262	262	527	527	0	0	1	29	7	3	3	868	36	41.47466	262	11.45038
2016	9	354	45	219	487	487	0	0	1	23	8	9	9	841	31	36.86088	219	41.09589
2016	10	317	191	191	482	482	0	0	1	28	10	4	4	799	38	47.55945	191	20.94241
2016	11	255	167	167	523	523	0	0	1	21	6	6	6	778	27	34.70437	167	35.92814
2016	12	338	137	137	440	440	0	0	1	22	12	3	3	778	34	43.7018	137	21.89781
2017	1	299	83	83	456	456	0	0	1	31	5	6	6	755	36	47.68212	83	72.28915
2017	2	381	108	108	419	419	0	0	1	22	7	5	5	800	29	36.25	108	46.2963
2017	3	388	265	265	399	399	0	0	1	33	9	7	7	787	42	53.36722	265	26.41509
2017	4	331	116	116	433	433	0	0	1	30	4	11	11	764	34	44.50262	116	94.82758
2017	5	428	245	245	424	424	0	0	1	32	9	18	18	852	41	48.12207	245	73.46939
2017	6	513	12	173	475	475	0	0	1	28	5	0	0	988	33	33.40081	173	0
2017	7	390	129	129	455	455	0	0	1	23	5	4	4	845	28	33.13609	129	31.00775
2017	8	493	245	245	597	597	0	0	1	18	7	8	8	1090	25	22.93578	245	32.65306
2017	9	433	172	172	431	431	0	0	1	34	9	5	5	864	43	49.76852	172	29.06977
2017	10	454	341	341	599	599	0	0	1	32	13	6	6	1053	45	42.73504	341	17.59531
2017	11	390	199	199	556	556	0	0	1	47	17	7	7	946	64	67.65327	199	35.17588
2017	12	604	155	155	529	529	0	0	1	38	5	8	8	1133	43	37.95234	155	51.6129

2018	1	417	212	212	576	576	0	0	1	30	13	9	9	993	43	43.30312	212	42.45283
2018	2	328	135	135	402	402	0	0	1	27	12	6	6	730	39	53.42466	135	44.44444
2018	3	524	109	109	544	544	0	0	1	33	10	4	4	1068	43	40.26217	109	36.69725
2018	4	385	173	173	519	519	0	0	1	30	14	5	5	904	44	48.67257	173	28.90173
2018	5	491	99	99	738	738	0	0	1	32	7	6	6	1229	39	31.73312	99	60.60606
2018	6	377	183	183	310	310	1	0	1	33	15	5	38	377	15	39.7878	493	77.07911
2018	7	522	99	99	689	689	1	0	1	28	5	3	31	522	5	9.578544	788	39.3401
2018	8	562	238	238	547	547	1	0	1	26	12	9	35	562	12	21.35231	785	44.58599
2018	9	271	99	99	381	381	1	0	1	28	2	3	31	271	2	7.380074	480	64.58334
2018	10	402	231	231	451	451	1	0	1	40	7	7	47	402	7	17.41294	682	68.91496
2018	11	422	251	251	508	508	1	0	1	30	8	10	40	422	8	18.95735	759	52.70092
2018	12	314	146	146	368	368	1	0	1	31	4	8	39	314	4	12.73885	514	75.87549
2019	1	437	258	258	714	714	1	0	1	31	8	18	49	437	8	18.30664	972	50.41152
2019	2	356	176	176	422	422	1	0	1	31	10	0	31	356	10	28.08989	598	51.83947
2019	3	442	161	161	486	486	1	0	1	25	2	10	35	442	2	4.524887	647	54.09583
2019	4	407	161	161	339	339	1	0	1	30	7	6	36	407	7	17.19902	500	72
2019	5	422	294	294	458	458	1	0	1	24	7	8	32	422	7	16.58768	752	42.55319
2019	6	287	200	200	287	287	1	0	1	13	17	3	16	287	17	59.23345	487	32.85421
2019	7	374	538	538	611	611	1	0	1	24	10	3	27	374	10	26.73797	1149	23.4987
2019	8	552	125	125	480	480	1	0	1	32	4	10	42	552	4	7.246377	605	69.42149
2019	9	314	189	189	364	364	1	0	1	22	7	10	32	314	7	22.29299	553	57.86618
2019	10	418	209	209	642	642	1	0	1	28	6	11	39	418	6	14.35407	851	45.82844
2019	11	519	68	195	337	337	1	0	1	24	8	10	34	519	8	15.41426	532	63.90977
2019	12	321	320	320	428	428	1	0	1	19	6	6	25	321	6	18.69159	748	33.42246
2020	1	406	225	225	481	481	1	0	1	18	9	7	25	406	9	22.16749	706	35.41077
2020	2	366	106	106	638	638	1	0	1	39	8	6	45	366	8	21.85792	744	60.48387
2020	3	282	235	235	207	207	1	0	1	26	8	8	34	282	8	28.36879	442	76.92308
2020	4	250	16	208	187	187	1	0	1	18	7	1	19	250	7	28	395	48.10126
2020	5	214	97	97	188	188	1	0	1	14	6	6	20	214	6	28.03738	285	70.17544
2020	6	206	143	143	741	741	1	0	1	19	2	9	28	206	2	9.708738	884	31.67421
2020	7	301	94	94	555	555	1	0	1	17	8	1	18	301	8	26.57807	649	27.73498
2020	8	193	114	114	515	515	1	0	1	23	12	1	24	193	12	62.17617	629	38.1558
2020	9	327	69	219	466	466	1	0	1	25	2	4	29	327	2	6.116208	685	42.33577
2020	10	331	119	119	584	584	1	0	1	27	8	3	30	331	8	24.16918	703	42.67425
2020	11	288	92	92	328	328	1	0	1	28	9	5	33	288	9	31.25	420	78.57143
2020	12	410	137	137	321	321	1	0	1	20	11	3	23	410	11	26.82927	458	50.21834
2021	1	305	153	153	439	439	1	0	1	16	9	8	24	305	9	29.5082	592	40.54054
2021	2	353	51	140	504	504	1	0	1	19	1	5	24	353	1	2.832861	644	37.26708
2021	3	358	231	231	528	528	1	0	1	25	5	10	35	358	5	13.96648	759	46.11331
2021	4	494	135	135	563	563	1	0	1	21	16	1	22	494	16	32.38866	698	31.51863
2021	5	328	124	124	491	491	1	0	1	19	11	12	31	328	11	33.53659	615	50.40651
2021	6	364	222	222	589	589	1	0	1	32	6	3	35	364	6	16.48352	811	43.1566
2021	7	306	82	82	637	637	1	0	1	17	8	6	23	306	8	26.14379	719	31.98887
2021	8	262	136	136	421	421	1	0	1	25	7	6	31	262	7	26.71756	557	55.6553
2021	9	454	185	185	511	511	1	0	1	22	10	3	25	454	10	22.02643	696	35.91954
2021	10	416	246	246	981	981	1	0	1	31	3	5	36	416	3	7.211538	1227	29.33985
2021	11	299	241	241	509	509	1	0	1	13	5	3	16	299	5	16.72241	750	21.33333
2021	12	527	145	145	310	310	1	0	1	25	6	14	39	527	6	11.3852	455	85.71429
2022	1	495	130	130	490	490	1	0	1	14	12	0	14	495	12	24.24242	620	22.58064
2022	2	308	128	128	260	260	1	0	1	12	5	7	19	308	5	16.23377	388	48.96907
2022	3	587	215	215	536	536	1	0	1	18	10	5	23	587	10	17.03577	751	30.62583
2022	4	438	207	207	360	360	1	0	1	15	9	7	22	438	9	20.54795	567	38.8007
2022	5	258	332	332	651	651	1	0	1	15	6	5	20	258	6	23.25581	983	20.34588
2022	6	355	120	120	435	435	1	0	1	20	9	11	31	355	9	25.35211	555	55.85585
2022	7	379	98	98	554	554	1	0	1	14	11	3	17	379	11	29.02375	652	26.07362
2022	8	257	209	209	577	577	1	0	1	29	11	4	33	257	11	42.80156	786	41.98473
2022	9	388	52	219	368	368	1	0	1	22	15	3	25	388	15	38.65979	587	42.58944
2022	10	456	124	124	457	457	1	0	1	31	9	1	32	456	9	19.73684	581	55.07745
2022	11	451	118	118	619	619	1	0	1	31	2	7	38	451	2	4.43459	737	51.56038
2022	12	244	236	236	404	404	1	0	1	45	7	2	47	244	7	28.68852	640	73.4375
2023	1	401	38	195	502	502	1	0	1	22	5	2	24	401	5	12.46883	697	34.43328

2023	2	266	67	140	466	466	1	0	1	28	6	1	29	266	6	22.55639	606	47.85479
2023	3	377	147	147	518	518	1	0	1	44	16	4	48	377	16	42.44032	665	72.18045
2023	4	452	117	117	455	455	1	0	1	40	6	3	43	452	6	13.27434	572	75.17483
2023	5	293	192	192	591	591	1	0	1	34	10	0	34	293	10	34.12969	783	43.42273
2023	6	185	34	173	108	482	1	0	1	32	3	3	35	185	3	16.21622	655	53.43512

year	Month	beddays	all_bsi_co	environme	non-envirc	bsi incider	environme	non-envirc	month nur	hospital
2005	Jan	618	10	2	8	16.18123	3.236246	12.94498	1	yorkhill
2005	Feb	493	16	4	12	32.45436	8.11359	24.34077	2	yorkhill
2005	Mar	672	11	1	10	16.36905	1.488095	14.88095	3	yorkhill
2005	Apr	521	5	1	4	9.596929	1.919386	7.677543	4	yorkhill
2005	May	621	11	5	6	17.71337	8.05153	9.661836	5	yorkhill
2005	Jun	503	14	7	7	27.833	13.9165	13.9165	6	yorkhill
2005	Jul	499	12	5	7	24.0481	10.02004	14.02806	7	yorkhill
2005	Aug	525	12	6	6	22.85714	11.42857	11.42857	8	yorkhill
2005	Sep	515	11	0	11	21.35922	0	21.35922	9	yorkhill
2005	Oct	553	9	3	6	16.27486	5.424955	10.84991	10	yorkhill
2005	Nov	527	22	9	13	41.74573	17.0778	24.66793	11	yorkhill
2005	Dec	543	19	7	12	34.99079	12.89134	22.09945	12	yorkhill
2006	Jan	618	13	5	8	21.0356	8.090615	12.94498	1	yorkhill
2006	Feb	493	5	1	4	10.14199	2.028398	8.11359	2	yorkhill
2006	Mar	672	11	3	8	16.36905	4.464286	11.90476	3	yorkhill
2006	Apr	521	14	3	11	26.8714	5.758157	21.11324	4	yorkhill
2006	May	621	9	2	7	14.49275	3.220612	11.27214	5	yorkhill
2006	Jun	503	15	9	6	29.82107	17.89264	11.92843	6	yorkhill
2006	Jul	499	14	5	9	28.05611	10.02004	18.03607	7	yorkhill
2006	Aug	525	14	1	13	26.66667	1.904762	24.76191	8	yorkhill
2006	Sep	515	11	3	8	21.35922	5.825243	15.53398	9	yorkhill
2006	Oct	553	14	1	13	25.31646	1.808318	23.50814	10	yorkhill
2006	Nov	527	13	1	12	24.66793	1.897533	22.7704	11	yorkhill
2006	Dec	543	8	1	7	14.73297	1.841621	12.89134	12	yorkhill
2007	Jan	618	13	2	11	21.0356	3.236246	17.79935	1	yorkhill
2007	Feb	493	7	0	7	14.19878	0	14.19878	2	yorkhill
2007	Mar	672	11	2	9	16.36905	2.976191	13.39286	3	yorkhill
2007	Apr	521	10	2	8	19.19386	3.838772	15.35509	4	yorkhill
2007	May	621	17	5	12	27.3752	8.05153	19.32367	5	yorkhill
2007	Jun	503	6	2	4	11.92843	3.976143	7.952286	6	yorkhill
2007	Jul	499	13	5	8	26.0521	10.02004	16.03206	7	yorkhill
2007	Aug	525	10	5	5	19.04762	9.523809	9.523809	8	yorkhill
2007	Sep	515	11	4	7	21.35922	7.76699	13.59223	9	yorkhill
2007	Oct	553	11	4	7	19.8915	7.233273	12.65823	10	yorkhill
2007	Nov	527	8	0	8	15.18027	0	15.18027	11	yorkhill
2007	Dec	543	9	1	8	16.57458	1.841621	14.73297	12	yorkhill
2008	Jan	934	11	3	8	11.7773	3.211992	8.56531	1	yorkhill

2008	Feb	439	10	2	8	22.77904	4.555809	18.22323	2 yorkhill
2008	Mar	953	12	1	11	12.59181	1.049318	11.5425	3 yorkhill
2008	Apr	585	10	2	8	17.09402	3.418803	13.67521	4 yorkhill
2008	May	379	19	6	13	50.13193	15.83113	34.30079	5 yorkhill
2008	Jun	746	13	9	4	17.42627	12.06434	5.36193	6 yorkhill
2008	Jul	767	20	0	20	26.07562	0	26.07562	7 yorkhill
2008	Aug	537	19	12	7	35.38175	22.34637	13.03538	8 yorkhill
2008	Sep	646	10	2	8	15.47988	3.095975	12.3839	9 yorkhill
2008	Oct	535	16	7	9	29.90654	13.08411	16.82243	10 yorkhill
2008	Nov	484	5	0	5	10.33058	0	10.33058	11 yorkhill
2008	Dec	651	4	0	4	6.144393	0	6.144393	12 yorkhill
2009	Jan	633	14	2	12	22.1169	3.159558	18.95735	1 yorkhill
2009	Feb	381	2	0	2	5.249344	0	5.249344	2 yorkhill
2009	Mar	799	11	4	7	13.76721	5.006258	8.760951	3 yorkhill
2009	Apr	710	14	1	13	19.71831	1.408451	18.30986	4 yorkhill
2009	May	583	8	2	6	13.72213	3.430532	10.2916	5 yorkhill
2009	Jun	590	16	2	14	27.11864	3.389831	23.72881	6 yorkhill
2009	Jul	778	7	2	5	8.997429	2.570694	6.426735	7 yorkhill
2009	Aug	449	10	6	4	22.27172	13.36303	8.908686	8 yorkhill
2009	Sep	638	18	7	11	28.21317	10.97179	17.24138	9 yorkhill
2009	Oct	802	17	4	13	21.19701	4.987531	16.20948	10 yorkhill
2009	Nov	465	14	2	12	30.10753	4.301075	25.80645	11 yorkhill
2009	Dec	471	16	4	12	33.97028	8.492569	25.47771	12 yorkhill
2010	Jan	505	5	2	3	9.90099	3.960396	5.940594	1 yorkhill
2010	Feb	506	4	1	3	7.905138	1.976285	5.928854	2 yorkhill
2010	Mar	481	9	2	7	18.71102	4.158004	14.55301	3 yorkhill
2010	Apr	517	11	2	9	21.2766	3.868472	17.40812	4 yorkhill
2010	May	719	10	4	6	13.90821	5.563282	8.344924	5 yorkhill
2010	Jun	516	11	0	11	21.31783	0	21.31783	6 yorkhill
2010	Jul	397	12	3	9	30.2267	7.556675	22.67002	7 yorkhill
2010	Aug	891	7	4	3	7.856341	4.489338	3.367003	8 yorkhill
2010	Sep	566	4	1	3	7.067138	1.766784	5.300354	9 yorkhill
2010	Oct	493	15	6	9	30.42596	12.17039	18.25558	10 yorkhill
2010	Nov	769	7	0	7	9.102731	0	9.102731	11 yorkhill
2010	Dec	724	10	2	8	13.81215	2.762431	11.04972	12 yorkhill
2011	Jan	615	9	1	8	14.63415	1.626016	13.00813	1 yorkhill
2011	Feb	274	5	1	4	18.24817	3.649635	14.59854	2 yorkhill
2011	Mar	570	10	1	9	17.54386	1.754386	15.78947	3 yorkhill

2011	Apr	428	10	3	7	23.36449	7.009346	16.35514	4	yorkhill
2011	May	562	15	4	11	26.69039	7.117438	19.57295	5	yorkhill
2011	Jun	392	9	1	8	22.95918	2.55102	20.40816	6	yorkhill
2011	Jul	453	4	1	3	8.830022	2.207505	6.622517	7	yorkhill
2011	Aug	399	5	0	5	12.53133	0	12.53133	8	yorkhill
2011	Sep	505	6	2	4	11.88119	3.960396	7.920792	9	yorkhill
2011	Oct	392	6	2	4	15.30612	5.102041	10.20408	10	yorkhill
2011	Nov	479	9	0	9	18.78914	0	18.78914	11	yorkhill
2011	Dec	402	6	1	5	14.92537	2.487562	12.43781	12	yorkhill
2012	Jan	670	6	1	5	8.955224	1.492537	7.462687	1	yorkhill
2012	Feb	566	10	5	5	17.66784	8.833922	8.833922	2	yorkhill
2012	Mar	954	9	3	6	9.433962	3.144654	6.289308	3	yorkhill
2012	Apr	490	5	1	4	10.20408	2.040816	8.163265	4	yorkhill
2012	May	994	8	2	6	8.048289	2.012072	6.036217	5	yorkhill
2012	Jun	389	9	3	6	23.13625	7.712082	15.42416	6	yorkhill
2012	Jul	492	10	2	8	20.3252	4.065041	16.26016	7	yorkhill
2012	Aug	312	14	6	8	44.8718	19.23077	25.64103	8	yorkhill
2012	Sep	542	8	3	5	14.76015	5.535055	9.225092	9	yorkhill
2012	Oct	583	7	0	7	12.00686	0	12.00686	10	yorkhill
2012	Nov	467	4	0	4	8.56531	0	8.56531	11	yorkhill
2012	Dec	533	4	1	3	7.504691	1.876173	5.628518	12	yorkhill
2013	Jan	434	5	0	5	11.52074	0	11.52074	1	yorkhill
2013	Feb	613	6	2	4	9.787929	3.262643	6.525285	2	yorkhill
2013	Mar	560	8	0	8	14.28571	0	14.28571	3	yorkhill
2013	Apr	451	10	3	7	22.17295	6.651885	15.52106	4	yorkhill
2013	May	552	6	0	6	10.86957	0	10.86957	5	yorkhill
2013	Jun	368	10	2	8	27.17391	5.434783	21.73913	6	yorkhill
2013	Jul	244	4	2	2	16.39344	8.196721	8.196721	7	yorkhill
2013	Aug	347	7	5	2	20.17291	14.40922	5.763689	8	yorkhill
2013	Sep	357	15	2	13	42.01681	5.602241	36.41457	9	yorkhill
2013	Oct	534	3	0	3	5.617978	0	5.617978	10	yorkhill
2013	Nov	509	2	0	2	3.929273	0	3.929273	11	yorkhill
2013	Dec	513	5	1	4	9.746589	1.949318	7.797271	12	yorkhill
2014	Jan	533	6	1	5	11.25704	1.876173	9.380863	1	yorkhill
2014	Feb	670	8	2	6	11.9403	2.985075	8.955224	2	yorkhill
2014	Mar	384	13	3	10	33.85417	7.8125	26.04167	3	yorkhill
2014	Apr	465	8	1	7	17.2043	2.150538	15.05376	4	yorkhill
2014	May	560	10	1	9	17.85714	1.785714	16.07143	5	yorkhill

2014	Jun	517	15	3	12	29.01354	5.802708	23.21083	6	yorkhill
2014	Jul	360	6	3	3	16.66667	8.333333	8.333333	7	yorkhill
2014	Aug	743	5	1	4	6.729475	1.345895	5.38358	8	yorkhill
2014	Sep	348	12	6	6	34.48276	17.24138	17.24138	9	yorkhill
2014	Oct	529	9	2	7	17.01323	3.780718	13.23251	10	yorkhill
2014	Nov	519	5	1	4	9.633911	1.926782	7.707129	11	yorkhill
2014	Dec	509	13	3	10	25.54028	5.893909	19.64637	12	yorkhill
2015	Jan	730	9	1	8	12.32877	1.369863	10.9589	1	yorkhill
2015	Feb	436	9	1	8	20.6422	2.293578	18.34862	2	yorkhill
2015	Mar	525	11	1	10	20.95238	1.904762	19.04762	3	yorkhill
2015	Apr	591	9	1	8	15.22843	1.692047	13.53638	4	yorkhill
2015	May	347	7	1	6	20.17291	2.881844	17.29107	5	yorkhill
2015	Jun	487	10	0	10	20.53388	0	20.53388	6	qeuh
2015	Jul	386	7	0	7	18.13471	0	18.13471	7	qeuh
2015	Aug	621	7	0	7	11.27214	0	11.27214	8	qeuh
2015	Sep	481	5	0	5	10.39501	0	10.39501	9	qeuh
2015	Oct	530	11	1	10	20.75472	1.886792	18.86792	10	qeuh
2015	Nov	791	8	0	8	10.11378	0	10.11378	11	qeuh
2015	Dec	501	7	1	6	13.97206	1.996008	11.97605	12	qeuh
2016	Jan	435	11	0	11	25.28736	0	25.28736	1	qeuh
2016	Feb	550	6	0	6	10.90909	0	10.90909	2	qeuh
2016	Mar	364	11	1	10	30.21978	2.747253	27.47253	3	qeuh
2016	Apr	664	9	0	9	13.55422	0	13.55422	4	qeuh
2016	May	801	10	1	9	12.48439	1.248439	11.23596	5	qeuh
2016	Jun	549	8	3	5	14.57195	5.464481	9.107468	6	qeuh
2016	Jul	563	22	2	20	39.07638	3.552398	35.52398	7	qeuh
2016	Aug	565	9	4	5	15.9292	7.079646	8.849558	8	qeuh
2016	Sep	546	10	2	8	18.31502	3.663004	14.65201	9	qeuh
2016	Oct	428	9	4	5	21.02804	9.345795	11.68224	10	qeuh
2016	Nov	879	16	3	13	18.2025	3.412969	14.78953	11	qeuh
2016	Dec	640	15	5	10	23.4375	7.8125	15.625	12	qeuh
2017	Jan	867	21	4	17	24.22145	4.61361	19.60784	1	qeuh
2017	Feb	599	22	3	19	36.72788	5.008347	31.71953	2	qeuh
2017	Mar	648	24	2	22	37.03704	3.08642	33.95062	3	qeuh
2017	Apr	983	28	5	23	28.48423	5.08647	23.39776	4	qeuh
2017	May	838	24	5	19	28.63962	5.966587	22.67303	5	qeuh
2017	Jun	632	24	3	21	37.97468	4.746835	33.22785	6	qeuh
2017	Jul	658	10	5	5	15.19757	7.598784	7.598784	7	qeuh

2017	Aug	617	19	5	14	30.79416	8.103727	22.69044	8 qeuh
2017	Sep	779	16	5	11	20.53915	6.418485	14.12067	9 qeuh
2017	Oct	624	16	4	12	25.64103	6.410256	19.23077	10 qeuh
2017	Nov	608	20	6	14	32.89474	9.868421	23.02632	11 qeuh
2017	Dec	664	18	4	14	27.10843	6.024096	21.08434	12 qeuh
2018	Jan	792	12	2	10	15.15152	2.525253	12.62626	1 qeuh
2018	Feb	525	7	1	6	13.33333	1.904762	11.42857	2 qeuh
2018	Mar	534	25	9	16	46.81648	16.85393	29.96255	3 qeuh
2018	Apr	426	12	5	7	28.16901	11.73709	16.43192	4 qeuh
2018	May	764	18	10	8	23.56021	13.08901	10.4712	5 qeuh
2018	Jun	526	13	7	6	24.71483	13.30798	11.40684	6 qeuh
2018	Jul	461	3	1	2	6.507592	2.169197	4.338395	7 qeuh
2018	Aug	576	11	4	7	19.09722	6.944445	12.15278	8 qeuh
2018	Sep	461	12	5	7	26.03037	10.84599	15.18438	9 qeuh
2018	Oct	733	7	3	4	9.549795	4.09277	5.457026	10 qeuh
2018	Nov	692	9	1	8	13.00578	1.445087	11.56069	11 qeuh
2018	Dec	436	13	2	11	29.81651	4.587156	25.22936	12 qeuh
2019	Jan	394	5	3	2	12.69036	7.614213	5.076142	1 qeuh
2019	Feb	575	7	1	6	12.17391	1.73913	10.43478	2 qeuh
2019	Mar	440	11	4	7	25	9.090909	15.90909	3 qeuh
2019	Apr	605	5	1	4	8.264462	1.652893	6.61157	4 qeuh
2019	May	529	9	2	7	17.01323	3.780718	13.23251	5 qeuh
2019	Jun	292	6	3	3	20.54795	10.27397	10.27397	6 qeuh
2019	Jul	579	7	3	4	12.08981	5.181347	6.908463	7 qeuh
2019	Aug	288	3	1	2	10.41667	3.472222	6.944445	8 qeuh
2019	Sep	406	9	3	6	22.16749	7.389163	14.77833	9 qeuh
2019	Oct	322	5	1	4	15.52795	3.10559	12.42236	10 qeuh
2019	Nov	668	6	3	3	8.982036	4.491018	4.491018	11 qeuh
2019	Dec	623	1	0	1	1.605136	0	1.605136	12 qeuh
2020	Jan	501	4	0	4	7.984032	0	7.984032	1 qeuh
2020	Feb	424	8	0	8	18.86792	0	18.86792	2 qeuh
2020	Mar	431	3	1	2	6.960557	2.320186	4.640371	3 qeuh
2020	Apr	192	3	1	2	15.625	5.208333	10.41667	4 qeuh
2020	May	277	2	0	2	7.220217	0	7.220217	5 qeuh
2020	Jun	250	3	1	2	12	4	8	6 qeuh
2020	Jul	483	3	1	2	6.21118	2.070393	4.140787	7 qeuh
2020	Aug	335	6	0	6	17.91045	0	17.91045	8 qeuh
2020	Sep	438	6	3	3	13.69863	6.849315	6.849315	9 qeuh

2020	Oct	300	2	1	1	6.666667	3.333333	3.333333	10	qeuh
2020	Nov	379	4	1	3	10.55409	2.638522	7.915567	11	qeuh
2020	Dec	380	4	0	4	10.52632	0	10.52632	12	qeuh
2021	Jan	390	1	0	1	2.564103	0	2.564103	1	qeuh
2021	Feb	316	1	0	1	3.164557	0	3.164557	2	qeuh
2021	Mar	557	2	0	2	3.590664	0	3.590664	3	qeuh
2021	Apr	444	3	1	2	6.756757	2.252252	4.504505	4	qeuh
2021	May	501	4	1	3	7.984032	1.996008	5.988024	5	qeuh
2021	Jun	349	4	1	3	11.46132	2.86533	8.595988	6	qeuh
2021	Jul	527	6	5	1	11.3852	9.487666	1.897533	7	qeuh
2021	Aug	533	3	0	3	5.628518	0	5.628518	8	qeuh
2021	Sep	511	6	3	3	11.74168	5.870842	5.870842	9	qeuh
2021	Oct	325	4	1	3	12.30769	3.076923	9.230769	10	qeuh
2021	Nov	376	4	0	4	10.6383	0	10.6383	11	qeuh
2021	Dec	350	1	0	1	2.857143	0	2.857143	12	qeuh
2022	Jan	390	4	2	2	10.25641	5.128205	5.128205	1	qeuh
2022	Feb	157	0	0	0	0	0	0	2	qeuh
2022	Mar	338	0	0	0	0	0	0	3	qeuh
2022	Apr	457	1	0	1	2.188184	0	2.188184	4	qeuh
2022	May	618	2	0	2	3.236246	0	3.236246	5	qeuh
2022	Jun	439	5	2	3	11.38952	4.555809	6.833713	6	qeuh
2022	Jul	654	3	1	2	4.587156	1.529052	3.058104	7	qeuh
2022	Aug	381	1	0	1	2.624672	0	2.624672	8	qeuh
2022	Sep	412	1	1	0	2.427185	2.427185	0	9	qeuh
2022	Oct	683	3	2	1	4.392386	2.928258	1.464129	10	qeuh
2022	Nov	439	6	0	6	13.66743	0	13.66743	11	qeuh
2022	Dec	436	2	0	2	4.587156	0	4.587156	12	qeuh

Year	Month	Admissions	Beddays
2005	Jan	84	618
2005	Feb	69	493
2005	Mar	88	672
2005	Apr	78	521
2005	May	81	621
2005	Jun	82	503
2005	Jul	74	499
2005	Aug	77	525
2005	Sep	75	515
2005	Oct	72	553
2005	Nov	76	527
2005	Dec	89	543
2006	Jan	84	618
2006	Feb	69	493
2006	Mar	88	672
2006	Apr	78	521
2006	May	81	621
2006	Jun	82	503
2006	Jul	74	499
2006	Aug	77	525
2006	Sep	75	515
2006	Oct	72	553
2006	Nov	76	527
2006	Dec	89	543
2007	Jan	84	618
2007	Feb	69	493
2007	Mar	88	672
2007	Apr	78	521
2007	May	81	621
2007	Jun	82	503
2007	Jul	74	499
2007	Aug	77	525
2007	Sep	75	515
2007	Oct	72	553
2007	Nov	76	527
2007	Dec	89	543
2008	Jan	99	934
2008	Feb	67	439
2008	Mar	72	953
2008	Apr	70	585
2008	May	72	379
2008	Jun	71	746
2008	Jul	81	767
2008	Aug	60	537
2008	Sep	63	646
2008	Oct	56	535
2008	Nov	76	484
2008	Dec	69	651
2009	Jan	64	633
2009	Feb	51	381
2009	Mar	91	799
2009	Apr	75	710
2009	May	61	583
2009	Jun	71	590

2009	Jul	57	778
2009	Aug	71	449
2009	Sep	71	638
2009	Oct	60	802
2009	Nov	71	465
2009	Dec	80	471
2010	Jan	73	505
2010	Feb	76	506
2010	Mar	84	481
2010	Apr	82	517
2010	May	87	719
2010	Jun	87	516
2010	Jul	74	397
2010	Aug	86	891
2010	Sep	86	566
2010	Oct	69	493
2010	Nov	73	769
2010	Dec	80	724
2011	Jan	68	615
2011	Feb	54	274
2011	Mar	84	570
2011	Apr	56	428
2011	May	80	562
2011	Jun	64	392
2011	Jul	64	453
2011	Aug	73	399
2011	Sep	67	505
2011	Oct	75	392
2011	Nov	70	479
2011	Dec	92	402
2012	Jan	105	670
2012	Feb	82	566
2012	Mar	108	954
2012	Apr	92	490
2012	May	85	994
2012	Jun	76	389
2012	Jul	84	492
2012	Aug	71	312
2012	Sep	72	542
2012	Oct	77	583
2012	Nov	86	467
2012	Dec	104	533
2013	Jan	81	434
2013	Feb	77	613
2013	Mar	93	560
2013	Apr	81	451
2013	May	90	552
2013	Jun	113	368
2013	Jul	83	244
2013	Aug	92	347
2013	Sep	92	357
2013	Oct	86	534
2013	Nov	86	509
2013	Dec	106	513
2014	Jan	98	533

2014	Feb	77	670
2014	Mar	84	384
2014	Apr	92	465
2014	May	90	560
2014	Jun	94	517
2014	Jul	78	360
2014	Aug	87	743
2014	Sep	71	348
2014	Oct	82	529
2014	Nov	69	519
2014	Dec	94	509
2015	Jan	81	730
2015	Feb	71	436
2015	Mar	102	525
2015	Apr	96	591
2015	May	85	347
2015	Jun	93	487
2015	Jul	73	386
2015	Aug	83	621
2015	Sep	92	481
2015	Oct	103	530
2015	Nov	102	791
2015	Dec	81	501
2016	Jan	94	435
2016	Feb	80	550
2016	Mar	101	364
2016	Apr	93	664
2016	May	78	801
2016	Jun	81	549
2016	Jul	105	563
2016	Aug	103	565
2016	Sep	116	546
2016	Oct	87	428
2016	Nov	100	879
2016	Dec	86	640
2017	Jan	112	867
2017	Feb	77	599
2017	Mar	108	648
2017	Apr	74	983
2017	May	95	838
2017	Jun	82	632
2017	Jul	84	658
2017	Aug	105	617
2017	Sep	88	779
2017	Oct	69	624
2017	Nov	103	608
2017	Dec	111	664
2018	Jan	122	792
2018	Feb	90	525
2018	Mar	95	534
2018	Apr	96	426
2018	May	106	764
2018	Jun	74	526
2018	Jul	115	461
2018	Aug	106	576

2018	Sep	92	461
2018	Oct	106	733
2018	Nov	105	692
2018	Dec	87	436
2019	Jan	81	394
2019	Feb	88	575
2019	Mar	101	440
2019	Apr	85	605
2019	May	96	529
2019	Jun	63	292
2019	Jul	89	579
2019	Aug	68	288
2019	Sep	77	406
2019	Oct	87	322
2019	Nov	96	668
2019	Dec	88	623
2020	Jan	85	501
2020	Feb	74	424
2020	Mar	74	431
2020	Apr	43	192
2020	May	43	277
2020	Jun	46	250
2020	Jul	57	483
2020	Aug	58	335
2020	Sep	60	438
2020	Oct	54	300
2020	Nov	61	379
2020	Dec	64	380
2021	Jan	72	390
2021	Feb	56	316
2021	Mar	89	557
2021	Apr	69	444
2021	May	83	501
2021	Jun	69	349
2021	Jul	81	527
2021	Aug	89	533
2021	Sep	89	511
2021	Oct	97	325
2021	Nov	97	376
2021	Dec	101	350
2022	Jan	97	390
2022	Feb	64	157
2022	Mar	75	338
2022	Apr	78	457
2022	May	104	618
2022	Jun	101	439
2022	Jul	67	654
2022	Aug	73	381
2022	Sep	96	412
2022	Oct	87	683
2022	Nov	85	439
2022	Dec	108	436

year	month	beddays_r	beddays_s	bedds_adj	beddays_b	beddb_adj	adm_northl	adm_southl	adm_bmt
2013	1	443	286	286	505	505	59	55	26
2013	2	316	165	165	225	225	44	36	14
2013	3	339	177	177	332	332	48	54	30
2013	4	489	284	284	441	441	58	89	26
2013	5	389	319	319	364	364	61	29	30
2013	6	396	180	180	355	355	56	35	34
2013	7	460	514	514	405	405	56	38	30
2013	8	450	95	95	398	398	49	21	21
2013	9	300	483	483	399	399	38	42	30
2013	10	379	204	204	552	552	46	40	36
2013	11	383	244	244	584	584	47	56	38
2013	12	461	205	205	452	452	40	36	28
2014	1	385	210	210	457	457	52	45	36
2014	2	398	96	96	405	405	52	29	26
2014	3	277	254	254	558	558	33	48	38
2014	4	363	447	447	429	429	35	56	31
2014	5	307	183	183	502	502	44	26	38
2014	6	495	234	234	526	526	60	25	35
2014	7	386	230	230	478	478	75	25	35
2014	8	472	365	365	476	476	50	45	49
2014	9	460	267	267	491	491	53	28	25
2014	10	488	214	214	472	472	51	28	33
2014	11	548	226	226	764	764	39	28	28
2014	12	251	334	334	563	563	44	38	34
2015	1	672	222	222	444	444	94	35	41
2015	2	328	406	406	356	356	46	34	21
2015	3	392	452	452	471	471	39	27	32
2015	4	335	193	193	405	405	42	28	35
2015	5	455	228	228	467	467	43	18	25
2015	6	221	166	166	725	725	44	30	34
2015	7	292	257	257	456	456	41	33	36
2015	8	411	178	178	385	385	45	21	34
2015	9	460	189	189	541	541	52	29	31
2015	10	438	157	157	649	649	48	27	42
2015	11	382	216	216	569	569	43	24	29
2015	12	427	206	206	444	444	57	26	27
2016	1	523	191	191	454	454	68	15	31

2016	2	483	145	145	467	467	62	21	27
2016	3	390	170	170	617	617	58	19	29
2016	4	304	246	246	522	522	53	29	30
2016	5	472	283	283	481	481	37	26	28
2016	6	404	197	197	424	424	41	19	25
2016	7	316	162	162	668	668	37	30	27
2016	8	341	262	262	527	527	48	24	25
2016	9	354	45	219	487	487	48	9	25
2016	10	317	191	191	482	482	46	17	26
2016	11	255	167	167	523	523	36	17	18
2016	12	338	137	137	440	440	39	23	21
2017	1	299	83	83	456	456	39	12	26
2017	2	381	108	108	419	419	49	10	28
2017	3	388	265	265	399	399	55	17	22
2017	4	331	116	116	433	433	51	12	31
2017	5	428	245	245	424	424	55	14	30
2017	6	513	12	173	475	475	54	3	32
2017	7	390	129	129	455	455	52	20	22
2017	8	493	245	245	597	597	62	22	25
2017	9	433	172	172	431	431	44	17	27
2017	10	454	341	341	599	599	56	18	25
2017	11	390	199	199	556	556	62	9	25
2017	12	604	155	155	529	529	67	21	20
2018	1	417	212	212	576	576	86	17	31
2018	2	328	135	135	402	402	46	6	24
2018	3	524	109	109	544	544	57	16	27
2018	4	385	173	173	519	519	62	21	29
2018	5	491	99	99	738	738	76	10	30
2018	6	377	183	183	310	310	62	18	22
2018	7	522	99	99	689	689	65	15	41
2018	8	562	238	238	547	547	72	25	34
2018	9	271	99	99	381	381	49	7	41
2018	10	402	231	231	451	451	61	17	23
2018	11	422	251	251	508	508	49	18	26
2018	12	314	146	146	368	368	51	17	25
2019	1	437	258	258	714	714	57	23	28
2019	2	356	176	176	422	422	49	24	24
2019	3	442	161	161	486	486	46	13	26

2019	4	407	161	161	339	339	57	11	24
2019	5	422	294	294	458	458	46	20	27
2019	6	287	200	200	287	287	50	16	15
2019	7	374	538	538	611	611	46	20	27
2019	8	552	125	125	480	480	43	12	29
2019	9	314	189	189	364	364	38	21	24
2019	10	418	209	209	642	642	55	22	36
2019	11	519	68	195	337	337	76	17	21
2019	12	321	320	320	428	428	77	21	25
2020	1	406	225	225	481	481	80	13	25
2020	2	366	106	106	638	638	214	17	32
2020	3	282	235	235	207	207	58	19	15
2020	4	250	16	208	187	187	35	9	9
2020	5	214	97	97	188	188	24	13	8
2020	6	206	143	143	741	741	31	18	14
2020	7	301	94	94	555	555	32	18	18
2020	8	193	114	114	515	515	26	21	22
2020	9	327	69	219	466	466	33	15	20
2020	10	331	119	119	584	584	34	13	20
2020	11	288	92	92	328	328	27	9	14
2020	12	410	137	137	321	321	38	13	12
2021	1	305	153	153	439	439	29	13	10
2021	2	353	51	140	504	504	38	10	18
2021	3	358	231	231	528	528	37	34	23
2021	4	494	135	135	563	563	44	32	16
2021	5	328	124	124	491	491	56	21	20
2021	6	364	222	222	589	589	51	18	23
2021	7	306	82	82	637	637	48	15	16
2021	8	262	136	136	421	421	56	17	19
2021	9	454	185	185	511	511	48	17	19
2021	10	416	246	246	981	981	39	12	16
2021	11	299	241	241	509	509	51	26	20
2021	12	527	145	145	310	310	48	24	15
2022	1	495	130	130	490	490	55	13	22
2022	2	308	128	128	260	260	55	15	14
2022	3	587	215	215	536	536	68	17	24
2022	4	438	207	207	360	360	51	17	18
2022	5	258	332	332	651	651	53	18	25

2022	6	355	120	120	435	435	51	13	22
2022	7	379	98	98	554	554	45	27	21
2022	8	257	209	209	577	577	52	17	25
2022	9	388	52	219	368	368	39	6	23
2022	10	456	124	124	457	457	38	15	19
2022	11	451	118	118	619	619	39	15	29
2022	12	244	236	236	404	404	48	15	17
2023	1	401	38	195	502	502	47	13	28
2023	2	266	67	140	466	466	38	15	20
2023	3	377	147	147	518	518	53	26	25
2023	4	452	117	117	455	455	50	20	21
2023	5	293	192	192	591	591	56	15	27
2023	6	185	34	173	108	482	63	11	24

year	month	beddays_t	beddays_s	beddays_l	bsi_bmt (c	bsi_north	bsi_south	environme	environme	enironmei	bsi incide	bsi incide	bsi incide	environme	environme	environme	non-envirc	non-envirc	non-envirc	non-envirc	non-environmental	bsi incidence rate_south
2013	1	443	286	505			3			1			10.48951			3.496504			2		6.993007	
2013	2	316	165	225			4			1			24.24242			6.060606			3		18.18182	
2013	3	339	177	332			3			0			16.94915			0			3		16.94915	
2013	4	489	284	441			1			0			3.521127			0			1		3.521127	
2013	5	389	319	364			4			0			12.53918			0			4		12.53918	
2013	6	396	180	355			2			1			11.11111			5.555555			1		5.555555	
2013	7	460	514	405			11			4			21.40078			7.782101			7		13.61868	
2013	8	450	95	398			6			0			63.15789			0			6		63.15789	
2013	9	300	483	399			4			1			8.281573			2.070393			3		6.21118	
2013	10	379	204	552			5			0			24.5098			0			5		24.5098	
2013	11	383	244	584			6			1			24.59016			4.098361			5		20.4918	
2013	12	461	205	452			2			0			9.756098			0			2		9.756098	
2014	1	385	210	457			7			2			33.33333			9.523809			5		23.80952	
2014	2	398	96	405			3			0			31.25			0			3		31.25	
2014	3	277	254	558	1		4	1		0	1.792115		15.74803	1.792115		0	0		4	0	15.74803	
2014	4	363	447	429	13	3	10	3	0	1	30.30303	8.264462	22.37136	6.993007	0	2.237136	10	3	9	23.31002	8.264462	20.13423
2014	5	307	183	502	11	3	10	1	0	0	21.91235	9.771987	54.64481	1.992032	0	0	10	3	10	19.92032	9.771987	54.64481
2014	6	495	234	526	17	2	7	3	0	4	32.31939	4.040404	29.91453	5.703422	0	17.09402	14	2	3	26.61597	4.040404	12.82051
2014	7	386	230	478	14	3	6	3	0	1	29.2887	7.772021	26.08696	6.276151	0	4.347826	11	3	5	23.01255	7.772021	21.73913
2014	8	472	365	476	24	5	8	5	0	2	50.42017	10.59322	21.91781	10.5042	0	5.479452	19	5	6	39.91597	10.59322	16.43836
2014	9	460	267	491	16	5	3	3	0	0	32.58656	10.86957	11.23596	6.10998	0	0	13	5	3	26.47658	10.86957	11.23596
2014	10	488	214	472	15	3	7	4	0	2	31.77966	6.147541	32.71028	8.474576	0	9.345795	11	3	5	23.30508	6.147541	23.36449
2014	11	548	226	764	18	7	3	3	1	0	23.56021	12.77372	13.27434	3.926702	1.824818	0	15	6	3	19.63351	10.9489	13.27434
2014	12	251	334	563	20	2	5	3	0	0	35.52398	7.968128	14.97006	5.328597	0	0	17	2	5	30.19538	7.968128	14.97006
2015	1	672	222	444	19	5	1	2	0	0	42.79279	7.440476	4.504505	4.504505	0	0	17	5	1	38.28829	7.440476	4.504505
2015	2	328	406	356	21	9	2	6	1	0	58.98877	27.43902	4.926108	16.85393	3.04878	0	15	8	2	42.13483	24.39024	4.926108
2015	3	392	452	471	14	3	11	0	0	0	29.72399	7.653061	24.33628	0	0	0	14	3	11	29.72399	7.653061	24.33628
2015	4	335	193	405	19	8	2	2	1	1	46.91358	23.8806	10.36269	4.938272	2.985075	5.181347	17	7	1	41.97531	20.89552	5.181347
2015	5	455	228	467	12	3	6	1	0	0	25.69593	6.593407	26.31579	2.141328	0	0	11	3	6	23.5546	6.593407	26.31579
2015	6	221	166	725	20	1	7	4	1	0	27.58621	4.524887	42.16867	5.517241	4.524887	0	16	0	7	22.06897	0	42.16867
2015	7	292	257	456	23	4	2	2	0	1	50.43859	13.69863	7.782101	4.385965	0	3.891051	21	4	1	46.05263	13.69863	3.891051
2015	8	411	178	385	19	9	2	1	0	0	49.35065	21.89781	11.23596	2.597403	0	0	18	9	2	46.75325	21.89781	11.23596
2015	9	460	189	541	27	12	7	3	2	0	49.90758	26.08696	37.03704	5.545287	4.347826	0	24	10	7	44.36229	21.73913	37.03704
2015	10	438	157	649	23	9	6	1	2	1	35.43914	20.54795	38.21656	1.540832	4.56621	6.369427	22	7	5	33.8983	15.98174	31.84713
2015	11	382	216	569	15	5	4	0	1	0	26.36204	13.08901	18.51852	0	2.617801	0	15	4	4	26.36204	10.4712	18.51852
2015	12	427	206	444	23	5	5	2	2	1	51.8018	11.7096	24.27184	4.504505	4.683841	4.854369	21	3	4	47.2973	7.025761	19.41748
2016	1	523	191	454	13	12	7	3	1	1	28.63436	22.94455	36.64922	6.60793	1.912046	5.235602	10	11	6	22.02643	21.03251	31.41361
2016	2	483	145	467	28	4	6	6	0	1	59.95717	8.281573	41.37931	12.84797	0	6.896552	22	4	5	47.10921	8.281573	34.48276
2016	3	390	170	617	20	7	4	6	1	1	32.41491	17.94872	23.52941	9.724473	2.564103	5.882353	14	6	3	22.69044	15.38461	17.64706
2016	4	304	246	522	19	1	3	0	0	0	36.39847	3.289474	12.19512	0	0	0	19	1	3	36.39847	3.289474	12.19512
2016	5	472	283	481	8	13	1	0	4	0	16.63202	27.54237	3.533569	0	8.474576	0	8	9	1	16.63202	19.0678	3.533569
2016	6	404	197	424	20	5	9	7	1	0	47.16981	12.37624	45.68528	16.50943	2.475248	0	13	4	9	30.66038	9.90099	45.68528
2016	7	316	162	668	35	11	3	2	1	0	52.39521	34.81013	18.51852	2.994012	3.164557	0	33	10	3	49.4012	31.64557	18.51852
2016	8	341	262	527	29	7	3	3	0	1	55.02846	20.52786	11.45038	5.6926	0	3.816794	26	7	2	49.33587	20.52786	7.633588
2016	9	354	45	487	23	8	9	1	1	1	47.22792	22.59887	200	2.053388	2.824859	22.22222	22	7	8	45.17454	19.77401	177.7778
2016	10	317	191	482	28	10	4	4	0	1	58.09129	31.54574	20.94241	8.298756	0	5.235602	24	10	3	49.79253	31.54574	15.70681
2016	11	255	167	523	21	6	6	1	1	2	40.15297	23.52941	35.92814	1.912046	3.921569	11.97605	20	5	4	38.24092	19.60784	23.9521
2016	12	338	137	440	22	12	3	3	0	1	50	35.50296	21.89781	6.818182	0	7.29927	19	12	2	43.18182	35.50296	14.59854
2017	1	299	83	456	31	5	6	4	0	1	67.98245	16.72241	72.28915	8.77193	0	12.04819	27	5	5	59.21053	16.72241	60.24096
2017	2	381	108	419	22	7	5	2	1	0	52.50597	18.3727	46.2963	4.77327	2.624672	0	20	6	5	47.7327	15.74803	46.2963
2017	3	388	265	399	33	9	7	2	1	1	82.70676	23.19588	26.41509	5.012531	2.57732	3.773585	31	8	6	77.69424	20.61856	22.64151
2017	4	331	116	433	30	4	11	3	0	1	69.28407	12.08459	94.82758	6.928406	0	8.620689	27	4	10	62.35566	12.08459	86.20689
2017	5	428	245	424	32	9	18	2	1	2	75.47169	21.02804	73.46939	4.716981	2.336449	8.163265	30	8	16	70.75471	18.69159	65.30612
2017	6	513	12	475	28	5	0	2	0	0	58.94737	9.746589	0	4.210526	0	0	26	5	0	54.73684	9.746589	0
2017	7	390	129	455	23	5	4	2	0	0	50.54945	12.82051	31.00775	4.395605	0	0	21	5	4	46.15385	12.82051	31.00775
2017	8	493	245	597	18	7	8	3	1	1	30.15075	14.19878	32.65306	5.025126	2.028398	4.081633	15	6	7	25.12563	12.17039	28.57143
2017	9	433	172	431	34	9	5	5	2	1	78.88631	20.78522	29.06977	11.60093	4.618937	5.813953	29	7	4	67.28539	16.16628	23.25581
2017	10	454	341	599	32	13	6	1	2	3	53.42237	28.63436	17.59531	1.669449	4.405286	8.797654	31	11	3	51.75292	24.22907	8.797654
2017	11	390	199	556	47	17	7	4	2	1	84.53237	43.58974	35.17588	7.194244	5.128205	5.025126	43	15	6	77.33813	38.46154	30.15075
2017	12	604	155	529	38	5	8	1	0	3	71.83365	8.278146	51.6129	1.890359	0	19.35484	37	5	5	69.94329	8.278146	32.25806

2018	1	417	212	576	30	13	9	2	1	0	52.08333	31.17506	42.45283	3.472222	2.398082	0	28	12	9	48.61111	28.77698	42.45283
2018	2	328	135	402	27	12	6	1	1	0	67.16418	36.58537	44.44444	2.487562	3.04878	0	26	11	6	64.67662	33.53659	44.44444
2018	3	524	109	544	33	10	4	0	0	1	60.66177	19.08397	36.69725	0	0	9.174312	33	10	3	60.66177	19.08397	27.52294
2018	4	385	173	519	30	14	5	2	0	0	57.80347	36.36364	28.90173	3.853565	0	0	28	14	5	53.94991	36.36364	28.90173
2018	5	491	99	738	32	7	6	2	1	0	43.36044	14.25662	60.60606	2.710027	2.03666	0	30	6	6	40.65041	12.21996	60.60606
2018	6	377	183	310	33	15	5	2	2	1	106.4516	39.7878	27.3224	6.451613	5.30504	5.464481	31	13	4	100	34.48276	21.85792
2018	7	522	99	689	28	5	3	4	0	0	40.63861	9.578544	30.30303	5.805515	0	0	24	5	3	34.83309	9.578544	30.30303
2018	8	562	238	547	26	12	9	4	4	1	47.53199	21.35231	37.81512	7.312614	7.117438	4.201681	22	8	8	40.21938	14.23488	33.61345
2018	9	271	99	381	28	2	3	5	1	0	73.49081	7.380074	30.30303	13.12336	3.690037	0	23	1	3	60.36745	3.690037	30.30303
2018	10	402	231	451	40	7	7	4	3	0	88.6918	17.41294	30.30303	8.86918	7.462687	0	36	4	7	79.82262	9.950249	30.30303
2018	11	422	251	508	30	8	10	0	2	1	59.05512	18.95735	39.84064	0	4.739336	3.984064	30	6	9	59.05512	14.21801	35.85658
2018	12	314	146	368	31	4	8	0	0	0	84.23913	12.73885	54.79452	0	0	0	31	4	8	84.23913	12.73885	54.79452
2019	1	437	258	714	31	8	18	5	2	3	43.41737	18.30664	69.76744	7.002801	4.576659	11.62791	26	6	15	36.41457	13.72998	58.13953
2019	2	356	176	422	31	10	0	3	0	0	73.45972	28.08989	0	7.109005	0	0	28	10	0	66.35071	28.08989	0
2019	3	442	161	486	25	2	10	3	1	0	51.44033	4.524887	62.1118	6.17284	2.262444	0	22	1	10	45.26749	2.262444	62.1118
2019	4	407	161	339	30	7	6	2	1	0	88.49557	17.19902	37.26708	5.899705	2.457002	0	28	6	6	82.59587	14.74201	37.26708
2019	5	422	294	458	24	7	8	1	2	0	52.40174	16.58768	27.21088	2.183406	4.739336	0	23	5	8	50.21834	11.84834	27.21088
2019	6	287	200	287	13	17	3	1	3	0	45.29617	59.23345	15	3.484321	10.45296	0	12	14	3	41.81185	48.78049	15
2019	7	374	538	611	24	10	3	2	1	0	39.27987	26.73797	5.576208	3.273322	2.673797	0	22	9	3	36.00655	24.06417	5.576208
2019	8	552	125	480	32	4	10	3	0	0	66.66666	7.246377	80	6.25	0	0	29	4	10	60.41667	7.246377	80
2019	9	314	189	364	22	7	10	3	0	0	60.43956	22.29299	52.91005	8.241758	0	0	19	7	10	52.1978	22.29299	52.91005
2019	10	418	209	642	28	6	11	3	0	3	43.61371	14.35407	52.63158	4.672897	0	14.35407	25	6	8	38.94081	14.35407	38.27751
2019	11	519	68	337	24	8	10	0	2	0	71.21661	15.41426	147.0588	0	3.853565	0	24	6	10	71.21661	11.56069	147.0588
2019	12	321	320	428	19	6	6	1	2	1	44.39252	18.69159	18.75	2.336449	6.23053	3.125	18	4	5	42.05608	12.46106	15.625
2020	1	406	225	481	18	9	7	1	1	1	37.42204	22.16749	31.11111	2.079002	2.463054	4.444445	17	8	6	35.34304	19.70443	26.66667
2020	2	366	106	638	39	8	6	4	1	0	61.12852	21.85792	56.60378	6.269592	2.73224	0	35	7	6	54.85893	19.12568	56.60378
2020	3	282	235	207	26	8	8	3	0	1	125.6039	28.36879	34.04255	14.49275	0	4.255319	23	8	7	111.1111	28.36879	29.78723
2020	4	250	16	187	18	7	1	0	0	0	96.25668	28	62.5	0	0	0	18	7	1	96.25668	28	62.5
2020	5	214	97	188	14	6	6	1	1	1	74.46809	28.03738	61.85567	5.319149	4.672897	10.30928	13	5	5	69.14893	23.36449	51.54639
2020	6	206	143	741	19	2	9	3	0	0	25.64103	9.708738	62.93706	4.048583	0	0	16	2	9	21.59244	9.708738	62.93706
2020	7	301	94	555	17	8	1	1	1	0	30.63063	26.57807	10.6383	1.801802	3.322259	0	16	7	1	28.82883	23.25581	10.6383
2020	8	193	114	515	23	12	1	1	0	0	44.66019	62.17617	8.77193	1.941748	0	0	22	12	1	42.71844	62.17617	8.77193
2020	9	327	69	466	25	2	4	0	0	1	53.64807	6.116208	57.97102	0	0	14.49275	25	2	3	53.64807	6.116208	43.47826
2020	10	331	119	584	27	8	3	1	2	3	46.23288	24.16918	25.21008	1.712329	6.042296	25.21008	26	6	0	44.52055	18.12689	0
2020	11	288	92	328	28	9	5	3	2	1	85.36585	31.25	54.34783	9.146341	6.944445	10.86957	25	7	4	76.21951	24.30556	43.47826
2020	12	410	137	321	20	11	3	0	2	1	62.3053	26.82927	21.89781	0	4.878049	7.29927	20	9	2	62.3053	21.95122	14.59854
2021	1	305	153	439	16	9	8	0	1	4	36.44647	29.5082	52.28758	0	3.278688	26.14379	16	8	4	36.44647	26.22951	26.14379
2021	2	353	51	504	19	1	5	1	0	0	37.69841	2.832861	98.03922	1.984127	0	0	18	1	5	35.71429	2.832861	98.03922
2021	3	358	231	528	25	5	10	0	2	0	47.34848	13.96648	43.29004	0	5.586592	0	25	3	10	47.34848	8.379889	43.29004
2021	4	494	135	563	21	16	1	2	5	0	37.30018	32.38866	7.407407	3.552398	10.12146	0	19	11	1	33.74778	22.26721	7.407407
2021	5	328	124	491	19	11	12	2	1	2	38.69654	33.53659	96.77419	4.07332	3.04878	16.12903	17	10	10	34.62322	30.4878	80.64516
2021	6	364	222	589	32	6	3	1	2	0	54.32937	16.48352	13.51351	1.697793	5.494505	0	31	4	3	52.63158	10.98901	13.51351
2021	7	306	82	637	17	8	6	0	1	0	26.6876	26.14379	73.17073	0	3.267974	0	17	7	6	26.6876	22.87582	73.17073
2021	8	262	136	421	25	7	6	1	2	3	59.38242	26.71756	44.11765	2.375297	7.633588	22.05882	24	5	3	57.00713	19.08397	22.05882
2021	9	454	185	511	22	10	3	2	2	0	43.05284	22.02643	16.21622	3.913894	4.405286	0	20	8	3	39.13894	17.62115	16.21622
2021	10	416	246	981	31	3	5	1	1	1	31.60041	7.211538	20.3252	1.019368	2.403846	4.065041	30	2	4	30.58104	4.807693	16.26016
2021	11	299	241	509	13	5	3	0	1	1	25.54028	16.72241	12.44813	0	3.344482	4.149378	13	4	2	25.54028	13.37793	8.298756
2021	12	527	145	310	25	6	14	2	1	3	80.64516	11.3852	96.55173	6.451613	1.897533	20.68966	23	5	11	74.19355	9.487666	75.86207
2022	1	495	130	490	14	12	0	0	1	0	28.57143	24.24242	0	0	2.020202	0	14	11	0	28.57143	22.22222	0
2022	2	308	128	260	12	5	7	1	1	2	46.15385	16.23377	54.6875	3.846154	3.246753	15.625	11	4	5	42.30769	12.98701	39.0625
2022	3	587	215	536	18	10	5	1	0	3	33.58209	17.03577	23.25581	1.865672	0	13.95349	17	10	2	31.71642	17.03577	9.302325
2022	4	438	207	360	15	9	7	0	1	1	41.66667	20.54795	33.81643	0	2.283105	4.830918	15	8	6	41.66667	18.26484	28.98551
2022	5	258	332	651	15	6	5	1	1	0	23.04148	23.25581	15.06024	1.536098	3.875969	0	14	5	5	21.50538	19.37984	15.06024
2022	6	355	120	435	20	9	11	0	1	1	45.97701	25.35211	91.66666	0	2.816901	8.333333	20	8	10	45.97701	22.53521	83.33334
2022	7	379	98	554	14	11	3	0	2	0	25.27076	29.02375	30.61225	0	5.277045	0	14	9	3	25.27076	23.7467	30.61225
2022	8	257	209	577	29	11	4	5	6	1	50.25996	42.80156	19.13876	8.665511	23.3463	4.784689	24	5	3	41.59446	19.45525	14.35407
2022	9	388	52	368	22	15	3	3	1	0	59.78261	38.65979	57.69231	8.152174	2.57732	0	19	14	3	51.63044	36.08247	57.69231
2022	10	456	124	457	31	9	1	3	1	0	67.83369	19.73684	8.064516	6.564551	2.192982	0	28	8	1	61.26915	17.54386	8.064516
2022	11	451	118	619	31	2	7	1	1	0	50.08078	4.43459	59.32203	1.615509	2.217295	0	30	1	7	48.46527	2.217295	59.32203
2022	12	244	236	404	45	7	2	3	2	0	111.3861	28.68852	8.474576	7.425743	8.196721	0	42	5	2	103.9604	20.4918	8.474576
2023	1	401	38	502	22	5	2	1	1	0	43.8247	12.46883	52.63158	1.992032	2.493766	0	21	4	2	41.83267	9.975062	52.63158

2023	2	266	67	466	28	6	1	2	0	0	60.08584	22.55639	14.92537	4.291845	0	0	26	6	1	55.79399	22.55639	14.92537
2023	3	377	147	518	44	16	4	0	4	1	84.94209	42.44032	27.21088	0	10.61008	6.802721	44	12	3	84.94209	31.83024	20.40816
2023	4	452	117	455	40	6	3	2	1	0	87.91209	13.27434	25.64103	4.395605	2.212389	0	38	5	3	83.51649	11.06195	25.64103
2023	5	293	192	591	34	10	0	2	0	0	57.52961	34.12969	0	3.384095	0	0	32	10	0	54.14552	34.12969	0
2023	6	185	34	108	32	3	3	1	1	0	296.2963	16.21622	88.23529	9.259259	5.405406	0	31	2	3	287.037	10.81081	88.23529

year	month	beddays_r	beddays_s	beddays_t	was BMT Q	Was North	Was South	bsi_bmt (c	bsi_north (	bsi_south	environme	environme	enironmer	beddays_c	bsi_qeuh_	bsi incider	beddays o	bsi count (	bsi incider	environme	environme	environme	environme	non-envirc	non-envirc	non-envirc	non-environmental	bsi incidence rate	other hospitals
2013	1	443	286	505	no	no	no			3			1				286	3	10.48951			1	3.496504			2	6.993007		
2013	2	316	165	225	no	no	no			4			1				165	4	24.24242			1	6.060606			3	18.18182		
2013	3	339	177	332	no	no	no			3			0				177	3	16.94915			0	0			3	16.94915		
2013	4	489	284	441	no	no	no			1			0				284	1	3.521127			0	0			1	3.521127		
2013	5	389	319	364	no	no	no			4			0				319	4	12.53918			0	0			4	12.53918		
2013	6	396	180	355	no	no	no			2			1				180	2	11.11111			1	5.555555			1	5.555555		
2013	7	460	514	405	no	no	no			11			4				514	11	21.40078			4	7.782101			7	13.61868		
2013	8	450	95	398	no	no	no			6			0				95	6	63.15789			0	0			6	63.15789		
2013	9	300	483	399	no	no	no			4			1				483	4	8.281573			1	2.070393			3	6.21118		
2013	10	379	204	552	no	no	no			5			0				204	5	24.5098			0	0			5	24.5098		
2013	11	383	244	584	no	no	no			6			1				244	6	24.59016			1	4.098361			5	20.4918		
2013	12	461	205	452	no	no	no			2			0				205	2	9.756098			0	0			2	9.756098		
2014	1	385	210	457	no	no	no			7			2				210	7	33.33333			2	9.523809			5	23.80952		
2014	2	398	96	405	no	no	no			3			0				96	3	31.25			0	0			3	31.25		
2014	3	277	254	558	no	no	no	1		4	1		0				812	5	6.157636			1	1.231527			4	4.926108		
2014	4	363	447	429	no	no	no	13	3	10	3	0	1				1239	26	20.98466			4	3.22841			22	17.75625		
2014	5	307	183	502	no	no	no	11	3	10	1	0	0				992	24	24.19355			1	1.008065			23	23.18548		
2014	6	495	234	526	no	no	no	17	2	7	3	0	4				1255	26	20.71713			7	5.577689			19	15.13944		
2014	7	386	230	478	no	no	no	14	3	6	3	0	1				1094	23	21.02377			4	3.656307			19	17.36746		
2014	8	472	365	476	no	no	no	24	5	8	5	0	2				1313	37	28.17974			7	5.331302			30	22.84844		
2014	9	460	267	491	no	no	no	16	5	3	3	0	0				1218	24	19.70443			3	2.463054			21	17.24138		
2014	10	488	214	472	no	no	no	15	3	7	4	0	2				1174	25	21.29472			6	5.110733			19	16.18399		
2014	11	548	226	764	no	no	no	18	7	3	3	1	0				1538	28	18.20546			4	2.60078			24	15.60468		
2014	12	251	334	563	no	no	no	20	2	5	3	0	0				1148	27	23.51916			3	2.61324			24	20.90592		
2015	1	672	222	444	no	no	no	19	5	1	2	0	0				1338	25	18.6846			2	1.494768			23	17.18984		
2015	2	328	406	356	no	no	no	21	9	2	6	1	0				1090	32	29.3578			7	6.422019			25	22.93578		
2015	3	392	452	471	no	no	no	14	3	11	0	0	0				1315	28	21.29278			0	0			28	21.29278		
2015	4	335	193	405	no	no	no	19	8	2	2	1	1				933	29	31.08253			4	4.287245			25	26.79528		
2015	5	455	228	467	no	no	yes	12	3	6	1	0	0	228	6	26.31579	922	15	16.26898	0	0	1	1.084599	6	26.31579	14	15.18438		
2015	6	221	166	725	yes	no	yes	20	1	7	4	1	0	891	27	30.30303	221	1	4.524887	4	4.489338	1	4.524887	23	25.81369	0	0		
2015	7	292	257	456	yes	no	yes	23	4	2	2	0	1	713	25	35.06311	292	4	13.69863	3	4.207573	0	0	22	30.85554	4	13.69863		
2015	8	411	178	385	no	no	yes	19	9	2	1	0	0	178	2	11.23596	796	28	35.17588	0	0	1	1.256281	2	11.23596	27	33.9196		
2015	9	460	189	541	no	no	yes	27	12	7	3	2	0	189	7	37.03704	1001	39	38.96104	0	0	5	4.995005	7	37.03704	34	33.96603		
2015	10	438	157	649	no	no	yes	23	9	6	1	2	1	157	6	38.21656	1087	32	29.43882	1	6.369427	3	2.75989	5	31.84713	29	26.67893		
2015	11	382	216	569	no	no	yes	15	5	4	0	1	0	216	4	18.51852	951	20	21.03049	0	0	1	1.051525	4	18.51852	19	19.97897		
2015	12	427	206	444	no	no	yes	23	5	5	2	2	1	206	5	24.27184	871	28	32.14696	1	4.854369	4	4.592422	4	19.41748	24	27.55453		
2016	1	523	191	454	no	no	yes	13	12	7	3	1	1	191	7	36.64922	977	25	25.58854	1	5.235602	4	4.094166	6	31.41361	21	21.49437		
2016	2	483	145	467	no	no	yes	28	4	6	6	0	1	145	6	41.37931	950	32	33.68421	1	6.896552	6	6.31579	5	34.48276	26	27.36842		
2016	3	390	170	617	no	no	yes	20	7	4	6	1	1	170	4	23.52941	1007	27	26.81231	1	5.882353	7	6.951341	3	17.64706	20	19.86097		
2016	4	304	246	522	no	no	yes	19	1	3	0	0	0	246	3	12.19512	826	20	24.21308	0	0	0	0	3	12.19512	20	24.21308		
2016	5	472	283	481	no	no	yes	8	13	1	0	4	0	283	1	3.533569	953	21	22.03568	0	0	4	4.197272	1	3.533569	17	17.83841		
2016	6	404	197	424	no	no	yes	20	5	9	7	1	0	197	9	45.68528	828	25	30.19324	0	0	8	9.661836	9	45.68528	17	20.5314		
2016	7	316	162	668	no	no	yes	35	11	3	2	1	0	162	3	18.51852	984	46	46.74797	0	0	3	3.04878	3	18.51852	43	43.69919		
2016	8	341	262	527	no	no	yes	29	7	3	3	0	1	262	3	11.45038	868	36	41.47466	1	3.816794	3	3.456221	2	7.633588	33	38.01843		
2016	9	354	45	487	no	no	yes	23	8	9	1	1	1	45	9	200	841	31	36.86088	1	22.22222	2	2.378121	8	177.7778	29	34.48276		
2016	10	317	191	482	no	no	yes	28	10	4	4	0	1	191	4	20.94241	799	38	47.55945	1	5.235602	4	5.006258	3	15.70681	34	42.55319		
2016	11	255	167	523	no	no	yes	21	6	6	1	1	2	167	6	35.92814	778	27	34.70437	2	11.97605	2	2.570694	4	23.9521	25	32.13367		
2016	12	338	137	440	no	no	yes	22	12	3	3	0	1	137	3	21.89781	778	34	43.7018	1	7.29927	3	3.856041	2	14.59854	31	39.84576		
2017	1	299	83	456	no	no	yes	31	5	6	4	0	1	83	6	72.28915	755	36	47.68212	1	12.04819	4	5.298013	5	60.24096	32	42.38411		
2017	2	381	108	419	no	no	yes	22	7	5	2	1	0	108	5	46.2963	800	29	36.25	0	0	3	3.75	5	46.2963	26	32.5		
2017	3	388	265	399	no	no	yes	33	9	7	2	1	1	265	7	26.41509	787	42	53.36722	1	3.773585	3	3.811944	6	22.64151	39	49.55527		
2017	4	331	116	433	no	no	yes	30	4	11	3	0	1	116	11	94.82758	764	34	44.50262	1	8.620689	3	3.926702	10	86.20689	31	40.57592		
2017	5	428	245	424	no	no	yes	32	9	18	2	1	2	245	18	73.46939	852	41	48.12207	2	8.163265	3	3.521127	16	65.30612	38	44.60094		
2017	6	513	12	475	no	no	yes	28	5	0	2	0	0	12	0	0	988	33	33.40081	0	0	2	2.024292	0	0	31	31.37652		
2017	7	390	129	455	no	no	yes	23	5	4	2	0	0	129	4	31.00775	845	28	33.13609	0	0	2	2.366864	4	31.00775	26	30.76923		
2017	8	493	245	597	no	no	yes	18	7	8	3	1	1	245	8	32.65306	1090	25	22.93578	1	4.081633	4	3.669725	7	28.57143	21	19.26605		
2017	9	433	172	431	no	no	yes	34	9	5	5	2	1	172	5	29.06977	864	43	49.76852	1	5.813953	7	8.101851	4	23.25581	36	41.66667		
2017	10	454	341	599	no	no	yes	32	13	6	1	2	3	341	6	17.59531	1053	45	42.73504	3	8.797654	3	2.849003	3	8.797654	42	39.88604		
2017	11	390	199	556	no	no	yes	47	17	7	4	2	1	199	7	35.17588	946	64	67.65327	1	5.025126	6	6.342494	6	30.15075	58	61.31078		
2017	12	604	155	529	no	no	yes	38	5	8	1	0	3	155	8	51.6129	1133	43	37.95234	3	19.35484	1	0.882613	5	32.25806	42	37.06973		
2018	1	417	212	576	no	no	yes	30	13	9	2	1	0	212	9	42.45283	993	43	43.30312	0	0	3	3.021148	9	42.45283	40	40.28197		
2018	2	328	135	402	no	no	yes	27	12	6	1	1	0	135	6	44.44444	730	39	53.42466	0	0	2	2.739726	6	44.44444	37	50.68493		
2018	3	524	109	544	no	no	yes	33	10	4	0	0	1	109	4	36.69725	1068	43	40.26217	1	9.174312	0	0	3	27.52294	43	40.26217		
2018	4	385	173	519	no	no	yes	30	14	5	2	0	0	173	5	28.90173	904	44	48.67257	0	0	2	2.212389	5	28.90173	42	46.46018		
2018	5	491	99	738	no	no	yes	32	7	6	2	1	0	99	6	60.60606	1229	39	31.73312	0	0	3	2.441009	6	60.60606	36	29.29211		
2018	6	377	183	310	yes	no	yes	33	15	5	2	2	1	493	38	77.07911	377	15	39.7878	3	6.085193	2	5.30504	35	70.99391	13	34.48276		
2018	7	522	99	689	yes	no	yes	28	5	3	4	0	0	788	31	39.3401	522	5	9.578544	4	5.076142	0	0	27	34.26396	5	9.578544		
2018	8	562	238	547	yes	no	yes	26	12	9	4	4	1	785	35	44.58599	562	12	21.35231	5	6.369427	4	7.117438	30	38.21656	8	14.23488		
2018	9	271	99	381	yes	no	yes	28	2	3	5	1	0	480	31	64.58334	271	2	7.380074	5	10.41667	1	3.690037	26	54.16667	1	3.690037		
2018	10	402	231	451	yes	no	yes	40	7	7	4	3	0	682	47	68.91496	402	7	17.41294	4	5.865103	3	7.462687	43	63.04985	4	9.950249		
2018	11	422	251	508	yes	no	yes	30	8	10	0	2	1	759	40	52.70092	422	8	18.95735	1	1.317523	2	4.739336	39	51.3834	6	14.21801		
2018	12	314	146	368	yes	no	yes	31	4	8	0	0	0	514	39	75.87549	314	4	12.73885	0	0	0	0	39	75.87549	4	12.73885		
2019	1	437	258	714	yes	no	yes	31	8	18	5	2	3	972	49	50.41152	437	8	18.30664	8	8.230453	2	4.576659	41	42.18107	6	13.72998		
2019	2	356	176	422	yes	no	yes	31	10	0	3	0	0	598	31	51.83947	356	10	28.08989	3	5.016722	0	0	28	46.82274	10	28.08989		

<u>Supplemental Table 19:</u> Data table for Figure 19 showing incidence rates of bacteraemia cases attributable to environemntally relevant microorganisms that may cluster edpidemiologically among haemato-oncology paediatric patients in GGC period (black font indicates Yorkhill, blue font indicates QEUH-RHC)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2005	3.24	4.06	0	1.92	8.05	9.94	8.02	9.52	1.94	1.81	11.39	11.05
2006	4.85	2.03	4.46	5.76	3.22	15.91	10.02	3.81	3.88	0	3.80	3.68
2007	3.24	0	2.98	3.84	4.83	3.98	10.02	9.52	5.83	5.43	0	1.84
2008	3.21	4.56	1.05	1.71	7.92	8.04	0	22.35	6.19	13.08	0	0
2009	3.16	0	6.26	0	0	3.39	1.29	11.14	6.27	3.74	4.30	6.37
2010	3.97	1.98	4.16	5.80	2.78	0	7.56	4.49	1.77	10.14	0	1.39
2011	1.63	3.65	1.75	7.01	5.33	2.55	2.21	0	0	2.55	0	2.49
2012	1.49	5.30	1.05	2.04	2.01	7.71	4.07	19.23	5.54	1.72	0	0
2013	0	0	0	4.44	0	5.44	8.20	11.53	2.80	1.87	0	0
2014	1.88	1.49	7.81	0	1.79	5.80	8.33	0	8.62	3.78	0	1.97
2015	0	2.29	1.91	1.69	2.88	0	0	0	0	1.89	0	0
2016	0	0	2.75	0	0	5.47	3.55	5.31	1.83	11.68	2.28	4.69
2017	3.46	3.34	1.54	2.04	2.39	4.75	6.08	8.10	6.42	4.81	6.58	6.02
2018	2.53	3.81	13.11	9.39	7.85	9.51	2.17	5.21	8.68	2.73	0	0
2019	5.08	1.74	9.09	1.65	3.78	6.85	1.73	3.47	4.93	3.11	1.50	0
2020	0	0	2.32	5.21	0	4.00	2.07	0	0	6.67	2.64	0
2021	0	0	0	2.25	0	2.87	7.59	0	1.96	3.08	0	0
2022	2.56	0	0	0	0	2.28	0	0	0	1.46	0	0

Notes to my calculations regarding cases of invasive aspergillosis

The data-set

In chapter 8 of our report, "Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen Elizabeth University Hospital and Royal Hospital for Children, Glasgow," there is a detailed explanation of how the parameters used to obtain the raw data from the Central Legal Office, on behalf of Greater Glasgow Health Board (NHSGGC).

For ease, this copied here:

Laboratory data

The NHS GGC laboratory services take samples from other health boards and primary care, so data was filtered based on allocation to either a haematology/haemato-oncology consultant or location.

All microbiological data that could indicate infection due to *A. spp*. were analysed. As *A. spp*. are never (with only vanishingly rare exceptions) cultured form blood, we looked at data from sputum and BAL with any *A. spp*. growth detected. In addition, three biomarkers that can indicate the presence of *A. spp*. were analysed:

- Galactomannan (GM), which is specific for *A. spp*.
- PCR for *A. spp*, which is also specific for *A. spp*.
- Beta-D-glucan (BDG), which can support a diagnosis of invasive aspergillosis, even though it is not *A. spp*. specific, but is a pan-fungal marker.

Time period

Results were available from 2013 to 2023 for the adult haemato-oncology service and from 2005 to 2022 for the paediatric service.

De-duplication

Where the same patient had multiple positive tests for the same admission episode, this was classified as one case.

Case definitions

In chapter 8, there is a detailed explanation of my definitions to define a case of invasive aspergillosis.

For ease, this is copied here:

Case definitions for this report

In this patient population, Proven cases of aspergillosis are exceptionally rare (as obtaining sterile tissue for diagnosis requires invasive procedures in an unwell patient). Outside of Proven cases, objective evidence for invasive aspergillosis comes from mycological data - culture or biomarkers – which is part of the Probable case definition but not Possible. Also, if BDG is the only mycological criterion, this is not indicative of an invasive aspergillosis as it is a pan-fungal marker.

Given these challenges of case definition, for the purposes of this evaluation, *any evidence of infection with A. spp. was counted as a suspected case (regardless of host and imaging factors)*. This approach may inflate the number of cases as mycological data alone is not regarded as a case by the EORTC/MSGERC criteria. On the other hand, Possible cases will be lost, as this category is based on host plus imaging criteria, but as explained above, Possibles lack any objective evidence of infection with an *A. spp*.

Data analysis

The file, "Cases over time_protected_updated_190624" shows the data I extracted from the original data-set of any case with any positive data based on the case definitions used.

Data is presented per sector, with each line a case and all relevant results for that case recorded.

I have made notes indicating where there was doubt about classification and why cases were accepted or rejected.

Date	Sample	Culture	GM	BDG	PCR	UPN	Year	Cases	
14/05/2013	Clotted Blood		P			[redacted]	2013	5	
25/07/2013	Bronchial Lavage	Aspergillus fumigatus				[redacted]			
25/07/2013	Bronchial Lavage	Aspergillus fumigatus				[redacted]			
26/07/2013	Clotted Blood		P			[redacted]			
02/10/2013	Sputum	Aspergillus fumigatus				[redacted]			
05/12/2013	Sputum	Aspergillus flavus				[redacted]			
20/12/2013	Bronchial Aspirate	Aspergillus flavus				[redacted]			
22/01/2014	Clotted Blood		P			[redacted]	2014	3	**NB 2 patients**
14/05/2014	Clotted Blood		P			[redacted]			
30/12/2014	Clotted Blood		P			[redacted]			
12/05/2016	Bronchio Alveolar Lavage		P			[redacted]	2016	2	
15/08/2016	Sputum	Aspergillus fumigatus				[redacted]			
16/08/2016	Sputum	Aspergillus fumigatus				[redacted]			
06/03/2017	Sputum	Aspergillus fumigatus				[redacted]	2017	3	**NB 2 patients. Possibly 3 if [redacted] regarded as 2 "indepenedent" episodes - on review, I have adjusted to 3, as I am now counting April and July as different episodes**
06/03/2017	Sputum	Escherichia coli				[redacted]			
27/04/2017	Sputum	Aspergillus fumigatus				[redacted]			
04/07/2017	Bronchio Alveolar Lavage	Aspergillus fumigatus				[redacted]			
04/07/2017	Bronchio Alveolar Lavage	Enterobacter asburiae				[redacted]			
19/10/2018	Sputum	Aspergillus species				[redacted]	2018	1	
19/10/2018	Sputum	Candida species				[redacted]			
30/05/2019	Bronchial lavage		P 1.27			[redacted]	2019	1	
15/06/2020	Sputum	Aspergillus fumigatus				[redacted]	2020	1	
15/06/2020	Sputum	Escherichia coli				[redacted]			
03/02/2021	Clotted Blood		P			[redacted]	2021	1	
12/03/2021	Clotted Blood		P			[redacted]			
29/03/2021	Clotted Blood		P			[redacted]			
02/04/2021	Clotted Blood		P			[redacted]			
08/04/2021	Clotted Blood		P			[redacted]			
15/04/2021	Clotted Blood		P			[redacted]			
19/04/2021	Clotted Blood		P			[redacted]			
22/04/2021	Clotted Blood		P			[redacted]			
26/04/2021	Clotted Blood		P			[redacted]			
06/05/2021	Clotted Blood		P			[redacted]			
11/08/2022	Clotted Blood			P		[redacted]	2022	1	**NB[redacted] is very unlikely to represent Aspergillus - ?more data**
11/08/2022	Clotted Blood		P			[redacted]			No imagign for this case, therefore NOT *Aspergillus - total remains 1*.
10/09/2022	Clotted Blood			P		[redacted]			
14/09/2022	Clotted Blood			P		[redacted]			
28/10/2022	Clotted Blood			P		[redacted]			
28/10/2022	Clotted Blood			P		[redacted]			
12/02/2023	Sputum	Aspergillus fumigatus				[redacted]	2023	1	
12/02/2023	Sputum	Pseudomonas aeruginosa				[redacted]			
12/02/2023	Sputum	Yeasts				[redacted]			

Date	Sample	Culture	GM	BDG	PCR	UPN	Year	Cases	
08/07/2013	Sputum	Aspergillus fumigatus				[redacted]	2013	1	1
08/07/2013	Sputum	Candida albicans				[redacted]			2
29/07/2013	Sputum	Aspergillus fumigatus				[redacted]			2
29/07/2013	Sputum	Yeast				[redacted]			2
12/08/2013	Sputum	Aspergillus fumigatus				[redacted]			3
12/08/2013	Sputum	Candida albicans				[redacted]			3
08/09/2013	Sputum	Aspergillus fumigatus				[redacted]			4
									1
03/04/2014	Clotted blood		P			[redacted]	2014	2	2
28/05/2014	Bronchio Alveolar Lavage	Aspergillus fumigatus				[redacted]			1
									2
04/06/2015	Sputum	Aspergillus flavus				[redacted]	2015	2	
10/06/2015	Sputum	Aspergillus flavus				[redacted]			
17/06/2015	Clotted Blood		P			[redacted]			
12/10/2015	Sputum	Aspergillus fumigatus				[redacted]			
04/05/2016	Sputum	Aspergillus fumigatus				[redacted]	2016	2	
29/08/2016	Sputum	Aspergillus fumigatus				[redacted]			
29/08/2016	Sputum	Candida albicans				[redacted]			
16/02/2017	Sputum	Aspergillus fumigatus				[redacted]	2017	3	
08/05/2017	Sputum	Aspergillus fumigatus				[redacted]			
25/08/2017	Sputum	Aspergillus fumigatus				[redacted]			
30/09/2017	Sputum	Aspergillus fumigatus				[redacted]			
30/11/2017	Sputum	Aspergillus niger				[redacted]			
30/11/2017	Sputum	Candida albicans				[redacted]			
06/12/2017	Sputum	Aspergillus fumigatus				[redacted]			
06/12/2017	Sputum	Candida albicans				[redacted]			
13/12/2017	Sputum	Aspergillus species				[redacted]			
13/12/2017	Sputum	Candida albicans				[redacted]			
05/07/2018	Sputum	Aspergillus fumigatus				[redacted]	2018	3	
26/07/2018	Sputum	Aspergillus fumigatus				[redacted]			
27/12/2018	Bronchio Alveolar Lavage		P			[redacted]			

13/08/2019	Clotted Blood		P 3.99	[redacted]		
06/11/2019	Clotted Blood		P 1.04	[redacted]	2019	4
26/11/2019	Clotted Blood		P 1.13	[redacted]		
19/11/2019	Sputum	Aspergillus fumigatus		[redacted]		
19/11/2019	Sputum	Candida albicans		[redacted]		
07/03/2019	Sputum	Aspergillus fumigatus		[redacted]		
07/03/2019	Sputum	Candida albicans		[redacted]		
15/01/2020	Sputum	Aspergillus fumigatus		[redacted]	2020	1
15/01/2020	Sputum	Candida albicans		[redacted]		
15/01/2020	Sputum	Candida species		[redacted]		
14/09/2021	Sputum	Aspergillus fumigatus		[redacted]	2021	2
14/09/2021	Sputum	Candida species		[redacted]		
29/12/2021	Sputum	Aspergillus fumigatus		[redacted]		
29/12/2021	Sputum	Staphylococcus aureus		[redacted]		
29/12/2021	Sputum	Stenotrophomonas maltophilia		[redacted]		
31/08/2022	Clotted Blood		P 1.64	[redacted]	2022	1
31/08/2022	PLASMA (EDTA BOTTLE)		D CT 32.76			
03/08/2023	Sputum	Aspergillus fumigatus		[redacted]	2023	2
23/08/2023	Sputum	Aspergillus fumigatus		[redacted]		
14/09/2023	Sputum	Aspergillus fumigatus		[redacted]		

Date	Sample	Culture	GM	BDG	PCR	UPN	Year	Cases	
23/01/2013	Bronchio Alveolar Lavage		P			[redacted]	2013	8	8
24/01/2013	Blood		P			[redacted]			4
03/06/2013	Clotted Blood		P			[redacted]			6
03/06/2013	Clotted Blood		P			[redacted]			2
11/07/2013	Blood		P			[redacted]			3
18/07/2013	Bronchio Alveolar Lavage		P			[redacted]			3
24/07/2013	Clotted Blood		P			[redacted]			6
23/10/2013	Clotted Blood		P			[redacted]			3
30/10/2013	Clotted Blood		P			[redacted]			2
23/10/2013	Clotted Blood		P			[redacted]			3
31/10/2013	Bronchio Alveolar Lavage		P			[redacted]			2
12/12/2013	Clotted Blood		P			[redacted]			
22/01/2014	Bronchio Alveolar Lavage		P			[redacted]	2014	4	
26/07/2014	Sputum	Aspergillus fumigatus				[redacted]			
30/07/2014	Sputum	Aspergillus fumigatus				[redacted]			
16/04/2014	Sputum	Aspergillus fumigatus				[redacted]			
16/04/2014	Sputum	Penicillium species				[redacted]			
05/03/2014	Clotted Blood		P			[redacted]			
05/03/2015	Bronchio Alveolar Lavage		P			[redacted]	2015	6	
05/03/2015	Bronchio Alveolar Lavage		P			[redacted]			
01/04/2015	Sputum	Aspergillus fumigatus				[redacted]			
01/04/2015	Sputum	Yeasts				[redacted]			
17/04/2015	Bronchio Alveolar Lavage		P			[redacted]			
26/05/2015	Sputum	Haemophilus influenzae				[redacted]			
26/05/2015	Sputum	Candida albicans				[redacted]			
26/05/2015	Sputum	Aspergillus fumigatus				[redacted]			
03/09/2015	Bronchio Alveolar Lavage		P			[redacted]			
08/02/2016	PLASMA (EDTA BOTTLE)				D	[redacted]	2016	2	
16/02/2016	Clotted Blood		P			[redacted]			
17/02/2016	Bronchio Alveolar Lavage		P			[redacted]			
19/02/2016	Bronchio Alveolar Lavage				D	[redacted]			
01/12/2016	Clotted Blood		P			[redacted]			
27/03/2017	Bronchio Alveolar Lavage		P			[redacted]	2017	3	**NB Case [redacted] in Sept cannot be reagrded as a true positive alone - see CTs plus negative Asp spp**
20/06/2017	Sputum	Aspergillus fumigatus				[redacted]			
07/09/2017	PLASMA (EDTA BOTTLE)				D	[redacted]			
02/10/2017	Bronchio Alveolar Lavage				D	[redacted]			
02/10/2017	PLASMA (EDTA BOTTLE)				D	[redacted]			
03/10/2017	Bronchio Alveolar Lavage		P			[redacted]			
11/01/2018	ENDOTRACHEAL SECR.				D CT 24.78	[redacted]	2018	3	**NB It is unclear if [redacted] and [redacted] are true positives, bit I am counting them as cases - need more info**
02/02/2018	PLASMA (EDTA BOTTLE)		P			[redacted]			
01/02/2018	Clotted Blood				D CT 32.52	[redacted]			
23/07/2018	PLASMA (EDTA BOTTLE)				D CT 36.24	[redacted]			
26/02/2019	Bronchio Alveolar Lavage		P			[redacted]	2019	6	
10/07/2019	PLASMA (EDTA BOTTLE)				D CT 34.15	[redacted]			
11/07/2019	Sputum	Aspergillus fumigatus				[redacted]			
11/07/2019	Alveolar lavage	Aspergillus fumigatus				[redacted]			
12/07/2019	Clotted Blood		P			[redacted]			
22/08/2019	Clotted Blood		P			[redacted]			
29/10/2019	Bronchio Alveolar Lavage		P			[redacted]			
31/10/2019	Bronchio Alveolar Lavage		P			[redacted]			

19/12/2019	Clotted Blood		P			[redacted]		
03/01/2020	Clotted Blood		P			[redacted]	2020	3
24/09/2020	PLASMA (EDTA BOTTLE)				D CT 35.85	[redacted]		
01/12/2020	Clotted Blood		P			[redacted]		
01/12/2020	Clotted Blood		P			[redacted]		
02/04/2021	Sputum	Aspergillus fumigatus				[redacted]	2021	2
02/04/2021	Sputum	Yeasts				[redacted]		
21/12/2021	Bronchio Alveolar Lavage		P			[redacted]		
20/01/2022	Bronchio Alveolar Lavage		P			[redacted]	2022	3
26/01/2022	Sputum					[redacted]		
26/01/2022	Sputum					[redacted]		
14/07/2022	Bronchio Alveolar Lavage		P			[redacted]		
14/07/2022	Bronchio Alveolar Lavage				D	[redacted]		
28/07/2022	Clotted Blood		P			[redacted]		
04/08/2022	PLASMA (EDTA BOTTLE)				D	[redacted]		
11/08/2022	Clotted Blood		P			[redacted]		
18/08/2022	Clotted Blood		P			[redacted]		
20/08/2022	Sputum				D	[redacted]		
01/09/2022	Clotted Blood		P			[redacted]		
08/09/2022	Clotted Blood		P			[redacted]		
22/09/2022	Clotted Blood		P			[redacted]		
29/09/2022	Clotted Blood		P			[redacted]		
05/10/2022	Clotted Blood		P			[redacted]		
20/10/2022	Clotted Blood		P			[redacted]		
03/11/2022	Clotted Blood		P			[redacted]		
10/11/2022	Clotted Blood		P			[redacted]		
23/08/2022	Bronchio Alveolar Lavage		P			[redacted]		
27/01/2023	Clotted Blood		P			[redacted]	2023	2
06/02/2023	Clotted Blood		P			[redacted]		
13/02/2023	Clotted Blood		P			[redacted]		
20/02/2023	Clotted Blood		P			[redacted]		
14/03/2023	Clotted Blood		P			[redacted]		
04/06/2023	Clotted Blood				P	[redacted]		

NB Case [redacted] with BDG posiitve only cannot be regarded as aspergillus infection

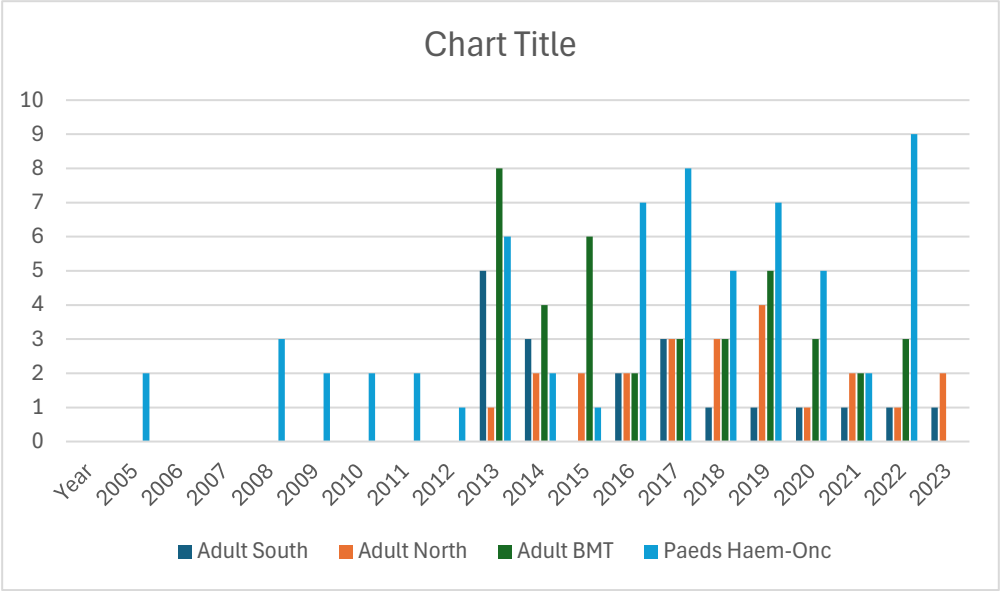
No imaging for this case, therefore NOT *Aspergillus - total remains 2*.

Date	Sample	Culture	GM	BDG	PCR	UPN	Year	Cases	
09/06/2005	Bronchio Alveolar Lavage	Stenotrophomonas maltophilia				[redacted]	2005	2	2
09/06/2005	Bronchio Alveolar Lavage	Aspergillus species				[redacted]			
16/10/2005	Sputum	Aspergillus species				[redacted]			
16/10/2005	Sputum	Candida albicans				[redacted]			3
16/10/2005	Sputum	Fungi				[redacted]			2
27/10/2005	Sputum	Aspergillus fumigatus				[redacted]			2
27/10/2005	Sputum	Haemophilus parainfluenzae				[redacted]			2
27/10/2005	Sputum	Penicillium species				[redacted]			1
									5
03/01/2008	Bronchio Alveolar Lavage	Aspergillus versicolor				[redacted]	2008	3	2
03/01/2008	Bronchio Alveolar Lavage	Aspergillus versicolor				[redacted]			0
27/11/2008	Bronchio Alveolar Lavage	Aspergillus fumigatus				[redacted]			6
11/12/2008	Sputum	Aspergillus fumigatus				[redacted]			8
									5
02/04/2009	Sputum	Aspergillus versicolor				[redacted]	2009	2	7
30/12/2009	Clotted Blood				D	[redacted]			4
31/12/2009	Clotted Blood				D	[redacted]			2
									3
09/02/2010	Blind BAL	Aspergillus fumigatus				[redacted]	2010	2	
09/02/2010	Blind BAL	Coagulase-Negative Staphylococcus				[redacted]			
22/11/2010	Clotted Blood				D	[redacted]			
25/11/2010	Clotted Blood				D	[redacted]			
30/11/2010	Bronchio Alveolar Lavage				D	[redacted]			
01/12/2010	Clotted Blood				E	[redacted]			
03/02/2011	Clotted Blood				D	[redacted]	2011	2	
04/02/2011	Clotted Blood				D	[redacted]			
05/02/2011	Bronchio Alveolar Lavage				D	[redacted]			
07/02/2011	Clotted Blood				D	[redacted]			
07/02/2011	Clotted Blood				D	[redacted]			
30/03/2011	Bronchio Alveolar Lavage				D	[redacted]			
08/10/2012	Sputum	Aspergillus fumigatus				[redacted]	2012	1	
08/10/2012	Sputum	Coliform Bacilli				[redacted]			
08/10/2012	Sputum	Escherichia coli				[redacted]			
11/10/2012	Clotted Blood				D	[redacted]			
15/10/2012	Sputum	Aspergillus fumigatus				[redacted]			
26/10/2012	Sputum	Aspergillus fumigatus				[redacted]			
30/10/2012	Sputum				D	[redacted]			
16/11/2012	Clotted Blood				D	[redacted]			
16/04/2013	Clotted Blood		P			[redacted]	2013	5	**NB Hard to be sure if [redacted] is 1,2 or more episodes - I have counted as 2**
22/04/2013	Clotted Blood		P			[redacted]			
29/04/2013	Clotted Blood		P			[redacted]			
04/06/2013	Clotted Blood		P			[redacted]			
18/06/2013	Clotted Blood				D	[redacted]			
27/06/2013	Clotted Blood		P			[redacted]			
01/07/2013	Clotted Blood		P			[redacted]			
11/07/2013	Clotted Blood		P			[redacted]			
07/08/2013	Clotted Blood		P			[redacted]			
16/08/2013	Clotted Blood		P			[redacted]			
26/08/2013	Clotted Blood		P			[redacted]			
12/09/2013	Clotted Blood		P			[redacted]			
16/10/2013	Clotted Blood		P			[redacted]			

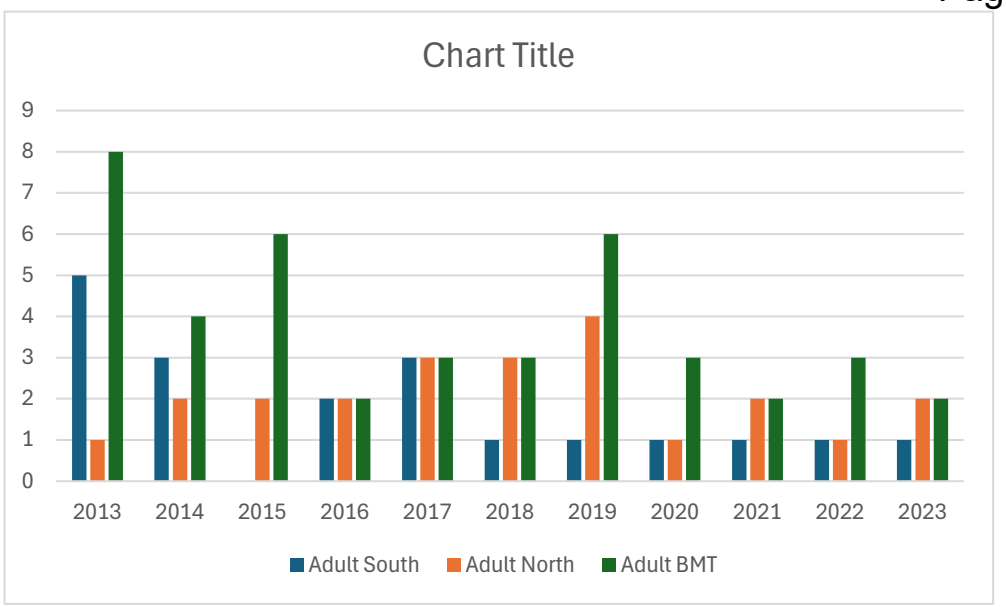
25/10/2013	Clotted Blood		P			[redacted]		
21/10/2013	Clotted Blood		P			[redacted]		
18/12/2013	Clotted Blood		P			[redacted]		
07/01/2014	Clotted Blood		P			[redacted]	2014	2 **NB Case [redacted] does not meet criteria for a case as one 'equivocal' blood PCR only**
10/01/2014	Clotted Blood		P			[redacted]		
13/03/2014	Bronchio Alveolar Lavage		P			[redacted]		
29/12/2014	Clotted Blood				E	[redacted]		
						[2015]		1
24/12/2015	Clotted Blood		P			[redacted]		
06/01/2016	Clotted Blood		P			[redacted]	2016	
12/01/2016	Clotted Blood		P			[redacted]		
29/06/2016	PLASMA (EDTA BOTTLE)				D	[redacted]		
29/06/2016	PLEURAL FLUID				D	[redacted]		
06/07/2016	Bronchio Alveolar Lavage		P			[redacted]		
14/07/2016	Blind BAL	Aspergillus fumigatus				[redacted]		
14/07/2016	Blind BAL	Pseudomonas aeruginosa				[redacted]		
14/07/2016	Bronchio Alveolar Lavage				D	[redacted]		
20/07/2016	Bronchio Alveolar Lavage				D	[redacted]	2016	4 **NB Case [redacted] is really one prolonged episode**
21/07/2016	PLEURAL FLUID				D	[redacted]		
23/07/2016	Bronchio Alveolar Lavage				D	[redacted]		
30/07/2016	Bronchio Alveolar Lavage				D	[redacted]		
30/07/2016	Bronchio Alveolar Lavage				D	[redacted]		
07/08/2016	Bronchio Alveolar Lavage				D	[redacted]		
20/09/2016	BRAIN TISSUE				D	[redacted]		
29/11/2016	Clotted Blood		P			[redacted]		
01/12/2016	Bronchio Alveolar Lavage		P			[redacted]		
29/12/2016	Clotted Blood		P			[redacted]		
08/12/2016	Clotted Blood		P			[redacted]		
17/01/2017	Clotted Blood		P			[redacted]	2017	7 **NB While I am counting all PCR "Ds" as cases, more data is needed to have confidence they should really be counted**
24/01/2017	Clotted Blood		P			[redacted]		
23/02/2017	Bronchio Alveolar Lavage				N	[redacted]		
02/03/2017	Bronchio Alveolar Lavage		P			[redacted]		
18/03/2017	Bronchio Alveolar Lavage		P			[redacted]		
20/03/2017	PLASMA (EDTA BOTTLE)				D	[redacted]		
26/04/2017	Bronchio Alveolar Lavage		P			[redacted]		
23/10/2017	Bronchio Alveolar Lavage				D	[redacted]		
07/11/2017	Clotted Blood		P			[redacted]		
08/12/2017	Clotted Blood		P			[redacted]		
23/12/2017	PLASMA (EDTA BOTTLE)				D	[redacted]		
09/02/2018	PLEURAL FLUID				D	[redacted]	2018	5
09/02/2018	BRONCHIAL ASPIRATE				D	[redacted]		
20/04/2018	Bronchio Alveolar Lavage				D	[redacted]		
21/06/2018	PLASMA (EDTA BOTTLE)				D	[redacted]		
17/07/2018	INDUCED SPUTUM				D	[redacted]		
27/09/2018	Clotted Blood		P			[redacted]		
				Pan PCR				
01/02/2019	PLASMA (EDTA BOTTLE)			N	D	[redacted]	2019	7 **NB Again, I am counting all of these as cases, but more data is needed to be sure**
27/02/2019	PLASMA (EDTA BOTTLE)			N	D	[redacted]		
19/08/2019	Bronchio Alveolar Lavage			D	N	[redacted]		
10/09/2019	PLASMA (EDTA BOTTLE)			N	D	[redacted]		
26/08/2019	PLASMA (EDTA BOTTLE)			N	D	[redacted]		
25/10/2019	PLASMA (EDTA BOTTLE)			N	D	[redacted]		

21/11/2019	PLASMA (EDTA BOTTLE)			D	N	[redacted]		
26/11/2019	Bronchio Alveolar Lavage		P			[redacted]		
			Pan PCR					
24/01/2020	PLASMA (EDTA BOTTLE)		D CT 38.37		N	[redacted]	2020	4 **NB I have not counted case [redacted] as there is only a solitary very high CT from blood**
04/02/2020	Endo-Tracheal Aspirate	Aspergillus fumigatus				[redacted]		
04/02/2020	Endo-Tracheal Aspirate	Candida albicans				[redacted]		
27/06/2020	PLASMA (EDTA BOTTLE)		D		N	[redacted]		
04/11/2020	Clotted Blood		P			[redacted]		
04/11/2020	PLASMA (EDTA BOTTLE)		N		D	[redacted]		
12/11/2020	Clotted Blood		P			[redacted]		
12/11/2020	PLASMA (EDTA BOTTLE)		N		D	[redacted]		
16/11/2020	Clotted Blood		P			[redacted]		
07/12/2020	Clotted Blood		P			[redacted]		
14/12/2020	Clotted Blood		P			[redacted]		
17/11/2020	Bronchio Alveolar Lavage		P			[redacted]		
2021						Both Ward 6A	2021	2
			BDG					
24/06/2022	Clotted Blood		P			[redacted]	2022	3 **NB Relying on BDG alone is problematic for diagnosing aspergillus infection, and by EORTC should NOT be counted**
28/07/2022	PLASMA (EDTA BOTTLE)				D	[redacted]		[redacted] - no imaging
15/08/2022	Clotted Blood		P			[redacted]		[redacted] extensive imaging, nil suggestive of fungal infection
18/08/2022	Clotted Blood		P			[redacted]		[redacted] CXR - lungs clear
22/08/2022	Clotted Blood		P			[redacted]		[redacted] CXRx2 and abdo US, nil suggestive of fungal infection
29/08/2022	Clotted Blood		P			[redacted]		[redacted], extensive imaging, lobar consolidation - nil suggestive of *Aspergillus*
16/09/2022	Clotted Blood		P			[redacted]		[redacted], imaging, including CT chest (only case!), nil suggestive of *Aspergillus*
27/09/2022	Clotted Blood		P			[redacted]		
05/10/2022	Clotted Blood		P			[redacted]		
20/10/2022	Clotted Blood		P			[redacted]		
22/08/2022	Clotted Blood		P			[redacted]		
30/08/2022	PLASMA (EDTA BOTTLE)				DL	[redacted]		
31/08/2022	Clotted Blood		P			[redacted]		
06/09/2022	Clotted Blood		P			[redacted]		
10/09/2022	Clotted Blood		P			[redacted]		
09/09/2022	Clotted Blood		P			[redacted]		
14/09/2022	Clotted Blood		P			[redacted]		
16/09/2022	Clotted Blood		P			[redacted]		
28/09/2022	Clotted Blood		P			[redacted]		
10/10/2022	Clotted Blood		P			[redacted]		
09/12/2022	Clotted Blood		P			[redacted]		
15/12/2022	Clotted Blood		P			[redacted]		

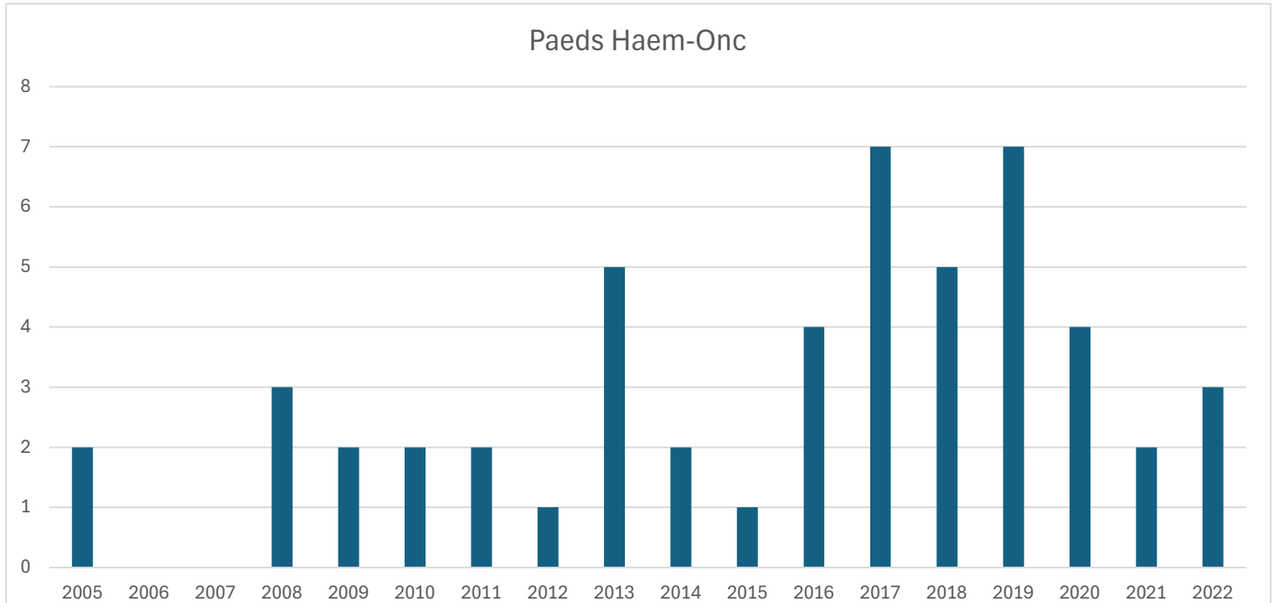
Year	Adult South	Adult North	Adult BMT	Paeds Haem-Onc
2005				2
2006				
2007				
2008				3
2009				2
2010				2
2011				2
2012				1
2013	5	1	8	6
2014	3	2	4	2
2015		2	6	1
2016	2	2	2	7
2017	3	3	3	8
2018	1	3	3	5
2019	1	4	5	7
2020	1	1	3	5
2021	1	2	2	2
2022	1	1	3	9
2023	1	2		



	Adult South	Adult North	Adult BMT
2013	5	1	8
2014	3	2	4
2015		2	6
2016	2	2	2
2017	3	3	3
2018	1	3	3
2019	1	4	6
2020	1	1	3
2021	1	2	2
2022	1	1	3
2023	1	2	2



	Paeds Haem-Onc			
2005	2	2		
2006				
2007				
2008	3	3		
2009	2	2		
2010	2	2		
2011	2	2		
2012	1	1	Adult BMT	
2013	5	5		8
2014	2	2		4
2015	1	1		6
2016	4	4		2
2017	7	7		3
2018	5	5		3
2019	7	7		6
2020	4	4		3
2021	2	2		2
2022	3	3		3
				2



SCOTTISH HOSPITALS INQUIRY

Rebuttal of the Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen Elizabeth University Hospital and Royal Hospital for Children, Glasgow

Authored by: Professor Peter Hawkey, Dr Samir Agrawal and Dr Lydia Drumright, Published: July 2024

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1. PREFACE:

As a result of continuing problems arising from patient infection incidents on the QEUH campus, in November 2019, the Scottish Government's Health and Social Care Management Board escalated NHS GGC to 'Stage 4' of its escalation ladder. As a result, an Oversight Board under the chair of the then Chief Nursing Officer, Professor Fiona McQueen, was established to address issues concerned.

As part of the work of the Oversight Board, the Cabinet Secretary for Health and Sport set out plans for a Case Note Review in a Parliamentary statement on 28 January 2020. We were commissioned to form the Independent Expert Panel for that Case Note Review with the charge that we would review the case notes of Paediatric Haemato-Oncology patients at the RHC and QEUH who, from 2015 to 2019, had experienced a Gram-negative environmental pathogen bacteraemia (and selected other organisms).

We commenced our work at the end of February 2020 and published the Case Note Review Overview Report in March 2021. We completed our work in July 2021 having communicated our findings to the families of all children and young people involved.

We gave evidence to the Scottish Hospitals Inquiry in October 2024 (Glasgow III hearing) but in March 2025 were requested to provide a written response to the Inquiry of an additional independent report, instructed by the Central Legal Office on behalf of NHS Greater Glasgow and Clyde, published in July 2024 and authored by Professor Hawkey, Dr Agrawal and Dr Drumright.

We have worked together to provide this rebuttal of the evidence presented in that report, and of the conclusions reached by its authors. Despite the criticisms made of our work by Hawkey et al., we remain confident that the conclusions reached and presented in the CNR Overview Report stand.

For simplicity, in places in our text we have informally referred to the report by Hawkey et al. as the 'HAD report'.

Michael Stevens
Gaynor Evans
Mark Wilcox
3 June 2025

2. CHAPTER 1: INTRODUCTION

This chapter commences with a short statement about the instruction given for the report and sets out its intent as follows:

"The purpose of this instruction is to consider the question of risk, specifically whether there is objective evidence/data that would support the proposition that since the QEUH and RHC opened in 2015 patients were exposed to a greater risk of infection than would be expected due to a widespread issue with the water and ventilations systems".

There then follows a discourse on the nature of infection risk and on the authors' planned approach to its investigation in the context given.

We identified a number of themes within this short chapter (also with reference to the Executive Summary) which are reiterated and developed elsewhere in the overall report but on which we make the following observations here.

1.1 Infection Prevention and Control (IPC)

Hawkey et al. state *"Infection risk is multifactorial and varies depending on: a micro-organisms ability to cause disease (pathogenicity), its natural habitat (source), ability to spread (mode of transmission), susceptibility of the host to infection (opportunity). If an increase in infection rates is observed, this is not necessarily attributable to the built environment given this complexity."*

There is a key omission here. As in much of the report, the effectiveness of infection prevention & control (IPC) measures is neither considered nor recognised. IPC measures are crucial to minimise the risk of infection, especially in vulnerable populations such as patients with haematological, and other, cancers undergoing intensive chemotherapy and/or haematopoietic stem cell transplants. IPC measures are also relevant in minimising the risks of acquiring exogenous microorganisms, including those from the environment.

Indeed, in the entirety of the HAD report, the terms IPC, infection prevention and control, infection prevention or infection control are infrequently mentioned, outside of

the chapter on ventilation. When infection control is mentioned, the context is sometimes confusing, as in the following example in Chapter 7, Page 97:

"Indeed, the most common source of Klebsiella pneumoniae infection in healthcare settings is person to person transmission, followed by contaminated intraven[e]ous catheters, which point to infection control practices rather than environmental concerns; improved infection control may be why we have seen a reduction in cases at QEUH compared Yorkhill."

The suggestion made by Hawkey et al of benefit from improved IPC practice is entirely contrary to the observations made in the Case Note Review (CNR) Overview Report about deficiencies in IPC process and practice at NHS GGC – a point to which we refer again in Chapter 2.

1.2 Source of Blood Stream Infections (BSIs) and routes of transmission

The Executive Summary (Page 1) includes this statement:

"6. The primary source for bacteria causing bloodstream infection (BSI) in neutropenic patients is the patient's own gut, particularly for Gram-negative bacteria."

Whilst this is true, it does not address the source of the bacteria in 'the patient's own gut'. So, where do Gram-negative bacteria come from? Are they truly the patient's own (endogenous) bacteria, and as such were they present when the patient entered the hospital, or were they bacteria acquired in the hospital? Bacteria causing BSIs may be acquired from the environment directly or via the hands of the patient, healthcare workers, visitors and/or other patients. These bacteria may colonise the intravascular catheters that form a mainstay of treatment (e.g. for administering drugs and blood products) in haematology/oncology patients, subsequently they may then enter the blood, typically whilst the catheters are manipulated. Alternatively, bacteria may colonise the gastrointestinal tract of these patients with the potential to transit the mucosa of the gut, damaged by chemotherapy, into the blood stream.

It is almost never established with certainty which routes of transmission have occurred in an individual patient. Nevertheless, in our critique of the HAD report, we

discuss how the epidemiology (clustering) of the BSIs seen in the CNR supports the possibility of exogenous / hospital-acquired infection.

The Executive Summary (Page 2) also includes the following statement:

"12 Attributing the source of a BSI pathogen to the built environment of the hospital can only be shown if: the strains identified are indistinguishable, a source is present, and a route of transmission and a portal of entry are identified."

This dogmatic statement is not true. In fact, it is well appreciated by IPC and infection epidemiology experts that molecular proof, as suggested by Hawkey et al. later in their report, is often not forthcoming. One can very reasonably attribute the origin of an infection to a source/sources if the available data/epidemiology are consistent with such a hypothesis.

Hawkey et al. place significant weight on the results of Whole Genome Sequencing (WGS) analysis (principally discussed in Chapter 7 but also referred to elsewhere) and in response we have set out in detail how sampling of the water environment and, the identification (typing) of the strains recovered from patients and water source were both incomplete.

In fact, in Chapter 2, Section 2.2, Hawkey et al., in discussing the definition of an outbreak, make this concession *"Practically, precise genetic subtyping of microorganisms rarely identifies the outbreak as it is a relatively slow and complicated process and therefore typically carried out retrospectively."*

1.3 Comparative incidence and Clusters

Hawkey et al. write *"As the hospital environment is not sterile, it will always pose a certain level of risk of infection. If the built environment poses an increased risk of infection, then this would manifest in an increased rate of infection."*

Whilst this statement may seem intuitively correct, as we discuss in Chapter 7, Section 7.2, there are many other factors that moderate infection rates and which challenge the use of whole population analyses to decide, as stated by Hawkey et al. (Page 12) *"…if there is no increase in infection rates then it can be concluded that there is no increased level of risk from the built environment or other potential factors".*

This is not true and there remains the necessity to look in detail at individual patient factors where epidemiological data suggest the possibility of a cluster of the same infection, as was done in the CNR. We reject the assertion made by Hawkey et al. that the CNR was not of assistance in relation to the question of whether the built environment at the QEUH and RHC posed an increased risk of infection to those in the cohort we were asked to assess in the CNR.

It must be emphasised that an increased risk of infection to an individual is not the same as an increased rate of infection in the population from which they come.

If the BSIs identified in the CNR were most likely endogenously acquired, for this to have been true, the clusters of infections we identified would need to have been independent events, i.e. were not related. Basic infection control analysis clearly showed clustering of some types of infections in time and place. The occurrence of repeated, unrelated infections with the same microorganism is very unlikely. What is the chance that clusters, occurring in time, of children managed in the same wards all developed BSIs caused by bacteria of the same species that had no common source(s)? It is implausible that, on repeated occasions, patients who developed a BSI were the source of BSIs in other patients. It is more likely that there were common sources of the bacteria involved that either colonised (and then infected) or directly infected the children.

3. CHAPTER 2: MICRO-ORGANISMS

Chapter 2 of the HAD report, entitled Micro-organisms, begins with an historical review of the standard classification of bacteria. It emphasises the normality of bacteria within our usual habitat and social environments whilst highlighting their potential as a cause for disease, particularly in immunocompromised individuals. The second part addresses the meaning and use of the term 'outbreak'.

in this chapter, we are critical of the authors' approach in three key areas:

- In their section on Naming and Classification of bacteria, we found much of the content is not directly relevant to the work and findings of the CNR; and the authors add little understanding to the real impact of Health Care Acquired Infection within the environment in which immunocompromised patients are managed.
- The discussion about 'What is meant by an outbreak?' does not utilise a standard definition as was used for the purposes of the CNR.
- The authors' failure to recognise the value of an investigation of the individual patient in terms of the origin of the infection.

2.1 How bacteria are named

Bundle 44 vol 1,Page 13, Section 2.1

It is difficult to understand the authors' intent in including this section in their report. Whilst it may be relevant in providing a textbook description of the historical evolution of bacterial characteristics and classification, it has no direct relevance to the HAD report's criticism of the CNR. Nor does it reference the impact of the organisms named within a clinical environment, and only superficially draws on infections that could particularly manifest in immunocompromised patients.

Perhaps Hawkey et al. could have linked their description of environmental bacterial exposure more directly to the fact that infections and outbreaks of infection occur in patients in hospital, and to a description of how such infections occur. This possibility applies not only to patients who are immunocompromised, although they are at especial risk. It may occur where there is a portal of entry and where infection prevention and control (IPC) practices are suboptimal; where there is contamination

of a medical device, such as an intravenous line, or of the patient environment; or cross infection from patient to patient. An investigative response to determine the possible source and mode of transmission is critical to reduce the risk for further cases of infection.

Standard precautions referenced in the National IPC Manual[1], describe measures that should be in place to mitigate for opportunistic pathogenic organisms and reduce the bioburden for patients. We identified suboptimal IPC practice at NHS GGC in the CNR Overview Report[2].

Section 2.1 of the HAD report mentions a number of Gram negative bacteria excluded from the CNR. Reference to Escherichia coli is also made in Chapter 4 of the report on which we comment further. It is nevertheless worth reiterating here that E.coli bacteraemia, the most prolific blood steam infection acquired in hospital with the potential for endogenous infection, was excluded from the CNR review.

2.2 What is meant by an outbreak?

Bundle 44 vol 1, Page 15, Section 2.2, Paragraph 1

The published definition of a health care associated infection outbreak is clearly stated in section 3.1 of the Health Protection Scotland National IPC Manual[3] as *"Two or more linked cases with the same infectious agent associated with the same healthcare setting over a specified time period, or a higher-than-expected number of cases of HAI in a given healthcare area over a specified time period".* This was the definition we accepted for our work in the CNR. However, the first definition of outbreak given in this Section of the HAD report is not the same and refers to infection *"…caused by genetically indistinct micro-organisms"*. We assert that this is aspirational and not the reality with which infection prevention and control practitioners work daily - a fact Hawkey et al. appear to acknowledge later in the same paragraph where they discuss

[1] Bundle 19, Document 24, Page 440.

[2] Bundle 6, Document 38, Page 1039, Section 5.3.4

[3] Bundle 19, Document 24, Page 458, Section 3.1.

the importance of recognising an increase beyond the normally expected numbers of a specific infection.

An apparent increase in numbers of cases of the same infection linked by time, place, and person (i.e. those in proximity such as within the same ward), should trigger an investigation, using defined outbreak management principles, to identify any potential commonality between cases as well as to consider suboptimal aspects of IPC practice that may have led to cross infection and/or the identification of a potential source.

In practice, even with contemporaneous investigation, it is not always possible to identify the source of an outbreak until later in an investigation, or if at all. The investigation is also dependent on the investigating team taking the most appropriate samples from locations or people and requesting the most appropriate testing to either confirm or exclude linked cases.

Whilst Hawkey et al. place great emphasis on the identification of genetically similar micro-organisms, in practice mutations are possible and there remains the potential for genetically very similar but not exact matches to be linked. We discuss this further in our critique of Section 7.3 of the HAD report.

In the latter part of Section 2.2, the authors' focus on the role of the gut as a site of endogenous spread for blood stream infections (BSI) in immunocompromised patients. This possibility is one to which we explicitly referred in the CNR and took into consideration, as far as was possible, when considering if there was the possibility of the origin of a blood stream infection from the hospital environment[4].

The final reference in this section is to the reality that environmental bacteria will be found in the general environment.

Hawkey et al. illustrate this with reference to a study which identified *Cupriavidus spp* and *Burkholderia spp* in domestic water supplies[5]. Apart from the obvious longer term implications for public health from findings of this kind, the identification of some bacterial species which may cause human disease in this way, and more especially in those who are immunocompromised, serves to underline the importance of IPC

[4] Bundle 6, Document 38, Page 1018, Section 3.6.6.

[5] Khan, S., Knapp, C.W. & Beattie, T.K. Antibiotic Resistant Bacteria Found in Municipal Drinking Water. *Environ. Process.* **3**, 541–552 (2016). https://doi.org/10.1007/s40710-016-0149-z

precautions in the environments in which such patients are managed. It is striking that Hawkey et al. do not reflect on this or on the critical relevance of methodologies available for reducing risk in water supply to the built environment[6].

2.3 Investigation of the individual patient

Bundle 44 vol 1, Page 17, Paragraph 2

Hawkey et al. make two important statements early in their report, the first at this point in Chapter 1 which states: *"A significant reliance appears to have been placed on the Queen Elizabeth University Hospital and Royal Hospital for Children Case Note Review Overview Report (Stevens, Evans and Wilcox, 2021) (CNR) in relation to whether the built environment at the QEUH and RHC posed an increased risk of infection. However, we do not consider that the CNR is of assistance in determining this for the reasons set out below."*

Bundle 44 vol 1, Page 13, Paragraph 2

At the beginning of Chapter 2, they write *"In order to be able to identify the sources of particular micro-organism that can cause infection, a number of factors need to be understood including, the natural habitats of that microorganism, the route of transmission by which micro-organisms get to the patient and enter the body. The identification of micro-organisms and what constitutes an outbreak must also be understood."*

It is therefore very strange that the authors seem both to recognise the complexity of attempts needed to determine how patients develop infection but at the same time imply that individual patient review, as undertaken by the CNR, is not helpful.

A prime requirement in determining the root cause of an infection is to consider, in detail, the clinical circumstances of the individual experiencing the infection, in addition to the microbiological and environmental factors involved. It is worth re-emphasising that the use of Root Cause Analysis was not introduced for the investigation of infection in paediatric haematology oncology patients at NHS GGC until late 2019,

[6] Bundle 1, Document 6, Page 287.

long after concerns about the pattern and frequency of gram-negative environmental BSI had been raised as a matter of concern[7].

In conducting the CNR we followed conventional IPC practice when attempting to determine the aetiology of the BSIs in the children we studied, and when categorising the likelihood that these might in some way be related to the hospital environment. We set out our approach in considerable detail in Chapter 3 of the Overview Report[8]. Furthermore, we invited the families of the children concerned to contribute their own observations about the circumstances of their child's infection in case this offered insights additional to our own deliberations. We maintain this to be the correct approach.

[7] Bundle 6, Document 38, Page 1064, Section 8.2.2.2.

[8] Bundle 6, Document 38, Page 1007, Chapter 3.

4. CHAPTER 3: CLINICAL SIGNIFICANCE AND EPIDEMIOLOGY OF THE MOST COMMON GROUPS OF PRESUMED ENVIRONMENTAL BACTERIA SELECTED FOR INCLUSION BY THE CNR

This chapter offers commentary on the *"particular bacterial species"* considered by the CNR. We largely address its content in the sequence in which it is presented but as our comments focus only on certain areas of the data provided in Chapter 3 of the HAD report, the sections in our chapter follow that sequence but are not necessarily named the same.

3.1 Klebsiella

Bundle 44 vol 1, Page 23, Section 3.1.1

After an introduction to the wider group (Enterobacterales) to which Klebsiella belongs, and a review of its characteristics in health care settings, Hawkey et al. make this general statement *"It is important to maintain a balanced view, as just because bacteria of the same species can be found in those sites they are not necessarily the source of infection."*

It is good to see this statement as that is the position we took in our approach to the CNR but we believe that the clustering epidemiology, which we discuss in Chapter 7, Section 7.2, supports the possibility of a common source(s).

At the end of this section, Hawkey et al. conclude, *"To summarise, Klebsiella are important nosocomial pathogens, the most common sources are the community colonised patients admitted to hospital and a colonised hospital patient with transfer to colonise other patients via contact with contaminated surfaces, hands of medical/nursing staff and sometimes food. On occasions wet sources such as sink traps, drains, medical equipment and cleaning cloths can act as primary sources for infection having been contaminated by material from colonised patients."*

It seems, therefore, they agree that the hospital environment could be a source of infection with these Gram-negative bacilli and we highlight next just a few of many reports in the published literature that confirm this to be the case.

In this UK publication[9], a sampling strategy was used to investigation the hospital water environment. It is notable that the level of stringency of environmental testing was typically absent when GGC investigated its clusters of BSIs in children. Three units (acute admissions, adult critical care, adult general medicine) within a single hospital (John Radcliffe Hospital, Oxford, UK) were screened four times on rotation every 3 weeks over 3 months, March–May 2017. Units were chosen to capture different patient populations, admission turnaround times and wastewater plumbing infrastructure. The haematology ward (on a separate hospital site) was also sampled on a single day (12 May 2017) subsequent to a small cluster of patient cases of three *OXA-48* carbapenemase-associated Enterobacterales bloodstream infections described and published previously[10].

The authors concluded: *"In this study, we have demonstrated that hospital sink drains are widely—and in many cases abundantly—contaminated with key Enterobacterales species causing healthcare-associated infections, and are potential reservoirs of multiple resistance genes encoding resistance to important clinical antimicrobials….."* and

"In conclusion, without conditioning on the presence of resistance markers, we have demonstrated that colonization of ward sink drains with diverse and abundant populations of Enterobacterales, including drug-resistant lineages, is common and persistent. The evidence linking contaminated, unmitigated wastewater reservoirs (including sink drains) in healthcare settings with outbreaks of colonization/disease with drug-resistant Gram-negative bacilli in patients seems clear but no study to our knowledge has focused on the potential risk posed by Enterobacterales in sinks in general. Screening of sinks is not carried out in the absence of observed outbreaks, making it difficult to quantify wider patient-associated risk from the studies available. We demonstrate that contaminated sinks may be contributing to a proportion of healthcare-associated infections caused by Enterobacterales, and further work to

[9] Constantinides B, Chau KK, Quan TP, Rodger G, Andersson MI, Jeffery K, Lipworth S, Gweon HS, Peniket A, Pike G, Millo J, Byukusenge M, Holdaway M, Gibbons C, Mathers AJ, Crook DW, Peto TEA, Walker AS, Stoesser N. Genomic surveillance of *Escherichia coli* and *Klebsiella* spp. in hospital sink drains and patients. Microb Genom. 2020 Jul;6(7): https://doi.org/10.1099/mgen.0.000391

[10] Chau K. WGS investigation of three OXA-48 carbapenemase bacteraemia and sink colonisation on a haematology unit. Poster presentation 2018.

investigate how to reduce the risk posed by this hospital environmental reservoir is warranted." [We have omitted the inclusion of references quoted in the relevant excerpts.]

Next, we highlight a pertinent paragraph in a publication from the Netherlands concerning the role of the environment in the transmission of Gram-negative bacteria in two consecutive outbreaks in a haematology-oncology department[11].

"In recent years, many studies have demonstrated that sink drains, shower drains and toilets in hospitals can serve as a reservoir of Gram-negative bacterial infections in patients. These bacteria reside in biofilms in and on water related devices, which are refractory to disinfection. Bacteria in the biofilm of drainage systems are under a prolonged antibiotic selective pressure, because antibiotics in faeces and urine of patients are absorbed by the biofilm. Within these biofilms, containing several bacterial species, acquired mobile resistance elements are frequently transferred, resulting in highly resistant microorganisms, such as extended-spectrum β-lactamase and carbapenemase-producing Enterobacterales and Pseudomonas species". [We have again omitted the inclusion of references quoted in the relevant excerpts.]

These authors ended: *"In conclusion, the drainage system of the hospital is an important reservoir of MDR bacteria, threatening the admitted patients."*

Finally, we also draw attention to the recommendations of a Joint Working Party that reported on the prevention and control of multi-drug-resistant Gram-negative bacteria; Professor Hawkey is a co-author of this report[12]. The following are direct quotes from that report:

[11] van der Zwet WC, Nijsen IEJ, Jamin C, van Alphen LB, von Wintersdorff CJH, Demandt AMP, Savelkoul PHM. Role of the environment in transmission of Gram-negative bacteria in two consecutive outbreaks in a haematology-oncology department. Infect Prev Pract. 2022 Feb 25;4(2):100209. https://doi.org/10.1016/j.infpip.2022.100209

[12] Wilson AP, Livermore DM, Otter JA, Warren RE, Jenks P, Enoch DA, Newsholme W, Oppenheim B, Leanord A, McNulty C, Tanner G, Bennett S, Cann M, Bostock J, Collins E, Peckitt S, Ritchie L, Fry C, Hawkey P. Prevention and control of multi-drug-resistant Gram-negative bacteria: recommendations from a Joint Working Party. J Hosp Infect. 2016 Jan;92 Suppl 1:S1-44. https://doi.org/10.1016/j.jhin.2015.08.007

"Environmental screening should be considered where there is unexplained transmission of MDR Gram-negative organisms or a possible common source for an outbreak".

"Transmission from patient to patient is believed to be mainly via hands of staff, although common environmental sources have occasionally been described and should be sought where no other plausible vectors can be found (e.g. ventilator equipment or water supply)".

"Several studies have found that environmental contamination with resistant K. pneumoniae is more common than contamination with resistant E. coli".

"Transmission of MDR Gram-negative bacteria, particularly non-fermenters, has been associated with contamination of the environment, water systems or equipment".

Returning to the HAD report, and with specific regard to *Klebsiella* spp, Hawkey et al. write:

"These careful early studies of the epidemiology of Klebsiella are often forgotten and the importance of the colonised patient is underestimated. Careful investigation of outbreaks of infection nowadays can reveal lapses in hand hygiene which probably represents the most common route of transmission in hospital wards."

No references are provided here to support this assertion.

The authors go on to discuss possible water-related sources of Klebsiella infections, and indeed highlight here *"Sink traps (U bends or P traps) which always contain water and therefore large bacterial populations, usually in a biofilm, are sometimes seen as the most important source of Gram-negative infections in wards with vulnerable patients (e.g. haematology/oncology, intensive care units, burns units etc.)."*

We have referenced, above, reports that emphasise the importance of water related nosocomial infections caused by *Klebsiella* spp. and other Gram-negative bacteria. Below, using a citation made by Hawkey et al in the HAD report, we provide further evidence of the importance of the hospital environment as a source of hospital-acquired infections.

When discussing the sources of nosocomial Klebsiella infections, Hawkey et al refer to a 2017 systematic review by Kizny Gordon et al. published in 2017[13]. To be clear, the conclusions of this review are applicable not just to carbapenem resistant bacteria; as Hawkey et al. themselves state *"the basic bacterial epidemiology is unaffected by the antibiotic resistance genes carried."*

Hawkey et al. concluded by quoting from Kizny Gordon et al. as follows: "*Pseudomonas spp. and Acinetobacter spp. are environmental colonisers surviving in low nutrient conditions whereas the Enterobacteriales are predominantly of human origin and their concentration in drains may represent a different ecosystem and inoculation from human waste."* Notably, however, the next sentence by Kizny Gordon et al. is omitted by Hawkey et al. This reads: *"However, all these organisms are capable of colonizing water system biofilms".*

The Kizny-Gordon et al. review also contains other important conclusions not mentioned by Hawkey et al. For example:

"Seventy-two percent of studies reported controlling outbreaks, of which just more than one-third eliminated the organism from the water environment. A combination of interventions seems to be most successful, including reinforcement of general infection control measures, alongside chemical disinfection."

"Within the hospital there are numerous opportunities for patient exposure to water and drainage reservoirs Transmission may result from direct or indirect water contact, or from droplets created during water activities, highlighting the importance of water delivery and wastewater design to minimize these risks. Seven studies recognized that poor design or use of sinks, drains, and sluice areas may have contributed to institutional outbreaks."

It is relevant to mention here is that the taps installed in the paediatric haematology oncology wards at NHS GGC were not of approved design. In the CNR Overview Report, we drew attention to our inability to find an evaluation of the implementation of recommendations that impacted the risk of infection to patients; for example, the

[13] Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, Peto TEA, Crook DW, Stoesser N. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature. Clin Infect Dis. 2017 May 15;64(10):1435-1444. https://doi.org/10.1093/cid/cix132

installation of point of use water filters to taps and linking these to the results of water testing[14].

Two of the seven institutional outbreaks cited by Kizny Gordon et al. involved *Klebsiella* spp[15] [16] but we contribute other examples include the following references[17] [18], the latter not referenced by the systematic review. In addition, multiple other potential water system related nosocomial outbreaks caused by Klebsiella are cited by Kizny Gordon et al., with the following sobering statement:

"General limitations of our study include potential publication bias and the language and literature source restrictions used in this review. Healthcare water environments are probably often neglected in outbreak assessments, which may result in an underestimation of the problem."

We have rehearsed the data for Klebsiella in significantly more detail than that for the other important types of GNE bacteria discussed in this chapter but this literature illustrates many of the general points that guided our understanding of potential risk to patients in the CNR that could have arisen from the hospital environment.

[14] Bundle 6, Document 38, Page 1065, Section 8.2.2.2.

[15] Leitner E, Zarfel G, Luxner J, Herzog K, Pekard-Amenitsch S, Hoenigl M, Valentin T, Feierl G, Grisold AJ, Högenauer C, Sill H, Krause R, Zollner-Schwetz I. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing Klebsiella oxytoca on a hematology ward. Antimicrob Agents Chemother. 2015 Jan;59(1):714-6. https://doi.org/10.1128/AAC.04306-14

[16] Kotsanas D, Wijesooriya WR, Korman TM, Gillespie EE, Wright L, Snook K, Williams N, Bell JM, Li HY, Stuart RL. "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. Med J Aust. 2013 Mar 18;198(5):267-9. https://doi.org/10.5694/mja12.11757

[17] Tofteland S, Naseer U, Lislevand JH, Sundsfjord A, Samuelsen O. A long-term low-frequency hospital outbreak of KPC-producing Klebsiella pneumoniae involving Intergenus plasmid diffusion and a persisting environmental reservoir. PLoS One. 2013;8(3):e59015. https://doi.org/10.1371/journal.pone.0059015

[18] Lowe C, Willey B, O'Shaughnessy A, Lee W, Lum M, Pike K, Larocque C, Dedier H, Dales L, Moore C, McGeer A; Mount Sinai Hospital Infection Control Team. Outbreak of extended-spectrum β-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1). Emerg Infect Dis. 2012 Aug;18(8):1242-7. https://doi.org/10.3201/eid1808.111268

3.2 Enterobacter

Bundle 44 vol 1, Page 26 (Section 3.1.2

Next, Hawkey et al. address the potential for environmental sources of hospital-acquired infections due to *Enterobacter* spp. Although they may seem to play down this risk, it is notable that five studies highlighting a role for *Enterobacter* spp. in hospital-acquired infections are included in the Kizny Gordon review, relating to water sources (drains, taps, showers, sinks).

Hawkey et al. write "*When outbreaks of Enterobacter cloac[e]ae involving environmental sources occur molecular typing using WGS usually demonstrates a clear cluster of a specific strain type.*"

This statement is unhelpful and misleading for two reasons. First, it presumes that only one strain type is causing an outbreak rather than multiple types as could happen if a water/wastewater system was contaminated with multiple bacteria as is normally found. Second, there is an assumption that sufficient samples have been obtained to enable a robust comparison to be made of bacteria present in water sources with those causing infections. Notably, in a study cited by Hawkey et al. in which Enterobacter infections were linked to the hospital water system[19], the authors state the following:

"To determine whether showering was the most likely route of acquisition, we performed a shower test in six bathrooms in which OXA-48-positive E. cloacae had been detected in the shower drains 48 h prior to testing. In two experiments, E. cloacae was detected on the sedimentation plates placed on the shower tap, on a soap dish, and on the rim of a sink. In the other four experiments, we did not detect any E. cloacae in the sedimentation plates by culture. Other water-carrying systems such as sinks, toilets, and shower heads were screened prior to the shower tests to exclude existing reservoirs." and:

[19] Nurjadi D, Scherrer M, Frank U, Mutters NT, Heininger A, Späth I, Eichel VM, Jabs J, Probst K, Müller-Tidow C, Brandt J, Heeg K, Boutin S. Genomic Investigation and Successful Containment of an Intermittent Common Source Outbreak of OXA-48-Producing Enterobacter cloacae Related to Hospital Shower Drains. Microbiol Spectr. 2021 Dec 22;9(3): e0138021. https://doi.org/10.1128%2FSpectrum.01380-21

"Wastewater from toilets and shower drains was collected at defined intervals, weekly during the peak of the outbreak and otherwise monthly, from all patient rooms of the three affected wards"

Again, it is clear that extensive sampling is required to find possible sources of infection and, in that context, we refer again to the deficiencies in the data from WGS investigation provided to the CNR[20].

3.3 Pseudomonas

Bundle 44 vol 1, Page 23, Section 3.2.1

Here Hawkey et al. discuss *Pseudomonas* spp., implicated as being potentially linked to the hospital environment in the CNR (defined as being in 'Strong Possible', 'Probable' & 'Strong Probable' groups)[21]. They comment:

"It is the most important bacterium when considering colonisation of hospital water systems which then can lead to infection in susceptible patients."

We agree. The authors say nothing here to refute our findings.

3.4 Acinetobacter

Bundle 44 vol, Page 30, Section 3.2.2

Hawkey et al. discuss *Acinetobacter* spp., which was also implicated as being potentially linked to the hospital environment in the CNR. They comment that: *"The epidemiology of Acinetobacter is therefore rather different to Pseudomonas and Klebsiella in that although there are reports of contamination of wet medical equipment together with isolates from sink traps as outbreak sources, surface contamination and resistance to drying is the most important source (Cruz- Lopez 2022)."*

[20] Bundle 6, Document 38, Page 1076, Section 8.3.1.

[21] Bundle 6, Document 38, Page 1044, Section 5.6, Table 5.4.

We agree but note that the authors say nothing here to refute our CNR findings and we are aware of published examples of water associated hospital outbreaks of infection caused by *Acinetobacter* spp.[22] [23].

[22] Umezawa K, Asai S, Ohshima T, Iwashita H, Ohashi M, Sasaki M, Kaneko A, Inokuchi S, Miyachi H. Outbreak of drug-resistant Acinetobacter baumannii ST219 caused by oral care using tap water from contaminated hand hygiene sinks as a reservoir. Am J Infect Control. 2015 Nov;43(11):1249-51. https://doi.org/10.1016/j.ajic.2015.06.016

[23] Wei L, Feng Y, Lin J, Kang X, Zhuang H, Wen H, Ran S, Zheng L, Zhang Y, Xiang Q, Liu Y, Wu X, Duan X, Zhang W, Li Q, Guo H, Tao C, Qiao F. Handwashing sinks as reservoirs of carbapenem-resistant *Acinetobacter baumannii* in the intensive care unit: a prospective multicenter study. Front Public Health. 2024 Oct 9;12:1468521. https://doi.org/10.3389/fpubh.2024.1468521

3.5 Stenotrophomonas

Bundle 44 vol 1, Page 30, Section 3.2.3

In this section, Hawkey et al. discuss *Stenotrophomonas maltophilia*, also implicated as being potentially linked to the hospital environment in the CNR. They comment that: *"This emphasises the fact that S. maltophilia can be found very widely in the hospital environment but comparatively rarely causes infections, even in the immune-compromised and careful strain typing is needed to identify any actual environmental source."*

Their comment *"comparatively rarely cause infections"* belies the fact that *S. maltophilia* is widely recognised as an increasingly important pathogen, especially in immunocompromised patients. In writing this response, we undertook a simple literature search via PubMed® using the terms 'stenotrophomonas, infection, immunocompromised': this provided 252 publications. There are multiple reports implicating environmental/water sources in hospital outbreaks caused by *S. maltophilia*, for example.[24] [25] [26]

3.6 Other GNE organisms

The remaining sections in Chapter 3 (Sections 3.1.3, 3.1.4 and 3.3.1 – 3.4.10) briefly describe a range of other Gram-negative bacteria that caused BSIs in the CNR.

Hawkey et al. discuss *Serratia* spp.(Section 3.1.3) and *Citrobacter* spp.(Section3.1.4). *Serratia* spp. were less frequently implicated as being potentially linked to the hospital environment in the CNR (defined as being in 'Strong Possible', 'Probable' & 'Strong Probable' groups)[27]. No *Citrobacter* spp. were found in these groups. Whilst we do

[24] Guyot A, Turton JF, Garner D. Outbreak of Stenotrophomonas maltophilia on an intensive care unit. J Hosp Infect. 2013 Dec;85(4):303-7. https://doi.org/10.1016/j.jhin.2013.09.007

[25] Yashar M, Basarir KE, Tanriverdi ES, Celep S, Sirekbasan L, Rakici E, Ejder N, Musellim E, Cicek AC, Yilmaz M. Stenotrophomonas maltophilia outbreak originating from a pull-out faucet in a pediatric intensive care unit in Turkey: Insights from clinical records and molecular typing. Am J Infect Control. 2024 May;52(5):605-610. https://doi.org/10.1016/j.ajic.2023.11.018

[26] Carbonell N, Oltra MR, Clari MÁ. *Stenotrophomonas maltophilia*: The Landscape in Critically Ill Patients and Optimising Management Approaches. Antibiotics (Basel). 2024 Jun 22;13(7):577. https://doi.org/10.3390/antibiotics13070577

[27] Bundle 6, Document 38, Page 1043, Section 5.6, Table 5.4.

not comment in detail here on these sections, these do not refute the possibility of an environmental source for infections cause by *Serratia* spp. or other rarer BSIs. Indeed, for one of these, *Cupriavidus* spp., NHS GGC told us that, in 2016, it had been possible to definitively link the environment to one Cupriavidus BSI. Although this case was not included in our review, it is referred to in our report[28].

3.7 Mycobacterium chelonae

Finally, we are surprised that Chapter 3 makes no mention of this organism despite it being the source of infection in 3 patients in the CNR. *M. chelonae* is ubiquitous in the environment and is found in soil, water, and aquatic animals. It is commonly associated with skin and soft tissue infection and also causes catheter-related infections and post-surgical infections after implants, transplants, and injections. Notably, NHS GGC told us that it was possible to definitively link the environment to infection by *M. chelonae* in a case that was included in the CNR but as we did not see the confirmatory data, we were unable to record this as definitely related to the environment in our report[29].

We maintain our position that it remains entirely plausible, supported by the epidemiology of the infections seen in the CNR, that there could have been an environmental source(s) for some of the BSIs identified.

[28] Bundle 6, Document 38, Page 1071, Section 8.3.1.

[29] Bundle 6, Document 38, Page 1044, Footnote 66.

5. CHAPTER 4: CASE NOTE REVIEW

4.1 Introductory comments

Chapter 4 of the HAD report, entitled Case Note Review, seeks to challenge aspects of the methodology used in the CNR and the validity of its conclusions. We are, however, critical of the authors' approach to their critique in the following key areas:

- Discussing the patient as a source of infection, with consequent risks of patient cross contamination, without acknowledging our own recognition of the possibility of endogenous infection and observations of less than adequate Infection Prevention Control (IPC) measures.
- Proposing a theory of infection selection in patients (relating specifically to the occurrence of *S. maltophilia*) from the use of specific antibiotics of the carbapenem group (notably Meropenem) without fairly representing the strength of the evidence on which this observation is based.
- Appearing to misunderstand the selection of bacteria chosen for study in the CNR and ignoring, without explanation, one specific group of infections included within it (*M. chelonae*).
- Criticism of our approach to the use of Whole Genome Sequencing (WGS) data in defining clusters but failing to recognise the limitations on the data made available to us by NHS GGC at the time.
- Criticising a lack of comparison of the overall rates of infection with other similar institutions without discussion of the data available from studies made by Health Protection Scotland in 2019, to which we referred in our report.

As references to some of these themes reappear at different points within this Chapter, we have taken the points on which we wish to comment as they first arise within the text, providing Page and Paragraph/line references to relevant text in the HAD report itself.

4.2 The patient as a source of infection and patient to patient colonisation

Bundle 44 vol 1, Page 40

The HAD report states that patients spend long periods at home (during treatment) when there is ample opportunity for colonisation by environmental bacteria.

The references offered in the HAD report were not all published at the time we undertook our work, and we suggest that those chosen to support the risk of bacterial colonisation in the home environment are selective and neither specific to the population of patients included in the CNR *(Denton et al. 1998)*[30] nor obviously relevant *(Tavares et al. 2020)*[31]. Nevertheless, we do not disagree that this is a possibility and one which we carefully considered in own deliberations[32]. Indeed, we specifically identified one patient in the CNR who almost certainly acquired his repeated blood stream infections from the home environment[33].

We wish to point out that, certainly in paediatric practice (and we note that none of the HAD report authors has experience in paediatric haematology oncology), the opportunity to maximise time for patients to spend out of hospital during treatment still requires frequent and recurrent exposures to the hospital environment, whether this is in an outpatient clinic, day care unit or during inpatient admission. We believe the frequency of a patient's separate encounters with the hospital environment may be as relevant as, for example, the duration of a single inpatient stay[34]. We also point out that the desire to maximise time for the patient to be at home is not merely for reasons of social benefit or family cohesion, important as these factors are, but also because of the recognised risk of hospital acquired infection.

[30] Denton M, Wilcox MH, Parnell P, Green D, Keer V, Hawkey PM, Evans I, Murphy P. Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii on a neurosurgical intensive care unit. J Hosp Infect. 2004 Feb;56(2):106-10. https://doi.org/10.1016/j.jhin.2003.10.017

[31] Tavares M, Kozak M, Balola A, Sá-Correia I. Burkholderia cepacia Complex Bacteria: aFeared Contamination Risk in Water-Based Pharmaceutical Products. Clin Microbiol Rev. 2020 Apr 15;33(3):e00139-19. https://doi.org/10.1128/cmr.00139-19

[32] Bundle 6, Document 38, Page 1043, Section 5.6.

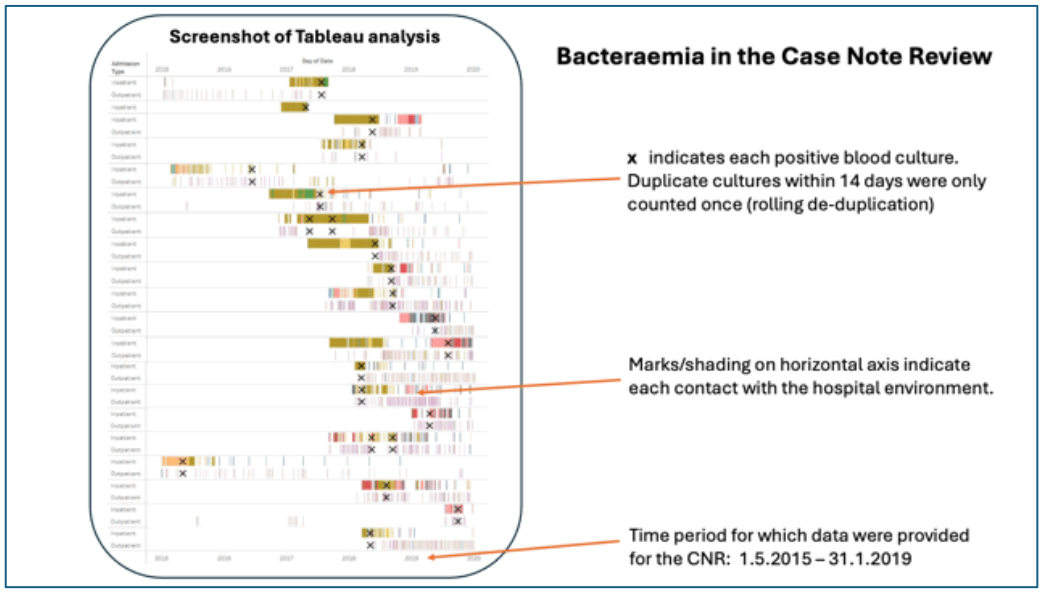
[33] Glasgow 3 - Prof Mike Stevens, Transcript, Transcript - Professor Mike Stevens - 30.10.2024 | Hospitals Inquiry, page 25.

[34] Glasgow 3 - Prof Mike Stevens, Transcript, Transcript - Professor Mike Stevens - 30.10.2024 | Hospitals Inquiry, page 65.

We had access, via Tableau data visualisation software, to each coded point of contact with the QEUH/RHC environment for every patient in the CNR. This included outpatient, day care and inpatient episodes which could be interrogated by date and location[35].

The relevant question posed of the CNR was whether it was possible to associate the infections identified within the CNR with the environment of the RHC and the QEUH. In order to address this, we took the dates, pattern and frequency of each patient's interaction with the hospital environment into consideration alongside details of clinical history and the proximity (by date and location) of the same infection arising in other patients. Figure 4.1 illustrates the complexity and utility of the location data available.

Figure 4.1: Screenshot of tableau analysis showing a selection of data illustrating bacteraemia in relation to contacts with the hospital environment



Bundle 44 vol 1, Page 40, line 11

Having raised the question of bacterial colonisation from the home environment and noting the conclusion drawn in the CNR that 34% of infectious episodes were categorised as 'most likely' to be derived from the hospital environment, Hawkey et al. suggest that a lower figure (20% or less) would be reasonable. This estimate still

[35] Bundle 6, Document 38, Page 1011, Section 3.3.3.

appears to us to be significantly high, particularly as we were describing bacteraemia and not merely evidence of colonisation. Their suggestion appears to be supported by reference to publications which compared bacterial isolates from patients with those obtained from the environment using Whole Genome Sequencing (WGS) and with a prospective approach to environmental sampling.

Whilst WGS will be discussed further in Section 4.5 of this Chapter and in more detail in our response to Section 7.3 of the HAD report, we wish to be clear that, in addition to transmission from the hospital environment, we considered the possibility of environmental origin outside the hospital site as well as the possibility of patient to patient spread. We agree that sequencing information, as provided by WGS, is the only way to determine if two microorganisms of the same species are genetically related, and therefore potentially transmitted between patients or the environment and patients. Yet during the era in which patients considered within the CNR were being treated, and up to the date of submission of our report, WGS data at NHS GGC were limited. Nor was there evidence of a systematic approach to prospective environmental microbiological surveillance.

We consider patient to patient infection to be a failure of protection within the hospital environment and we are surprised that the authors do not reference the importance of Infection Prevention Control (IPC) measures in reducing the risk of patient to patient and environment to patient transmission. The absence of comment on this point is particularly relevant as we described less than optimal IPC measures in the environment in which care was delivered at QEUH/RHC[36]. Our concerns included poor quality data and lack of governance and assurance relating to improvements to IPC based on audit submissions.

[36] Bundle 6, Document 38, Page 1036, Section 5.3.

4.3 The Meropenem theory

Bundle 44 vol 1, Page 40, line 26

The authors move from a discussion of the risk of the home environment to consider the impact of broad spectrum antibiotic use and its risk for the development of multi-resistant Gram-negative bacteria.

We agree that broad spectrum antibiotic use has the potential for dominant colonisation of the microbiological environment of the gastrointestinal tract in patients being treated for cancer and similar conditions.

The HAD report pays particular attention to *Stenotrophomonas maltophilia* and we agree *that S. maltophilia* is a bacteria of rising concern that has been reported as a cause of healthcare-associated infection with significant morbidity and mortality. It is a non-fermentative, aerobic, Gram-negative bacillus usually isolated from humid places such as water, soil and sewage. It can form biofilms and colonise surfaces of medical devices.

Hawkey et al. present data from a study in adult patients with acute myeloid leukaemia [Aitken et al. 2021][37]. This is an aggressive malignancy also seen in children and adolescents although significantly less frequently than the more typical form of acute lymphoblastic leukaemia found in the paediatric age group.

The authors use the Aitken study to highlight the theory that prior administration of carbopenem antibiotics (typically, meropenem) may drive the risk of patient colonisation with *S. maltophilia* and with it, the risk of subsequent blood stream infection. There are, however, weaknesses in the Aitken study which the study authors themselves identify, notably that: the study was small (only 8/90 patients studied developed *S.maltophilia*); it was based in a single treatment centre; and it lacked the power to permit a full multivariate analysis. Indeed, the impact of prior meropenem exposure was found not to be significant in the exploratory multivariate analysis the authors report. In addition, Aitken et al. referenced at least three other studies that had not identified meropenem exposure as a risk factor for *S. maltophilia* colonisation or

[37] Bundle 27 Volume 18, Document 4, page 32.

blood stream infection. These authors concluded that their own results should be viewed as '*hypothesis generating'*.

Bundle 44 vol 1, , 45, Paragraph 2, line 5

In contrast, Hawkey et al. suggest that the work reported by Aitken et al. offers a *"highly plausible explanation for the apparent increase in S. maltophilia BSI …"*. This might seem a premature and over-optimistic assessment of a risk which cannot be considered as proven at this stage. However, as we recognise an increased interest in this arena and we have reviewed more recent literature (from 2022) to further illuminate the hypothesis.

We identified 5 studies of patients with *S. maltophilia* BSI published since that time which appear to be of relevance. Two are relatively large adult studies and two are from paediatric populations. We also identified one systematic review although this addressed *S. maltophilia* pneumonia rather than BSI.

These publications are briefly summarised here:

Kanchanasuwan et al.[38]. This retrospective study, from Thailand characterised the previous antibiotic exposure and outcome of 117 adult patients with *S. maltophilia* blood stream infections (BSI) of whom 112 (96%) had received prior carbapenem antibiotics. The authors concluded that *S. maltophilia* mostly affects patients with a history of the prior use of antibiotics, especially carbapenem, reflecting the widespread but potentially problematic use of carbapenem in hospitalized patients. Unfortunately, they did not study a parallel population of equivalently ill patients without *S. maltophilia* infections to determine if prior use of carbapenem was equally prevalent. This study cannot be considered informative to the matter in question.

Chung Yi Koh et al.[39]. A retrospective study of adults from Singapore reported a total of 349 distinct encounters (from 322 unique patients, one third of whom were in

38 Kanchanasuwan S, Rongmuang J, Sirapaitoon P et al. Clinical Characteristics, Outcomes, and Risk Factors for Mortality in Patients with Stenotrophomonas maltophilia Bacteremia. J Clin Med. 2022;11(11):3085. https://doi.org/10.3390/jcm11113085

[39] Koh Mcy, Ngiam Jn, Chew Kl et al., Clinical Presentation And Outcomes Of Bloodstream Infection With Intrinsically Carbapenem-Resistant Non-Fermenting Gram-Negative organisms:*Stenotrophomonas maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium spp*. in Singapore, from 2012 to 2024. BMC Infect Dis. 2025;25(1):273. https://doi.org/10.1186/s12879-025-10636-9

intensive care) with a BSI over a 12 year period. *S. maltophilia* was the commonest (56.4%), followed by *Elizabethkingia spp*. (36.4%) and *Chryseobacterium spp*. (7.2%). Prior carbapenem exposure (defined as within 1 month prior to infection) was observed in 59.9% of all cases but with no significant differences observed among the three pathogens.

Franco et al.[40]. This is a retrospective matched case-control study from a neonatal intensive care unit in New York, published this year. It reported a total of 15 cases with *S. maltophilia* cultured from any site, and 35 controls. This was a small study and it was not restricted to BSI; it could therefore include patients who were colonised rather than with established infection. Nevertheless, significant risk factors identified for the culture of *S. maltophilia* included days of any prior antibiotics (median 24 vs. 18, *p = 0.036*) and days of all broad-spectrum antibiotics (median 19 vs. 12 days, *p = 0.027*), as well as duration of meropenem exposure (median 9 vs. 6 days, *p = 0.018)* and of any meropenem exposure (100% vs. 22%, *p = 0.005*). There was no multivariate analysis to allow for confounding variables in the analysis.

Bilen et al.[41]. This was a retrospective observational study from a tertiary children's hospital in Turkey, performed between January 2014 and December 2021. All patients aged under 18 years with *S. maltophilia* (n= 73) and *P. aeruginosa* (n=80) BSI were included in the study. Over 30% of patients in each group were being treated for haematology oncology conditions. 81% of *S. maltophilia* and 95% of *P. aeruginosa* infections were nosocomial in origin (identified after 48 hours in hospital).

Prior glycopeptide (teicoplanin or vancomycin) and carbapenem (meropenem, ertapenem or imipenem) antibiotic use was significantly higher in patients both with *S. maltophilia* BSI (41% vs. 21.2%; OR, 2.544; 95% CI: 1.250–5.180; *p= 0.009* for glycopeptide exposure, and 52% vs. 23.7%; OR, 3.429; 95% CI: 1.719–6.840; *p= 0.001* for carbapenem exposure). Multivariate analysis showed that only prior

[40] Franco S, Abdelhemid A, Fordjour L et al.. *Stenotrophomonas maltophilia* Associated Factors and Outcomes in a Neonatal Intensive Care Unit: A Retrospective Matched Case-control Study. Pediatr Infect Dis J. 2025;44(1):69-73. https://doi.org/10.1097/INF.0000000000004530

[41] Bilen NM, Sahbudak Bal Z, Güner Özenen G, Yildirim Arslan S, Ozek G, Ozdemir Karadas N, Yazici P, Cilli F, Kurugöl Z. Risk Factors for Infection and Mortality Associated With Stenotrophomonas maltophilia Bloodstream Infections in Children; Comparison With Pseudomonas aeruginosa Bloodstream Infections. Pediatr Infect Dis J. 2023 May 1;42(5):374-380. https://doi.org/10.1097/INF.0000000000003845

carbapenem use was associated with *S. maltophilia* BSI (adjusted odds ratio [AOR], 2.710; 95% CI: 1.225–5.992; *p= 0.014*). Further multivariate analysis showed PICU admission because of BSI and prior glycopeptide use were independently associated with mortality in patients with *S. maltophilia* BSI (AOR, 19.155; 95% CI: 2.337–157.018; *p = 0.006* and AOR, 9.629; 95% CI: 1.053–88.013; *p= 0.045*, respectively) but not prior carbapenem exposure.

Wang N et al.[42] This meta-analysis, the only recently published study using this methodology that we were able to identify, included a total of eight studies with a sample size of 2,320 cases. Meta-analysis of prior antibiotic exposure showed that β-Lactamase inhibitors (OR = 9.98, 95% CI: 1.51 ~ 65.96), aminoglycosides (OR = 4.01, 95% CI: 2.06 ~ 7.80), carbapenems (OR = 2.82, 95% CI: 1.49 ~ 5.31), and quinolones (OR = 2.17, 95% CI: 1.21 ~ 3.89) were all significant risk factors for ICU-acquired *S. maltophilia* pneumonia. It is unclear whether this result can translate to a similar analysis of patients with BSI but the study serves to illustrate that other antibiotic exposure, not just carbapenem, may represent risk.

Whilst the meropenem hypothesis cannot be dismissed, nor is it proven and the published experience remains inconsistent. However, given the emphasis placed by Hawkey et al. on 'meropenem risk' (our terminology) regarding *S. maltophilia,* we have undertaken a) a review of antibiotic policy for treating haematology oncology patients at RHC during the era of the CNR and b) looked again at data available on the use of antibiotics in the CNR.

We had access to all versions of the policy guiding the use of antibiotics for febrile neutropenia[43] (fever in occurrence with low white cell count, a state which carries a significant risk of serious infection in immunocompromised patients) in paediatric haematology oncology patients issued from August 2010 to March 2020, i.e. encompassing the era of the CNR. There was no significant change across the four versions of the policy issued during this time. Each specified Tazocin (piperacillin-

[42] Wang N, Tang C, Wang L. Risk Factors for Acquired *Stenotrophomonas maltophilia* Pneumonia in Intensive Care Unit: A Systematic Review and Meta-Analysis. Front Med (Lausanne). 2022;8:808391. https://doi.org/10.3389/fmed.2021.808391

[43] Bundle 44 Volume 1, Documents 33, 34, 35, 36, Pages 597 – 634.

tazobactam) and Gentamicin as first line antibiotic therapy for suspected infection, with the addition of Vancomycin or Teicoplanin if there was concern about infection of the central venous line. Meropenem was to be substituted for Tazocin (continuing also with Gentamicin) if there was persistent fever at 72 hours. This guidance could be (and was) modified by microbiological advice and the reporting of antibiotic sensitivities from proven positive cultures.

Hawkey et al. refer to a worldwide shortage of piperacillin-tazobactam in 2017-2018 and suggest that this sometimes required the substitution of meropenem. They provide no evidence that this occurred at NHS GGC. They also state that *"This resulted in an increase usage of meropenem around the time of the increase(d) occurrence of S maltophilia"* before referring again to the work of Aitken et al.

No evidence is provided for this assertion.

Although we collected data on antibiotic usage in our clinical summaries of each patient prior to and during the treatment of each infection in the CNR, we do not have a fully comprehensive dataset across the whole CNR for the entire duration of the period under study. This would be necessary to identify any change in meropenem use with time.

Nevertheless, we have undertaken an analysis of meropenem exposure in periods of 30 and 60 days prior to infection in patients in the CNR who experienced *S. maltophilia* BSI.

We used patients in the CNR who experienced Enterobacter cloacae infections as a similar sized comparator group.

The analysis shows no difference. (Table 4.1).

Table 4.1 The use of meropenem within periods of 30 and 60 days prior to the occurrence of infections with *Stenotrophomonas maltophilia* and *Enterobacteria cloacae* in the CNR

Organism	Patients with informative	Meropenem use documented within 30 days prior to infection	Patients with informative	Meropenem use documented within 60 days prior to infection

	records* (Total)	Yes	No	records* (Total)	Yes	No
S. maltophilia	18 (23)	5	13	14 (23)	5	9
E. cloacae	22 (24)	4	18	12 (24)	5	7
Uncorrected χ^2		0.523			0.097	
p (2 tail)		0.50 (not significant)			0.76 (not significant)	

*The term 'Informative Records' implies that these patients had hospital contact records spanning periods of 30 or 60 days prior to infection *and* adequate clinical information about antibiotic use in these periods from data extracted for the CNR.

4.4 Defining the population within the CNR

Bundle 44 vol, Page 41, Paragraph 2

Hawkey et al. claim that they were not clear exactly how many BSI occurred in the period studied, and what the causative bacteria were. We this this statement may refer to data for all types of BSI in patients included over the entire period of the CNR, as data describing the infections included in the CNR are provided in the Overview Report[44].

Their concern also appears to relate to whether some bacterial species regarded as having an environmental origin were included or not, and yet we believe our report to be quite clear on this point[45] . We worked to a pre-defined protocol[46]; the HAD team say this was not available to them although it could have been requested.

It is of note that the infections selected for the CNR included three patients with infections caused by acid fast environmental organisms (*Mycobacterium chelonae)*. It is interesting to note that Hawkey et al. do not address these infections in their report at all. We understood that NHS GGC established a direct link to the hospital environment in one of these patients – a point to which we referred in the CNR Overview Report[47]. However, as we were not provided with direct evidence for this link during our work, we were unable to classify this case as 'definitely' linked to the hospital environment as would otherwise have been the case.

Bundle 44 vol 1, Page 44, Paragraph 2

Hawkey et al. question the exclusion of *E coli* from the CNR but *E coli* was described as a Gram negative, non-environmental organism in the databases used to define the cases selected[48]. The HAD report refers to one study *(Woodford et al. 2007)*[49] which

[44] Bundle 6, Document 38, Page 1028, Section 4.3, Tables 4.2 and 4.3 *and* Page 1105, Appendix B.

[45] Bundle 6, Document 38, Page 1008, Section 3.2.

[46] To be Bundled - A53008955 - Case Note Review - Paediatric Haemato-Oncology Patients RHC, NHSGGC - Epidemiological & Clinical Outcomes Protocol - April 2020 v1.0.

[47] Bundle 6, Document 38, Page 1044, Footnote no. 66 *and* Page 1071, Section 8.3.1.

[48] To be Bundled - A53008955 - Case Note Review - Paediatric Haemato-Oncology Patients RHC, NHSGGC - Epidemiological & Clinical Outcomes Protocol - April 2020 v1.

[49] Woodford N, Kaufmann ME, Karisik E, Hartley JW. Molecular epidemiology of multiresistant Escherichia coli isolates from community-onset urinary tract infections in Cornwall, England. J Antimicrob Chemother. 2007;59(1):106-9. https://doi.org/10.1093/jac/dkl435

suggests that a strain of *E coli* may have caused a cluster of infections on a ward in one hospital over a period of several months. However, detailed reading of this publication reveals that only one isolate was available for testing and we believe this, and the very small size of the study, is insufficient to challenge the more commonly held view that *E coli* causes systemic infection by translocation from gut colonisation.

4.5 Defining clusters and WGS

Bundle 44 vol 1, Page 41, Paragraph 3

The HAD report discusses the terminology of a 'cluster' and makes the following statement: *"We do not think it appropriate to group different species of bacteria as a cluster despite the CNR panel suggesting that the putative environmental sources had multiple types of many different species…."*. We can confirm that we separately distinguished different species of the same bacteria in our considerations of possible clustering.

Bundle 44 vol1, Page 41, Paragraph 4

This leads on towards a discussion of the relevance of WGS, both in this paragraph and in the next, as well as in Chapter 7. We will respond to this more fully in our critique of their Section 7.3.

Initially, however, Hawkey et al. discuss the importance of other factors in determining the likelihood of clustering. Whilst they suggest the identification of a species of bacteria from an environmental site in spatial and temporal association with a patient who has a similar bacteraemia does not unequivocally establish it as a source, we assert that it substantially raises the possibility that this may be the case.

The authors suggest that a route of transmission and portal of entry need also to be identified but do not specify how a specific route of transmission or a specific portal of entry might be determined with any certainty except by undertaking a detailed root cause analysis.

It is self-evident that patients such as those in the CNR receive care in an environment in which there are multiple opportunities for exposure to water supply, water and drainage, including taps, sinks, lavatories, drains and all surfaces that might be wet from cleaning or splashing. All these represent opportunities for transmission of bacteria. Furthermore, virtually all patients have interventions on multiple occasions involving venous access, sometimes at multiple sites and most often via central venous catheters, as well as other invasive procedures, all of which would have afforded potential portals of entry.

Defining a specific route of transmission or a specific portal of entry may therefore be less relevant than the recognition that the nature of the patient's diagnosis and treatment raise these as a strong possibility.

All that said, it is of interest in this context that the HAD report does not comment on our observations about the frequency of environmental repairs to both ventilation and plumbing in the clinical environment[50]; the lack of proactive action following IMT meetings regarding investigation of potential outbreaks of infection[51]; or the failure to update the ICNet system to provide additional alerts which would have ensured greater engagement by IPC nurses with their investigation[52]. We also commented on the surprisingly late agreement (in the latter part of 2019) that Root Cause Analysis (RCA) should form the basis for future IMT investigation[53].

Bundle 44 vol1, Page 42, Paragraph 1

At the end of this Paragraph the authors describe the CNR's method of defining outbreaks/clusters as *"unusual, suboptimal and a limited method of investigation"*. They go on to suggest that we did not wish to consider WGS strain typing and, in doing so, reference Section 8.3.1 in the CNR report[54]. We will consider this further in our critique of Section 7.3 of the HAD report. Their prior suggestion is, however, incorrect:

[50] Bundle 6, Document 38, Page 1035, Section 5.2.

[51] Bundle 6, Document 38, Page 1066, Section 8.2.2.3 and Example 8.5.

[52] Bundle 6, Document 38, Page 1071, Section 8.4.2 and Page 97, Example 8.7

[53] Bundle 6, Document 38, Page 1064, Section 8.2.2.2.

[54] Bundle 6, Document 38, Page 1069, Section 8.3.1.

we would have been pleased to utilise more data about WGS had this been available to us.

Hawkey et al. fail to acknowledge our description of the inadequacy of the data with which we were provided. We referenced this both with regard to environmental sampling[55], and to data about WGS[56]. We made the point that not only were there limited data on patient isolates, the number of environmental samples available for analysis was particularly small. We highlighted, for example, in Section 8.3.1 of our report, that only 6 water samples were included in the database provided to us for environmental isolates in 2019, of which only 3 could be successfully analysed by WGS. Furthermore, 140 other samples from the total of 550 samples in the database for that year had incomplete location information, so any results that might have been obtained could not be accurately related to sites of patient care.

We also described our general concerns about the quality of data we had received from NHS GGC[57]. In the Executive Summary[58] we made a statement about data from WGS analysis which included these words "*…despite over 5 years of experience in investigation of outbreaks of GNE bacteria and concerns about the hospital environment, NHS GGC had not established an electronic database of microbiological typing results…. and consequently had no ability to easily relate potentially linked bacterial isolates".* We also went on to comment on *"an inconsistent approach to environmental sampling…".*

We stand by these criticisms and reiterate that it is important to understand the approach we took to address the second of the key objectives for the CNR, namely, 'Is it possible to associate these infections with the environment of the RHC and QEUH?'. Our deliberations and final decisions were based on all the information available to us at the time including that describing the clinical circumstances of the patient prior to and at the time of the infection; the temporal relationship between

[55] Bundle 6, Document 38, Page 1039, Sections 5.4 and 5.5.

[56] Bundle 6, Document 38, Page 1069, Section 8.3.1.

[57] Bundle 6, Document 38, Page 1058, Section 8.1

[58] Bundle 6, Document 38, Page 979, Executive Summary.

infections of the same bacterial species; and the overall frequency and pattern of GNE infections within the period of the CNR.

Bundle 44 vol 1, Page 44, Paragraph 4

Hawkey et al. revisit the issue of WGS in this Paragraph (and in Paragraph 2 on Page 40). We will address this again in our response to Section 7.3 of their report.

They also repeat their conclusions about the publication [Aitken et al., 2021] which they suggest supports a theory of 'meropenem risk' and reiterate the unsubstantiated suggestion that meropenem usage increased within NHS GGC because of a shortage of piperacillin-tazobactam. These points are further rehearsed in Paragraph 3 on Page 40 of the HAD report and have already been addressed in Section 4.3 of this Chapter.

4.6 Comparison of the overall rates of infection with other institutions

Bundle 44 vol 1, Page 45, Paragraph 4

Hawkey et al. suggest that a comparison with overall rates of infection in other similar institutions should have been undertaken as part of the work for the CNR.

Although this was not directly within the Terms of Reference for our review, we had access to relevant work undertaken by Health Protection Scotland (HPS), published in November 2019[59] (although this document is still dated October 2019 which is when, we believe, a first draft was published).

This work had previously informed the protocol for the CNR (see Section 4.4 above) and was referenced in the CNR Overview Report[60]. It includes an analysis of rates of infection at NHS GGC during the era of the CNR compared with similar data from the two other paediatric haematology oncology units in Scotland (Edinburgh and Aberdeen) over the same period of time. It included the following as one of its recommendations:

[59] Bundle 7, Document 7, Page 250.

[60] Bundle 6, Document 38, Page 1009, Section 3.2.1 and Page 1068, Section 8.2.3.

"NHS GG&C should consider current control measures around restriction on services for newly diagnosed patients as there is no evidence from the HPS review of the data that supports the continued restriction of services".

However, our own conclusion about the data presented in this report and included in the CNR Overview Report read as follows[61]:

"We do not see that this report would have provided any clear message of either reassurance or concern about past events. Nor do we see that it offered a clearly interpretable and favourable comparison with other Scottish children's hospitals (not least because of the size of the paediatric haematology oncology services in these three hospitals varies substantially – NHS GGC being easily the largest)".

It is interesting to note that this final version of the HPS report (published in November 2019) was redacted in parts for reasons we do not know. We requested an unredacted copy of the report (which we believe to have been first distributed in October 2019 [62]) in preparation for giving evidence to the Glasgow III hearings in October 2024. The text of the same recommendation simply reads *"NHS GG&C should consider current control measures around restriction on services for newly diagnosed patients".* We are unclear why the additional assurance was added to the redacted version and do not believe the data supports this addition.

The redacted version of the 2019 HPS report[63] is still publicly available and would have been accessible to Hawkey et al., but it is not referred to in their own report despite their assertion that such a comparison would have been important for the CNR. Nor did they undertake a similar comparison themselves, relying instead on an analysis of data comparing rates of infection at RHC during and beyond the era of the CNR, with those at Yorkhill (the previous home of RHC). We will address the findings relating to this analysis in our critique of Section 7.2 of the HAD report.

A comparison of incidence data for infections at NHS GGC with that at other Scottish paediatric haematology oncology units was also included in another document,

[61] Bundle 6, Document 38, Page 1068, Section 8.2.3.

[62] Bundle 7, Document 6, Page 214.

[63] Bundle 7, Document 7, Page 250

published earlier in 2019[64]. This was not referred to in the CNR Overview report but shows that the rate of environmental bacteria cultures was significantly higher at Yorkhill and RHC (combined) than at the other Scottish paediatric hospitals over the 5 years from 2014-2018. Reasonably, in our view, the report advises that the comparison with the paediatric hospitals in other health boards should be interpreted with caution as differences in the patient population may introduce bias to the comparison.

In conclusion, Hawkey et al. assert that comparative studies with other institutions would have been important for our work but although we did not undertake such comparisons ourselves, such data do exist. This position was also elaborated with reference to Mr Mookerjee's expert witness report[65], as part of Professor Stevens evidence to the Inquiry in October 2024[66].

Our view remains that comparisons of populations from different units offer limited assistance in responding to the question asked of the CNR – "Is it possible to associate these infections with the environment of the RHC and QEUH?"

[64] Bundle 7, Document 5, Page 194.

[65] Bundle 21, Volume 1, Page 3.

[66] Glasgow 3 - Prof Mike Stevens, Transcript, Transcript - Professor Mike Stevens - 30.10.2024 | Hospitals Inquiry Pages 60 et seq.

6. CHAPTER 5: WATER

We highlight below specific points and shortcomings in this chapter. In doing so, we note that it contains multiple examples of important observations that reinforce the plausibility for the hospital environment providing potential sources of infections in hospital patients, particularly in immunocompromised individuals.

5.1 Are hospital water systems sterile?

Bundle 44 vol 1, Page 47

This offers a brief introduction to the reality that hospital water systems are not sterile and that high quality supply is typically only supplied to specialist areas and now to augmented care areas, particularly with respect to *P. aeruginosa*.

We have no comments to offer.

5.2 What constitutes a contaminated water system?

Bundle 44 vol 1, Page 47

After a general discussion of bacteria in water, Hawkey et al. turn towards the clinical setting and state:

"Probably the most common method of the regression type of contamination occurs when the tap outlet is contaminated by immersion in water containing human derived material (e.g. wash water from a patient which may contain small amounts of urine, faeces, or worse, infected urine being emptied into a sink). Hospitals have strict rules on the disposal of such waste to prevent colonisation of water outlets in clinical areas, but under pressure they may not always be followed. The bacteria encountered in this setting are gut bacteria and sometimes skin bacteria although skin bacteria such as Staphylococcus aureus do not survive well in the environment whereas E. coli, Klebsiella spp. and other such organisms may survive as transient colonisers of the biofilm on the tap nozzle. The significance of this is that the tap in turn can act as a source to contaminate water that is drawn from the tap for use on patients or staff hands during hand washing."

We note and agree with this statement and highlight the admission by Hawkey *et al.*. about Gram-negative bacteria such a *Klebsiella* spp. having a potential environmental source(s) for transmission to patients e.g. via the contaminated hands of healthcare workers, and suboptimal IPC and environmental cleaning practice.

A little further on the same page, we encounter a more contentious statement:

"Furthermore, taps in domestic premises and non-clinical areas of hospitals are usually colonised. However, some groups of patients (such as those with neutropenia, immunosuppression, long term indwelling intravascular cannulas, respiratory tract intubation with mechanical ventilation, etc.) can infrequently become infected from these sources. It should be remembered that though one can find a source in a hospital environment of potentially pathogenic bacteria, without a route of transmission and a portal of entry into the patient these sources are of little consequence."

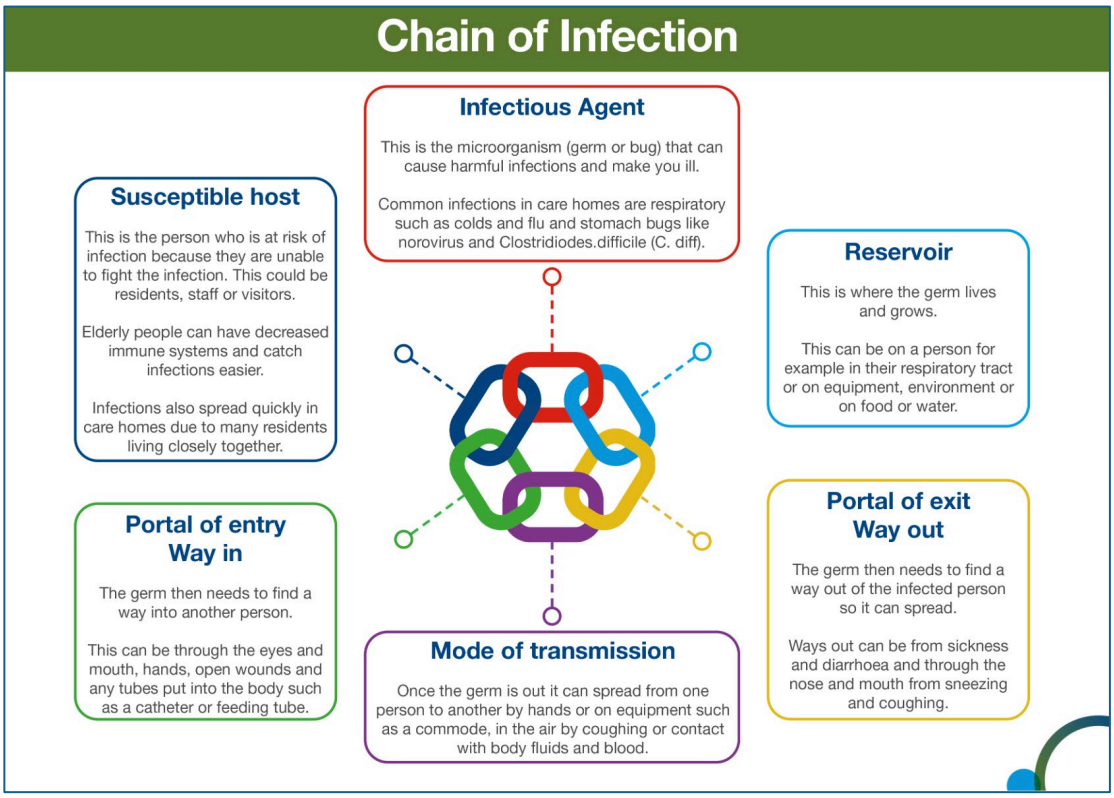
Whilst we note and agree with the first section of this paragraph, particularly that immunocompromised patients in hospitals can become infected from contaminated taps, the last part of this statement is odd. There are multiple potential ways in which bacteria may be transmitted to immunocompromised patients in hospitals and then cause infection.

Most strikingly, these patients typically have intravascular catheters for much of their hospital stay and these provide direct access into the bloodstream for drugs, blood products, fluids and (unwittingly) for microbes that can be encountered in the environment and on the hands of patients, healthcare workers and visitors.

The image below (Figure 5.1) from the NIPCM[67], originally designed for use in care home settings, is helpful in explaining the link:

[67] National Infection Prevention and Control Manual. Chain of infection https://www.nipcm.scot.nhs.uk/care-home-infection-prevention-and-control-manual-ch-ipcm/print?section=2820

Figure 5.1: The Chain of Infection



Hawkey et al.'s focus on determining the route of transmission and portal of entry is something also discussed in Chapter 4 of our rebuttal (Section 4.5).

Bundle 44 vol, Page 48

At the end of Section 5.2, Hawkey et al. conclude *"In summary, although sink traps have a diverse and sometimes profuse bacterial population which is enriched sometimes by human derived materials and consequently bacteria associated with human colonisation or infection, they are not the dominant source of bloodstream infection when compared to gut and or oral microbiomes of immune compromised patients themselves."*

We did not claim this to be the case. However, as Hawkey et al. state, and many reports have shown, sinks are clear potential sources of bacteria that can cause infections in patients. Gram-negative bacteria that are found either predominantly in the environment, e.g. in water systems, and/or in humans and which contaminate sinks via discarded fluids, are the key microbes found in sinks/sink traps.

The epidemiological clustering of BSIs identified in the CNR, noting how we categorised the likelihood of each infection being linked to the hospital environment, could certainly be linked to sink contamination.

Characterisation using WGS methodology is the tool with which links between patient and environment can be determined. We comment elsewhere in this report (Chapter 7, Section 7.3) about the interpretation of the WGS data available from NHS GGC and the sporadic and inconsistent sampling and storage of isolates from environmental sites when looking for such links.

5.3 What if there was 'widespread contamination of the hospital water system?

Bundle 44 vol 1, Page 48

In Section 5.3 of their report, which deals with questions that would be pertinent if there was indeed widespread contamination of the water system, Hawkey et al. make the following statement

"In our experience significant problems of increased rates of infection caused by bacteria capable of coming from an environmental source are identified when intensive testing of water is undertaken when an outbreak is suspected, rather than from routine or prospective testing.'

We agree and believe that NHS GGC clearly suspected that the hospital environment, and specifically the water system, could be linked to infections in children. They subsequently took mitigating action in treating the water system and taking the major decision to remove patients from the paediatric haematology oncology unit (Wards 2a and 2b) to permit environmental improvements. Yet, as we have detailed in the CNR Overview Report[68] [69] and in our critique elsewhere in this rebuttal (Chapter 7, Section 7.3), the short-comings of the environmental sampling and WGS investigations that were carried out at the time, and since, to determine if an environmental source(s) could explain the clusters of BSIs investigated in the CNR, are significant.

[68] Bundle 6, Document 38, Page 1041, Section 5.5.2

[69] Bundle 6, Document 38, Page 1042, Example 5.2

When discussing the possibility that a water system is a source of environmental contamination, Hawkey et al. return to the theme of WGS investigation:

"*The definitive evidence for linkage is to demonstrate that the DNA sequences (or part thereof - see below) of a micro-organism causing a BSI in a patient is indistinguishable to that of the same species of micro-organism found in the built microenvironment. This is not only essential for the understanding/identifying of an environmental source of risk to patients but also for ensuring there is not an overreaction to these possible sources of infection."*

We agree that DNA based typing can indeed provide definitive evidence linking infections in patients with the built environment. However, it is important to acknowledge two key issues here. Firstly, the 'needle in a haystack' analogy may apply, given the numerous opportunities for microbial contamination of a water system and the very many different bacteria that can be found which will vary in time and place. Secondly, especially if coupled with low frequency, sporadic and/or missed opportunities to establish linkages (see further our comments Chapter 7, Section 7.3), establishing DNA based matches between bacteria in patients with those in the environment may not be achieved.

Earlier in their report (Chapter 3), Hawkey et al. cite an authoritative systematic review published by Kizny Gordon et al.[70], and do so again here. This was a review of published studies investigating the hospital water environment as a source for antibiotic-resistant organisms causing hospital-acquired infections. Despite placing significant weight on the findings of this review, Hawkey et al. did not include the following statements from this publication:

"Screening of patients and the environment varied widely with regard to the methods used, frequency, and site".

"Differences in environmental sampling, such as collecting water samples before or after tap flushing and use of varying water volumes, may affect sampling sensitivity. Microbiological methods differed between studies, and although the use of selective

[70] Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, Peto TEA, Crook DW, Stoesser N. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature. Clin Infect Dis. 2017 May 15;64(10):1435-1444. https://doi.org/10.1093/cid/cix132

culture media may have improved detection, organisms with near-breakpoint minimum inhibitory concentrations could have been missed, underestimating the true prevalence and extent of environmental contamination. Molecular tests may increase sensitivity."

Experience from the literature also suggests that published studies will tend to have more complete information and, by contrast, unpublished investigation of links between the hospital environment and patient infections can be expected to have more gaps in data, increasing the chances that linkages will occur but go unrecognised

We concur with the caveat implied in the quotations from Kizny Gordon et al. (above) and believe that, put simply, the absence of DNA based typing links between microbes in the hospital environment and those causing patient infections does not exclude the possibility that the former is a source of the latter.

Conventional IPC practice, when there is a suspected source of infection, is to eliminate or modify the source. For this reason, Kizny Gordon et al. noted the following: *"Seventy-two percent of studies reported controlling outbreaks, of which just more than one-third eliminated the organism from the water environment. A combination of interventions seems to be most successful, including reinforcement of general infection control measures, alongside chemical disinfection."*

In interesting contrast, Hawkey et al. comment that *"In our experience, when clusters of infection occur caused by potentially environmentally sourced bacteria in immune supressed patients, investigation with detailed typing very often reveals a homogeneous cluster of strains and helps tremendously in identifying the source and introducing control measures."*

This unreferenced statement downplays the realities of such investigations. Interestingly, we note the following published investigation[71] of which Professor Hawkey was a co-author. This reports the use of intensive sampling to investigate the water systems in four UK hospitals as the source of infections in patients.

[71] Halstead FD, Quick J, Niebel M, Garvey M, Cumley N, Smith R, Neal T, Roberts P, Hardy K, Shabir S, Walker JT, Hawkey P, Loman NJ. Pseudomonas aeruginosa infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals. J Hosp Infect. 2021 May;111:162-168. DOI:10.1016/j.jhin.2021.01.020

The authors noted that *"In order to understand the extent and dynamics of the colonization of ICU tap water outlets, a prospective longitudinal study is required. Only one such study has been published …"* and concluded *"The overall findings suggest that water outlets are the most likely source of P. aeruginosa nosocomial infections in some settings, and that widespread introduction of control measures would have a substantial impact on infections."*

Thus, intensive sampling to an extent that is infrequently practiced in routine settings is needed to identify hospital water systems as a source of infection in patients.

5.4 If patients were at an increased risk of infection as a result…?

Sections 5.4.1 and 5.4.2 add little to earlier to data in sections of Chapter 3, on which we have commented in detail.

Bundle 44 vol 1, Page 52, Section 4.3

Here Hawkey et al. use a study by Kampmeier et al.[72] to attempt to refute that the clusters of cases of *S. maltophilia* BSIs we described in the CNR could have had an environmental source. Notably this study examined a single cluster of 4 cases of BSI over a 6 month period using WGS and concluded that their assumed cluster of cases did not constitute 'an outbreak'.

This scenario differs somewhat from the findings of the CNR in which 21 *S. maltophilia* BSIs occurred in 19 children. There were 12 episodes of *S. maltophilia* bacteraemia in 11 children during 2018, but none after September 2018 (when Ward 2A closed in September and patients were transferred to Ward 6A), until the first of 4 episodes in 4 children between April - September 2019 [we acknowledge that there is an error in Section 4.3.2 of the CNR – there were 4, not 5, cases of *S.maltophilia* BSI between April – September 2019].

Referencing this one report has no particularly useful bearing on the scenario we investigated in the CNR. In fact, Kampmeier et al. indicated that while the 4 isolates

[72] Kampmeier S, Pillukat MH, Pettke A, Kossow A, Idelevich EA, Mellmann A. Evaluation of a *Stenotrophomonas maltophilia* bacteremia cluster in hematopoietic stem cell transplantation recipients using whole genome sequencing. Antimicrob Resist Infect Control. 2017 Nov 13;6:115. DOI:10.1186/s13756-017-0276-y

causing BSIs were not genetically related to each other, this clearly did not remove the possibility of an environmental source(s) for these infections. Indeed, they found that one environmental isolate from a shower outlet was closely related (by WGS) to a single patient's isolate. They concluded:

"WGS analysis can be used to precisely refute an outbreak of S. maltophilia. Additionally, WGS- based typing documented sanitary installations in HSCT units to be an actual source or result of transmission between environmental and human habitats. Hence, in addition to classical infection control strategies, monitoring of aqueous habitats has to be established within the rooms of HSCT recipients in order to prevent transmission of these multidrug resistant organisms, as recently also shown for P. aeruginosa."

Bundle 44 vol, Page 53

Towards the end of this chapter, Hawkey et al. make a statement with which we don't disagree *"The important lesson here is that not all clusters of S. maltophilia infections in immunocompromised patients are necessarily spread from an environmental source."* and go on to say *"It also demonstrates the power of WGS typing to identify highly related clusters of organisms coming from single sources*." with which it is also possible to agree but, as we argue repeatedly in this rebuttal, WGS can only do so when sampling of both patient and environment is adequate.

With regard to their specific attention to the study by Kampmeier, we suggest that they inappropriately compare the larger clusters in time and place that we identified in the CNR, and the possibility/probability of them having an environmental source(s), with one small cluster of cases in a hospital in Germany, and whether a cluster of BSIs was caused by a single bacterial strain. Clusters of cases may be caused by one or more strains of a particular bacterial species, noting that the environment can contain multiple different strains of that species, especially over weeks/months.

This chapter ends with a return to the 'Meropenem theory' placing particular emphasis on the study by Aitken et al.[73], the findings of which even the authors expressed

[73] Aitken SL, Sahasrabhojane PV, Kontoyiannis DP, Savidge TC, Arias CA, Ajami NJ, Shelburne SA, Galloway-Peña JR. Alterations of the Oral Microbiome and Cumulative Carbapenem Exposure Are Associated With Stenotrophomonas maltophilia Infection in Patients With Acute Myeloid Leukemia

caution. We have addressed this issue in some detail in Chapter 4 and do not believe the case to be proven.

Receiving Chemotherapy. Clin Infect Dis. 2021 May 4;72(9):1507-1513.
https://doi.org/10.1093/cid/ciaa778

7. CHAPTER 6: VENTILATION

We did not evaluate the involvement of the hospital ventilation systems or the airborne transmission of pathogens in the CNR but offer a few observations.

In an introduction to Chapter 5 in the Case Note Review Overview Report[74] we mentioned 'chilled beam' systems (a type of radiation/convection heating, ventilation, and air conditioning system designed to heat and cool buildings) in passing only.

We also cited the findings of a 2020 Independent Review,[75] which highlighted deficits in the hospital environment that included (but were not limited to) issues such as: the design and maintenance of the water system; lower than required air exchange in patient rooms and inadequate positive pressure protection of patient rooms; the lack of provision of particulate (HEPA) filtration in some higher risk patient areas; and uncertainties around the appropriate utilisation of chilled beams for temperature control in rooms used for immunocompromised patients[76].

We referred further to chilled beams when reviewing the built environment and its maintenance[77], describing problems with *"….maintenance of chilled beams following reports about leaks or condensation, or both, and where additional cleaning was required for control of dust".*

We note that Hawkey et al. do not reference the 2020 Independent Review and nor are chilled beams mentioned.

We have no further comments to offer on this chapter.

[74] Bundle 6, Document 38, Page 1033, Section 5.1.

[75] Bundle 27, Volume 9, Document 11, Page 145.

[76] Inkster T, Peters C, Soulsby H. Potential infection control risks associated with chilled beam technology: experience from a UK hospital. J Hosp Infect. 2020 Nov;106(3):613-616. https://doi.org/10.1016/j.jhin.2020.08.011

[77] Bundle 6, Document 38, Page 1036, Section 5.2

8. CHAPTER 7: WATER – ANALYSIS OF INFECTION RATES AND DATA FROM QEUH & RHC

Despite the title of this chapter, its three sections deal with quite different issues. Section 7.1 examines the issue of water testing for one bacterium, *P. aeruginosa*, and what can be inferred from such results; Section 2, despite its title, is devoted to a detailed epidemiological review of bacteraemia from available NHS GGC data for both adults and children; and Section 3 focuses on Whole Genome Sequencing.

We address our comments to each of these sections in turn, providing Page and Section/Paragraph references to relevant text in the HAD report itself.

7.1 Learning from water testing for *P.aeruginosa*

Bundle 44 vol 1, Page 62

Hawkey et al. state: "*Inspection of water testing results for Pseudomonas aeruginosa should give a good indication as to whether there was extensive contamination of the hospital water system and particularly in the high risk areas which includes the paediatric haematology-oncology wards."*

No robust evidence is provided to support this claim, especially the assertion that results for one bacterium give 'a good indication' about contamination levels in general. In fact, Hawkey et al. admit that: *"In trying to assess whether there is widespread contamination of the water system we are faced with the problem that all water systems have a bacterial load and there is no published and accepted threshold value for contamination that correlates with the development of an outbreak."* The levels of *P. aeruginosa* in water tell us about one bacterium and not about others.

Having claimed that water testing results for *P. aeruginosa* can be used as a proxy to determine if extensive contamination of the hospital water system occurred, they then point out that for much of the period covering the CNR only low-level testing for *P. aeruginosa* occurred: *"At the QEUH, low level (frequency) testing for Pseudomonas aeruginosa of approximately an average of 20 samples per month was undertaken over the whole time only in the high risk areas. There was intensive sampling in*

December 2015 to March 2016 with 450 samples taken." Higher rates and wider sampling were introduced in November 2018.

The NHS England Health Technical Memorandum (HTM)[78] provides guidance on the legal requirements, design applications, maintenance and operation of hot and cold water supply, storage and distribution systems in all types of healthcare premises. Importantly, the following statement is made on page 25 of the HTM:

"**When water treatment and control programmes are effectively maintained to control Legionella, there will be less likelihood of colonisation of other opportunistic microorganisms. However, in the case of P. aeruginosa, where the origin may be due to retrograde contamination from patients and/or staff, other local management control measures and monitoring will be necessary.**"

Scottish guidance[79] notes that total bacterial burden in water, as opposed to levels of *P. aeruginosa*, can be indicative of levels of specific bacterial pathogens.

"Levels of TVC organisms in water samples give an indication of the effectiveness of residual disinfection and consequently the likelihood of finding potentially pathogenic micro-organisms."

The Gram-negative bacteria causing BSIs in patients in the CNR clearly can also enter water systems via 'retrograde contamination' from patients and/or staff. Thus, sampling and testing for one bacterium, i.e. *P. aeruginosa*, neither tells whether other bacteria were present nor how prevalent they were.

In the CNR Overview Report we commented on the confusion we identified at NHS GGC regarding water testing in general and for *P. aeruginosa* specifically. We stated[80]:

"There did not appear to be a systematic water sampling process in place, or a consistent water system related response to clusters of infections caused by (often

[78] A48115612 - NHS England. Health Technical Memorandum 04-01: Safe water in healthcare premises. Document first published: 20 May 2016. Page updated: 27 August 2024. Available at: Health Technical Memorandum 04-01: Safe water in healthcare premises. Part A: Design, installation and commissioning Accessed 26 May 2025.

[79] Bundle 25, Document 56.3, Page 787

[80] Bundle 6, Document 38, Page 1042

unusual/uncommon) GNE bacteria. We are not assured that there was adequate communication about what sampling and testing occurred and the results obtained. We have been told that some key staff involved in IPC at NHS GGC were denied access to water sampling/testing information despite multiple requests. As the concerns increased about whether the bacteraemias occurring in children on the Haematology Oncology wards at NHS GGC might be related to environmental/water contamination, the lack of a clear step change in the organisation's approach to water sampling, testing, reporting and strategy is of concern."

We stand by this statement. We also conclude that the levels of *P. aeruginosa* reported by Hawkey et al. have little or no bearing on the likelihood of water contamination by other Gram-negative bacteria relevant to our case series.

7.2 BSI incidence rates and the likelihood of clustering

This section of Chapter 7 does not directly address the question identified in its title. It largely consists of analyses of BSI incidence rates at NHS GCC and an examination of the likelihood that clustering, defined in time and space (described by Hawkey et al. as epidemiological clustering), occurred in haematology oncology patients, including those from the paediatric service.

Bundle 44 vol 1, Page 64. Comparing infection rates

At the start of this section, Hawkey et al. write *"It is difficult, and often inappropriate, to compare numbers of cases of a possible healthcare associated infections (HCAI) between different hospitals, locations and/or wards without taking into account the volume of activity in a given hospital/location/ward."*

We were not asked to, and did not compare infection rates at NHS GGC with levels elsewhere. We agree with the statement above and would have argued as such had we been asked to do so. In Chapter 4, Section 4.6 of this rebuttal, we have already addressed the suggestion made by Hawkey et al. that we should have undertaken such an analysis and critiqued the data that were available to us when we wrote the our report from the work undertaken by HPS in 2019[81].

[81] Bundle 7, Document 7, Page 250.

It is also the case that inter-institution comparison could lead to 'normalisation' of higher than desirable rates if the comparison offers similar or better results than elsewhere, whereas the focus should be on searching for ways to reduce rates to the minimum and identify any source.

There are many reasons why comparison of apparent infection rates between hospitals, and especially between units/wards can be flawed, and indeed misleading. The ways in which infections are diagnosed vary as does the intensity of sampling and testing patients. The types of patients, including their underlying diseases and severities of conditions, the way they are managed, including the use of anti-infectives to prevent or treat infections, may vary considerably. Routine surveillance to compare infection rates across hospitals for target bacteria, such as MRSA and *C. difficile*, has occurred for over a decade. However, hospital level results may also hide/mask the infection rates seen within individual units/specialties/wards in those hospitals.

Hawkey et al. argue that bed days are the most appropriate denominator for calculating incidence rates for HCAIs but go on to discuss the many other factors that may affect the risk and detection of infection within a hospital population (including those we describe above) and which will influence comparative incidence rates.

We suggest that a further factor to be considered is the frequency with which patients attend the hospital environment. This is discussed in Chapter 4, Section 4.2 of this rebuttal and visually illustrated in Figure 4.1. Are, for example, 20 day case attendances the same as 20 inpatient days in terms of the risk of environmental exposure? This point was addressed in evidence given by Professor Stevens as part of the Glasgow III hearings[82]. We do not claim to know how this pattern of care affects environmental risk but argue that this is another factor that also needs to be taken into consideration when assessing the utility of incidence analysis across a whole population without also considering the specific circumstances of an individual patient and the infection concerned. This latter approach being, we believe, the strength of the CNR process.

Bundle 44 vol 1, Page 66. Selection of micro-organisms

[82] Glasgow 3 - Prof Mike Stevens, Transcript, Transcript - Professor Mike Stevens - 30.10.2024 | Hospitals Inquiry, page 126.

Hawkey et al. here set out their approach to a review of *"all potential bacteraemia in adult and paediatric settings in the NHS GGC for Queen Elizabeth University Hospital (QEUH) and comparable hospitals"*. Their aim was to examine bacterial incidence and compare any difference in trends.

Tables 1 and 2 set out the micro-organisms grown in blood samples between 2008 and 2023 for adult and paediatric patients at NHS GGC hospitals that either could be, or are not usually, environmentally transmitted respectively.

We note some differences from the list in Table 1 when compared with the protocol used to select patients for the CNR[83], notably the absence of *Mycobacterium chelonae* and, less importantly, *Herbaspirillum spp*. The absence of any consideration given within the HAD report to the patients with *M. chelonae* infections is unclear and represents a serious omission. Table 1 also includes a number of bacteria that were not included in the CNR protocol, perhaps most notably *Proteus spp*. Interestingly, Table 2 (non-environmental BSI) includes *Escherichia spp*. which seems inconsistent with the criticism made by Hawkey et al. for the exclusion of *E. coli* from within the CNR cohort. This is discussed in Chapter 4, Section 4.4. of our rebuttal.

Bundle 44 vol 1, Page 67. Methodology

In Section 7.2.2, Hawkey et al. describe methodology for analysing their bacteraemia data. There is a lack of detail in the text which we observe as follows:

Nowhere is it explicit that the populations studied in the analyses provided are exclusively haematology oncology patients: we have assumed this to be the case.

It is likely the patient records were extracted by consultant name and/or ward. In our experience, hospital coding by consultant name can be inaccurate and we reported inaccurate patient location coding by NHS GGC in the CNR Overview Report[84].

No definition is provided for the selection of the paediatric population: we assume this is likely to have been based on location of care and / or consultant name rather than

[83] To be Bundled - A53008955 - Case Note Review - Paediatric Haemato-Oncology Patients RHC, NHSGGC - Epidemiological & Clinical Outcomes Protocol - April 2020 v1.

[84] Bundle 6, Document 38, Page 1075, Section 8.5

by age; but, if age has been a criteria, it is important to note that Teenage/Young Adult (TYA) patients are also under the care of the paediatric service.

There is no definition of how bacteraemias were classified: the protocol agreed for the CNR defined this as follows: Hospital associated infection (HAI) – a positive blood culture in a patient who has been hospitalised for at least 48 hours; and Healthcare associated infection (HCAI) – a positive blood culture in a patient within 48 hours of admission but who has had specified healthcare contact or intervention in the prior 30 days. It is not clear if the same rules guided the inclusion of patients and infections in the populations studied by Hawkey et al.

Finally, Table 1 and 2 in this section state that data included in the analyses were collected from 2008 – 2023, a substantially longer period than that we considered in the CNR. There is no discussion why these dates were chosen or any consideration given to changes in the nature of the relevant clinical services over that period of time, apart from reference to geographical re-location. Confusingly, later in this chapter, graphs provided in Section 7.2.3, addressing adult patients, show that the period of data collection extended from 2013 – 2023; and in Section 7.2.7, dealing with paediatric patients, that data collection was from 2005 to the end of 2022.

This lack of precision and the observed deficits in explanation given to the description of how the cohorts were established for these studies is surprising.

Bundle 44 vol 1, Page 67. Definition of Clustering:

Hawkey et al. explain that they examined epidemiological clusters in the list of organisms with the potential to be environmentally transmitted (Table 1) by bacterial species, dates and locations. They go on to provide definitions for different levels of likelihood of clustering which they summarise in Table 3.

We agree with their observation that a ‘probable’ epidemiological cluster is a ‘qualitative definition’ that can only be definitively confirmed with the molecular evidence that the microorganisms concerned are closely genetically related. They go on to suggest that these epidemiological clusters are those *“we would recommend investigating”* in this way. They fail at this point to acknowledge that this is what the CNR attempted to do, but that only limited data from molecular studies were available and there was very little evidence of systematic/robust environmental sampling that

could provide the parallel assessment required to identify a likely source. We discuss the issue of Whole Genome Sequencing later in our review of Section 7.3.

Hawkey et al. further develop their approach to the analysis of clustering and we agree that it would be reasonable to look at the number in each clustering category (Probable, Possible etc. as per Table 3) by location over a period of time (years) but question the validity of expressing the data as 'rate per clustering type per hospital'. We also question the use of a cumulative scoring system.

The approach taken by the HAD report fails to recognise that important clusters in a small number of patients (which might in time and with appropriate investigation be shown to be related to the environment) could be obscured by the 'noise' generated by much larger amounts of non-informative data.

Bundle 44 vol 1, Page 69. Adult patient data:

We reviewed data provided for adult patients in Sections 7.2.3 to 7.2.6 with interest but concluded that the principal focus of our rebuttal should remain with the paediatric population (Sections 7.2.7 to 7.2.9) as these data are of direct interest to the findings of the CNR. Moreover, we have no knowledge of the nature and delivery of adult haematology oncology services in NHS GGC. There are, however, some general points of comparison between the adult and paediatric analyses that are worth considering.

Hawkey et al. provide no data on the size of the patient populations from which their incidence data derive but, based on the age distribution of the general population and the rapidly increasing frequency of malignant disease with age, it is certain that the number of adults in the adult dataset would be substantially larger than the number of children and young people contributing to the paediatric dataset. This is apparent when comparing, for example, Figure 1 (adult) and Figure 17 (children). Similarly, the number of bed days of exposure will be substantially different.

It was therefore of interest to us that the number of BSI caused by selected environmentally relevant bacteria in the paediatric group compare with the adult group as follows (Table 7.1):

Table 7.1: Number of environmentally relevant bacteria in the HAD report adult and paediatric series, with added comparison to data from the CNR[85].

	HAD report Adult data (Data from 2013-2023)	HAD report Paediatric data (Data extracted only for the CNR era 2015-219)	Data from CNR Overview Report
Acinetobacter baumanni	0	5	6
Acinetobacter ursingii	13	5	6
Klebsiella oxytoca	27	8	9
Klebsiella pneumoniae	No data given	22	21
Klebsiella oxytoca	27	8	9
Pseudomonas aeruginosa	32	8	9
Enterobacter cloacae	36	24	25
Stenotrophomonas maltophilia	45	22	23

Apart from small discrepancies between the numbers of cases identified in the CNR and those identified within the same date range as the CNR from the HAD report paediatric database, it is striking that the relative number of BSI caused by, in particular, *E. cloacae and S. maltophilia* are disproportionately far greater in the paediatric setting than would be expected. This, of itself, would have been a useful observation for Hawkey et al to have discerned and explored.

Bundle 44 vol 1, Page 9. Paediatric patient data:

The relevant sections relating to the HAD paediatric dataset commence with Section 7.2.7. It is our assumption that Hawkey et al. chose to collect data from the Yorkhill

[85] Bundle 6, Document 38, Page 1029, Table 4.3

site from 2005 as this offered 10 years of patient observation prior to the move of the RHC to the QEUH site, but this is not explicit.

Figure 17 shows a plot of the incidence of all bacteraemia per 1,000 bed days [we note that the Y axis is incorrectly labelled] over their entire study period (2005-2022). The authors comment that this shows an obvious decrease in incidence rate from early 2018 onwards. They attribute this to changes in IPC practice and to antimicrobial or other policy change.

We have two important observations about this graph. First, there was indeed a concerted effort to reduce CLABSI (Central Line Associated Bacterial Infections) in the paediatric haematology oncology unit at NHS GGC during the era of the CNR. This work derived from a Quality Improvement Group established in 2017 and there was subsequent evidence of a significant reduction in CLABSI rates from a previously very high level to that of 0.77/1000 line days at the time of the writing of the CNR report. This is an impressive achievement, and we highlighted this as good practice in our report[86]. Nevertheless, we caution that, although good central line care can reduce the incidence of Gram-negative BSI, its principal benefit is in driving down the frequency of staphylococcal line site infections and infected line site associated BSI. Without access to data on changes in the incidence of line associated BSI we cannot assess the impact this had on overall BSI rate, but it is likely to have been considerable.

Second, the X axis of Figure 17 extends for a period of 18 years whilst the era of the CNR was 4.5 years (June 2015-December 2019). Were the data in Figure 17 to be plotted only to show those years, we suggest the visual interpretation would be different. It is more likely that it would conclude a rise in incidence after the move from Yorkhill, peaking in 2017-2018, followed by a decline. It was at this time that concern about the water system reached a critical point, mitigating measures were taken and wards 2a and 2b were closed for substantial alterations.

Moreover, comparing the pattern of BSI from bacteria of environmental concern in Figure 18 [we note that the legend for the X axis is incorrect], we suggest it is even less likely that one could state with certainty that there was a reduction in incidence

[86] Bundle 6, Document 38, Page 1085, Section 9.4

rate over the era of the CNR, as this is somewhat obscured by a very obvious peak in incidence during 2018.

These changes in perspective challenges the strength of the conclusion reached by Hawkey et al. when they state *"This suggests that whatever factors are influencing bacteraemia incidence overall are also having an impact on bacteraemia attributable to environmentally relevant pathogens".*

Bundle 44 vol 1, Page 95, Section 7.2.8. Environmentally relevant BSI:

The preliminary observations described above on the overall incidence of BSI lead on to a more detailed analysis of the type of bacterial species identified and the possible clustering of BSIs in the paediatric dataset.

As we have already pointed out, Hawkey et al. chose to present and analyse data from a much wider range of years than those covered by the CNR. This creates two problems. First, as we have argued in section 7.2 of this rebuttal, comparison of incidence rates across different sites is not straightforward. Second, the inclusion of data beyond the years studied in the CNR may obscure or dilute trends of significance within a shorter period. Crucially, clusters of infection occurring over shorter time periods can clearly be obscured/missed by such analysis.

Much of section 7.2.8 is a narrative description of species identified, but it includes informative tables (11a, b, c, d, e, f; 12; 13; & 14) which include assessments of clustering probability.

In response, we have re-visited our original data to reassess the likelihood of epidemiological clustering using the criteria established by Hawkey et al. themselves (Table 3 of their report). We also compared this with the final decisions we took in the CNR about the likelihood of a relationship to the environment.

In presenting these data, we wish to reiterate that the methodology used by the CNR not only took into consideration evidence for epidemiological clustering but also the clinical and other data with which we had been supplied. This included a small amount of WGS data and we address this more specifically in relation to the extended WGS dataset provided to Hawkey et al. for their work.

Table 7.2 summarises our application of the criteria used by Hawkey et al.

Table 7.2: Clustering criteria and score coding used in Table 3 to 7

CLUSTERING CRITERIA & SCORE CODING (derived from HAD report Table 3)		
Coding against HAD definition: same hospital, **and same wards**	PROBABLE	2 or more cases 0-30 days apart
	POSSIBLE	2 or more cases 31-120 days apart
	UNLIKELY	2 or more cases 121-365 days apart
	NOT CLUSTERED	2 or more cases more than 365 days apart
Coding against HAD definition: same hospital, **but different wards**	PROBABLE	2 or more cases 0-30 days apart
	POSSIBLE	2 or more cases 31-60 days apart
	UNLIKELY	2 or more cases 61-365 days apart
	NOT CLUSTERED	2 or more cases more than 365 days apart

The following Tables 7.3 to 7.7 show data from the CNR for the following micro-organisms: *Klebsiella oxytoca*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Enterobacter cloacae*; and *Stenotrophomonas maltophilia*. The time interval (in days) was calculated between BSIs of the same bacterial species and the criteria shown in Table 7.2 were applied to determine the likelihood of a cluster with either a preceding or succeeding BSI. These data are shown alongside the final decisions of the CNR, for the same BSI, expressing the likelihood of a relationship to the environment.

Notes:

1. Clustering at room level is rarely identified and only possible where complete data were available. Bed spaces were not coded in day care areas (Ward 2b and Ward 4b). We also encountered mis-coding where clinical activity was coded to Ward 2b after it's closure. In our experience there is considerable and frequent movement of patients, carers and staff within and between inpatient and day care settings.

2. Numbers in red in the columns showing days of separation between BSIs highlight the shortest intervals and indicate a Probable cluster.

3. One patient with multiple GNE infections was included in the CNR dataset but the child's parents did not consent for the clinical analysis. Infection dates were included for clustering purposes but no clinical data was reviewed and no assessment made of

likely linkages to the environment. These BSIs are excluded from Tables 7.4, 7.6 & 7.7.

Table 7.3: Clustering analysis for BSI with Klebsiella oxytoca

Klebsiella oxytoca clustering analysis							
Location at time of culture or at last hospital attendance (Ward / Bed)	**Clustering by ward and room with another BSI of the same species within 90 days**	**Days from preceding infection**	**Days from succeeding infectione**	**Likelihood of clustering by interval from preceding infection using HAD report clustering criteria**	**Likelihood of clustering by interval from succeeding infection using HAD report clustering criteria**	**Highest likelihood of clustering - interval from either preceding or succeeding infection using HAD report clustering criteria**	**Final CNR decision (whether the BSI was likely to be related to the hospital environment)**
2a/7	no	n/a	104	n/a	POSSIBLE	POSSIBLE	POSSIBLE
2a/15	no	104	205	POSSIBLE	UNLIKELY	POSSIBLE	POSSIBLE
2a/?	n/a	205	20	UNLIKELY	PROBABLE	PROBABLE	PROBABLE
2a/9	no	20	2	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/10	no	2	324	PROBABLE	UNLIKELY	PROBABLE	PROBABLE
2a/12	no	324	72	UNLIKELY	UNLIKELY	UNLIKELY	POSSIBLE
opd	n/a	72	274	UNLIKELY	UNLIKELY	UNLIKELY	POSSIBLE
2a/?	n/a	274	n/a	UNLIKELY	n/a	UNLIKELY	POSSIBLE
	Median interval (days)	138	104				

Table 7.4: Clustering analysis for BSI with Klebsiella pneumoniae

Klebsiella pneumoniae clustering analysis							
Location at time of culture or at last hospital attendance (Ward / Bed)	Clustering by ward and room with another BSI of the same species within 90 days	Days from preceding infection	Days from succeeding infectione	Likelihood of clustering by interval from preceding infection using HAD report clustering criteria	Likelihood of clustering by interval from succeeding infection using HAD report clustering criteria	Highest likelihood of clustering - interval from either preceding or succeeding infection using HAD report clustering criteria	Final CNR decision (whether the BSI was likely to be related to the hospital environment)
2a/16	no	n/a	36	n/a	POSSIBLE	POSSIBLE	POSSIBLE
2a/?	n/a	36	32	POSSIBLE	POSSIBLE	POSSIBLE	POSSIBLE
2a/11	yes	32	53	POSSIBLE	POSSIBLE	POSSIBLE	POSSIBLE
2a/18	no	53	37	POSSIBLE	POSSIBLE	POSSIBLE	PROBABLE
2a/11	yes	37	158	POSSIBLE	UNLIKELY	POSSIBLE	POSSIBLE
2b/-	n/a	158	92	UNLIKELY	UNLIKELY	UNLIKELY	POSSIBLE
2a/26	no	92	18	UNLIKELY	PROBABLE	PROBABLE	UNLIKELY
2a/16	no	18	57	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
2a/3	no	57	2	POSSIBLE	PROBABLE	PROBABLE	POSSIBLE
2a/?	n/a	2	31	PROBABLE	POSSIBLE	PROBABLE	n/a - excluded
2a/18	no	31	12	POSSIBLE	PROBABLE	PROBABLE	POSSIBLE
2a/23	no	12	36	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
2a/?	n/a	36	32	POSSIBLE	POSSIBLE	POSSIBLE	UNRELATED
2a/?	n/a	32	35	POSSIBLE	POSSIBLE	POSSIBLE	UNRELATED
2a/10	no	35	16	POSSIBLE	PROBABLE	PROBABLE	PROBABLE
cdu	n/a	16	11	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/22	no	11	48	PROBABLE	POSSIBLE	PROBABLE	PROBABLE
2a/1	no	48	147	POSSIBLE	UNLIKELY	POSSIBLE	PROBABLE
6a/?	n/a	147	86	UNLIKELY	UNLIKELY	UNLIKELY	n/a - excluded
6a/25	no	86	60	UNLIKELY	POSSIBLE	POSSIBLE	POSSIBLE
6a/?	n/a	60	n/a	POSSIBLE	n/a	POSSIBLE	POSSIBLE
	Median interval (days)	36	36				

Table 7.5: Clustering analysis for BSI with Pseudomonas pneumoniae

Pseudomonas aeruginosa clustering analysis							
Location at time of culture or at last hospital attendance (Ward / Bed)	Clustering by ward and room with another BSI of the same species within 90 days	Days from preceding infection	Days from succeeding infectione	Likelihood of clustering by interval from preceding infection using HAD report clustering criteria	Likelihood of clustering by interval from succeeding infection using HAD report clustering criteria	Highest likelihood of clustering - interval from either preceding or succeeding infection using HAD report clustering criteria	Final CNR decision (whether the BSI was likely to be related to the hospital environment)
2a/11	no	n/a	155	n/a	UNLIKELY	POSSIBLE	POSSIBLE
2a/2	no	155	131	UNLIKELY	UNLIKELY	UNLIKELY	POSSIBLE
2a/25	no	131	22	UNLIKELY	PROBABLE	PROBABLE	POSSIBLE
2a/?	n/a	22	31	PROBABLE	POSSIBLE	PROBABLE	PROBABLE
2a/?	n/a	31	20	POSSIBLE	PROBABLE	PROBABLE	PROBABLE
2a/12	no	20	28	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2a/25	no	28	290	PROBABLE	UNLIKELY	PROBABLE	PROBABLE
6a/?	n/a	290	5	UNLIKELY	PROBABLE	PROBABLE	POSSIBLE
6a/?	n/a	5	n/a	PROBABLE	n/a	PROBABLE	POSSIBLE
	Median interval (days)	29	29				

Table 7.6: Clustering analysis for BSI with Enterobacter cloacae

Enterobacter cloacae clustering analysis							
Location at time of culture or at last hospital attendance (Ward / Bed)	Clustering by ward and room with another BSI of the same species within 90 days	Days from preceding infection	Days from succeeding infectione	Likelihood of clustering by interval from preceding infection using HAD report clustering criteria	Likelihood of clustering by interval from succeeding infection using HAD report clustering criteria	Highest likelihood of clustering - interval from either preceding or succeeding infection using HAD report clustering criteria	Final CNR decision (whether the BSI was likely to be related to the hospital environment)
2a/7	no	n/a	32	n/a	POSSIBLE	POSSIBLE	POSSIBLE
2a/20	no	32	198	POSSIBLE	UNLIKELY	POSSIBLE	POSSIBLE
2a/16	yes	198	21	UNLIKELY	PROBABLE	PROBABLE	POSSIBLE
2a/18	no	21	14	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/16	yes	14	70	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
2a/16	yes	70	19	POSSIBLE	PROBABLE	PROBABLE	POSSIBLE
cdu	n/a	19	138	PROBABLE	UNLIKELY	PROBABLE	NOT CLUSTERED
2a/3	no	138	0	UNLIKELY	PROBABLE	PROBABLE	PROBABLE
2a/26	no	0	7	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/12	no	7	9	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2a/?	n/a	9	13	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2a/10	no	13	15	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/23	no	15	28	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a/11	no	28	42	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
2a/16	no	42	51	POSSIBLE	POSSIBLE	POSSIBLE	POSSIBLE
6a/?	n/a	51	97	POSSIBLE	UNLIKELY	POSSIBLE	n/a - excluded
opd	n/a	97	37	UNLIKELY	POSSIBLE	POSSIBLE	POSSIBLE
6a/24	no	37	21	POSSIBLE	PROBABLE	PROBABLE	POSSIBLE
6a/23	no	21	76	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
6a/11	no	76	14	POSSIBLE	PROBABLE	PROBABLE	POSSIBLE
6a/2	no	14	9	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
6a/?	n/a	9	37	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
6a/?	n/a	37	9	POSSIBLE	PROBABLE	PROBABLE	PROBABLE
opd	n/a	9	n/a	PROBABLE	n/a	PROBABLE	POSSIBLE
	Median interval (days)	21	21				

Table 7.7: Clustering analysis for BSI with Stenotrophomonas maltophilia

Stenotrophomonas maltophilia clustering analysis							
Location at time of culture or at last hospital attendance (Ward / Bed)	Clustering by ward and room with another BSI of the same species within 90 days	Days from preceding infection	Days from succeeding infectione	Likelihood of clustering by interval from preceding infection using HAD report clustering criteria	Likelihood of clustering by interval from succeeding infection using HAD report clustering criteria	Highest likelihood of clustering - interval from either preceding or succeeding infection using HAD report clustering criteria	Final CNR decision (whether the BSI was likely to be related to the hospital environment)
2a / ?	n/a	n/a	316	n/a	UNLIKELY	UNLIKELY	POSSIBLE
2a / 25	no	316	21	UNLIKELY	PROBABLE	PROBABLE	PROBABLE
2a / 26	no	21	37	PROBABLE	POSSIBLE	PROBABLE	POSSIBLE
2b / -	n/a	37	24	POSSIBLE	PROBABLE	PROBABLE	n/a (excluded)
2a / 15	no	24	10	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2a / 18	no	10	63	PROBABLE	POSSIBLE	PROBABLE	PROBABLE
2a / 16	no	63	168	POSSIBLE	UNLIKELY	POSSIBLE	POSSIBLE
2a / 11	no	168	4	UNLIKELY	PROBABLE	PROBABLE	PROBABLE
2b / -	n/a	4	1	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a / 15	no	1	18	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2b / -	n/a	18	31	PROBABLE	POSSIBLE	PROBABLE	n/a (excluded)
2a / 1	yes	31	8	POSSIBLE	PROBABLE	PROBABLE	PROBABLE
2b / -	no	8	4	PROBABLE	PROBABLE	PROBABLE	POSSIBLE
2a / 1	yes	4	17	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2a / 25	no	17	64	PROBABLE	POSSIBLE	PROBABLE	PROBABLE
2a / 15	no	64	32	POSSIBLE	POSSIBLE	POSSIBLE	PROBABLE
2a / 23	no	32	3	POSSIBLE	PROBABLE	PROBABLE	PROBABLE
2a / ?	n/a	3	5	PROBABLE	PROBABLE	PROBABLE	PROBABLE
2b / -	n/a	5	211	PROBABLE	UNLIKELY	PROBABLE	PROBABLE
Miscoded to 2b	n/a	211	37	UNLIKELY	POSSIBLE	POSSIBLE	POSSIBLE
opd	n/a	37	75	POSSIBLE	UNLIKELY	POSSIBLE	PROBABLE
6a / 7	no	75	56	UNLIKELY	POSSIBLE	POSSIBLE	POSSIBLE
Miscoded to 2b	n/a	56	n/a	POSSIBLE	n/a	POSSIBLE	POSSIBLE
	Median interval (days)	27	27				

We then reviewed the clustering status for these five groups of micro-organisms, comparing results from the HAD data (taken from their Tables 11 c, d & e, 12, 13 & 14 but extracting data for BSI *only* within the era of the CNR) with our own data from the CNR, coded according to the HAD criteria, and with the final decisions taken by the CNR for the same BSI episodes (Table 7.8).

In considering the data in Table 7.8, it should be emphasised that a comparison of the data for epidemiological clustering with that for the final decisions reached by the CNR is not a directly equivalent relationship. The final decisions provided, and discussed in the CNR Overview Report reflected our integrated view of the likelihood that a BSI was linked to the hospital environment; these decisions were not simply a statement of the likelihood of clustering based on time and space.

Table 7.8: Clustering probabilities determined as Possible or Probable comparing CNR data with that from the HAD database and with the final CNR decision for the five principal environmentally relevant micro-organisms over the era of the CNR (June 2015-December 2019)

	CNR data scored by HAD criteria				HAD data				CNR final decision			
	Total BSI	Possibly cluster (&)	Probably cluster (&)	Total Probably + Possibly (%)	Total BSI	Possibly cluster (%)	Probably cluster (%)	Total Probably + Possibly (%)	Total BSI	Possibly related (%)	Probably related (%)	Total Probably + Possibly (%)
K. oxytoca	8	2 (25%)	3 (37%)	5 (62%)	8	2 (25%)	4 (50%)	6 (75%)	8	5 (62%)	3 (37%)	8 (100%)
K. pneumoniae	21	10 (48%)	9 (43%)	19 (90%)	22	9 (41%)	11 (50%)	20 (91%)	19 (2 excluded from assessment)	11 (58%)	5 (26%)	16 (84%)
P. aeruginosa	9	1 (11%)	7 (78%)	8 (89%)	8	4 (50%)	3 (37%)	7 (87%)	9	6 (67%)	3 (33%)	9 (100%)
E. cloacae	24	5 (21%)	19 (79%)	24 (100%)	24	12 (50%)	12 (50%)	24 (100%)	23 (1 excluded from assessment)	16 (69%)	6 (26%)	22 (96%)
S. maltophilia	23	6 (26%)	16 (69%)	22 (96%)	22	15 (68%)	6 (27%)	21 (95%)	21 (2 excluded from assessment)	9 (43%)	12 (57%)	21 (100%)

Interpreting these data is not straightforward and variation is likely attributable to the numbers in some individual categories being very small.

There are some minor discrepancies between the total numbers in the CNR and HAD data groups and some of the BSI were excluded from assessment in the CNR final decision process (as also shown in Tables 7.4, 7.6 & 7.7). However, some general observations can be offered.

Overall, when considering the combined total of those Possibly and Probably clustered, there is little distinction between the HAD data and the CNR data scored by the HAD criteria, with substantial alignment of both with the final decisions from the CNR. In particular, the data for *E. cloacae* and *S. maltophilia* are all very close. These are the two largest groups. The total (Possibly + Probably) for *K. pneumonia* varies to a greater extent, but differences are still small and the lesser degree of alignment with the final outcomes from the CNR may reflect a tendency for us to have been more conservative in assigning the probability of a link with the environment to BSI with this organism.

There is some inconsistency in the assignation of 'Probably' clustered when comparing the HAD data and the CNR data scored by the HAD criteria. This is particularly obvious for *S. maltophilia* (27% vs 69%) and to a lesser extent for *E. cloacae* (50% vs 79%). This is difficult to explain without more specific data from the HAD database to calculate time separation in days. This is, of course, unless Hawkey et al moderated the data shown in their Tables 11 c, d & e, 12, 13 & 14 with the results of the available WGS data (shown in their Tables 15a, b and c) but this is not made clear in their text.

There is a tendency for the final CNR decisions to be rated Probably (linked to the environment) less frequently than the CNR data, scored by the HAD. Visually, this can be seen more obviously in Tables 7.3 to 7.7 where the colour coding assists in highlighting the differences between the penultimate column (highest likelihood of clustering) and the final column (final CNR decision). This would be in line with a moderating effect of other factors taken into consideration when we undertook our final assessment, in which we considered each case in detail, reviewing all available risk factors for each individual infection and patient.

Overall, however, the finding of 100% Probable + Possible environmental clustering for *E. cloacae;* ≥95% for *S. maltophilia;* ≥90% for *K. pneumoniae;* ≥87% for *P. aeruginosa* ; and ≥62% for *K. oxytoca,* in data looking at the same infections in the CNR and HAD datasets, together with a very close alignment to the final decisions made by the CNR, is hard to dismiss as circumstantial. This strongly supports the potential for an environmental link in the origin of at least some of these BSI.

It is also worth making a specific comment about Table 16 in the HAD report. This shows a statistical analysis of data for epidemiological clustering between BSI at Yorkhill and at RHC/QEUH. We have already indicated our general caution about comparisons between different hospitals, especially so over a period of 18 years when many other influencing factors will have changed, not least the mitigations put in place to the water supply by NHS GGC at the QEUH site from late 2018. We believe these influences challenge the validity of the statistical comparison being made.

Furthermore, looking carefully at Figure 19 [not specifically referenced in the text by Hawkey et al. and in which the X axis is incorrectly labelled] and Figure 20, showing an analysis of BSI incidence per 1,000 bed days across the 18 years of HAD data, it is difficult to be convinced that there is any diminution in the incidence of environmental BSI before the end of 2019, when the CNR ended. The most obvious feature of the section of the chart relating to the CNR era is what appears to be a clear increase in incidence from the time that the Children's Hospital relocated until at least the latter part of 2017.

In assessing the trend line fitted to the data shown in Figure 22, we visually compared the patterns of spikes and troughs with those shown in Figure 19, both apparently using the same data for environmentally relevant microorganisms. There appear to be some differences, the most obvious being that the highest peaks in incidence shown in 2014, 2018 and 2019 are all higher in Figure 22 than in Figure 19. This merits review by Hawkey et al but, even with these differences, the trend line fitted to the data in Figure 22, purporting to show a declining incidence of environmental BSI from mid 2015, is almost certainly affected by the inclusion of data after the end of 2019.

We therefore challenge the conclusions drawn from Figure 22 and, even with any needed corrections to the presentation of the data, suggest that a similar analysis for the specific period of the CNR would not be interpreted in the same way.

We are aware that Hawkey et al. had the opportunity to review more WGS data than was available to us and our comments on this aspect of their analyses (their Tables 15a, b & c) are further elaborated in Section 7.3.

In conclusion, we disagree with the conclusion made by Hawkey et al. that *"There have been large reductions in incident BSI overall and by microorganisms [with] environmental attribution at QEUH overtime, suggesting overall increasing effective prevention strategies*" in so far as this might apply to the period of the CNR.

We remain firm in our belief that the conclusions of the CNR Overview Report stand.

7.3 What does the available sequencing data show?

Bundle 44 vol 1, Page 119

Hawkey et al. refer to reports by Professor Tom Evans on multiple occasions in this chapter of their report but do not formally cite these and do not provide source data for conclusions made by Prof. Evans. They repeatedly draw from the *"detailed reports by Professor Tom Evans"* to assert that the WGS investigations undertaken at NHS GGC do not support environmental sources for the BSIs investigated in the CNR.

For example, they write

"The detailed reports by Professor Tom Evans do[es] not identify any matching isolates between the environment and patients for Stenotrophomonas. In the case of Enterobacter spp. all 29 blood culture isolates were typed together with 60 environmental isolates. The only paired indistinguishable isolates in the clinical collection were from isolates obtained at different times from the same patient. This confirms the sensitivity and specificity of WGS SNP typing. No patients shared indistinguishable strains which is what would be expected if a common environmental source was potentially responsible."

We identified the Evans' reports in evidence given to the Glasgow II hearings in June 2023. They consist of four documents - three reports on Cupriavidus[87], Enterobacter[88] and Stenotrophomonas[89] respectively, and a Technical Appendix[90] to these reports. The contents describe what was found when WGS was carried out on a range of bacteria obtained from humans and the environment.

As we were concerned to understand more fully the importance of the reports, we have reviewed each to understand what was done, and notably what was not included, so as to be able to comment on the robustness of these investigations. Our critique follows for each microorganism in turn.

Cupriavidus

We shall not comment in detail on Evans' report on Cupriavidus as there were only 2 BSIs with this microorganism in the CNR. We found the report difficult to unravel and we also reviewed a parallel report from Leanord and Brown[91]. We believe there are important caveats that suggest a lack of robustness of the WGS investigation and its conclusions. In summary, we note specifically that:

- It is not clear from the report how many isolates in total were examined in the WGS investigation, (sections 3.1-3.3, page 225 of his report).
- The report does not set out whether isolates from children were included.
- While the report by Leonard & Brown does specify that isolates from children were included in the WGS investigation, when showing the relationships of these isolates (e.g. page 1208, Figure 6 of their report) 'human' isolates are not distinguishable as to whether they were from children or adults.
- The numbers in the separate reports on *Cupriavidus* spp. by Evans and by Leonard & Brown do not tally. For example, Evans states (page 225 of his report) *"8 isolates were from human blood cultures. Of the 8 human samples, there were duplicate*

[87] Bundle 8, Document 44, Pages 223-229

[88] Bundle 8, Document 45, Pages 230-238

[89] Bundle 8, Document 46, Pages 239-246

[90] Bundle 8, Document 47, Pages 247-248

[91] Bundle 6, Document 40, Pages 1195-1235

sets of samples from 2 patients on the same occasion. Accounting for these duplicates gives a total of 6 human isolates." Leonard & Brown (page 1207 of their report) indicate that *"A total of 9 human clinical isolates were also sequenced. These included two isolates from the original human case described by Inkster et al. (2021), and 7 other human isolates from different hospitals in NHS GG&C, isolated between 2016 and 2021"*

- It is clear that no environmental *Cupriavidus* spp. isolates prior to March 2018 were included in the WGS investigation.
- One of the two *Cupriavidus* spp. BSIs in the CNR occurred in September 2017, the other in January 2018. Thus, both BSIs occurred before the dates when any of environmental isolates included in the WGS investigation were obtained.

We conclude therefore that the WGS investigation of *Cupriavidus* spp. lacks sufficient robustness to exclude the possibility of matches between isolates from the hospital environment and the BSIs found in the CNR.

Enterobacter

In his report on Enterobacter infection, Evans makes statements that show the lack of robustness of the WGS investigation and its conclusions. For example, on Page 223 of his report he says:

"As outlined in the separate report on water sampling, between 2015 and 2020 10,311 water samples were tested by the Environmental laboratory (not counting those tested only for Legionella). 6 of these samples contained a species of Enterobacter and they were not analysed further. Enterobacter spp. are not one of the alert organisms specified by the National Infection Prevention and Control Manual Appendix 13."

This statement confirms that none of the isolates from water samples during 2015-2020 that were identified as showing *Enterobacter* spp. were included in the WGS investigation. Furthermore, it is highly probable that there were other isolates of *Enterobacter* spp. recovered from water samples during this period but, as pointed out by Evans, these are not one of the alert organisms specified by the National Infection Prevention and Control Manual Appendix 13, and the laboratory would typically not routinely aim to identify *Enterobacter* spp. in water samples.

On the same page, Evans also reveals that:

"In addition to water testing, some environmental sampling was carried out in the QEUH and RHC, including swabs of sinks, drains, and other surfaces. This was not performed in a standardised or structured fashion. Samples were processed in the routine microbiological laboratories and in accordance with standard practice were not necessarily subsequently stored."

We conclude therefore that many of the *Enterobacter* spp. in samples from sinks, drains, and other surfaces would also not have been available for the WGS investigation. Indeed, from the table on page 233-4 of his report, we can see that there was a total of 59 *Enterobacter* spp. isolates. Again, depending on how and why the various samples from sinks, drains, and other surfaces were processed, and the bacteria grown were/were not identified, it is highly probable that the actual total number of *Enterobacter* spp. isolates from these environmental sites was much higher than this.

These findings about environmental sampling are entirely consistent with the observations we made in the Case Note Review Oversight Report in which we stated:

"There did not appear to be a systematic sampling process in place or a consistent water system related response to clusters of infections caused by (often unusual/uncommon) GNE bacteria" [92] and *"We conclude these data do not support a systematic approach to water sampling (i.e. frequent, repeated sample collection) certainly for wards 2A and 2B and in the context of concern regarding possible environmental sources of bacteraemias."* [93]

Thus, the sporadic nature of sampling means that any such isolates are, at best, a narrow snapshot of the true prevalence of *Enterobacter* spp. in the hospital environment.

Using the numbers in Evans' report, even if we were to assume that the total of *Enterobacter* spp. isolates from water samples (n=6) and sinks, drains, and other surfaces (n=59) was therefore 65, the report states on page 234 that: *"6 isolates of*

[92] Bundle 6, Document 38, Page 1042

[93] Bundle 6, Document 38, Page 1042, Example 5.2

Enterobacter from environmental samples were serendipitously retained and whole genome sequences obtained." It seems, therefore, that approximately 90% of the *Enterobacter* spp. isolates recovered from the hospital environment were not included in the WGS investigation of these bacteria.

Furthermore, Evans also states on page 234 of his report that: *"Over this period, 29 patient samples were identified from blood cultures from patients within the QEUH/RHC. These were subjected to whole genome analysis. The species identified were E. hormaechei (14), E. asburiae (5), E. roggenkampii (4), E. kobei (2), E. chengduensis (1), E. genomosp. O (2), and E. ludwigii (1)."*

Almost all of the *Enterobacter* spp. in the CNR (n=27) were *E* cloacae, which is not described in Evans list, and only 2 were *E. hormaechei*, one from 2017 and one from 2019. At best, we conclude only these 2 patients could have been included in this WGS investigation.

It is inconceivable, therefore, that Evans' statement (on page 236 of his report) *"On that basis, I conclude on balance of probabilities that the human infections from these Enterobacter species were not acquired from environmental sources within the QEUH/RHC."* could apply to the CNR.

We disagree with his conclusion and suggest that the WGS investigation falls substantially short of being an analysis robust enough to define the true relatedness of *Enterobacter* spp. recovered from patients with BSIs and/or from the hospital environment.

<u>Stenotrophomonas</u>

Finally, and similarly, in his report on Stenotrophomonas infection, Evans makes statements that illustrate the lack of robustness of the WGS investigation and its conclusions.

For example, Page 242 of his report states: *"Out of this total, 76 isolates of S. maltophilia were found. The number of isolates of this organism versus the number of water samples in each year were: 2015, 0/127 samples; 2016, 1/286 samples; 2017, 0/383 samples; 2018, 26/2490 samples; 2019, 8/2147 samples; and 2020, 41/4878 samples. Of these 10,311 water samples, 6,183 looked specifically for Gram negative organisms including S. maltophilia, of which 71 (1.1%) were positive for this species.*

40 isolates of S. maltophilia were isolated from other environmental sources such as drains. Although as outlined, different locations and extents of testing were carried out, there is no reason to suppose that this level of the presence of S. maltophilia in water samples is not representative. Over this time period, there were 23 isolates of clinical relevance isolated from blood cultures."

So, we see that, despite Evans assertion that *"there is no reason to suppose that this level of the presence of S. maltophilia in water samples is not representative"*, approximately 40% of the water samples referred to were not specifically examined for the presence of Gram-negative bacteria such as *S. maltophilia*. We disagree with his statement, as it is clear that the sampling of water, sinks, drains and other sites was sporadic/not systematic, and the degree of scrutiny to which these samples were subjected to look specifically for *Stenotrophomonas* spp. will have been variable.

Next, Evans describes (on page 242 of his report) that *"We were able to carry out a detailed analysis of the genomes of 84 these organisms and related them to available epidemiological data, notably time and place of isolation. 79 of these sequences were from samples at the QEUH campus (including the Royal Children's Hospital). Of these, 23 of the isolates were from blood cultures, and 56 from isolates of water samples and environmental samples taken from drains from a variety of outlet points (which represents 48% (56/116) of the total water/environmental S. maltophilia isolated at the QEUH campus). The human samples were collected between 2015-2020, the water and environmental samples from 2018-2020. For the purposes of this report, the term "environmental" is used below to include isolates obtained from the water supply or from other environmental sources."*

From this statement we see that:

- only some of the (sporadically sought/obtained) *Stenotrophomonas* spp. isolates from environmental samples were included in the investigation;
- the environmental isolates in the investigation did not cover the period 2015-early 2018; approximately one third of the *Stenotrophomonas* spp. BSIs in the CNR occurred before the period when environmental isolates were included in the WGS investigation.

- there is a lack of detail provided on precisely how many of the blood culture isolates from children in the CNR were included; 23 *S. maltophilia* BSIs were reported in our case series. We suggest again, from the numbers provided and the whole hospital approach to the inclusion of blood culture isolates here, that it is likely, at best, that only a few such isolates from the CNR will have been included in the WGS investigation.

In an attempt to obtain greater clarity, we also reviewed the report provided by Leanord & Brown. Specifically, we looked at *'Figure 15: Maximum Likelihood Tree of Stenotrophomonas maltophilia isolates generated from SNP analysis'*. On face value, these appear to be the results of the WGS analysis discussed by Evans in his report – both cover 84 bacteria – here, 25 are from humans, whereas Evans refers to 23 from blood cultures. No dates for the human isolates are provided in Figure 15, which hampers our understanding of its relevance to the CNR. It is possible that some were from other children at the RHC but not in the CNR. We also see that a total of 11 hospital ward drain isolates were included; 8/11 were from the children's wards relevant to the CNR. However, none of these were obtained in 2018, which was the peak year for *S. maltophilia* BSIs in the CNR (n=12). This further challenges the value of the WGS investigation in relation to the conclusions of our work in the CNR.

Evans concludes (Page 245 of his report) that *"Overall, therefore, in my opinion, on balance of probability, none of the clinical cases of infections with S. maltophilia originated from water systems within the QEUH/RHC."*

We do not agree with this assertion and alternatively conclude the WGS investigation of *Stenotrophomonas* spp. isolates has important shortcomings that mean it lacks sufficient robustness to exclude the possibility of a match between isolates from the hospital environment with some of the BSIs in the CNR.

Conclusions

Based on our in-depth analysis of how these investigations were conducted, we do not agree with assertions made by Hawkey et al. that the WGS data do not support environmental sources for BSIs, including those included in the CNR. Instead, we assert that these investigations were flawed and cannot be relied on to refute matches

between isolates present in the hospital environment and those causing infections patients.

Searching for matches between bacteria in the hospital environment with those causing infections in patients is fraught with challenges, analogous to looking for a needle in a haystack. Noting the protracted period represented by our case series, to increase the odds of finding evidence to link source(s) in the environment to patient infections requires systematic searching, i.e. both in sampling and in the laboratory investigations of those samples.

Systematic sampling of the environment did not happen, and isolates recovered between 2015 and early 2018 were not even included in the WGS investigations. Similarly, and surprisingly, some bacteria causing BSIs in children were not included in the WGS investigations, yet it is routine practice to store blood culture isolates for years. Given the heightened concerns emerging about infections in children on the haematology oncology unit from 2015-2019, we do not understand why the bacteria causing the BSIs were not stored, and as a result important gaps were created in the WGS investigations subsequently undertaken.

To conclude, in their own commentary on the Evans' reports, Hawkey et al. acknowledge their recognition that not all clinical and environmental isolates were subjected to WGS strain typing but present a view that disagrees with our position that inconsistent and incomplete inclusion of both patient and environmental isolates in these studies is a significant shortcoming.

Interestingly, however, after stating *"We do not believe in the face of the published experience and the levels of infection with marker organisms seen at QEUH that the environment was a significant source of infection"* they go on to insert an important concession *"We conclude that whilst some infections may have been acquired from the hospital environment,* [our underlining] *acquisition of colonisation in other settings can be considered as the probable cause for many of the infections encountered."*

Perhaps, therefore, the disagreement rests not on the principle but on the quantum – how many of the infections in the CNR were related to the environment?

We are clear that the WGS data available do not provide the answer, and that it remains difficult to dismiss the very marked epidemiological clustering described in Section 7.2, with its close relationship to the final decisions reported by the CNR.

9. CHAPTER 8: VENTILATION – ANALYSIS OF INFECTION RATES AND DATA FROM QEUH & RHC

As stated in Chapter 6, we did not evaluate the involvement of the hospital ventilation systems or airborne transmission of pathogens in the CNR.

We have no comments to make on this chapter

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ORIGINAL ARTICLE

Antibiotic Resistant Bacteria Found in Municipal Drinking Water

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Abstract Multidrug resistant bacteria in water supply systems have been emerging as a growing public health concern. Many factors affect the source and fate of these bacteria. However, conditions in the plumbing systems may contribute to the dispersion of resistance genes among bacterial populations. Through the process of lateral gene transfer, resistance genetic material can be exchanged between species in the microbial population, intensifying the problem of resistance genes. The main aim of this study was to investigate the diversity of microorganisms in tap water in Glasgow, Scotland, and the occurrence of certain antibiotic resistance genes and gene-transfer mechanisms. Results show that antibiotic resistant bacteria exist at the consumers' end of the distribution system, some of which also contain integrase genes, which can aid in the dispersion of resistance genes. Presence of such microorganisms indicates that further investigations should be taken to assess the risks to public health.

Keywords Antimicrobial resistance · Water distribution system · *intI* · *qac* · *sul*

1 Introduction

Supply of safe drinking water to the global population remains one of the major concerns for public health. Many factors impact this provision, including increasing world population, limited freshwater resources, and pollution. Contemporary issues affecting supply include

Highlights • Plumbing systems contain antibiotic resistance bacteria
• *sul*1 and *sul*2 genes are found in bacteria which show resistance against sulfonamides drugs
• The presence of integrons suggests that resistance traits can be transferred to other bacteria

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contamination with chemical compounds, e.g. pharmaceutical and personal care products, and biological agents, which can contribute to increased antimicrobial resistance in bacteria (Batt et al. 2006; Larsson et al. 2007; Fick et al. 2009; Pruden et al. 2013; Hartmann et al. 2014). The use of these chemicals has been increased to a point that propagation of antimicrobial resistance has become unavoidable, and it is now considered an emerging contaminant of concern in the environment (Pruden et al. 2006). In urban areas, water treatment plants utilise multiple technologies to remove many pollutants (Xu et al. 2007), while integrated constructed wetlands are used in rural areas for removal of pollutants and resistance genes (Chen et al. 2015a), however, these systems are never entirely effective (Xu et al. 2007).

Various processes in the treatment plants (Armstrong et al. 1981), and the physical and chemical nature of the long distribution systems, facilitate the enrichment of bacteria and their genes. Attachment to particulates, capsule formation and increased tolerance of bacteria to chemical disinfectants help in the survival and spread of resistant organisms in water supply systems (Ridgway and Olson 1982; LeChevallier et al. 1984; LeChevallier et al. 1988; Bridier et al. 2011, Wingender and Flemming 2011).

The presence of antimicrobial resistant bacteria and their genes in water bodies is not limited to under-developed countries (Khan et al. 2013; Ahammad et al. 2014); they have been found in developed nations including Australia, Germany and the United Kingdom (Stoll et al. 2012). Many enteric bacteria with multiple drug resistance (MDR), e.g., *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella* and *Shigella* species, have been found in drinking and recreational water resources (Kumar et al. 2013). Previously, both Gram-positive and Gram-negative bacteria, including *Staphylococcus*, *Alcaligenes*, and *Acinetobacter* species, have been reported in drinking-water distribution systems (Armstrong et al. 1981). Antibiotic resistant *Pseudomonas* species have also been isolated from drinking water (Vaz-Moreira et al. 2012; Ribeiro et al. 2014). The presence of MDR in the clinical environment makes the treatment of diseases difficult (Ashbolt et al. 2013); their presence in water bodies, and increased exposure risk, may necessitate enhanced water treatment and increase expense.

Bacteria isolated from drinking water may not only contain antibiotic resistance, they may also express resistance to commonly used disinfectants, including chlorine and monochloramine (Ridgway and Olson 1982; Chiao et al. 2014, Khan et al. 2016). These disinfectants are indispensable in water treatment plants to eradicate bacteria and minimise the growth of bacteria in the distribution system. Resistance develops upon exposure to sub-inhibitory concentrations over time, and a number of bacterial species with monochloramine resistance have been reported in drinking water, including: *Coxiella*, *Desulfuromonas*, *Desulfomonile*, *Escherichia*, *Geobacter*, *Legionella*, *Mycobacterium*, and *Sphingomonas* species (Chiao et al. 2014).

Besides physical and chemical processes, genetic factors contribute to the dispersion of resistance genes in drinking water distribution systems. Horizontal gene transfer (HGT) mechanisms move resistance genes from resistant to susceptible populations on mobile genetic elements like integrons (Mazel 2006; Gillings et al. 2008; Stalder et al. 2012; Jechalke et al. 2013). HGT spreads genetic elements and their genes among dissimilar groups of bacteria (Gaze et al. 2011), rapidly transferring antibiotic and disinfectant resistance genes (Boucher et al. 2007; Stokes and Gillings 2011; Mokracka et al. 2012). Integrons have a recombination system, which captures genes and serves as a reservoir of resistance genes (Stokes and Hall 1989; Demarre et al.

2007; Xu et al. 2011). The transfer of multiple genes on integrons via HGT intensifies the problem of resistance in bacterial communities, as it can link resistance traits between environmental bacteria and human pathogens in drinking water systems (Ribeiro et al. 2014).

Among multidrug resistance bacteria, class 1 integrons are most common (Shearer and Summers 2009), and they are related to the presence of resistance to quaternary ammonium compounds (QACs), *qac*EΔ1 genes and sulphonamide resistance *sul*1 determinants (Kucken et al. 2000). Almost half of the class 1 integrons contain a *qac* resistance genes cassette (Gillings et al. 2009), and the prevalence of class 1 integrons in bacteria having previous exposure to QACs is greater than those without previous exposure (Gaze et al. 2005). Moreover, bacteria that possess or acquired plasmids, transposons or integrons, have greater QACs resistance than those which do not have any of these genetic elements (Bjorland et al. 2003). Bacteria often show cross resistance for QACs and antibiotics (Morente et al. 2013), including the use of efflux-pump systems to generate resistance to unrelated broad-range antimicrobials (Buffet-Bataillon et al. 2012). Enzyme based mechanisms of antibiotic resistance is also found in bacteria. In the presence of antibiotics, bacteria may acquire genes encoding enzymes which can destroy the antibiotic before reaching the target, resulting in the development of resistant bacteria (Tenover 2006).

This study aimed to determine the prevalence of bacteria and resistance genes in drinking water. Assays included genes responsible for resistance to a wide variety of quaternary ammonium compounds (*qac* genes) and sulphonamide antibiotics (*sul*1 and *sul*2 genes), as these genes commonly co-occur. Mobile genetic integrons were detected via their integrase gene *intI*1 and *intI*2. The co-occurrence of *sul* and *qac* genes in bacteria from the water distribution system was also examined.

2 Materials and Methods

2.1 Sampling and Processing

Tap-water samples were collected from residences in Glasgow in sterile screw capped bottles, and were processed within 2–4 h of collection. One hundred millilitres water samples were vacuum-filtered through 0.22 μm pore size cellulose nitrate gridded membranes (Millipore, UK), which were then aseptically placed on Standard Plate Count Agar APHA (Oxoid, UK). The plates were incubated for 48 h at 37 ± 2 °C. Selected bacterial isolates from the resultant growth were streaked on Nutrient Agar (Oxoid, UK) plates to isolate colonies; 4–5 colonies of each strain were preserved in glycerol using a Bacterial Beads Preservation Kit (Cryo vials TS/71-MX, Technical Service Consultants Ltd., UK) and stored at −80 °C.

2.2 DNA Extraction and PCR Amplification

DNA of the bacterial isolates were thermally extracted by mixing strains with 100 μL of PBS (pH 7.4) and undergoing a series of freeze thaw cycles at −80 and 70 °C with continuous shaking between each cycle. The contents were centrifuged at 10,000 rpm for 5 min at the end of the fourth thermal cycle, and DNA from the supernatant was stored at −80 °C.

PCR reactions were performed with a Bio-Rad iQ5 Real-Time PCR Detection System for the presence of 16S-rRNA, *intI*1, *intI*2, *sul*1, *sul*2 and *qac* genes using previously described primers (Table 1; Pei et al. 2006; Luo et al. 2010; Caporaso et al. 2011; Jechalke et al. 2013). Twenty microliter PCR reactions consisted of 10 μL of MegaMix-Blue-PCR Mastermix with dye (Microzone Limited, UK), 1 μL of each primer (500 ηM final concentration; Sigma-Aldrich Life Science, UK), 6 μL of nuclease-free water, and 3 μL of DNA sample. Each PCR run consisted of initial denaturation at 95 °C for 3 min; this was followed by 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at annealing temperature (Table 1), extension at 72 °C for 30 s, and then a final extension at 72 °C for 10 min. PCR products were further verified with 2 % agarose gel in 1× Tris Acetate-EDTA buffer; the size of amplified products was determined against a 50-bp incrementing DNA ladder (Fisher BioReagent, UK).

2.3 DNA Purification and Sequencing

PCR products from 16S-rRNA gene amplification were purified using the QIAquick PCR Purification Kit (Qiagen, UK) according to the manufacturer's instructions. Purified and cleaned amplicon concentration was determined by the EPOCH™ Microplate spectrophotometer system (BioTek, UK). Amplicon was mixed with 5 μM forward primer solution used in PCR in a 1:1 ratio in a total volume of 10 μL and sent to LightRun Sequencing Service (GACT Biotech Ltd, London, UK) for sequencing. Bacteria were identified by comparing the sequences using the BLAST program through the National Centre for Biotechnology Information (NCBI).

Table 1 PCR Primers for targeting different genes

Primer	Sequence (5′–3′)	PCR annealing temperature (°C)	Amplicon size	Reference
V4-16S-515F	TGTGCCAGCMGCCGCGGTAA	50	312	(Caporaso et al. 2011)
V4-16S-806R	GGCTACHVGGGTWTCTAAT			
qacEaIIF	CGCATTTTATTTTCTTTCTCTGGTT	60	Not detected	(Jechalke et al. 2013)
qacEaIIR	CCCGACCAGACTGCATAAGC			
int1-F	GGCTTCGTGATGCCTGCTT	57	148	(Luo et al. 2010; Chen et al. 2015a)
int1-R	CATTCCTGGCCGTGGTTCT			
int2-F	GTTATTTTATTGCTGGGATTAGGC	56	166	(Luo et al. 2010; Chen et al. 2015a)
int2-R	TTTTACGCTGCTGTATGGTGC			
sul1-F	CGCACCGGAAACATCGCTGCAC	56	163	(Pei et al. 2006; Chen et al. 2015a)
sul1-R	TGAAGTTCCGCCGCAAGGCTCG			
sul2-F	TCCGGTGGAGGCCGGTATCTGG	60.8	191	(Pei et al. 2006; Chen et al. 2015a)
sul2-R	CGGGAATGCCATCTGCCTTGAG			

F forward, *R* reverse

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3 Results

3.1 Identification of Bacteria by16S-rRNA Sequencing: Bacterial Community Structure

Bacteria were isolated from tap water by a membrane filtration method ($n = 148$) and 87 colonies were identified up to genus level by sequencing the V4 region of 16S-ribosomal RNA gene. The water distribution system harboured three phyla of bacteria: Proteobacteria, Actinobacteria and Firmicutes. Among them, 54 (62.1 %) belonged to the phylum Proteobacteria. Sub grouping of this phylum indicated the presence of 10 alpha-proteobacteria (11.5 %), 38 beta-proteobacteria (43.7 %), 5 gamma-proteobacteria (5.7 %) and 1 epsilon-proteobacterium (1.2 %). Firmicutes were the second largest phyla found in drinking water and 18 (20.7 %) bacteria belonged to this group, while 15 (17.2 %) bacteria were from Actinobacteria.

The presence of both Gram-negative and Gram-positive bacteria was confirmed in the tap water; among them some of the bacteria can be pathogenic. Species of *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Kocuria*, *Staphylococcus*, *Arthrobacter*, *Comamonas*, *Acidovorax*, *Blastomonas*, *Variovorax*, *Escherichia* and pathogenic *Burkholderia* were found in the water distribution system.

3.2 Presence of Antibiotic Resistance Genes

Detection of the *sul*1 and *sul*2 genes specific for sulfonamide resistance was performed on 148 isolates and these genes were detected in 12 (8.1 %) isolates, thus confirming the presence of antibiotic resistant bacteria in the water distribution system; none contained both genes. *Sul*1 genes were detected in 8 (5.4 %) bacteria, while *sul*2 genes were present in 4 (2.7 %) isolates (Table 2). In two (1.4 %) isolates, these genes were also positive for integrons, while in 10 (6.8 %) isolates they were found singly without integrase genes indicating that *sul* genes did not always correspond to *intI* genes as expected. This suggests that the *sul* genes in these bacteria might either be present on chromosome, or associated with other genetic elements (Gundogdu et al. 2011) other than *intI* genes. The distribution of bacteria containing antibiotic resistance genes (ARGs) was widespread among organisms including *Bacillus*, *Cupriavidus*, *Variovorax*, *Kocuria*, *Ralstonia*, *Dermacoccus*, *Micrococcus* and *Staphylococcus* species from the samples.

3.3 Presence of Disinfectant Resistance Genes

Disinfectant resistance genes *qac* were not found in any of the isolates. *Qac* genes associated with class 1 integrons have a high occurrence rate in the environment both in Gram-positive and Gram-negative bacteria (Jaglic and Cervinkova 2012); in this study, the presence of *qac* genes was not detected.

3.4 Presence of Transferable Markers

PCR amplification analyses indicated that class-1 integrons existed in eight bacteria (9.2 %), while *intI*2 genes were not detected in any isolate. In this study, *Dermacoccus* sp. had both *sul*1 and *intI*1 genes, while *Micrococcus* sp. had *sul*2 and *intI*1 genes simultaneously. Presence

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Table 2 Detection of *intI*1, *intI*2, *sul*1, *sul*2 and *qac* genes in bacteria isolated from the drinking water distribution system

N#	Phylum	Genus	Genes					Isolate identification
			*intI*1	*intI*2	*sul*1	*sul*2	*qac*	
1	Actinobacteria	*Arthrobacter*	−	−	−	−	−	DW(518)
1	Actinobacteria	*Arthrobacter*	+	−	−	−	−	DW(509)
2	Actinobacteria	*Dermacoccus*	−	−	−	−	−	DW(597, 603)
1	Actinobacteria	*Dermacoccus*	−	−	+	−	−	DW(608)
1	Actinobacteria	*Dermacoccus*	+	−	+	−	−	DW(607)
1	Actinobacteria	*Dietzia*	−	−	−	−	−	DW(625)
1	Actinobacteria	*Janibacter*	−	−	−	−	−	DW(644)
1	Actinobacteria	*Kocuria*	−	−	−	−	−	DW(565)
1	Actinobacteria	*Kocuria*	−	−	+	−	−	DW(620)
1	Actinobacteria	*Kocuria*	+	−	−	−	−	DW(513)
2	Actinobacteria	*Micrococcus*	−	−	−	−	−	DW(505, 637)
1	Actinobacteria	*Micrococcus*	+	−	−	−	−	DW(638)
1	Actinobacteria	*Micrococcus*	+	−	−	+	−	DW(512)
9	Alphaproteobacteria	*Blastomonas*	−	−	−	−	−	DW(525, 526, 547, 551, 553, 554, 556, 559, 599)
1	Alphaproteobacteria	*Sphingomonas*	−	−	−	−	−	DW(576)
8	Betaproteobacteria	*Acidovorax*	−	−	−	−	−	DW(516, 521, 537, 539, 540, 541, 544, 569)
5	Betaproteobacteria	*Burkholderia*	−	−	−	−	−	DW(530, 615, 617, 626, 643)
1	Betaproteobacteria	*Comamonas*	−	−	−	−	−	DW(503)
10	Betaproteobacteria	*Cupriavidus*	−	−	−	−	−	DW(501, 502, 504, 511, 522, 570, 578, 580, 587, 591)
1	Betaproteobacteria	*Cupriavidus*	−	−	−	+	−	DW(515)
2	Betaproteobacteria	*Cupriavidus*	−	−	+	−	−	DW(610, 622)
1	Betaproteobacteria	*Cupriavidus*	+	−	−	−	−	DW(604)
5	Betaproteobacteria	*Ralstonia*	−	−	−	−	−	DW(609, 613, 616, 618, 619)
1	Betaproteobacteria	*Ralstonia*	−	−	−	+	−	DW(614)
2	Betaproteobacteria	*Variovorax*	−	−	−	−	−	DW(546, 549)
2	Betaproteobacteria	*Variovorax*	+	−	−	−	−	DW(557, 600)
1	Epsilonproteobacteria	Not identified						DW(533)
5	Firmicutes	*Bacillus*	−	−	−	−	−	DW(514, 527, 529, 531, 640)
1	Firmicutes	*Bacillus*	−	−	−	+	−	DW(507)
1	Firmicutes	*Bacillus*	−	−	+	−	−	DW(532)
1	Firmicutes	*Brevibacillus*	−	−	−	−	−	DW(535)
5	Firmicutes	*Paenibacillus*	−	−	−	−	−	DW(552, 623, 634, 635, 641)
1	Firmicutes	*Paenibacillus*	−	−	+	−	−	DW(536)
3	Firmicutes	*Staphylococcus*	−	−	−	−	−	DW(538, 542, 632)
1	Firmicutes	*Staphylococcus*	−	−	+	−	−	DW(631)
2	Gammaproteobacteria	*Enhydrobacter*	−	−	−	−	−	DW(506, 508)
2	Gammaproteobacteria	*Escherichia*	−	−	−	−	−	DW(560, 611)
1	Gammaproteobacteria	*Pantoea*	−	−	−	−	−	DW(595)

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of *intI*1 genes confirms the presence of transferable genetic element integrons in the bacteria of the water supply system which could involve in the dispersion of antibiotic and disinfectant resistance genes in the environment.

4 Discussion

The presence of antibiotic resistant bacteria and their genes in water is a major public health concern and a global challenge (Pruden et al. 2012; Pruden et al. 2013). They are a major contributing factor in water pollution, found in natural water bodies (Ahammad et al. 2014), treated drinking water (Pruden et al. 2006), and drinking water distribution systems (Xi et al. 2009), can cause infectious diseases (Pruden et al. 2012), and are difficult to treat (Levy 2002). Currently, waterborne diseases are not only related to the presence of faecal bacteria, but also the occurrence of opportunistic pathogens in water systems (Wang et al. 2013). Around 30 different bacterial genera have been isolated from drinking water in different studies including genera of *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Escherichia* and *Proteus* (Norton and LeChevallier 2000; Allen et al. 2004; Gallego et al. 2005, Chiao et al. 2014). In developed countries, outbreaks of waterborne infections due to the drinking water have been reported (Kilvington et al. 2004; Brunkard et al. 2011). Antibiotic resistant infections are also not uncommon in these countries. In the USA, two million people suffered from antibiotic resistant infections every year and among them 23,000 died (CDC 2013). Drinking water distribution systems host a variety of microorganisms (Simoes et al. 2010), and bacteria having antibiotic resistance can find their way into drinking water in these systems. From drinking water, they can colonize the gastrointestinal tract and serve as a potential health risk for the population, especially immune-compromise individuals (Lee et al. 2010).

Isolation of bacteria by membrane filtration demonstrates the presence of viable organisms, so it is a good indicator of the presence of living bacteria in the water environment, which can be actively involved in gene transfer among bacteria and spread of disease in humans. 16S-rRNA gene information is generally used for the identification of bacteria from different environments (Chakravorty et al. 2007). While a useful housekeeping genetic marker to classify bacteria, 1–14 % of organisms remain unidentified as it has low phylogenetic power at species level and cannot discriminate some genera properly (Drancourt et al. 2000; Woo et al. 2003; Mignard and Flandrois 2006; Janda and Abbott 2007). In this study, we identified 87 % of bacteria from the tap water, while 13 % of bacteria were not characterized as no significant similarity was found. These bacteria belong to 22 genera (Table 2), and also included *Burkholderia*, some species of which are waterborne pathogens and may cause melioidosis in humans (Howard and Inglis 2003). The isolation and identification of multiple types of bacteria at the point of use indicates that the distribution network or plumbing systems might be playing some role in the existence of these bacteria, and the ecology of the system could be a contributing factor in their incidence (Hong et al. 2010).

A well-recognized factor which could contribute to the existence of antibiotic resistance bacteria at the consumer end is the presence of biofilms in the distribution system or plumbing system of the buildings from where water samples have been taken. Prevalence of antibiotic resistant bacteria in a water supply system, even in the presence of disinfectant, could be exacerbated by aging infrastructure, in which

biofilms have formed on surfaces (Abe et al. 2012). The multiple layers of microorganisms in these biofilms decrease the residual disinfectant levels to the inner layers; further, bacteria in these layers could develop and transfer resistance traits against antimicrobials to other susceptible populations present in these biofilms (Molin and Tolker-Nielsen 2003; Bridier et al. 2011). Water storage in tanks and cisterns also causes a decrease in the amount of residual disinfectant as water retention time increases, thus allowing bacteria to grow in water. Disinfection itself could concentrate the antibiotic resistant bacteria and their genes in drinking water, as was found in the work of Shi et al. (2013), where chlorination caused the enrichment of *tet*, *amp* and *erm* genes.

The emergence of antibiotic resistance in the environment is not only due to physiological factors, but also depends on genetic factors like horizontal gene transfer (HGT) rate (Andersson and Hughes 2010). Co-selection of two different antibiotic resistance genes occurs through HGT due to the genetic linkage of these genes. For example, sulfonamide resistance genes are plasmid borne and often linked with other antibiotic resistance genes. It has also been found that *sul* and *intI* genes co-exist in water, which might be due to the presence of *sul*1 genes on the class 1 integrons (Chen et al. 2015b). This contributes to the reason that sulphonamide resistance has not declined even when the use of the antibiotics has been reduced (Enne et al. 2004).

Quaternary ammonium compounds resistant genes are also present on mobile genetic elements, such as class 1 integrons (Chapman 2003). Bacterial strains, which acquire genetic units like plasmids, transposons or integrons, show a higher resistance to QACs (Bjorland et al. 2003). The selective pressure by quaternary ammonium compounds (Stalder et al. 2012) disperses *qac* genes and antibiotic resistance genes associated with the integrons (Paulsen et al. 1993; Paulsen et al. 1996; Jeong et al. 2009). This indicates that cross resistance for QACs and antibiotics is possible (Morente et al. 2013). Other mechanisms like a multidrug efflux pump and modification of the cell wall also induce resistance in bacteria (Jaglic and Cervinkova 2012), which allow them to survive in the presence of disinfectant. This could be a reason that in the current study, *qac* genes were not detected in any of the bacteria despite the fact that *intI*1 genes were present with antibiotic resistance genes. This suggests that in the absence of disinfectant resistant genes, other mechanisms of resistance might help bacteria persist in the water distribution system.

5 Conclusions

Isolation of antibiotic resistant bacteria from drinking water demonstrates a need for greater awareness of ecological interactions in drinking water and increased monitoring of distribution systems and plumbing. Presence of those genera, some species of which could cause human diseases, indicates that water quality could not be guaranteed at the consumer end, and future studies should focus on treatment considerations at point of use to guarantee safety.

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Compliance with ethical standards

Conflict of Interest No conflict of interest is found.

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Case Note Review

Paediatric Haemato-Oncology Patients

Royal Hospital for Children

NHS Greater Glasgow and Clyde

Epidemiological & Clinical Outcomes Protocol

April 2020 v1.0

Introduction

National Antimicrobial Resistance & Healthcare Associated Infection (ARHAI) Scotland (formally Health Protection Scotland) has been supporting NHS Greater Glasgow and Clyde in the investigation of a suspected increase in the number of cases of patients with unusual environmentally associated Gram-negative bacteria blood cultures.

Previous epidemiological investigations have focused on microbiological laboratory data. These investigations recommended that systematic collection of clinical and epidemiological information on cases be carried out allowing the cases to be described in the context of risk including, where possible, in the context of environmental risks and incidents.

In January 2020, the Scottish Government announced that a case note review would be undertaken to include all children with Gram negative environmental bacteraemia and selected other infections between May 2015 and December 2019. An internationally validated Paediatric Trigger Tool (PTT) has been selected to provide data to the independent review team. In addition to the PTT review, a supplementary epidemiological review of the cases is required to collect patient, outcome and risk data systematically using agreed definitions and for the findings to support the incident investigation.

The objectives of this epidemiological investigation are to:

- Determine a timeline for each of the cases
- Characterise the cases in terms of time, place and person
    - Time: describe the episodes of bloodstream infection over time and create a timeline for outbreak, including plotting of control measures against number of cases
    - Place: describe the location of patients (hospital, ward, bed/bay) and describe their movements in the hospital
    - Person: characterise the patients with infection in terms of intrinsic and extrinsic risk factors; outcomes; antimicrobial prophylaxis and treatment; and individual infection prevention and control measures in place.
- Describe the cases in the context of environmental risks and incidents including use environmental microbiological data and HAI-SCRIBE/other estates data (where possible)

Methods

Study design

This protocol describes the methodology for a retrospective review of cases using patient cases notes. The epidemiological study design is descriptive rather than analytical and no control group will be selected to make comparisons between patients with and without infection to determine risk (at this time).

The study population includes all cases that meet one of the following definitions:

- A patient being cared for in the paediatric haematology or oncology specialty in the Royal Hospital for Children, NHS GGC with:
    - at least one positive blood culture of a Gram negative organism associated with the environment* between May 2015 and December 2019 OR
    - at least one positive culture of an atypical Mycobacterium (Acid Fast Environmental bacteria, see Appendix 1) between May 2015 and December 2019

Note: The expert panel agreed to a flexible approach to inclusion of patients in the review. There may be instances when there is a request to review the case notes of a patient who does not meet the above inclusion criteria. There may also be instances where parents request that their child is not included in the review. Both these circumstances will need to be considered during the analysis and when the final report is being prepared.

A number of datasets were available from previous epidemiological review of the microbiology data. Assessment of these datasets indicated that, whilst there were overlaps, it was necessary for optimal case ascertainment to combine the datasets and create a list of patients that appear in one or more of the datasets. These datasets were two extracts from NHS GGC's LIMS system, a central line associated bloodstream infection surveillance dataset and the Electronic Communication of Surveillance in Scotland (ECOSS).

* Details of the organisms in each of the datasets are provided in Appendix 1.

Data collection methods

A team of 3-4 epidemiologists from National ARHAI Scotland will undertake the case note review using all available patient information in the GGC Clinical Portal. It is expected that this will include:

- Nursing notes
- Medical notes
- Temperature charts
- Paediatric early warning scores
- Drug charts
- Surgery/operation notes
- Laboratory report e.g. microbiology results
- Other relevant charts e.g. wound charts, stool charts, care plans.

Clinical Portal holds all of the patient notes with the exception of those held in Critical Care. Admission and movement data are captured in the GGC Trakcare system and an extract will be provided for ease of processing the data.

Further information relating to each of these categories is provided in supplementary document “Expert Panel Dataset V1.0”.

Linkage to the Paediatric Trigger Tool Review (PTT)

The epidemiological review will be undertaken in collaboration with the clinical team undertaking the PTT review (‘ground team’) thus ensuring that the clinical expertise available provides support to the epidemiological and clinical outcomes review. The PTT will assess admissions associated with the infections that are included in the review.

Data handling

All data will be linked by the patient’s CHI number and a timeline will be created for each case. All data will be collected in a bespoke relational database from which data can be extracted into a template for use by the Expert Review Panel.

Appendix 1: Prior microbiology groupings in the 4 datasets

Organisms included in Environmental groupings

NHSGGC CLABSI surveillance	NHSGGC ECOSS selected Gram-negative organisms (GGC Selected GNeg)	NHSGGC Microbiology LIMS Surveillance	National ARHAI Scotland ECOSS Under18 bloods RHC HaemOnc
Gram Negative Environmental (GN ENV)	**Gram Negative Environmental (GN ENV)**	**Gram Negative Environmental (GN ENV)**	**Gram Negative Environmental (GN ENV)**
Achromobacter spp. *Acinetobacter baumannii* *Acinetobacter ursingii* *Aeromonas hydrophila* *Burkhold cepacia* *Chryseomonas indologenes* *Chryseob. spp* *Cupriavidis pauculus* *Eliz. meningoseptica* *Elizabethkingia spp.* *Delftia acidovorans* *Pseudomonas spp.* *Rhiz. radiobacter* *Roseomonas mucosa* *Sphingomonas spp.* *Steno. maltophilia*	*Acinetobacter baumannii* *Acinetobacter ursingii* *Aeromonas hydrophila* *Brevundimonas spp.* *Burkholderia cepacia* *Chryseobacterium indologenes* *Chryseobacterium spp.* *Cupriavidus pauculus* *Delftia acidovorans* *Elizabethkingia meningoseptica* *Elizabethkingia spp.* *Pseudomonas spp.* *Rhizobium radiobacter* *Sphingomonas paucimobilis* *Steno. maltophilia*	*Achromobacter sp* *Acinetobacter baumannii* *Acinetobacter ursingii* *Aeromonas spp* *Brev. spp.* *Burk. cepacia group* *Chryseob. spp* *Chryseobacterium indologenes* *Chryseomonas sp p.* *Cup. pauculus* *Del. acidovorans* *Delftia spp.* *Elizabethkingia. spp.* *Herbaspirillum sp* *Pseudomonas spp* *R. planticola* *Rh. radiobacter* *R. mucosa* *Sph. paucimobil* *Steno. maltophilia*	*Achromobacter spp.* *Acinetobacter spp.* *Aeromonas hydrophila* *Brevundimonas spp.* *Burkholderia cepacia* *Chryseobacterium indologenes* *Chryseobacterium spp.* *Cupriavidus pauculus* *Delftia acidovorans* *Elizabethkingia meningoseptica* *Elizabethkingia miricola* *Elizabethkingia spp.* *Pseudomonas spp.* *Raoultella planticola* *Rhizobium radiobacter* *Roseomonas mucosa* *Sphingomonas paucimobilis* *Steno. maltophilia*
Gram Negative Enteric /Environmental (GN ENT/ENV)	**Gram Negative Enteric /Environmental (GN ENT/ENV)**	**Gram Negative Enteric /Environmental (GN ENT/ENV)**	**Gram Negative Enteric /Environmental (GN ENT/ENV)**
Citrobacter spp. *Enterobacter cloacae* *Klebsiella spp.* *Pantoea spp.* *Serratia liquefaciens* *Serratia marcesens*	*Citrobacter spp.* *Enterobacter spp.* *Klebsiella spp.* *Pantoea spp.* *Serratia liquefaciens* *Serratia marcescens*	*Citrobacter spp.* *Enterobacter spp.* *Klebsiella spp.* *Pantoea spp.* *Ser. liquefac.* *Ser. marcescens*	*Citrobacter spp.* *Enterobacter spp.* *Klebsiella spp.* *Pantoea spp.* *Serratia liquefaciens* *Serratia marcescens*
Gram Positive Environmental (GP ENV)	**Gram Positive Environmental (GP ENV)**	**Gram Positive Environmental (GP ENV)**	**Gram Positive Environmental (GP ENV)**
Gordonia polyisoprenivorans	N/A	*Gordonia polyisoprenivorans*	*Gordonia bronchialis*
Acid Fast Environmental (AF ENV)	**Acid Fast Environmental (AF ENV)**	**Acid Fast Environmental (AF ENV)**	**Acid Fast Environmental (AF ENV)**
Mycobacterium chelonae	N/A	*Myc. chelonae group* *Myco fortuitum* *Mycobacterium chelonae*	*Mycobacterium chelonae* *Mycobacterium spp.*
Fungi Environmental (Fungi ENV)	**Fungi Environmental (Fungi ENV)**	**Fungi Environmental (Fungi ENV)**	**Fungi Environmental (Fungi ENV)**
Rhodotorula mucilaginosa	N/A	*Rhod. mucilaginosa*	*Rhodotorula mucilaginosa*

Organisms included in Non-environmental groupings

NHSGGC CLABSI surveillance	NHSGGC ECOSS selected Gram-negative organisms (GGC Selected GNeg)	NHSGGC Microbiology LIMS Surveillance	National ARHAI Scotland ECOSS Under18 bloods RHC HaemOnc
Gram Negative Non-environmental (GN NON-ENV)	**Gram Negative Non-environmental (GN NON-ENV)**	**Gram Negative Non-environmental (GN NON-ENV)**	**Gram Negative Non-environmental (GN NON-ENV)**
Escherichia coli *Fusobacterium nucleatum* *Proteus mirabilis*	N/A	*Bact. uniformis* *Cap. sputigena* *Escherichia coli* *Fuso. nucleatum* *Haemophilus influenzae* *Mor. catarrhalis* *Moraxella nonliquefaciens* *Moraxella osloensis* *Neis. subflava* *Proteus mirabilis*	*Bacteroides uniformis* *Capnocytophaga sputigena* *Escherichia coli* *Escherichia fergusonii* *Fusobacterium nucleatum* *Haemophilus influenzae* *Moraxella spp.* *Neisseria spp.* *Ochrobactrum anthropi* *Proteus mirabilis*
Gram Positive Non-environmental (GP NON-ENV)	**Gram Positive Non-environmental (GP NON-ENV)**	**Gram Positive Non-environmental (GP NON-ENV)**	**Gram Positive Non-environmental (GP NON-ENV)**
Aerococcus viridans *Clostridium spp.* *Corynebacterium spp.* *Dermacoccus nishinomiyaens* *Diphtheroids* *Enterococcus spp.* *Gemella Sanguinis* *Gordonia polyisoprenivorans* *Gram +ve bacilli* *Gram Pos B* *Gram Pos C* *Gran Adiac* *Granulicatella Adiacens* *Kocuria rhizophilia* *Lactobacilus* *Micrococcus spp.* *Paenibacillus durus* *Propionibacterium acnes* *Rot. mucilaginosa* *Staphylococcus spp.* *STCNS* *Streptococcus spp.*	N/A	*Aerococcus viridans* *Alpha strep* *Bacillus spp.* *C. perfiringens* *Coag Neg Staph.* *Corynebacterium spp* *Derm. nishinomiyaens* *Diphtheroids* *Enterococcus spp.* *Gemella.sanguinis* *GPC-Strep* *Gram +ve bacilli* *Gram positive cocci* *Gran. adiacens* *K. rhizophila* *Lactobacillus spp* *Micrococcus spp.* *Paenibacillus spp.* *Propionibacterium acnes* *Rothia mucilaginosa* *Staphylococcus spp.* *Streptococcus spp.*	*Abiotrophia defectiva* *Aerococcus viridans* *Bacillus spp.* *Clostridium perfringens* *Clostridium septicum* *Corynebacterium spp.* *Dermacoccus spp.* *Enterococcus spp.* *Gemella sanguinis* *Granulicatella adiacens* *Kocuria spp.* *Lactobacillus spp.* *Lactococcus lactis* *Leuconostoc lactis* *Micrococcus spp.* *Paenibacillus spp.* *Propionibacterium spp.* *Rothia spp.* *Staphylococcus spp.* *Streptococcus spp.*
Acid Fast Non-environmental (AF NON-ENV)	**Acid Fast Non-environmental (AF NON-ENV)**	**Acid Fast Non-environmental (AF NON-ENV)**	**Acid Fast Non-environmental (AF NON-ENV)**
Nil	N/A	Nil	Nil
Fungi Non-environmental (Fungi NON-ENV)	**Fungi Non-environmental (Fungi NON-ENV)**	**Fungi Non-environmental (Fungi NON-ENV)**	**Fungi Non-environmental (Fungi NON-ENV)**
Candida spp. *Yeasts*	N/A	*Candida spp.*	*Candida spp.*

Appendix 2: Origin of Infection (mandatory enhanced surveillance protocol)

Hospital acquired infection (HAI): Positive blood culture obtained from a patient who has been hospitalised for ≥48 hours. If the patient was transferred from another hospital, the duration of in-patient stay is calculated from the date of the first hospital admission. If the patient was a neonate/baby who has never left hospital since being born.

OR

The patient was discharged from hospital in the 48hr prior to the positive blood culture being taken.

OR

A patient who receives regular haemodialysis as an out-patient.

OR

Contaminant if the blood aspirated in hospital.

OR

If infection source/entry point is surgical site infection (SSI).

Healthcare associated infection (HCAI): Positive blood culture obtained from a patient within 48 hours of admission to hospital and fulfils one or more of the following criteria:

Was hospitalised overnight in the 30 days prior to the positive blood culture being taken.

OR

Resides in a nursing, long term care facility or residential home.

OR

IV, or intra-articular medication in the 30 days prior to the positive blood culture being taken, but **excluding IV illicit drug use**.

OR

Had the use of a registered medical device in the 30 days prior to the positive blood culture being taken e.g. intermittent self-catheterisation or percutaneous endoscopic gastrostomy (PEG) tube with or without the direct involvement of a healthcare worker (excludes haemodialysis lines see HAI).

OR

Underwent any medical procedure which broke mucous or skin barrier i.e. biopsies or dental extraction in the 30 days prior to the positive blood culture being taken.

OR

Underwent care for a medical condition by a healthcare worker in the community which involved contact with non-intact skin, mucous membranes or the use of an invasive device in the 30 days prior to the positive blood culture being taken e.g. podiatry or dressing of chronic ulcers, catheter change or insertion.

Community infection: Positive blood culture obtained from a patient within 48 hours of admission to hospital who does not fulfil any of the criteria for healthcare associated bloodstream infection.

Not known: Only to be used if the bacteraemia is not an HAI, and unable to determine if Community or HCAI

Appendix 3: Devices – Vascular access devices

Controlled list from enhanced national bacteraemia surveillance:

Device (A) Arterial Line

Device (B) PVC

Device (C1) CVC non-tunnelled

Device (C2) CVC tunnelled

Device (D1) Dialysis line – non-tunnelled

Device (D2) Dialysis line – tunnelled

Device (D3) Dialysis line – fistula

Device (E) PICC/Midline

Appendix 4: Devices - Non-VAD

Controlled list from enhanced national bacteraemia surveillance:

Device (F) Invasive ventilation

Device (G) CAPD

Device (H) Surgical drain

Device (I) Chest drain

Device (J) Urinary catheter

Device (K) Suprapubic catheter

Device (L) PEG

Device (M) External shunt

Clinical Infectious Diseases

REVIEW ARTICLE

The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections—A Systematic Review of the Literature

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Over the last 20 years there have been 32 reports of carbapenem-resistant organisms in the hospital water environment, with half of these occurring since 2010. The majority of these reports have described associated clinical outbreaks in the intensive care setting, affecting the critically ill and the immunocompromised. Drains, sinks, and faucets were most frequently colonized, and *Pseudomonas aeruginosa* the predominant organism. Imipenemase (IMP), *Klebsiella pneumoniae* carbapenemase (KPC), and Verona integron-encoded metallo-β-lactamase (VIM) were the most common carbapenemases found. Molecular typing was performed in almost all studies, with pulse field gel electrophoresis being most commonly used. Seventy-two percent of studies reported controlling outbreaks, of which just more than one-third eliminated the organism from the water environment. A combination of interventions seems to be most successful, including reinforcement of general infection control measures, alongside chemical disinfection. The most appropriate disinfection method remains unclear, however, and it is likely that replacement of colonized water reservoirs may be required for long-term clearance.

Keywords. carbapenem-resistant; carbapenemase; healthcare-associated infections; outbreak; water.

Over the last 10–15 years, clinically relevant carbapenem-resistant organisms (CROs), such as *Pseudomonas aeruginosa, Acinetobacter baumannii,* and the Enterobacteriaceae, have disseminated globally [1]. Genes encoding for important carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and the metallo-β-lactamases, are often transmitted between organisms by mobile genetic elements, such as plasmids, contributing to their spread. Limited treatment options and high mortality in those infected are particularly worrying. Hence, understanding reservoirs and transmission of common CROs and transmissible carbapenemases is a research priority.

Hospitals present a unique opportunity for bacteria to interact, proliferate, and infect vulnerable populations. The healthcare water environment, including potable water, faucets, sink surfaces and wastewater drainage systems (drains, sink/shower traps, toilets, drainage pipes), can be a reservoir for nosocomial pathogens, such as drug-resistant Enterobacteriaceae, *Pseudomonas* spp., and *A. baumannii* [2–4]. The prevalence of these multidrug-resistant organisms (resistant to ≥3 antimicrobial classes) is rising [5], and they will increasingly dominate the hospital environmental microbiome. Determining effective infection control (IC) measures to decontaminate environmental reservoirs and prevent cross-transmission of multidrug-resistant organisms between patients and the environment may minimize potentially lethal outbreaks. The aim of this literature review was to summarize studies identifying common CROs in the hospital water environment, the evidence for CRO transmission between this environment and patients, and successful IC interventions to terminate outbreaks and eliminate CROs from this environment.

METHODS

PubMed was searched using the following Medical Subject Headings (MeSH) and text terms: (enterobacter* OR pseudomon* OR acinetobacter) AND (drain OR sink OR shower OR faucet OR hospital water) (search date: 7–9 March, 2016). All abstracts in English, French, Spanish, and German from 1967 to the search date were screened. Articles were excluded if the full article was unavailable through PubMed or the

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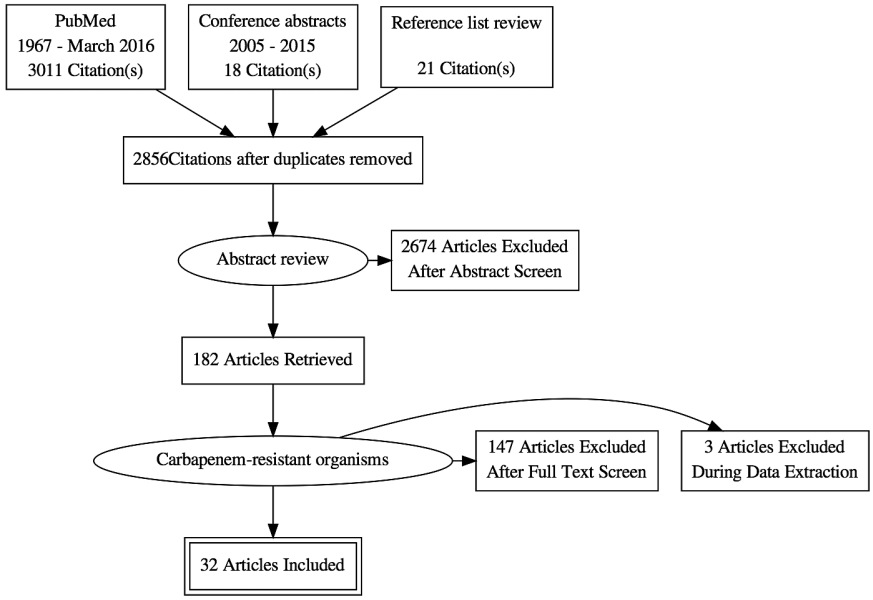

Figure 1. Flowchart of study selection process. Three studies were excluded during data extraction because the outbreak organism was not found in the water environment.

University of Oxford, if the study did not occur in the acute healthcare setting, if it involved hospital wastewater remote to patient care areas, or if the organisms of interest were not identified. Citations in the selected articles were reviewed for additional relevant reports.

Additional searches of conference abstracts were undertaken (9–10 March 2016) for the European Congress of Clinical Microbiology and Infectious Diseases (2005–2015), Interscience Conference on Antimicrobial Agents and Chemotherapy (2013–2014), Infectious Diseases Society of America ID Week (2004–2015), and the Australian Society of Infectious Diseases (ASID) meetings (2013–2015).

All studies involving CROs (organisms phenotypically nonsusceptible to ≥1 carbapenem or producing a carbapenemase) were then included. The ORION (Outbreak Reports and Intervention Studies of Nosocomial Infection) framework was used to assess outbreak reports [6]. Data were collected for the following variables: author, publication year, study design,

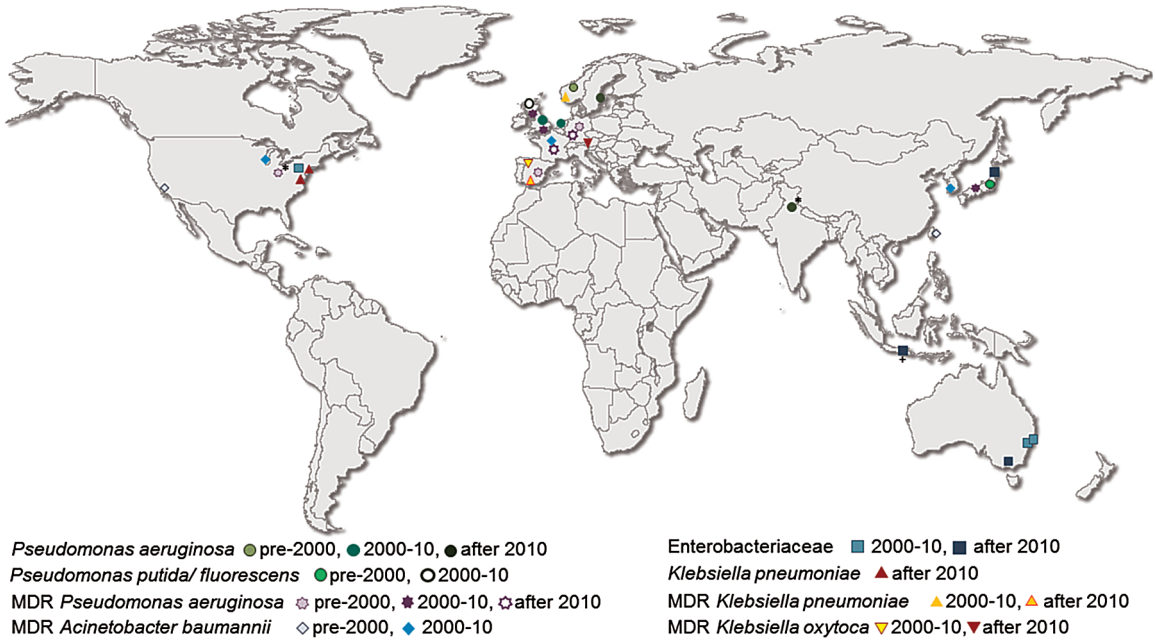

Figure 2. Geographic and temporal distribution of carbapenem-resistant organisms (CROs). The geographic and temporal distribution of the included studies based on the organism reported. MDR, multidrug-resistant (resistant to ≥3 antimicrobial classes). *Study period unknown. †Dewi et al [10] report *Acinetobacter* spp. and *Aeromonas* spp. along with Enterobacteriaceae.

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Table 1. Study Settings and Populations[a]

Patient population	Studies, No. (N = 32)	Intensive Care Unit	High-risk (Hematology, Nephrology, Burns Unit)	Multiple Wards	Other[b]
Adult	25	Knoester et al [25], Kotsanas et al [26], Durojaiye et al [16], Wang et al [27], La Forgia et al [28], Wendel et al [29], Bukholm et al [31], Tofteland et al [32], Vergara-López et al [33], Leitner et al [20], Snitkin et al [22], Podnos et al [23], Odom et al [11], Peña et al [35], Pitten et al [36], Biswal et al [8], Landelle et al [37]	Wong et al [19], Leitner et al [20], Breathnach et al [21], Leung et al [24], Betteridge et al [7], Ambrogi et al [18], Odom et al [11]	Peña et al [35], Pitten et al [36], Kouda et al [38], Landelle et al [37], Seara et al [34], Yomoda et al [9], Breathnach et al [21], Biswal et al [8]	None
Pediatric/ neonatal	3	Hong et al [30], Alter et al [15]	None	None	Ito et al [14]
Not given	4	Majumdar et al [17], Kaiser et al [13]	None	Stjarne Aspelund et al [12]	Dewi et al [10]

[a]Some studies included multiple different settings, so categories are not mutually exclusive.

[b]Single non–high-risk wards or not specified.

organism, carbapenemase mechanism, reservoir(s), evidence of environment-patient transmission, and type/success of interventions (Supplementary Table 1). Authors of studies reporting terminating outbreaks were contacted (August 2016) regarding the ongoing success of any interventions.

RESULTS

Study Settings and Populations

Search and screening strategy results are shown in Figure 1. Thirty-two studies were included (Supplementary Table 1). Of these, 27 were outbreak investigations, and 5 were epidemiological surveillance studies [7–11]. Twenty-three were full articles, 6 were conference abstracts [10–15], 2 were letters [16, 17] and 1 was a short report [18]. The studies included data from Europe (n = 16), Asia (n = 7), North America (n = 6), and Australia (n = 3) (Figure 2). Thirty studies occurred between 1996 and 2015, with 50% of these since 2010. Two studies did not document the study time frame [8, 15]. Most were conducted in adult inpatients, with 7 in pediatric/neonatal populations or unspecified (Table 1). Eleven studies involved immunocompromised patients, including those with hematological malignancies [12, 19–22], solid tumors [22], primary immunodeficiencies [22], renal disease [18], burns [7, 8, 23, 24], or unspecified diagnoses [11]. Fifteen studies occurred solely in the intensive care setting [13, 15–18, 23, 25–33], and the others involved various medical/surgical wards, intensive care units, operating theaters, or the whole hospital (Table 1).

Patient Sampling Strategy and Risk Factors for CRO Infection/Colonization

In total, 926 patients from 31 studies were CRO colonized (n = 184), infected (n = 189), or unspecified (n = 553). In 22 studies, active patient screening was performed, varying in site (swab samples from the rectum, nose, throat, groin, axilla, perineum, perianal, wound, and catheter sites; samples of sputum, urine, stool, tracheal aspirate, blood, and gastric tubes) and frequency (twice weekly, weekly, or fortnightly). The prevalence of patient infection or colonization ranged from 1.6% to 26.7% (reported in 8 studies [8, 14, 19, 22, 24, 30, 31, 33]). Risk factor analysis for colonization/infection was performed in 4 studies [14, 24, 25, 31], and risks included preceding surgery, patient location, prolonged mechanical ventilation, older

Table 2. Water Reservoirs Containing Carbapenem-Resistant Organisms[a]

Water Reservoir	Studies, No. (N = 32)	References
Drains/drainage systems	17	Peña et al [35], Kotsanas et al [26], La Forgia et al [28], Betteridge et al [7], Leitner et al [20], Wendel et al [29], Breathnach et al [21], Leung et al [24], Snitkin et al [22], Tofteland et al [32], Vergara-López et al [33], Yomoda et al [9], Stjarne Aspelund et al [12], Odom et al [11], Knoester et al [25], Landelle et al [37], Seara et al [34]
Sink surfaces	14	Betteridge et al [7], Wendel et al [29], Knoester et al [25], Podnos et al [23], Wang et al [27], Biswal et al [8], Hong et al [30], Bukholm et al [31], Kouda et al [38], Landelle et al [37], Dewi et al [10], Kaiser et al [13], Ito et al [14], Leung et al [24]
Faucets	8	Odom et al [11], Knoester et al [25], Majumdar et al [17], Pitten et al [36], Hong et al [30], Bukholm et al [31], Alter et al [15], Leung et al [24]
Water	3	Knoester et al [25], Ambrogi et al [18], Bukholm et al [31]
Inflatable hair wash basin	2	Wendel et al [29], Knoester et al [25]
Sensor mixer taps	1	Durojaiye et al [16]
Water/tea dispenser	2	Wong et al [19], Ito et al [14]
Shower/shower equipment	3	Betteridge et al [7], Leung et al [24], Seara et al [34]
Toilet bowl/brush	2	Breathnach et al [21], Kouda et al [38]

[a]Some studies had multiple water reservoirs, so categories are not mutually exclusive.

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Table 3. Carbapenem-Resistant Organisms and Carbapenemase Type[a]

Organism	Studies, No. (N = 32)	Reservoirs	IMP	KPC	VIM	NDM	GIM	MIC/Phenotype only
Pseudomonas aeruginosa	13	Sinks, drains, faucets, hair wash basins, water samples, sensor mixer taps, toilet bowls/brushes	None	None	Knoester et al [25], Ambrogi et al [18], Breathnach et al [21], Stjarne Aspelund et al [12]	None	Wendel et al [29]	Peña et al [35], Durojaiye et al [16], Majumdar et al [17], Pitten et al [36], Biswal et al [7], Bukholm et al [31], Kouda et al [38], Alter et al [15]
Other *Pseudomonas* spp.	2	Drinking water dispenser, water pipes	Yomoda et al [9]	None	None	None	None	Wong et al [19]
Acinetobacter baumannii	5	Sinks, drains, faucets	None	None	None	None	None	Podnos et al [23], Wang et al [27], La Forgia et al [28], Hong et al [30], Landelle et al [37]
Klebsiella pneumoniae	7	Drains, faucets, showers, sinks	Kotsanas et al [26], Leung et al [24], Ito et al [14]	Snitkin et al [22], Tofteland et al [32], Odom et al [11]	None	Seara et al [34]	None	None
Klebsiella oxytoca	3	Drains, faucets, showers, sinks	Leung et al [24], Vergara-López et al [33]	Leitner et al [20]	None	None	None	None
Enterobacter spp.	5	Drains, faucets, showers, sinks	Kotsanas et al [26], Betteridge et al [7], Leung et al [33]	Tofteland et al [32]	None	None	None	Dewi et al [10]
Escherichia coli	3	Drains, cold tea dispenser	Kotsanas et al [26], Betteridge et al [7], Ito et al [14]	None	None	None	None	None
Serratia marcescens	3	Drains, faucets, showers, sinks	Kotsanas et al [26], Betteridge et al [7] , Leung et al [24]	None	None	None	None	None
Other (*Leclercia* spp., *Pantoea* spp., *Citrobacter freundii, Raoutella planticola, Escherichia hermannii, Aeromonas hydrophilia, Proteus mirabilis* or not specified)	4	Drains, faucets, showers, sinks	Betteridge et al [7] , Leung et al [24]	Kaiser et al [13]	None	None	None	Dewi et al [10]

Abbreviations: GIM, German imipenemase; IMP, imipenemase; KPC, *Klebsiella pneumoniae* carbapenemase; MIC, minimum inhibitory concentration; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase.

[a]Some studies included multiple different species, so categories are not mutually exclusive.

age, burns, longer hospital stay, and drinking tea from a contaminated dispenser. Length of stay before colonization/infection was documented in only 10 studies, varying from 1 to 134 days [19, 24–26, 28–30, 32–34]. Mortality was assessed in 18 studies, with a mean rate of 25.7% (range, 0%–85%) [12, 16, 19–24, 26, 28–36]. However, 2 studies did not comment on whether deaths were attributable to the study organism(s) [28, 36].

Environmental Sampling Strategies

Hospital water environment investigations included sampling from faucets (n = 18), drainage systems (n = 17), sink surfaces (n = 16), and water (n = 14). Other environmental samples were taken in 26 studies, including medical equipment, patient environment, antiseptic solutions/liquid soaps, enteral nutrition, staff areas, and air samples. Sampling methods varied but typically included moist sterile swab/water samples of varying volumes. Only 5 studies detailed sink and drainage system design [18, 20, 21, 26, 30].

All studies identified the relevant CROs in the water environment, mostly in drains/traps, sink surfaces, and faucets (Table 2). CROs were found in other sites in 15 of 32 studies. The hands of healthcare workers (HCWs) were sampled in 6 studies [8, 17, 23, 27, 33, 36]; additional pharyngeal, rectal, and nasal swab samples were obtained in 2 [8, 33], 1 [33], and 1 [8] study respectively. The study CRO was not colonizing HCWs in any studies.

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CROs Investigated

Organisms studied included *P. aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *A. baumannii*, and various Enterobacteriaceae (Table 3). Molecular carbapenemase identification was performed in 17 studies. The genes identified included bla_{IMP} (n = 6), bla_{KPC} (n = 5), bla_{VIM} (n = 4), bla_{NDM} (n = 1), and bla_{GIM} (n = 1) (Table 3). Multiple species of Enterobacteriaceae were involved in 7 studies, suggesting interspecies/intergenus resistance gene transfer [7, 10, 13, 14, 24, 26, 32], specifically demonstrated in vitro for bla_{IMP} and bla_{KPC} [7, 9, 32].

Microbiological and Typing Methods Deployed

Microbiological methods for culture and identification were clearly described for 11 studies [11, 19, 20, 22, 24–26, 29, 32, 33, 37], with no methods given in 7 [12–15, 17, 28, 35]. Methods differed in the media and biochemical tests used. Samples from patients in 2 studies [25, 34] were incubated in an enrichment broth; however, only 1 of these studies supplemented this with a carbapenem disc [34], following Centers for Disease Control and Prevention recommendations [39]. Antimicrobial susceptibility testing methods were reported in 26 studies and included disc diffusion, microbroth dilution, Etest and PCR. Seven of 32 studies did not report susceptibilities [7, 10–14, 24]. CROs were susceptible only to polymixin B/colistin in 8 of 16 studies that reported testing for colistin susceptibility [18, 21, 23, 27, 28, 30, 36, 37]. In 4 studies that did not test for colistin susceptibility, CROs remained susceptible only to amikacin [15, 16, 26, 35]. Variable susceptibility to fluoroquinolones, tigecycline, fosfomycin, other aminoglycosides, and β-lactams was found in the remaining 13 studies. One study reported that the outbreak organism (KPC–*Klebsiella pneumoniae*) became resistant to all antibiotics after initially being susceptible to gentamicin, tigecycline, and colistin [22]. Molecular typing was performed in all but 2 studies [8, 10], mostly using pulsed-field gel electrophoresis (Table 4).

CRO Transmission and IC Interventions

The organism under investigation was detected in both patients and the environment in all but 1 study where only the environment was assessed [10]. All studies found evidence of cross-transmission between patients and the environment based on epidemiological links and/or identical antimicrobial susceptibility phenotypes/molecular typing (Table 4).

Nine studies reported IC breaches that probably contributed to outbreaks. These included poor sink design [18, 20, 21, 26, 30], use of sinks for contaminated clinical waste disposal [20, 26, 37], storage of clean patient materials around sinks/sluices [21, 29], reuse of nonsterile surgical drapes and open drainage in the cystoscopy room [35], use of a single brush to clean sinks without between-site disinfection [26], blocked sewage pipes and waste pipe leaks [21], and failure to clean shower drains [21]. Nontouch sensor taps were a reservoir in 1 study [16].

Interventions to eliminate CROs were reported in 27 studies. Twenty-five studies included water environment decolonization interventions, including chemical disinfection (alcohol, chlorination, aldehydes, biguanides, sodium hypochlorite [bleach], acetic acid, hydrogen peroxide, silver nitrate, hot water, and pressurized steam), sterile water for high-risk patient care, assignment of sinks to hand hygiene only, and replacement of contaminated equipment, faucets, sinks or drainage systems (Figure 3). Two studies reported cleaning or disinfecting the water environment, but without details [17, 23]. Two studies described agents used to clean rooms but did not provide

Table 4. Bacterial Strain/Mobile Genetic Element Typing Methods[a]

Typing Method	Studies, No. (N = 32)	Reference(s)
PFGE	20	Breathnach et al [21], Snitkin et al [22], Tofteland et al [32], Seara et al [34], Wendel et al [29], Wong et al [19], Podnos et al [23], Yomoda et al [9], Stjarne Aspelund et al [12], Kotsanas et al [26], Kouda et al [38], Peña et al [35], Majumdar et al [17], Pitten et al [36], Wang et al [27], Ambrogi et al [18], Landelle et al [37], Vergara-López et al [33], Kaiser et al [13], Alter et al [15]
Resistance gene PCR	8	Knoester et al [25], Seara et al [34], Wendel et al [29], Betteridge et al [7] , Kotsanas et al [26], Kouda et al [38], Leung et al [24], Odom et al [11]
MLST	5	Tofteland et al [32], Seara et al [34], Hong et al [30], Wendel et al [29], Leitner et al [20]
Rep-PCR	4	Snitkin et al [22], Leitner et al [20], Betteridge et al [7], Stjarne Aspelund et al [12]
Plasmid/MGE profiling	3	Tofteland et al [32], Betteridge et al [7] , Yomoda et al [9]
RAPD	2	Wong et al [19], Podnos et al [23]
AFLP	2	Knoester et al [25], Bukholm et al [31]
VNTR	2	Durojaiye et al [16], Breathnach et al [21]
REA	1	La Forgia et al [28]
WGS	2	Ito et al [14], Snitkin et al [22]
Antimicrobial susceptibility profile only	1	Biswal et al [7]

Abbreviations: AFLP, amplified fragment length polymorphism; MGE, mobile genetic element; MLST, multilocus sequence typing; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; RAPD, random amplification of polymorphic DNA; REA, restriction enzyme analysis; Rep-PCR, repetitive element sequence-based PCR; VNTR, variable-number tandem repeat; WGS, whole-genome sequencing.

[a]Some studies used multiple methods, so categories are not mutually exclusive. One study did not perform any typing (Dewi et al [10]).

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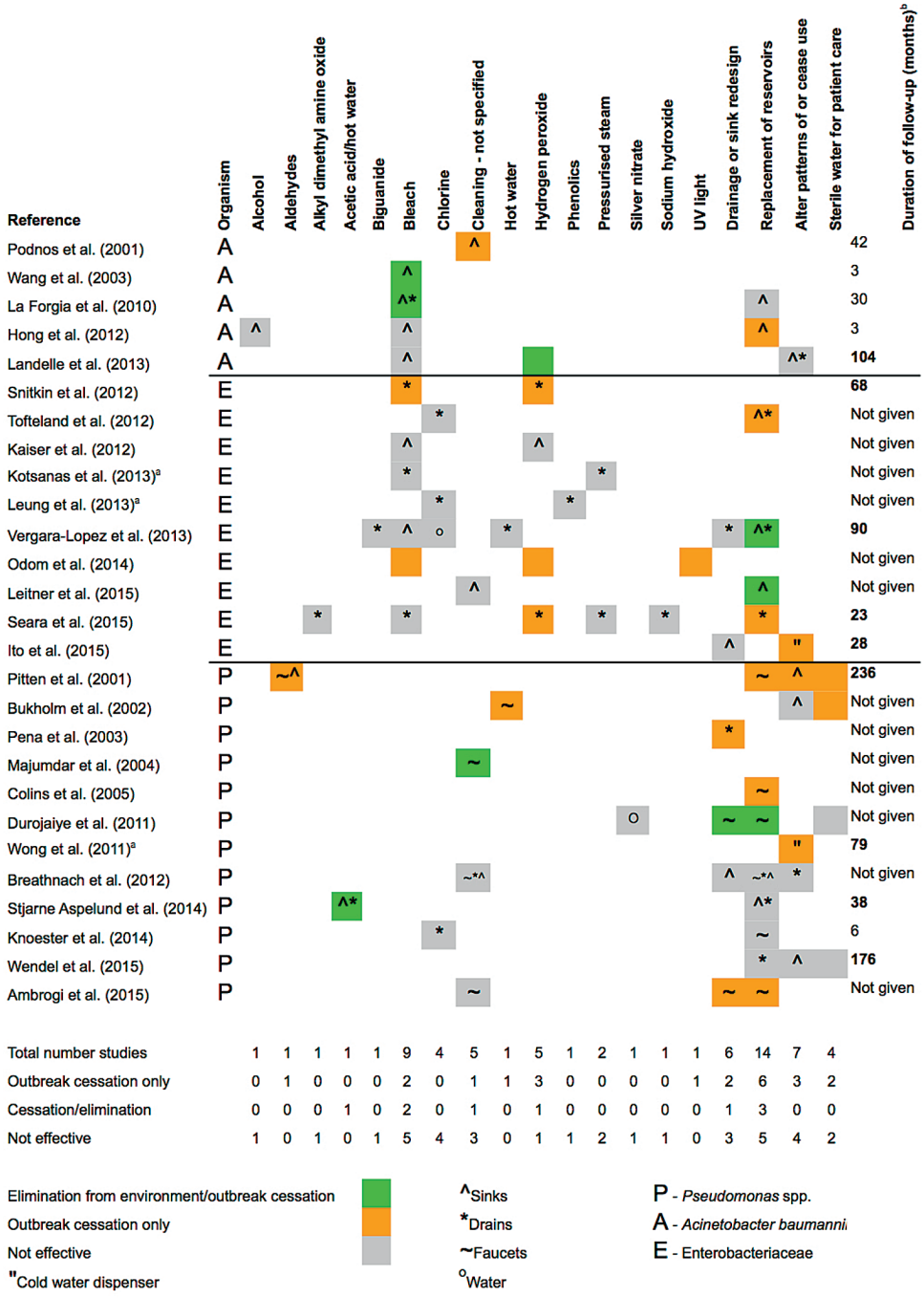

Figure 3. Infection control interventions and outcomes. Five studies did not report any interventions (Betteridge et al [7], Biswal et al [7], Kouda et al [38], Yomoda et al [9], Dewi et al [10]), and 5 reported only plumbing interventions (Wong et al [19], Wendel et al [29], Alter et al [15], Peña et al [35], and Ito et al [14]). For duration of follow-up (*right axis*), months shown in bold denote studies that responded to our communication.

specific information on disinfecting the water environment [11, 22].

Twenty-two studies reported enhancing general IC measures, including contact isolation, strict hand hygiene, active surveillance, reinforcement of cleaning and disinfection procedures, audits, and education sessions. Of the 25 studies that reported specific environmental interventions, 22 reported success in terminating the clinical outbreak, whereas just more than one-third of these managed to eliminate the organism from environmental reservoirs (Figure 3) [12, 16, 17, 20, 27, 28, 33, 37]. Interventions successful at disinfecting water reservoirs included cleaning of sinks and taps (details not given) [17], daily cleaning of sink surfaces with 0.1% sodium hypochlorite [27], weekly cleaning of sinks and plumbing with acetic acid/hot water [12], transferring all patients to a dedicated isolation unit and hydrogen peroxide vapor disinfection [37], replacing

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nontouch sensor taps with conventional taps [16], and replacing sinks or drainage systems [20, 33]. Kouda et al [38] reported success without giving details of interventions.

Only 7 of 22 studies reporting success stated the duration of follow-up after the intervention [23, 27–30, 33, 34], which ranged from 2 months to 3.5 years. The authors of 10 studies responded to our communication regarding further follow-up. Seven of these reported no further cases of the outbreak organism [14, 19, 22, 33, 36, 37, 38]. Kouda et al [38] attributed their success to ceasing use of a urinal and changes to mopping practices (details not given). Snitkin et al [22] occasionally isolated CROs from drains, but not the original outbreak organism. Wendel et al [29] reported ongoing sporadic patient cases and environmental isolation despite enhanced IC measures and banning storage of patient material around sinks, however further strain typing has not been done. Seara et al [34] reported 7 additional cases in 2015, but none in 2016. They found hydrogen peroxide vapor to be the most effective disinfectant. Stjarne Aspelund et al [12] reported re-emergence of the outbreak organism after sink replacement, but no further cases or environmental isolation since July 2015, a change thought attributable to weekly flushing of sink drains and waste pipes with acetic acid/hot water.

DISCUSSION

Hospital Water Environment as a Reservoir of CROs

Drains/traps, sinks and faucets were the most common reservoirs of CROs identified. Others included portable hair washing basins, water samples, drink dispensers, toilet bowls/brushes, and shower equipment (Table 2). Initial seeding of these reservoirs was potentially due to contamination from affected patients [20, 22, 25, 32, 33, 36, 37]. Large, complex premise plumbing systems could have areas of stagnation and corrosion, variable nutrient and microbiology loads, and water temperatures ideal for promoting bacterial colonization and biofilm formation [3, 4]. Once colonized, there may be further propagation via the wastewater drainage system to distal sink drains connected to the initial reservoir, and via direct or indirect water contact to other patients [2].

P. aeruginosa was the most frequent organism (41% of studies), and distributed across all water reservoirs. *A. baumannii* was found predominantly in sink basins, and Enterobacteriaceae most commonly in drains. These findings may reflect biological differences in which *Pseudomonas* spp. and *Acinetobacter* spp. are environmental colonizers surviving in low-nutrient conditions, whereas Enterobacteriaceae are predominantly of human origin and their concentration in drains may represent a different ecosystem and inoculation directly from patient waste. However, all these organisms are capable of colonizing water system biofilms [3]. Knowing the predominant site for each organism may help guide surveillance strategies, outbreak investigations, and IC interventions.

Because they can be horizontally transferred on mobile genetic elements, carbapenemase genes represent the most concerning mechanism of carbapenem resistance and may be readily transmitted in environmental reservoirs. Tofteland et al [32] found evidence of possible environmental bla_{KPC}-plasmid transfer between *K. pneumoniae* strains, and Betteridge et al [7] concluded there was likely environmental intergenera plasmid exchange between Enterobacteriaceae. Resistance genes and associated mobile genetic elements should therefore be characterized in CRO outbreak investigations. Despite this, only 56% of studies assessed carbapenemase production. The most common enzymes identified were bla_{IMP}, bla_{KPC}, and bla_{VIM}, consistent with previous prevalence reports [1]. Phenotypic surveillance may facilitate the detection of novel carbapenemases.

CRO Transmission Between the Hospital Water Environment and Patients

There was evidence of CRO transmission between the environment and patients based on phenotypic [8] or genotypic methods in all studies assessing this, but most studies used relatively low-resolution methods. Fourteen studies used >1 genetic typing method (Table 4), potentially allowing for greater discrimination. Despite this, transmission routes were difficult to characterize, possibly due to both limited sampling and typing resolution. Whole-genome sequencing (WGS) is a highly discriminatory typing tool increasingly used in outbreak/transmission investigations. Two studies used WGS, with Snitkin et al [22] highlighting its advantages, including confirmation of a monoclonal outbreak, identification of unexpected modes of transmission, and tracking several potential resistance mutations in newly colistin-resistant isolates.

Previous studies have found that HCWs may facilitate nosocomial transmission of multidrug-resistant organisms [3]. Notably, there was no evidence of HCW colonization in the studies reviewed. However, only 19% assessed HCWs, perhaps underestimating the role of HCWs in CRO transmission. Other confounders could include inappropriate timing of sampling and observer effects if staff were aware of surveillance during sampling. Despite the absence of HCW colonization, 10 studies concluded that HCWs were probably implicated in CRO transmission [17, 18, 20, 22, 23, 25, 27, 32, 36, 37]. Colonization of a sink in a medication room attended only by HCWs was cited as evidence for this in 1 study [20]. Most studies also reported a significant reduction in transmission with enhancement of general IC measures, including reinforcement of hand hygiene and contact precautions. However, the relative contribution of HCWs to CRO transmission remains undefined.

Within the hospital there are numerous opportunities for patient exposure to water and drainage reservoirs [4]. Transmission may result from direct or indirect water contact,

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or from droplets created during water activities, highlighting the importance of water delivery and wastewater design to minimize these risks. Seven studies recognized that poor design or use of sinks, drains, and sluice areas may have contributed to institutional outbreaks [18, 20, 21, 26, 29, 30, 37]. Guidelines for design of hand-wash basins generally include a large basin to contain splashes, taps that are not aligned directly over drains to minimize aerosols, no plugs or overflows, and ensuring that basins are not used for disposal of patient-related waste [40]. This guidance varies by country and application of the recommendations probably varies between institutions.

Effective IC Strategies

IC strategies used mostly included bundled approaches involving enhanced general IC measures, disinfection, and replacing reservoirs, making it difficult to ascertain the relative contribution of individual approaches. Enhancing general IC measures led to a reduction in clinical cases in most studies but often did not completely terminate outbreaks.

Chemical disinfection was most useful for environmental colonization with *A. baumannii* and *P. aeruginosa*. Faucet contamination with *P. aeruginosa* was successfully treated with aldehydes [36] or hot water [31], and Enterobacteriaceae with bleach, hydrogen peroxide vapor or UV light [11]. Eradication of sink colonization with *A. baumannii* was possible with bleach [27] or hydrogen peroxide vapor [37], however chemical disinfection was not effective for Enterobacteriaceae. In drain colonization, some success with eradicating Enterobacteriaceae and *A. baumannii* was reported using bleach [11, 22, 28] or hydrogen peroxide vapor [11, 22, 34, 37]. One study reported ongoing suppression of *P. aeruginosa* with weekly disinfection of sinks and drainage systems with acetic acid/hot water [12]. Use of hydrogen peroxide vapor was most promising, but this method is expensive and was ineffective in liquid and foam form [13, 34]. Bleach, hot water, UV light, and aldehyde-based disinfectants were effective in some studies, but other studies reported failures with these agents. Other agents were also used without success (Figure 3). Notably, the concentrations and deployment of agents varied widely; standardizing approaches would allow for more comparable outcome assessment.

Overall, the most successful intervention was replacing reservoirs (Figure 3). Replacing taps contained 67% of outbreaks associated with *P. aeruginosa* affecting faucets [15, 16, 18, 36]. Replacement of drains/drainage systems and sinks was always successful for Enterobacteriaceae colonization [20, 32–34] but failed with *P. aeruginosa* [12, 21, 29]. Other effective strategies included drainage or sink redesign, sterile water for care of high-risk patients, and prohibiting the storage of clean patient material around a sink. These findings may also be relevant for water-environment associated outbreaks with organisms that harbor other resistance mechanisms, such as extended-spectrum- β-lactamases. A list of current recommendations from international and national organizations on the prevention and control of CROs can be found in Supplementary Table 2; no organization makes recommendations regarding environmental sampling or chemical disinfectant.

Review and Study Limitations

General limitations of our study include potential publication bias and the language and literature source restrictions used in this review. Healthcare water environments are probably often neglected in outbreak assessments, which may result in an underestimation of the problem.

The heterogeneity in study methods also limits the generalizability of conclusions. Measuring the clinical impact of outbreaks was difficult; 23 studies did not give prevalence data, and 13 did not assess mortality rates. Screening of patients and the environment varied widely with regard to the methods used, frequency, and site. Rectal swab/fecal samples are recommended for patient CRO colonization screening [5], but 10 studies only sampled other body sites, and 9 did not screen patients at all. Consequently patient colonization may have been underestimated in up to 60% of studies.

Differences in environmental sampling, such as collecting water samples before or after tap flushing and use of varying water volumes, may affect sampling sensitivity. Microbiological methods differed between studies, and although the use of selective culture media may have improved detection, organisms with near-breakpoint minimum inhibitory concentrations could have been missed, underestimating the true prevalence and extent of environmental contamination. Molecular tests may increase sensitivity [29].

CONCLUSIONS

Water environment-associated CRO outbreaks have been reported increasingly in the last 5 years. Critically ill and immunocompromised patients are particularly at risk. Drains/traps, sinks and faucets were most frequently colonized, and *P. aeruginosa* was the predominant organism. Standardizing patient and environmental screening, and microbiological methods, when investigating CRO outbreaks may allow earlier detection of reservoirs. Carbapenemases should be characterized, and strains typed to confirm relatedness. WGS is a promising tool for determining transmission routes, reservoirs, and resistance mechanisms.

The incorrect design and use of hand-wash basins and other water areas may propagate outbreaks. Various IC interventions have been used with mixed results for each organism and reservoir. A combination of interventions is probably required to terminate outbreaks and eradicate CROs from the environment. Useful approaches include reinforcing general IC measures,

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combined with chemical disinfection using hydrogen peroxide vapor. Further studies assessing the effectiveness of different chemical disinfection strategies are needed. Replacement and improved management of colonized water reservoirs is also probably an important component of control.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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"Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks

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Abstract

Objectives: Clinical utility of carbapenem antibiotics is under threat because of the emergence of acquired metallo-β-lactamase (MBL) genes. We describe an outbreak in an intensive care unit (ICU) possibly associated with contaminated sinks.

Design, setting and participants: Four clusters of gram-negative bacteria harbouring the MBL gene bla_{IMP-4} were detected in the ICU at Dandenong Hospital between November 2009 and July 2012. Epidemiological investigations were undertaken in order to identify a common point source. During September 2012, screening using rectal swabs for all ICU patients, and environmental swabs targeting all ICU handwashing sinks and taps were collected. Samples were cultured onto selective carbapenem-resistant Enterobacteriaceae (CRE) agar. Suspected CRE isolates were further characterised using the modified Hodge test and VITEK 2 and confirmed by polymerase chain reaction and sequencing of MBL genes. Clinical and environmental CRE isolates were typed by pulsed-field gel electrophoresis.

Results: Ten clinical isolates and one screening isolate of CRE (consisting of *Klebsiella pneumoniae* [5], *Serratia marcescens* [4], *Enterobacter cloacae* [1] and *Escherichia coli* [1]) were detected with the bla_{IMP-4} gene over the 30-month period. *S. marcescens* was isolated persistently from the grating and drain of eight central sinks. Molecular typing confirmed that clinical and environmental isolates were related. Tap water cultures were negative. Several attempts to clean and decontaminate the sinks using detergents and steam cleaning proved unsuccessful.

Conclusion: This report highlights the importance of identification of potential environmental reservoirs, such as sinks, for control of outbreaks of environmentally hardy multiresistant organisms.

One of the most concerning emerging resistance traits among gram-negative bacteria is the ability of these organisms to produce carbapenem-hydrolysing β-lactamases, which confer resistance to almost all β-lactams.[1] Carbapenem-resistant Enterobacteriaceae (CRE) are increasing in prevalence worldwide, causing growing concern, as they are often combined with non-β-lactam resistance to produce isolates that are multidrug resistant, have few treatment options available and are associated with high mortality rates.[2]

Although multiple resistance mechanisms have been identified, carbapenem resistance is often plasmid-encoded, allowing gene dissemination and a propensity to cause nosocomial outbreaks.[3-6]

We describe a CRE outbreak due to the presence of the metallo-β-lactamase gene bla_{IMP-4} in an intensive care unit (ICU) associated with contaminated sinks. This report highlights the key role of bacterial environmental contamination and sink design and usage in the propagation of CRE outbreaks.

Methods

Dandenong Hospital is a 440-bed tertiary referral hospital in Melbourne, Australia. The ICU has a 14-bed capacity, admitting both medical and surgical patients and averaging 1400 admissions yearly.

Ten ICU patients were found to have clinical specimens with Enterobacteriaceae harbouring the bla_{IMP-4} gene between November 2009 and July 2012. The ICU routinely screens for vancomycin-resistant enterococci (VRE) carriage using rectal swabs on admission, weekly and on discharge. During the 4-week period from 6 September to 4 October 2012, CRE screening was performed in addition to the routine screen for VRE.

Environmental screening for CRE targeting all 28 wet area locations (including 11 sinks and taps, as well as water fountains and ice machines) was performed. Samples were recollected from CRE positive locations after successive decontamination attempts. A total of 97 samples were collected from taps, water, grates and drains (10 cm down and above the water trap interface where biofilm may be expected to persist).

All environmental and patient screening swabs were cultured onto chromogenic agar (*Brilliance* CRE Agar, Oxoid). Suspect colonies were further characterised using the modified Hodge test and VITEK 2 (Biomérieux). All isolates with a meropenem minimum inhibitory concentration (MIC) ≥ 0.25 mg/L and a positive modified Hodge test result were forwarded for confirmation by polymerase chain reaction and molecular sequencing. Molecular typing using pulsed field gel electrophoresis (PFGE) was conducted on clinical and environmental isolates and interpreted as per the reference guidelines.[7]

Guidelines on ICU handwashing sink styles were reviewed to establish whether sinks met clinical design standards.[8]

Results

Ten clinical isolates (*Klebsiella pneumoniae* [$n = 5$], *Serratia marcescens* [$n = 4$] and *Enterobacter cloacae* [$n = 1$]) and one screening isolate (*Escherichia coli*) containing the bla_{IMP-4} gene were detected over the 30-month period. There were four distinct CRE clusters, commencing with three cases of *K. pneumoniae* in November 2009, followed by two cases of *S. marcescens* 6 months later, three cases (two *S. marcescens* and one *E. cloacae*) after another 11 months, and three cases of *K. pneumoniae* in July 2012.

Clinical characteristics, patient demographics and ICU admission details of all 11 patients are summarised in Box 1. Patients acquired CRE after a median length of stay in ICU of 10 days (range, 3–134 days). No

1 Demographics and intensive care unit (ICU) admissions for patients with carbapenem-resistant Enterobacteriaceae isolates

Patient	Underlying condition	Year of first positive culture	Culture type	Organism	Total hospital stay (days)	ICU stay (days)	ICU admissions in past 12 months	Total carbapenem therapy (days)	Outcome
1	Small bowel perforation, post-hernia repair	2009	Blood	*Klebsiella pneumoniae*	229	134	0	14	Discharged
2	Urosepsis, multiorgan failure	2010	Urine	*K. pneumoniae*	44	16	0	0	Discharged
3	Bilateral pneumonia	2010	Bronchial washings	*K. pneumoniae*	61	33	0	0	Discharged
4	Pneumonia	2010	Intercostal catheter swab	*Serratia marcescens*	44	32	0	2	Discharged
5	Pneumonia	2010	Endotracheal aspirate	*S. marcescens*	46	6	0	0	Discharged
6	Buttock abscess	2011	Urine	*S. marcescens*	49	10	0	0	Discharged
7	Pneumonia	2011	Penile swab	*S. marcescens*	46	22	0	3	Discharged
8	Congestive cardiac failure	2011	Sputum	*Enterobacter cloacae*	57	4	0	0	Died
9	Ischaemic stroke	2012	Sputum	*K. pneumoniae*	31	3	0	3	Died
10	Periprosthetic hip fracture	2012	Urine	*K. pneumoniae*	46	8	0	0	Discharged
11	Ketoacidosis	2012	Rectal screen swab	*Escherichia coli*	11	4	2	0	Discharged

patient died due to clinical CRE infection. Patient 1 had a prolonged CRE bacteraemia that responded to removal of a central venous catheter, the presumed source of infection.

A total of 111 rectal swabs were collected from 71 patients. Only one patient (Patient 11) was CRE-positive with an *E. coli* isolate detected.

Antibiotic resistance profiles of clinical isolates indicated resistance to β-lactams and meropenem with MICs ⩾1 mg/L. All *S. marcescens* clinical isolates were sensitive to piperacillin/tazobactam, ciprofloxacin and amikacin, while *K. pneumoniae* and *E. coli* were only sensitive to amikacin. *S. marcescens* environmental isolates showed a higher meropenem MIC of ⩾16 mg/L.

S. marcescens was the only species recovered from environmental samples and was isolated persistently, even after six attempts to decontaminate the grates and drains of eight of the 11 central sinks in the ICU. Tap spout and water cultures were negative for CRE.

Three of the four *S. marcescens* clinical isolates from 2010 and 2011 were indistinguishable or closely related by PFGE to four isolates from sinks. *S. marcescens* was isolated from an intercostal catheter swab of Patient 4, matching two sink isolates; Patient 5 had an endotracheal aspirate identical to two different sink locations; and the urine isolate from Patient 6 was closely related to a different sink. The isolate from Patient 7 was unable to be typed by PFGE.

Sink inspection revealed aged and deteriorating porcelain, even though sinks were only installed in 2005. Sink design did not comply with Australasian clinical design standards,[8] with a small, shallow sink and a tap that directed water over the drain (Box 2). The design of the ICU sinks led to poor use for hand hygiene (although measured hand hygiene compliance using alcoholic hand rub was around 70% within the unit) and the potential for organisms residing down the drain to be splashed back onto staff hands or contaminate patient areas. It was also revealed that the handwashing sinks had been used incorrectly, with staff disposing clinical waste and residual antibiotics directly into drains. Further enquiry also revealed that a single brush had been used to clean down the drains of all sinks in the ICU without disinfection between sinks.

Cleaning was attempted in an effort to rid the organisms from the sinks.

2 Existing design of intensive care unit sink compared with a design that complies with Australasian standards[8]

A: Existing sink, showing water spray directly over drain. **B:** Compliant sink design, showing larger basin and less forceful water flow directed away from drain, to prevent splash back and contamination with drain contents. ◆

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First, cleaning of grates and drains using single-use, soft brushes was attempted, but repeat screening revealed continued CRE growth. Next, in addition to the brushes, hypochlorite deep cleaning was used after the scrub; however, heavy CRE growth was again evident 1 week later. Finally, an attempt using pressurised steam decontamination (Jetsteam Maxi with plunger tool attachment, Duplex) for 1 minute at 170°C on grates and drains appeared to eradicate almost all CRE at Day 1 (one sink remained colonised); however, repeat testing 3 days after steam treatment showed re-emergence of CRE in all previously affected sinks.

Discussion

We report an outbreak of CRE in an ICU with identical organisms isolated from patients and an environmental source (sinks).

Dissemination of carbapenemase-resistant bacteria has been reported previously in an ICU in Australia, with 62 patients infected or colonised with gram-negative bacteria from multiple genera containing the bla_{IMP-4} gene.[9] The gene is carried on a highly mobile plasmid making it efficient for nosocomial transmission. The gene can reside in multiple genera of hardy environmental organisms that can establish a reservoir within biofilm.

Identifying the outbreak using molecular methods allowed us to establish clonality between clinical and environmental isolates and to propose a mechanism whereby patient contamination may have occurred. Although we cannot prove that the sinks were the source of patient infection, the persistence of the organism within the environment was a concern. Attempts at sink sterilisation were futile, and complete eradication will require future sink removal and replacement with appropriately designed sinks.

Others have reported outbreaks of multiresistant bacteria such as extended-spectrum β-lactamase-producing *K. pneumoniae* linked to imperfect sink design requiring sink cleaning, replacement and/or improved sink practices to successfully control the outbreaks.[10]

Our data should act as a reminder on the importance of appropriate hospital design, adequate environmental cleaning and antimicrobial stewardship in the hospital setting.

Competing interests: No relevant disclosures.

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Stamps of greatness

Karl Landsteiner (1868–1943)

LANDSTEINER was born on 14 June 1868 in Vienna, Austria. He studied medicine at the University of Vienna and graduated in 1891. For 5 years he studied chemistry in Wurzburg (with Emil Fischer) and Munich, but then returned to Vienna and transferred his interest to pathological anatomy.

In 1898 he became an assistant to the institute of pathological anatomy in Vienna, in 1908 the prosector at the Wilhelminaspital, and in 1911 professor of pathology at the University of Vienna. After the upheavals of World War I he went first to Holland and then, in 1922, to the Rockefeller Institute for medical research in New York, where he continued work even after being made Emeritus Professor in 1939.

From 1900 he studied the agglutination of blood from different individuals, and in 1909 he outlined the human blood types, thus making blood transfusion possible. Landsteiner received the Nobel Prize in Medicine in 1930 for this work, which he continued at the Rockefeller Institute.

During his time in Vienna he also studied (in collaboration with others) poliomyelitis, first showing that the disease was infectious, and then isolating the causative virus.

In the early 1940s at the Rockefeller Institute, he was part of the team that discovered the Rh factor in blood, adding to his work on blood transfusion and compatibility of donors.

Throughout his career he contributed many fundamental principles to the science of immunology and knowledge of antigens and anaphylaxis, and was the recipient of many academic honours and awards. He died on 26 June 1943 after suffering a heart attack while working in his laboratory two days earlier.

Philatelically he was honoured on the centenary of his birth in 1968 by both Austria and Germany.

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PLOS ONE

A Long-Term Low-Frequency Hospital Outbreak of KPC-Producing *Klebsiella pneumoniae* Involving Intergenus Plasmid Diffusion and a Persisting Environmental Reservoir

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Abstract

Background: To study the molecular characteristics of a long-term, low frequency outbreak of bla_{KPC-2} in a low prevalence setting involving the hospital environment.

Methodology/Principal Findings: KPC-producing bacteria were screened by selective chromogenic agar and Real-Time PCR. The presence of antibiotic resistance genes was ascribed by PCRs and subsequent sequencing, and the KPC-producing isolates were phylogenetically typed using PFGE and multi-locus sequence typing. Bla_{KPC-2}-plasmids were identified and analysed by S1-nuclease-PFGE hybridization and PCR based replicon typing. A ~97 kb IncFII plasmid was seen to carry bla_{KPC-2} in all of the clinical isolates, in one of the isolates recovered from screened patients (1/136), and in the *Klebsiella pneumoniae* and *Enterobacter asburiae* isolates recovered from the environment (sinks) in one intensive care unit. The *K. pneumoniae* strain ST258 was identified in 6 out of 7 patients. An intergenus spread to *E. asburiae* and an interspecies spread to two different *K. pneumoniae* clones (ST27 and ST461) of the bla_{KPC-2} plasmid was discovered. *K. pneumoniae* ST258 and genetically related *E. asburiae* strains were found in isolates of both human and environmental origins.

Conclusions/Significance: We document a clonal transmission of the *K. pneumoniae* ST258 strain, and an intergenus plasmid diffusion of the IncFII plasmid carrying bla_{KPC-2} in this outbreak. A major reservoir in the patient population could not be unveiled. However, the identification of a persisting environmental reservoir of strains with molecular determinants linked to human isolates, suggests a possible role of the environment in the maintenance of this long-term outbreak.

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Introduction

The *Klebsiella pneumoniae* carbapenemase (KPC) was first identified in the USA in a *Klebsiella pneumoniae* isolate dated from 1996[1] and has subsequently been reported worldwide.[2] High prevalence rates of KPC have been reported from regions and countries such as USA, Israel, China, and Greece, while a number of countries have reported hospital outbreaks and sporadic cases of KPC-producing *K. pneumonia.* [2]

KPC has been identified in various species of Enterobacteriaceae and non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* [2] Further, bla_{KPC} has been identified on plasmids differing in size and structure. This broad species distribution and plasmid diversity is likely due to the location of bla_{KPC} in a functional Tn*3*-based transposon structure (Tn*4401*) with a high transposition frequency.[3] With respect to *K. pneumoniae*, multilocus sequence typing (MLST) has shown that the global dissemination of KPC-producing *K. pneumoniae* is dominated by isolates belonging to a hyperepidemic clonal complex including sequence type (ST) 258.[4] Although the dissemination and outbreaks of KPC seems to be associated with specific clones several reports are describing outbreaks where several clones and different species are involved [5,6] as well as individual patients containing different species harbouring $bla_{KPC.}$[7,8,9]

In many countries the emergence of KPC-producing bacteria has been associated with import of isolates from high prevalent areas. In Norway, the first case of *K. pneumoniae* with KPC was associated with import from Greece in 2007 at a hospital in the southern part of Norway.[10] Subsequently, five additional clinical isolates were identified at the same hospital and a nearby hospital from patients with no recent history of travel or hospitalisation abroad. In this study we describe this long-term outbreak with regards to the molecular characteristics of these isolates, plasmid

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content and dissemination, and the identification of KPC-producing isolates in the hospital environment.

Materials and Methods

Hospital setting

Sørlandet Hospital (SH) is a 683-bed general hospital enterprise located in three different cities in the southern part of Norway. Due to sharing of some specialized medical functions a certain degree of interchange of patients between the hospitals occurs. Two hospitals belonging to Sørlandet Hospital, SH-Arendal (SH-A), and SH-Kristiansand (SH-K), as well as a tertiary hospital (Oslo University Hospital – Rikshospitalet (OUH-RH)) where involved in this outbreak. A screening programme was implemented during outbreak investigation involving a 12-bed (8 single rooms and one 4-bed room) surgical/medical intensive care unit (ICU-A) at SH-A.

Faecal and environmental screening programme

Faecal screening of ICU-A patients was initiated after the identification of clinical case number 6 (K66-62). Prospective screening was performed on patients ($n = 50$) on admittance and discharge from the ICU-A during a first screening period from 8th of May to 19th of October 2010, and then only on discharge from the ICU-A during a second screening period from 19th of October to 1st of April 2011 ($n = 65$). Retrospective screening was attempted on 29 patients that had been hospitalized in the ICU-A at overlapping intervals with patient 6. Seventeen of these were still hospitalized and screened, whereas 4/12 discharged patients were subjected to screening. Patients staying in the ICU-A for more than 14 days were screened repeatedly every second week.

Screening of the environment was undertaken on two occasions, from the 7th to the 9th of June 2010 and on the 27th of December 2010. The screenings involved sink drains in the ICU-A (n = 19), the neighbouring post-operative unit, the coronary unit, and taps for water to dialysis machines in the ICU-A. Environmental samples were obtained with sterile, cotton-tipped swabs (COPAN swabs®). Most areas examined were moist, but the cotton-tip was occasionally moistened when the area examined appeared relatively dry. Screening positive locations were disinfected and then controlled by monthly screening.

Isolation of KPC-producing bacteria and detection of bla_{KPC} during the screening programme

Screening for KPC-producing bacteria was performed using ChromID ESBL agar medium (bioMerieux, la Balme-les-Grottes, France) and an enrichment medium (tryptic-soy broth containing 2 mg/L cefpodoxime). Real-Time PCR of bla_{KPC} was performed directly on suspensions from the swab, enrichment medium, and colonies from the ChromID ESBL agar[11].

Bacterial strains and clinical data

Clinical data and data on bacterial strains investigated in this study are listed in Table 1 and 2.

Species identification

Bacterial identification was performed using the VITEK2 ID-GNB system (bioMérieux, Marcy l'Etoile, France) and/or MALDI-TOF (Microflex LT, Bruker Daltonics) with the MALDI Biotyper 3.0 software version.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined using Etest (bioMérieux) according to the manufacturer's instruction. The results were interpreted using breakpoints from the European Committee for Antimicrobial Susceptibility Testing.[12]

Molecular characterisation of KPC-producing isolates

PCR and sequencing for bla_{KPC}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and a multiplex PCR for the most prevalent plasmid-borne bla_{AmpC} -genes was performed as previously described (Samuelsen JAC 09). Isolates were typed by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested total genomic DNA.[10] Strain relatedness was analysed using BioNumerics version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium) using the band-based dice coefficient and the unweighted pairs geometric-matched analysis dendrogram with a position tolerance of 1% for optimization and band comparison. Final comparison of bands for the interpretation of relatedness was performed in accordance with Tenover criteria.[13] Multi-locus sequence typing (MLST) was performed on *K. pneumoniae* isolates according to the protocol described on the *K. pneumoniae* MLST web site http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Plasmid analysis

Plasmid profiling was performed using S1-nuclease digested plasmid DNA, separated by PFGE.[10] Subsequently, plasmid DNA was blotted onto nylon membrane and hybridised with bla_{KPC} specific probes to identify carrier plasmids. Furthermore, plasmids were classified into incompatibility groups using the PCR based replicon-typing (PBRT) scheme of Carattoli[14] and hybridisation with replicon FII specific probes as previously described.[10]

Transfer of resistance

Conjugal-transfer experiment was carried out in Luria-Bertani broth at 37°C for clinical strain K47-25 as donor and rifampicin-resistant E. coli J53-2 as recipient, mixed in a 1:9 ratio respectively. Transconjugants were selected on LB-agar plates (Becton Dickinson, Sparks, MD) supplemented with 100 mg/L rifampicin (Sigma-Aldrich, St. Louis, MO) and 2 mg/L ceftazidime (Sigma-Aldrich).

Ethics statement

This study focuses on the molecular characteristics of bacterial isolates collected as part of the clinical management and microbiology routine work. Faecal screening was performed according to the guidelines from the local hospital and collection of clinical data as part of outbreak investigations for implementation of appropriate infection control measurements. Consequently, ethical approval was not obtained for the study.

Results

Clinical cases

Six KPC-producing *K. pneumoniae* isolates from clinical samples were recovered in SH from November 2007 to April 2010 (Table 1 and Table 2). The first two isolates had been characterised previously.[10] The first patient had been hospitalized on Crete in September 2007 prior to hospital admission in Norway. The other patients had no history of recent travel abroad. 5 isolates were recovered from SH-A, whereas the isolate from patient 2 was recovered from SH-K. After the identification of KPC-producing *K. pneumoniae*, measures for contact isolation were initiated for all

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Table 1 Molecular characteristics of outbreak strains.

Isolate	Source (P/E/F) [1]	Month of isolation	Species	PFGE	MLST	bla_{KPC} [2]	bla_{SHV} [2]	bla_{TEM} [2]	Plasmid profiling (kB) [3]	Plasmid hybridization bla_{KPC} [4]
K47-25	P1	Nov 07	*K. pneumoniae*	A2	258	KPC-2	SHV-11/-12	TEM-1	40, 97, 160	97,160
K48-58	P2	March 08	*K. pneumoniae*	A2	258	KPC-2	SHV-11/-12	TEM-1	97, 242	97
K52-74	P3	Oct 08	*K. pneumoniae*	A2	ND	KPC-2	SHV-11/-12	TEM-1	40, 97, 194	97
K54-05	P4	Jan 09	*K. pneumoniae*	A2	ND	KPC-2	SHV-11	TEM-1	40, 97/100	97
K57-33	P5	March 09	*K. pneumoniae*	B	461	KPC-2	SHV-1	TEM-1	48, 97, 225	97
K66-62	P6	April 10	*K. pneumoniae*	A1	ND	KPC-2	SHV-11/-12	TEM-1	40, 97, 194	97
K66-73	P7/F	May 10	*K. pneumoniae*	A1	258	KPC-2	SHV-11/-12	TEM-1	40, 97, 194	97
K66-74	P7/F	May 10	*E. asburiae*	C1	-	KPC-2	NEG	TEM-1	97,145	97
K67-04	P1	Jan 10	*K. pneumoniae*	A1	258	NEG	SHV-11/-12	NEG	40, 194	NEG
K67-05	P4	May 09	*K. pneumoniae*	A2	258	NEG	SHV-11/-12	NEG	40, 194	NEG
K67-06	P3	March 10	*K. pneumoniae*	A2	258	NEG	SHV-11/-12	NEG	40, 194	NEG
K67-11	E5	June 10	*K. pneumoniae*	A2	ND	KPC-2	SHV-11/-12	TEM-1	40, 97, 194	97
K67-12	E5	June 10	*E. asburiae*	C2	-	KPC-2	NEG	TEM-1	97,145	97
K67-13	E6	June 10	*K. pneumoniae*	D	27	KPC-2	NEG	TEM-1	45, 97, 200	97
K67-14	E6	June 10	*K. pneumoniae*	D	27	KPC	NEG	TEM-1	45, 97, 200	97
K67-15	E5	June 10	*K. pneumoniae*	A1	ND	KPC	SHV-11/-12	TEM-1	40, 97, 194	97
K67-16	E5	June 10	*E. asburiae*	C2	-	KPC	NEG	TEM-1	97,145	97

[1)]P1–6 = clinical specimen from patients 1–6, P7/F = fecal screen patient 7, E = specimen from environmental screen (rooms 5, 6).
[2)]bla_{CTX-M} was negative in all isolates. bla_{pAmpC} was negative in isolates from patients 1–6, others ND.
[3)]Plasmid profiling (S1-nuclease digested DNA (kB)).
[4)]Plasmid DNA hybridization with bla_{KPC} specific probes.
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patients. When patient 1 was readmitted at SH-K in March 2008 measurements for contact isolation was however not implemented. Retrospective analysis of patient histories revealed that all SH-A patients had been hospitalized in the same ICU (ICU-A) as the index patient prior to detection of the KPC-producing isolates. However, during their stay the patients had been admitted to 12 different wards. Further, conventional epidemiological evaluation[15] was able to establish epidemiological links between patient 1 and 2, and 3 and 4, while a possible link was suggested between patient 4 and 5 (Table 2). Time and space overlaps between the clinical cases 1 and 3 as well as 5 and 6 could not be provided for, representing time intervals of 10 and 11 months, respectively.

Several recognized risk factors for ESBL colonization and infection[15,16,17,18] were present in all the patients that had been admitted to the ICU-A prior to isolation of KPC-producing *K. pneumoniae* (patient 1, 3–6) (Table 2). All patients were above 65 years of age. KPC-producing *K. pneumoniae* bacteraemia was recognized in one patient, whereas the findings in the other patients were regarded as most likely to be colonisations associated with artificial ventilation or urinary catheter. One patient died, but the KPC-producing *K. pneumoniae* was not considered to be attributable to infection or death in this patient (Table 2).

ESBL-producing bla_{KPC}-negative *K. pneumoniae* were also recovered in ambulatory urinary samples due to urinary tract infections (UTI) from three of the of the patients 26, 16, and 4 months after the initial isolation of the KPC-producing *K. pneumoniae* isolates, respectively (Table 1).

Patient screening

In the patient screening programme only 1/136 patients were positive for KPC-producing Enterobacteriaceae (patient 7). Patient 7, being hospitalized in room 5 for his entire ICU-A stay, was identified with KPC-producing *K. pneumoniae* (K66-73) and *Enterobacter asburiae* (K66-74) upon discharge (Table 1), while negative upon admission five days earlier. Room 5 was disinfected using a persulfate based disinfectant after having been occupied by patient 6 and then patient 7 was the first to be admitted two days later.

Environmental screening

KPC-producing bacteria were detected in 4/19 environmental locations in the ICU-A (sink drains in room 5, 6, 9, and the rinsing room). Bla_{KPC}-positive *K. pneumoniae* were identified in June 2010 from the sink drains in room 5 (K67-11 and K67-15) and room 6 (K67-13 and K63-14), and bla_{KPC}-positive *E. asburiae* from the sink drain in room 5 (K67-12 and K67-16) (Table 1). The sinks and sink traps were decommissioned and the connecting pipe elbows were disinfected using a chlorine disinfectant before new sinks and sink traps were installed. Monthly environmental screening of these positive locations was then undertaken. Bla_{KPC}-positive *K. pneumoniae* was again recovered from the sink in room 6 on two occasions in December 2010 triggering a new environmental screening in the ICU-A (data not shown). Additional sink drains in the ICU-A was investigated, and two new locations that were not subjected to investigation in June 2010 were now identified with bla_{KPC}-positive *K. pneumoniae* (room 9) and bla_{KPC}-positive *E. asburiae* (room 9 and the rinsing room, data not shown).

Although bla_{KPC}-positive isolates have been identified in the environment no additional clinical cases has been identified since patient 6.

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Table 2 Clinical data and risk factors for outbreak patients.

Isolate	Pat-ient	Species	Specimen	Hospita-lization abroad (country)	Month admitted	LOS[6]/LOS prior to diagnosis	Patient overlap[7]	Antibiotic treatment[8]	Other risk factors (I/S/R/D)	Role in infection	Discharged to
K47-25	P1	*K. pneumoniae*	Expectorate	Greece	Nov 07	51/6	Yes[3)]	MEM, PTZ	I/R	Uncertain	Home
K48-58	P2	*K. pneumoniae*	Urine	No	March 08	20/8	Yes[3)]	None	None [1)]	None	Nursing home
K52-74	P3	*K. pneumoniae*	Blood	No	Sep 08	36/23	No[5)]	MEM, TOB, CTX	I/R/D	Yes	Physical rehabilitation
K54-05	P4	*K. pneumoniae*	Expectorate	No	Nov 08	106/47	Yes[4,5)]	MEM,TOB	I/R/D	Uncertain	Physical rehabilitation
K57-33	P5	*K. pneumoniae*	Urine	No	Nov 08	178/131	Yes[4)]	MEM, IMI	I/S/R	Uncertain	Nursing home
K66-62	P6	*K. pneumoniae*	Expectorate	No	April 10	24†/17	No	MEM	I/S/R	None	Diseased (in hospital)
K66-73 K66-74	P7	*K. pneumoniae E. asburiae*	Feces/fecal screening	No	May 10	13/5	No	CTX	I/R	None	Home

[1)]Except urinary catheter.
[2)]I = admission to ICU, S = recent surgery (laparotomi), R = artificial ventilator use, D = subjected to haemodialysis.
[3)]Patient 1, being readmitted to SH-K, and patient 2 had been hospitalized in the same corridor for two days in March 2008 at SH-K although at separately staffed wards (Med. 2A-K and Med. 2C-K). Measures for contact isolation were not implemented for patient 1 on this occasion.
[4)]Patient 4 and patient 5 had been admitted simultaneously to the ICU-A, and had also been referred to a tertiary hospital (OUS-RH) at overlapping intervals.
[5)]Patient 3 and patient 4 were hospitalized in the ICU-A two days apart.
[6)]LOS = length of stay (days).
[7)]Overlap between patients in time and wards.
[8)]Anti G-negative antibiotics prior to diagnosis
doi:10.1371/journal.pone.0059015.t002

Molecular characterisation of the isolates

PFGE and MLST typing revealed that 14 *K. pneumoniae* isolates from both patients and the environment, including the three bla_{KPC}-negative *K. pneumoniae* UTI-isolates, belonged to two clonally related pulsotypes (A1 and A2), that by MLST were typed to ST258 (Figure 1, Table 1). Distinct *K. pneumoniae* PFGE/MLST-types were recovered in patient 5 (K57-33; pulsotype B/ST461) and isolates from the sink drain in room 6 (K67-13 and K67-14; pulsotype D/ST27). PFGE of the *E. asburiae* isolates revealed two closely related pulsotypes corresponding to the *E. asburiae* (pulsotype C1) isolate from the screening patient (patient 7; K66-47) and the two *E. asburiae* (pulsotype C2) isolate from room 5 (K67-12 and K67-16). The bla_{KPC}-gene was sequenced in all isolates from patients and from the environmental in June 2010 and found to be bla_{KPC-2} (Table 1). The bla_{KPC}-positive *K. pneumoniae* ST258 isolates harboured bla_{SHV-11}, bla_{SHV-12}, and bla_{TEM-1}, whereas the three bla_{KPC}-negative *K. pneumoniae* ST258 UTI-isolates, harboured bla_{SHV-12} and bla_{SHV-11}, but were negative for bla_{TEM}. The distinct *K. pneumoniae* STs in patient 5 (K57-33) and from room 6 (K67-13 and K67-14) harboured bla_{SHV-1} and lacked the bla_{SHV}-gene, respectively. The bla_{KPC}-positive *E. asburiae* isolates were devoid of bla_{SHV} but harboured bla_{TEM-1} (Table 1). PCRs for bla_{CTX-M} and the most prevalent plasmid-borne bla_{AmpC} genes were negative.

Plasmid analysis

S1-nuclease-PFGE profiles revealed the presence 2-3 plasmids in all isolates ranging from~40–~240 kb (Table 1, Figure 2). Hybridization with bla_{KPC}-specific probes identified a bla_{KPC}-carrier plasmid to approximately 97 kb in all bla_{KPC}-positive isolates of *K. pneumoniae* and *E. asburiae*. In the bla_{KPC}-positive *K. pneumoniae* isolate from patient 1 (K47-25) an additional bla_{KPC}-plasmid of ~160 kb was identified. PBRT and hybridization characterized the ~97 kb plasmid as an IncFII replicon plasmid. Except for the 97 kb bla_{KPC}-plasmid, separate plasmid profiles were recognized in the other *K. pneumoniae* pulsotypes/STs and the closely related *E. asburiae* clones. The plasmid profile among the *E. asburiae* isolates were identical (Table 1, Figure 2).

Interestingly, the plasmid profile of the pulsotype A/ST258 bla_{KPC}-negative *K. pneumoniae* clinical UTI isolates was devoid of the ~97 kb bla_{KPC}-plasmid, indicating loss of this plasmid (Table 1).

Transferability of bla_{KPC} resistance

In vitro plasmid-transfer experiments using K47-25 as the donor strain to *E. coli* resulted in transconjugants with a frequency of 2.5×10^{-7} per donor cells.

Antimicrobial susceptibility

The antimicrobial susceptibility profile of the isolates is shown in Table 3. Variable levels of reduced susceptibility to carbapenems were observed in all the bla_{KPC}-positive isolates whereas the three ESBL-positive bla_{KPC}-negative *K. pneumoniae* ST258 UTI isolates were susceptible to carbapenems (Table 1). A similar susceptibility profile with high-level resistance to penicillins and cephalosporins, resistance to ciprofloxacin, tobramycin, and amikacin, but susceptibility to gentamicin, were common to all *K. pneumoniae* ST258/pulsotype A isolates. In contrast, the other bla_{KPC}-positive *K. pneumoniae* pulsotypes were susceptible to ciprofloxacin, tobramycin, amikacin, and the gentamicin MIC was lower. The bla_{KPC}-positive *E. asburiae* isolates found in the screening positive patient (patient 7) and the environment showed resistance to ciprofloxacin, but were susceptible to the aminoglycosides.

All bla_{KPC}-positive *K. pneumoniae* ST258 isolates were resistant to colistin. 2 of 3 bla_{KPC}-negative *K. pneumoniae* UTI isolates were susceptible to colistin, whereas one isolate was resistant (K67-05) displaying a clearly visible double zone (MIC 0.5 mg/L and MIC 16 mg/L).

Discussion

The dissemination of multidrug resistance plasmids among Gram-negative bacteria is one of the major factors contributing to

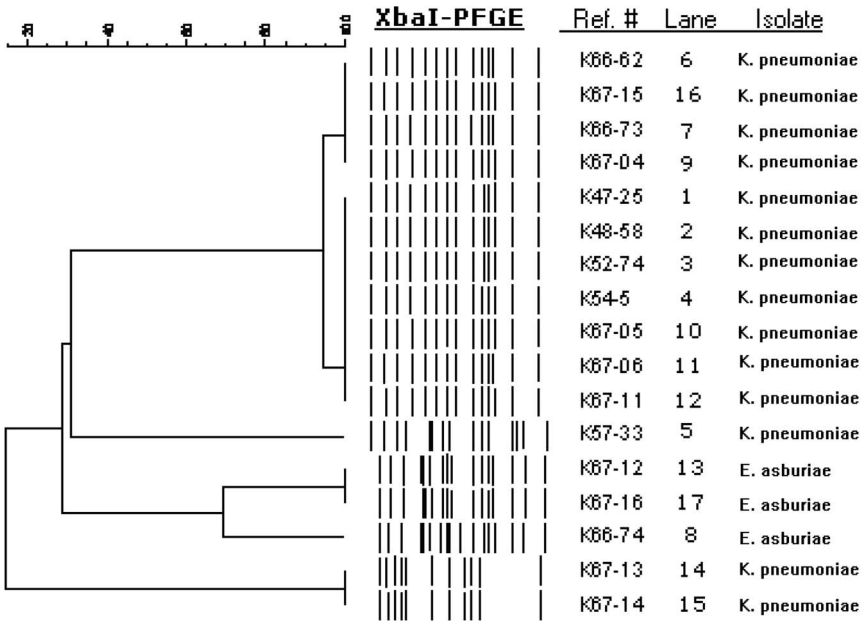

Figure 1. Dendrogram of XbaI-digested genomic DNA. Strains of KPC-producing *K. pneumoniae*- and *E.asburiae*-isolates and $ESBL_A$-producing *K. pneumonia* are shown with percentages of similarity to the right of the dendrogram.
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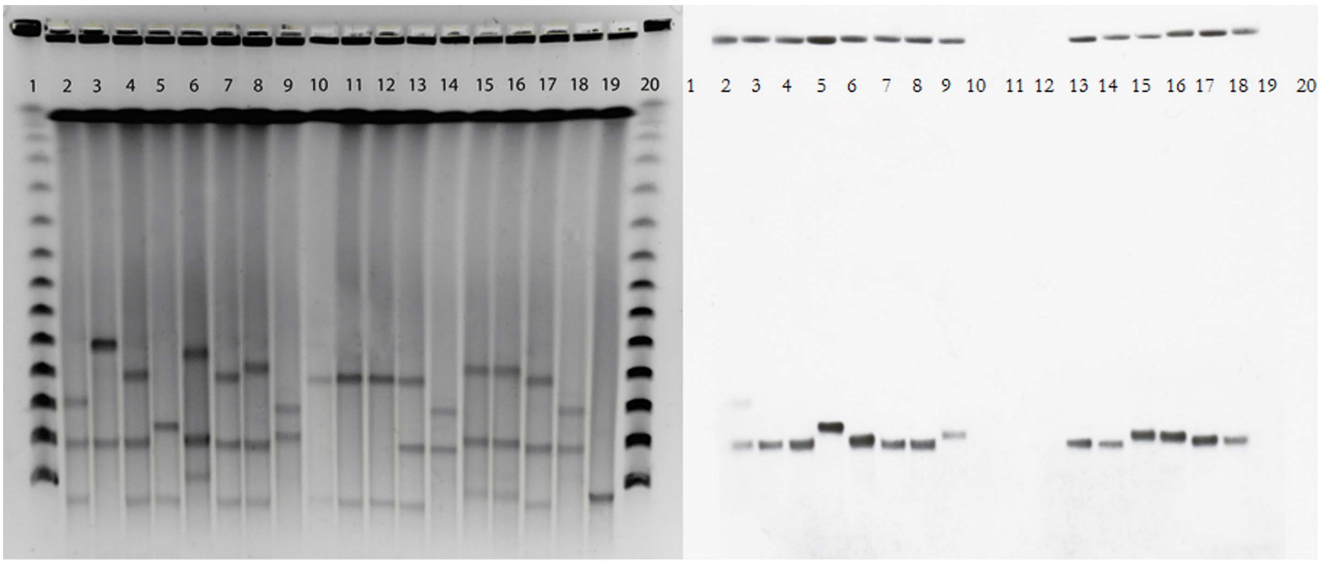

Figure 2. PFGE of S1 nuclease-digested total DNA. Lanes 1 and 20, phage λ DNA ladder (concatemers of 48.5 kb); lane 2, K47-25; lane 3, K48-58; lane 4, K52-74; lane 5, K54-05; lane 6, K57-33; lane 7, K66-62; lane 8, K66-73; lane 9, K66-74; lane 10, K67-04; lane 11, K67-05; lane 12, K67-06; lane 13, K67-11; lane 14, K67-12; lane 15, K67-13; lane 16, K67-14; lane 17, K67-15; lane 18, K67-16; lane 19, *K. pneumoniae* bla_{KPC} -negative control strain.
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the spread of antimicrobial resistance.[19] However, information about plasmid-dissemination during outbreaks is relatively limited. Often the focus is restricted to specific bacterial species and the dissemination of plasmids into different species might be overlooked. Highly mobile plasmids resulting in 'plasmid-borne outbreaks' may delay the recognition of an outbreak, and adds an additional layer of complexity to the molecular investigation of such outbreaks.[20] Plasmids have previously been linked to outbreaks of multi-drug resistant (MDR) Gram-negative bacteria[21,22,23,24,25], including nosocomial outbreaks of bla_{KPC}-containing plasmids in some studies.[5,7,8,20,26,27,28,29] Here we describe the dissemination of a KPC-plasmid between strains and species during a small long-term nosocomial outbreak in a low-prevalence setting as well as the possible role of the environment in this context. To the best of our knowledge this is the first nosocomial outbreak report of KPC-producing bacteria from the Nordic countries.

I*n vivo* mobility is indicated by identification of the ~97 kb IncFII bla_{KPC-2}-plasmid in four distinct MLST/PFGE types of *K. pneumoniae* and *E. asburiae*. This observation was supported by successful *in vitro* conjugation of bla_{KPC}-plasmids. bla_{KPC} – containing Tn*4401* has been documented on plasmids of different sizes belonging to different incompatibility groups.[30,31,32,33,34] While mostly being reported on broad host IncN plasmids[31], bla_{KPC} has recently been reported on narrow host range IncFII plasmids as well, promoting efficient spread within members of Enterobacteriaceae.[33,35,36]

It is well known that plasmid-transfer can occur *in vivo*, but less is known about plasmid-transfer occurring in the hospital environment. We can only speculate were the plasmid-transfer has occurred in this setting. The first clinical cases were associated with the hyperepidemic *K. pneumoniae* ST258 strain before plasmid-dissemination were observed into other *K. pneumoniae* STs (ST27 and ST461) and *E. asburiae*, all of them devoid of non-β-lactam resistances except the latter displaying low-level ciprofloxacin resistance. The KPC-producing *E. asburiae* remained clinically silent throughout the outbreak and first appeared in faecal screening of patient 7. Faecal screening was not performed on any of the clinical patients, thus whether KPC-producing *E. asburiae* was carried by any of these patients remains elusive. The finding of a distinct *K. pneumoniae* (ST27) with the ~97 kb KPC-plasmid in the hospital environment but not in patients could either be due to the unrecognized presence in patients and *in vivo* plasmid-transfer or that the plasmid-transfer have occurred in the environment before acquisition by a patient.

Interestingly, loss of bla_{KPC}-plasmids, ~160 kb plasmid in patient 1 and the ~97 kb plasmid in the three bla_{KPC}-negative *K. pneumoniae* UTI isolates, was observed in *K. pneumoniae* ST258 indicating either a high fitness cost or reduced plasmid stability. Experiments to determine the fitness cost related to the ~97 kb plasmid and its stability are ongoing.

Reservoirs in the patient or health care worker populations and the environment represent principle modes of spread in nosocomial outbreaks with the patient population being the most important reservoir in high-frequent outbreaks.[37] Introduction of the *K. pneumoniae* ST258 clone has caused major outbreaks in many hospitals.[38] It is possible that a high level of adherence to standard precautions prevented the establishment of a major gastrointestinal reservoir in our patients, and thus, the prerequisite of a major high-frequent outbreak, were not present.[37,39,40,41] The lack of a patient reservoir and the persistence of the outbreak despite the long interval between known clinical cases could indicate other possible reservoirs contributing to occasional transmissions and maintenance of the outbreak. Health care workers were not screened for faecal or hand carriage partly as no individuals with additional risk factors including dermatitis[37] could be identified and partly due to the fact that the current Norwegian health authority guidelines advocates against screening of health care workers in nosocomial outbreaks of multidrug resistant Gram-negative bacteria.[42] KPC-producing bacteria were detected in as much as 21% of environmental locations in screening samples from sink drains in the ICU-A. Moist surfaces and especially sink drains have been focused in several studies as a possible environmental source of transmission of Enterobacteriaceae, especially *Klebsiella* spp, to patients.[43,44,45,46,47,48] Although the environment were decontaminated and sinks were

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replaced, KPC-producing *E. asburiae* and *K. pneumoniae* were recovered during further environmental screening, suggesting that these strains can survive well in that environment. Contamination of the hands of health care workers due to occasional backsplash during hand washing in a contaminated sink and sink drains[44,46,47,49] or through moist surfaces near sinks and faucets[45] has been suggested as a possible mode of transmission to health care workers and subsequently to patients in the ICU setting, facilitating low-frequent transmissions. The epidemiological link between patient 6 and 7 through their succeeding use of room 5 and the isolation of pulsotype identical KPC-producing *K. pneumoniae* ST258 from both patients and the sink drain in that room as well as the recovery of closely related KPC-producing *E. asburiae* from faecal screening in patient 7 and the sink drain could suggest a possible environmental source of colonization of patient 7.

Investigation revealed that several sinks were heavily contaminated by bacteria presumably from waste water washed down the sink after cleaning of patients. A 'bed bath'-system for cleaning of patients were implemented to shortcut this opportunity. Repetitive disinfection with a biofilm active disinfectant was considered, but disinfection was only performed on two occasions; in June 2010 and January 2011.

In conclusion; the outbreak involved clonal spread of the hyperepidemic *K. pneumoniae* ST258 stain as well as interspecies plasmid transmission of a ~97 kb bla_{KPC}-plasmid into two other distinct *K. pneumoniae* strains and intergenus spread to *E. asburiae*. The establishment of a local environmental reservoir was documented. The spread among patients has probably occurred partly as a result of transmission between patients in some of the clinical cases, but we infer the possibility that the outbreak was maintained and prolonged with additional clinical cases added due to spread from environmental sources.

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Author Contributions

Conceived and designed the experiments: ST OS AS. Performed the experiments: OS UN JHL. Analyzed the data: ST OS UN. Contributed reagents/materials/analysis tools: ST OS UN JHL AS. Wrote the paper: ST OS.

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Short Report

Potential infection control risks associated with chilled beam technology: experience from a UK hospital

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SUMMARY

Energy efficiency technologies are now a feature in hospital design, with active chilled beams an example of one in use worldwide. Such innovations have clear benefits but there is a paucity of information with respect to any infection control risks. We describe our experience of chilled beam technology from one of our hospitals where we faced challenges with cleaning and episodes of water ingress including condensation. We highlight the importance of infection control risk assessment in relation to new technologies and the implementation of appropriate risk mitigation.

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Introduction

While active chilled beam technology is in use in hospitals globally including Scandinavia, Australia, and USA, their introduction has been largely driven by energy-saving advantages [1]. Active chilled beam technology delivers fresh air to the patient bedroom and is designed to integrate into suspended ceiling systems. Primary air from the supply ducting passes through nozzles into the room. The air movement creates low pressure and draws air back in from the room over the cooling coils. This is termed secondary (entrained) air and mixes with the primary air to be redelivered through diffusers (Figure 1).

In addition to energy conservation, advantages include thermal comfort and quiet operation [2]. Acknowledged disadvantages are the production of condensation and cleanliness issues [2]. Furthermore the large amounts of bedding used in healthcare settings can lead to the abundance of lint, which can become trapped within the beams [2]. There is a paucity of published data on infection control risks of chilled beam technology with no infection outcome-based clinical trials. Concerns remain regarding the impact of the recycled pathway of airborne pathogens intrinsic to the active chilled beam concept [3,4]. The American Society of Heating, Refrigerating and Air-Conditioning Engineers (2017) notes that chilled beams should not be used in intensive care units, protective isolation or source isolation rooms, toilets, procedure rooms, delivery rooms, endoscopy rooms, or clean rooms due to 'cleaning difficulty and potential for build-up of contamination'. The recirculation within the room interferes with the concept of a clean-to-dirty pathway for clean diluting air to remove pathogens from the contaminated air. There is potential for recirculation of pathogens within the breathing zone of patients and staff but this is an area of active research with computer modelling and other technologies and may depend on factors such as aerosol size, other heat sources in the room and human plume influences on air movements [5].

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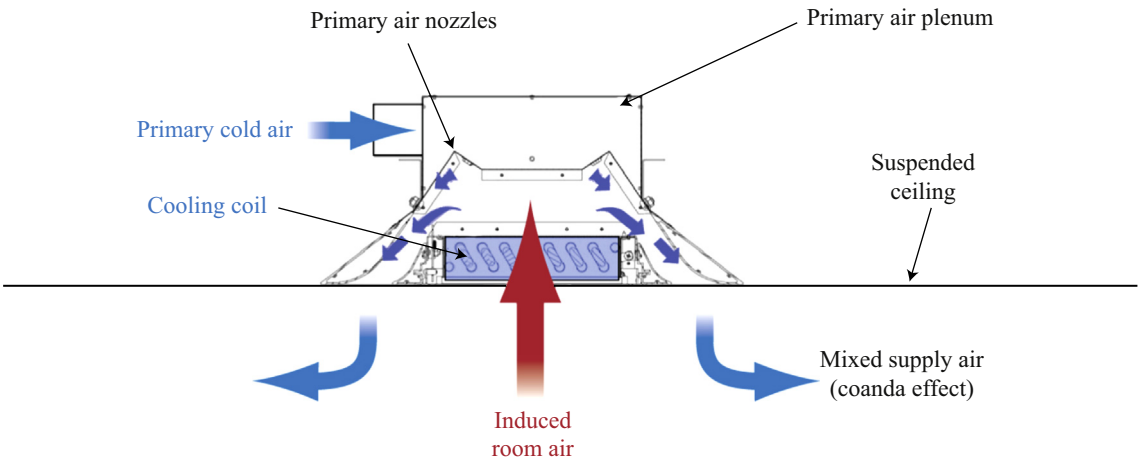

Figure 1. Active chilled beam technology. From: https://tacoadvancedhydronics.wordpress.com/category/radiant-cooling-chilled-beams/.

Current UK and Scottish Health Technical Memorandum guidelines for ventilation in healthcare premises are non-specific and give little guidance as to the suitability of chilled beam technology within healthcare premises [6]. However, the neutropenic and source isolation room concept are incompatible with chilled beams by token of the mandatory unimpeded clean-to-dirty pathway. Where chilled beams are in use in hospitals, guidance states that control settings should ensure that external components of the beam are always above the dew point. With reference to cleaning, access for cleaning and the impact of maintenance requirements should be considered [6]. We would recommend that water safety groups include chilled beam technology as a water source and that these are identified as a risk during the design phase as well as during maintenance. Our hospital has 1677 beds with active chilled beams installed in all patient rooms apart from the critical care and bone marrow transplant units.

Methods

Since our hospital opened in 2015 we have made a number of observations in relation to chilled beam technology which may have infection control implications.

Results and discussion

Cleaning

There have been issues with both access to clean and cleaning frequency. This is a clear disadvantage to the technology in a large hospital. The risk is intrinsic to the design which relies on entrained air from the room space, carrying fibres, skin squames and aerosols passing over the fins of the coils to be cooled/heated. This particulate matter tends to collect and stick to the metal (Figure 2). Others state that as the fin surfaces of an active chilled beam unit are relatively well gapped, dust and dirt particles tend not to bridge the fin surface; this does not reflect our experience [7].

The models in use in our hospital are not of the kind that allow easy removal of the entire beam for full-scale cleaning and decontamination. Cleaning is cumbersome and required on a six-week rotation due to visible build-up of dust, despite manufacturers suggesting six months to yearly. Access to clean can be problematic when patients are occupying the room, as the chilled beam is situated directly above the patient's bed. High patient turnover makes chilled beam cleaning impracticable as part of the standard discharge clean.

No filter is present, so all recirculated air will either carry with it the pathogens back into the room or deposit on to the beam. This poses an even greater risk when condensation drips

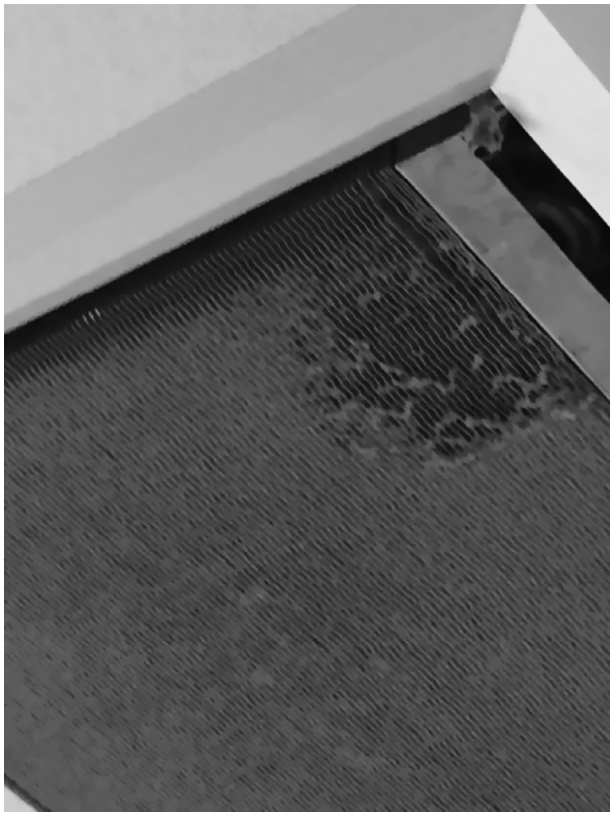

Figure 2. Photograph of chilled beam.

Box 1
Organisms isolated from dust in chilled beams

Micrococcus sp.
Coagulase-negative staphylococci
Bacillus sp.
Moraxella catarrhalis
Exophiala dermatitidis
Penicillium sp.
Rhodotorula sp.
Hyaline fungi
Aspergillus sp.
Panteoa agglomerans
Veillonella sp.
Stenotrophomonas sp.
Acinetobacter sp.
Paenibacillus pabuli
Klebsiella pneumoniae
Psychrobacter phenylpyruvicus

through the accumulated dust, as has occurred on several occasions.

Surface swabs from the chilled beams in our hospital have grown multiple organisms (Box 1). These include: (i) skin commensals in keeping with skin squame contamination (raising the possibility of meticillin-resistant and -susceptible *Staphylococcus aureus* also being present and recirculated); (ii) fungi, in keeping with moisture and a carbon source being present in the collected dust; (iii) enteric organisms: in keeping with possible airborne particles of bacteria entering into patient room from toilet and collecting on beams; (iv) environmental Gram-negative bacteria, in keeping with circulating moisture- and dust-borne environmental organisms. Surface swabs were undertaken in one of our general wards following reports of leaking from the chilled beams and observations of dust. These were plated directly on to blood agar, cysteine–lactose and electrolyte-deficient (CLED) agar and Sabouraud plates. In total, areas of the chilled beams from 19 rooms were swabbed. Prior to cleaning, 22 out of 49 (45%) samples were positive. In the two weeks post cleaning, four swabs out of 42 were positive (9%).

This is evidence of the potential biohazard posed by pathogens in the accumulated dirt on the fins of the beam and in keeping with the theoretical risk of airborne pathogens stagnating in the beam instead of being removed from a clean-to-dirty pathway. The likelihood is that as time goes on there will be an increasing bioburden of pathogens building up on the coils presenting an increasing hazard, particularly when combined with condensation events but not restricted to these [5].

Condensation, water leaks, and water contamination

Condensation should not occur on chilled beams and this is a prerequisite for safe functioning in healthcare [1,5,8]. The design and monitoring should be set up to eliminate this risk. Riffat states that 'when the panel surface temperature is lower than the dew point of room air, condensation on panel and pipe surfaces occurs, which can lead to damage from dripping water and an unacceptable room air condition' [1]. This is potentially serious in healthcare settings, particularly with immunocompromised patients. Condensation encourages mould and bacterial growth posing an infection hazard even in small amounts [9]. The degree of hazard depends on the bioburden, the environmental conditions, patient characteristics and effectiveness of the air change dilution effect. We have noted frequent condensation events: most have involved two or three beams in one ward area at a time, but on one occasion 106 rooms throughout the building were affected at the same time. It is not clear if this is due to temperature settings being too low for the dew point or if the dehumidification was planned to be in the air handling unit and is not present in some. Alteration to the dew point to operate above it has been undertaken to mitigate this risk and we have not been made aware of any further issues.

Our experience is not unique, with condensation issues being documented outside healthcare settings in office buildings and a school [10]. These were due to leaks in the duct system preventing sufficient air from reaching the beams [10].

As chilled beams use a water supply system within the hospital, with potential for leakage into clinical areas, the water supply requires temperature monitoring and attention to biocide usage if the water becomes contaminated. The circulating water system poses a *Legionella* spp., *Pseudomonas* spp. and other waterborne pathogen risk.

Leakage of supply water in our hospital has occurred from fittings. This phenomenon is supported by microbiology testing which isolated *Pseudomonas oleovorans* from the beam water system and from swabs from the leakage, external to the beam. This is also crucially backed by a history of water ingress to rooms. Whereas condensation on the beam causes 'indoor rain' as tiny droplets of condensation coalesce on the fins and fall into the room, leakage from the piping drips into the rim and drips from a corner in a gravity-directed pathway with the fins remaining dry [7]. *Pseudomonas aeruginosa* has also been isolated from the circulating water in our hospital chilled beam system.

There is no prospectively designed clinical trial based on infection rates that would give evidence for suitability of the use of this chilled beam technology in healthcare settings. Comparison for the UK context would need to be between full fresh air supply with and without chilled beams with standard cleaning methods and measuring healthcare-associated infection rates prospectively. The basis for the advantage of the chilled beam system in the published validation based on computer modelling and prototype testing was that conventional air-conditioning in hospitals included recirculation and indefinite pressure regimes, which is not the starting point in the UK [5]. The validation did not consider infectious aerosols emitting from a patient or infectious doses of various pathogens, nor did it include a comparison of different air change rates.

The proper functioning of a chilled beam system has been described as a balancing act, with temperature, humidity, volumes of outside air and occupancy all playing a role. Hospitals using chilled beam technology should be aware of the potential issues with access to clean, cleaning frequency, and the possibility of condensation or water leaks and subsequent risks from contaminated dust and water. Staff should be encouraged to report any condensation or water leakage events. Chilled beams should be included on a cleaning

schedule and frequency should be determined according to levels of dust build up. Infection control teams should consider chilled beams as a potential environmental source when investigating incidents/outbreaks.

A limitation of our report is that our experience is based on observations from a single centre only and may not reflect experience elsewhere. Further studies are required to delineate the infection control risks associated with such energy-saving efficiency methods which are likely to feature more commonly in future hospital builds.

In conclusion, when designing new hospitals, consideration should be given to the potential infection control risks associated with such energy-saving initiatives, with early involvement of the infection control team to ensure that risks are fully delineated and risk mitigation measures implemented. We would value more detailed reference to chilled beam and other energy efficiency technologies within national specifications for healthcare premises.

Conflict of interest statement
None declared.

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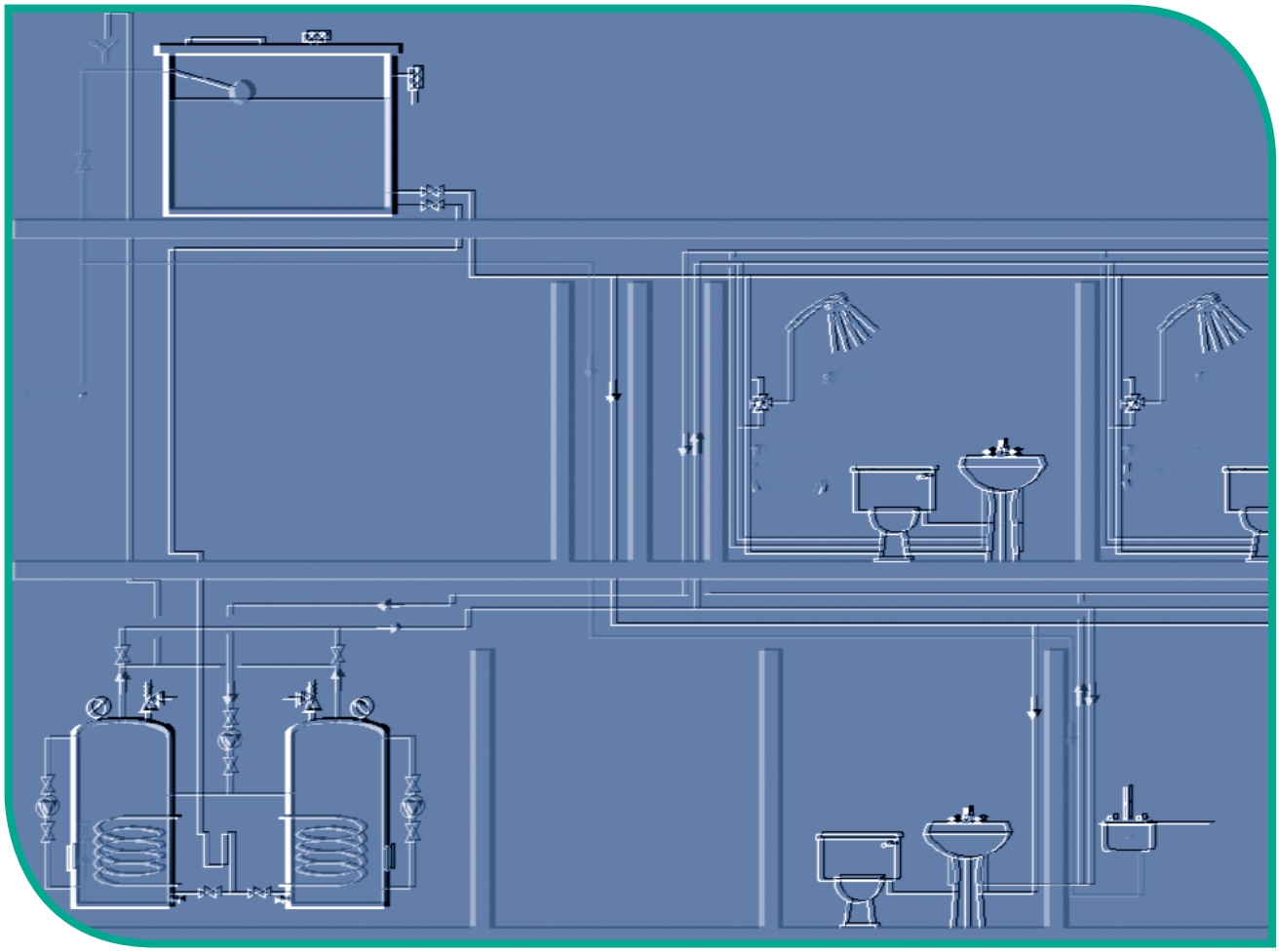
Department of Health

Health Technical Memorandum 04-01: Safe water in healthcare premises

Part A: Design, installation and commissioning



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Health Technical Memorandum 04-01 Safe water in healthcare premises

Part A: Design, installation and commissioning

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Executive summary

Preamble

This current review and update of HTM 04-01 is intended to move users of the document towards a holistic management of water systems via Water Safety Groups (WSGs), Water Safety Plans (WSPs) and other initiatives.

This version draws together and updates the previous guidance and includes recommendations for the safe management of water systems, via the integration of the principle of WSGs and WSPs (first introduced in the HTM 04-01 *Pseudomonas aeruginosa* addendum – published in March 2013), and how to manage and minimise the risks to health from various aspects, ranging from clinical risks, microbial and chemical contamination, changes to the water system, resilience of the water supply etc. It also introduces a stronger emphasis on staff competencies and the implementation of water hygiene awareness training.

Introduction

The development, construction, installation, commissioning and maintenance of hot and cold water supply systems are vital for public health. Healthcare premises are dependent upon water to maintain hygiene and a comfortable environment for patients and staff, and for treatment and diagnostic purposes.

Interruptions in water supply can disrupt healthcare activities. The design of systems should ensure that sufficient reserve water storage is available to minimise the consequence of disruption, while at the same time ensuring an adequate turnover of water to prevent stagnation in storage vessels and distribution systems.

This Health Technical Memorandum (HTM) gives comprehensive advice and guidance to healthcare management, design engineers, estate managers, operations managers, contractors and the supply chain on the legal requirements, design applications, maintenance and operation of hot and cold water supply, storage and distribution systems in all types of healthcare premises. It is equally applicable to both new and existing sites.

Aims of this guidance

The current review and update of HTM 04-01 is intended to move users of the document towards a holistic management of water systems via WSGs, WSPs and other initiatives.

It has been written to promote good practice for those responsible for the design, installation, commissioning, operation and maintenance of water services in healthcare premises, by:

- highlighting the need for robust governance and management;
- outlining the remit of the WSG and how this relates to the provision of safe water in healthcare premises;
- outlining key criteria and system arrangements to help stop the ingress of chemical and microbial contaminants and microbial colonisation and bacteria proliferation;
- illustrating temperature regimes for sanitary outlets to maintain water hygiene;

- ensuring the safe delivery of hot water;
- outlining how the correct selection of system components and correct use by occupants can help preserve the quality and hygiene of water supplies;
- providing a point of reference to legislation, standards and other guidance pertaining to water systems;
- providing a basic overview of possible potential waterborne pathogens;
- giving an overview of some of the different water systems (including components) and their safe installation, commissioning and operation and maintenance;
- providing typical system layouts and individual component location;
- providing information on thermostatic mixing valve configurations, appropriate usage and maintenance requirements;
- identifying key commissioning, testing and maintenance requirements for referral by designers, installers, operators and management;
- identifying key commissioning, testing and maintenance requirements for referral by designers, installers, commissioners, operators and management.

Controlling waterborne pathogens

The guidance gives comprehensive guidance on measures to control waterborne pathogens. While *Legionella* control is, in the main, associated with poor engineering configuration and maintenance, with no evidence of patient-to-patient or patient-to-outlet transfer, *P. aeruginosa* may be transferred to and from outlets and the water from both patients and staff. Suspected *P. aeruginosa* waterborne infections require additional investigations to determine the source and interventions from infection control specialists and microbiologists. Therefore, a temperature control regime is the traditional strategy for reducing the risk from *Legionella* and for reducing the growth and colonisation of other waterborne organisms within water systems. To prevent growth of *P. aeruginosa* and other waterborne pathogens, controls are necessary to manage the water system before and after the outlet.

As with all control measures, temperatures should be monitored at regular intervals to verify effective control.

Because of the complexity of hot and cold water distribution systems and the difficulty of maintaining a temperature control regime in some healthcare facilities, this guidance suggests that additional chemical, physical and other water control methods that have been shown to be capable of controlling microbial colonisation and growth may also be considered.

Main changes from the 2006 edition of HTM 04-01

- This 2016 edition of HTM 04-01 provides comprehensive guidance on measures to control waterborne pathogens such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Mycobacteria* as well as *Legionella*.
- This edition has been updated to align with the Health and Safety Executive's (HSE's) recently revised Approved Code of Practic e for Legionella (L8) and its associated HSG274 guidance documents.
- The Addendum to HTM 04-01 published in 2013 (and now Part C of HTM 04-01) introduced the concept of WSGs and WSPs. Part B of the HTM now includes updated guidance on the remit and aims of the WSG and WSP.
- New guidance has been included on the hygienic storing and installation of fittings and components and on the competency of installers/plumbers working on healthcare water systems. The guidance also outlines that any person working on water distribution systems or cleaning water outlets needs to have completed a

water hygiene awareness training course. An example course outline is included.

- Guidance on sampling techniques for, testing for, and the microbiological examination of *Pseudomonas aeruginosa* samples – originally in the HTM 04-01 Addendum – is now included in Part B to complement similar guidance on *Legionella*.

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Glossary and abbreviations

In addition to the definitions listed below, other definitions can be found in the Water Supply (Water Fittings) Regulations 1999; BS 6100; BS 8558; and BS EN 806.

Augmented care units/settings – There is no fixed definition of "augmented care"; individual providers may wish to designate a particular service as one where water quality must be of a higher microbiological standard than that provided by the supplier. While this document provides broad guidance, the water quality required will be dependent on both the type of patient and its intended use. Most care that is designated as augmented will be that where medical/nursing procedures render the patients susceptible to invasive disease from environmental and opportunistic pathogens such as *Pseudomonas aeruginosa* and other alert organisms. In broad terms, these patient groups will include:

a. those patients who are severely immunosuppressed because of disease or treatment: this will include transplant patients and similar heavily immunosuppressed patients during high-risk periods in their therapy;

b. those cared for in units where organ support is necessary, for example critical care (adult paediatric and neonatal), renal, respiratory (may include cystic fibrosis units) or other intensive care situations;

c. those patients who have extensive breaches in their dermal integrity and require contact with water as part of their continuing care, such as in those units caring for burns.

Backflow – Flow upstream, that is in a direction contrary to the intended normal direction of flow, within or from a water fitting.

Biofilm – a complex layer of microorganisms that have attached and grown on a surface. This form of growth provides a niche environment for a wide range of microorganisms to interact and where the secretion of exopolysaccharides by bacteria will form an extracellular matrix for both bacteria and other unicellular organisms such as amoebae and flagellates to remain in a protected state.

Dead-leg – a length of water system pipework leading to a fitting through which water only passes infrequently when there is draw off from the fitting, providing the potential for stagnation.

Healthcare-associated infections (HCAI) – encompasses any infection by any infectious agent acquired as a consequence of a person's treatment or which is acquired by a healthcare worker in the course of their duties.

Healthcare facility/building – all buildings, infrastructure, equipment, plant, embedded systems and related items that support the delivery of healthcare and services of all types, irrespective of their ownership or operation by third parties.

Healthcare organisations: organisations that provide or intend to provide healthcare services for the purposes of the NHS.

Point-of-use (POU) filter – a filter with a maximal pore size of 0.2 µm applied at the outlet, which removes bacteria from the water flow.

Redundant pipework (also known as blind end): a length of pipe closed at one end through which no water passes.

Thermostatic mixing valve: valve with one outlet, which mixes hot and cold water and automatically controls the mixed water to a user-selected or pre-set temperature.

Waterborne pathogen: microorganism capable of causing disease that may be transmitted via water and acquired through ingestion, bathing, or by other means.

Water outlet: (In this document) refers mainly to taps and showerheads, but other outlets, as indicated by risk assessments, may be considered important.

Water Safety Group (WSG): A multidisciplinary group formed to undertake the commissioning and development and ongoing management of the water safety plan (WSP). It also advises on the remedial action required when water systems or outlets are found to be contaminated and the risk to susceptible patients is increased.

Water safety plan (WSP): A risk-management approach to the safety of water that establishes good practices in local water distribution and supply. It will identify potential hazards, consider practical aspects, and detail appropriate control measures.

Water supply [to the healthcare facility]: The water supplied can be via:

- the mains water supply from the local water undertaker;
- a borehole (operated by the healthcare organisation as a private water supply);
- a combination of mains water and borehole supply;
- emergency water provision (bulk tankered water or bottled drinking water).

Water undertaker – the role of a water undertaker is defined in a number of sections of the Water Industry Act 1991.

Wholesomeness: standards of wholesomeness are defined in section 67 of the Water Industry Act 1991. Separate legislation for public and private supplies sets out the prescribed concentrations and values for water and are detailed in the following legislation: the Water Supply (Water Quality) Regulations 2000 for water from a public supply; or the Private Water Supplies Regulations 2009 for water from a private supply.

List of abbreviations

COSHH – Control of Substances Hazardous to Health [Regulations]

CQC – Care Quality Commission

DWI – Drinking Water Inspectorate

EA – Environment Agency

EPDM – ethylene propylene diene monomer

HBN – Health Building Note

HSE – Health & Safety Executive

HSG274 Part 2 – The Health & Safety Executive's technical guidance on the control of Legionnaires' disease in hot and cold water systems

HTM – Health Technical Memorandum

POU – point of use

PWTAG – Pool Water Treatment Advisory Group

SHTM – Scottish Health Technical Memorandum

UKWIR – UK Water Industry Research

WRAS – Water Regulations Advisory Scheme

WSG – Water Safety Group

WSP – Water safety plan

0 Policy and regulatory overview: water safety and the healthcare estate

Introduction

0.1 The National Health Service (NHS) has a corporate responsibility to account for the stewardship of its publicly funded assets. This includes the provision, management and operation of an efficient, safe estate that supports clinical services and strategy.

0.2 This corporate responsibility is carried by all accountable officers, directors with responsibility for estates & facilities and their equivalents, chairs, chief executive officers and non-executive board members. Together they have a responsibility to enact the principles set out in this document, provide leadership and work together to implement the necessary changes to provide a safe, efficient high quality healthcare estate.

0.3 To achieve this, quality and fitness-for-purpose of the healthcare estate is vital. Health Technical Memorandum (HTM) 04-01 seeks to set out the quality of, and standards for, water safety in the healthcare estate.

0.4 A healthcare organisation's Water Safety Group (WSG) (see Part B) is pivotal in ensuring that decisions affecting the safety and integrity of the water systems and associated equipment do not go ahead without being agreed by them. This includes consultations relating to decisions on the procurement, design, installation and commissioning of water services, equipment and associated treatment processes.

0.5 The quality and fitness-for-purpose of the estate are assessed against a set of legal requirements and governance standards.

Adhering to the guidance outlined in this HTM will be taken into account as evidence towards compliance with these legal requirements and governance standards.

Compliance of the healthcare estate

0.6 Principles related to the safety of healthcare estates and facilities are enshrined in the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014, specifically Regulation 12(2)(h) and Regulation 15 of the Act.

Note

There are numerous other statutes and legal requirements that NHS organisations, supporting professionals, contractors and suppliers must comply with. These are covered in the respective Health Building Notes (HBNs), Health Technical Memoranda (HTMs) and the NHS Premises Assurance Model (NHS PAM).

Health and Social Care Act 2008 (Regulated Activities) Regulations 2014

0.7 Regulation 12(2)(h) decrees that registered providers must assess:

- the risk of, and prevent, detect and control the spread of, infections, including those that are health care associated.

0.8 Appropriate standards of cleanliness and hygiene should be maintained in premises used for the regulated activity. DH (2015) issued 'The Health and Social Care Act 2008 Code of Practice on the prevention and control of infections and related guidance' (the HCAI Code of Practice), which contains statutory guidance about compliance with regulation 12(2)(h) (see paragraphs 0.15–0.17).

0.9 Regulation 15 of the Act states that:

(1) All premises and equipment used by the service provider must be–

a. clean,

b. secure,

c. suitable for the purpose for which they are being used,

d. properly used

e. properly maintained, and

f. appropriately located for the purpose for which they are being used.

(2) The registered person must, in relation to such premises and equipment, maintain standards of hygiene appropriate for the purposes for which they are being used.

Note

The "registered person" means, in respect of a regulated activity, the person who is the service provider or a registered manager in respect of that activity.

A "service provider" means a person registered with the CQC under Chapter 2 of Part 1 of the Health and Social Care Act 2008 as a service provider in respect of that regulated activity.

Regulator requirements

0.10 The CQC independently regulates all providers of regulated health and adult social care activities in England. The CQC's (2015) 'Guidance for providers on meeting the regulations' explains how to meet regulations 12(2)(h) and 15 outlined above.

0.11 Failure to comply with the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014 and the Care Quality Commission (Registration) Regulations (2009) is an offence, and the CQC has a wide range of enforcement powers that it can use if a provider is not compliant. These include the issue of a warning notice that requires improvement within a specified time, prosecution, and the power to cancel a provider's registration, removing its ability to provide regulated activities.

Examples of governance and assurance mechanisms arising from primary legislation (not exhaustive)

NHS Constitution

0.12 The Health Act 2009 places a duty on bodies providing and commissioning NHS services to have regard to the NHS Constitution. The Health and Social Care Act 2012 further applied this duty to the new bodies created by that Act or by amendments to the 2012 Act.

0.13 The NHS Constitution "sets out rights to which patients, public and staff are entitled". It also outlines "the pledges which the NHS is committed to achieve, together with responsibilities that the public, patients and staff owe to one another to ensure that the NHS operates fairly and effectively".

0.14 It commits the NHS to ensuring **"that services are provided in a clean and safe environment that is fit for purpose, based on national best practice (pledge)".** In order to deliver on this pledge, the NHS should take account of:

- the NHS Premises Assurance Model (NHS PAM) – the NHS PAM identifies where the NHS Constitution needs to be considered and where assurance is required;
- national best practice guidance for the design and operation of NHS healthcare facilities (such as HBNs and HTMs).

The HCAI Code of Practice

0.15 A complex range of issues distinguishes healthcare environments from most other building types. One of the most important of these relates to the control of infection. Infection prevention and control teams should be consulted on any design decisions and a risk analysis conducted on the many issues of design involving water systems (see Health Building Note 00-09 – 'Infection control in the built environment'). To manage and monitor the prevention and control of infections effectively, the HCAI Code of Practice requires a WSG and a water safety plan (WSP) to be in place.

0.16 The information outlined in HBN 00-09 follows the general principles given in the HCAI Code of Practice, which sets out criteria against which a registered provider will be judged on how it complies with Regulation 12(2)(h) of the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014 on the prevention, detection and control of infections, particularly waterborne infections in relation to this document.

0.17 The law states that the HCAI Code of Practice must be taken into account by the CQC when it makes decisions about registration against the cleanliness and infection control requirement. The regulations also say that providers must have regard to the Code when deciding how they will comply with registration requirements. Therefore, by following the Code, registered providers will be able to show that they meet the requirement set out in the regulations. However, the Code is not mandatory. A registered provider may be able to demonstrate that it meets the regulations in a different way (equivalent or better) from that described in this document. The Code aims to exemplify what providers need to do in order to comply with the regulations.

Figure 1 illustrates how DH estates and facilities best practice guidance (such as the NHS PAM and HTMs) aligns with the statutory and policy framework. This guidance is fundamental to ensuring that NHS trusts are able to deliver on their commitments under the NHS Constitution and to comply with the CQC's fundamental standards.

Never events

0.18 NHS England's never events policy framework defines "never events" as serious, largely preventable patient safety incidents that should not occur if the available preventative measures have been implemented by healthcare providers. On the list of never events is scalding of patients. The risk of scalding for vulnerable patients (children and young people, older people, and disabled people) is a particular problem in healthcare premises. HTM 04-01 provides guidance on reducing the risk of scalding.

Health and safety legislation

0.19 In the UK, the control of legionellae falls within the requirements of the Health and Safety at Work etc. Act. This Act also places duties on design teams, suppliers and installers to ensure that articles or substances for use at work are safe and without risks to health and that any information related to the article or substance is provided. The Management of Health and Safety at Work Regulations 1999 provide a broad framework for controlling health and safety at work. The Control of Substances Hazardous to Health Regulations 2002 (COSHH) provide a framework of actions designed to assess, prevent or control the risk from bacteria like *Legionella* and take suitable precautions.

0.20 The Health and Safety Executive's (2013) Approved Code of Practice 'Legionnaires' disease: The control of legionella bacteria in water systems (L8)' contains practical guidance on how to manage and control the

risks in water systems. The HSE has published complementary technical guidance in HSG274, which is split into three specific areas:

- Part 1 – evaporative cooling systems;
- Part 2 – hot and cold water systems; and
- Part 3 – other risk systems.

0.21 In addition, under the Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (RIDDOR), there is a duty for employers to report any cases of legionellosis in an employee who has worked on hot and cold water systems that are likely to be contaminated with *Legionella*. Cases of legionellosis are reportable under RIDDOR if:

a. a doctor notifies the employer; and
b. the employee's current job involves work on or near cooling systems that are located in the workplace and use water; or work on water-service systems located in the workplace which are likely to be a source of contamination.

0.22 With regard to enforcement responsibilities, the Health & Safety Executive will take the lead with regard to incidents involving *Legionella*. See the 'Memorandum of understanding between the Care Quality Commission, the Health and Safety Executive and local authorities in England'.

Security

0.23 Accessibility to all plant and equipment should be limited to authorised personnel only (see NHS Protect's (2012) 'Guidance on the security and management of NHS assets').

Water regulations

0.24 As well as complying with the recommendations outlined in this document, the design and installation of the hot and cold water services, new or extended, in any healthcare premises should also comply with:

- the Water Supply (Water Fittings) Regulations 1999;
- Defra's guidance to the Water Supply (Water Fittings) Regulations;
- recommendations of the water suppliers in the Water Regulations Advisory Scheme's (WRAS) 'Water Regulations Guide', and
- any other requirements of the local water undertaker.

Water Supply (Water Fittings) Regulations 1999

0.25 These Regulations set legal requirements for the design, installation, operation and maintenance of plumbing systems, water fittings and water-using appliances. They have a specific purpose to prevent misuse, waste, undue consumption or erroneous measurement of water and, most importantly, to prevent contamination of drinking water.

0.26 These Regulations apply in all types of premises supplied, or to be supplied, with water from a water undertaker. They apply from the point where water enters the property's underground pipe, to where the water is used in plumbing systems, water fittings and water-using appliances. However they do not apply in premises that have no provision of water from the public mains supply.

Water Supply (Water Quality) Regulations 2000

0.27 These Regulations cover the quality of water supplied by water undertakers for public distribution which is intended for domestic purposes; these purposes include drinking, cooking, food preparation, washing and sanitation. Water supplied meeting these quality requirements is referred to as wholesome water.

Private Water Supplies Regulations 2009

0.28 These Regulations cover private sources of water intended for human consumption including drinking, cooking, food preparation or

other domestic purposes, such as boreholes and wells. Water meeting these quality requirements is referred to as wholesome water. These Regulations also place duties for monitoring and control of the quality of public water supplies where these are then further distributed to other users on separate premises by the water company's bill payer (this arrangement is often referred to as onward distribution).

0.29 Local authorities are the regulators for private water supplies and have a number of statutory duties under the Private Water Supplies Regulations.

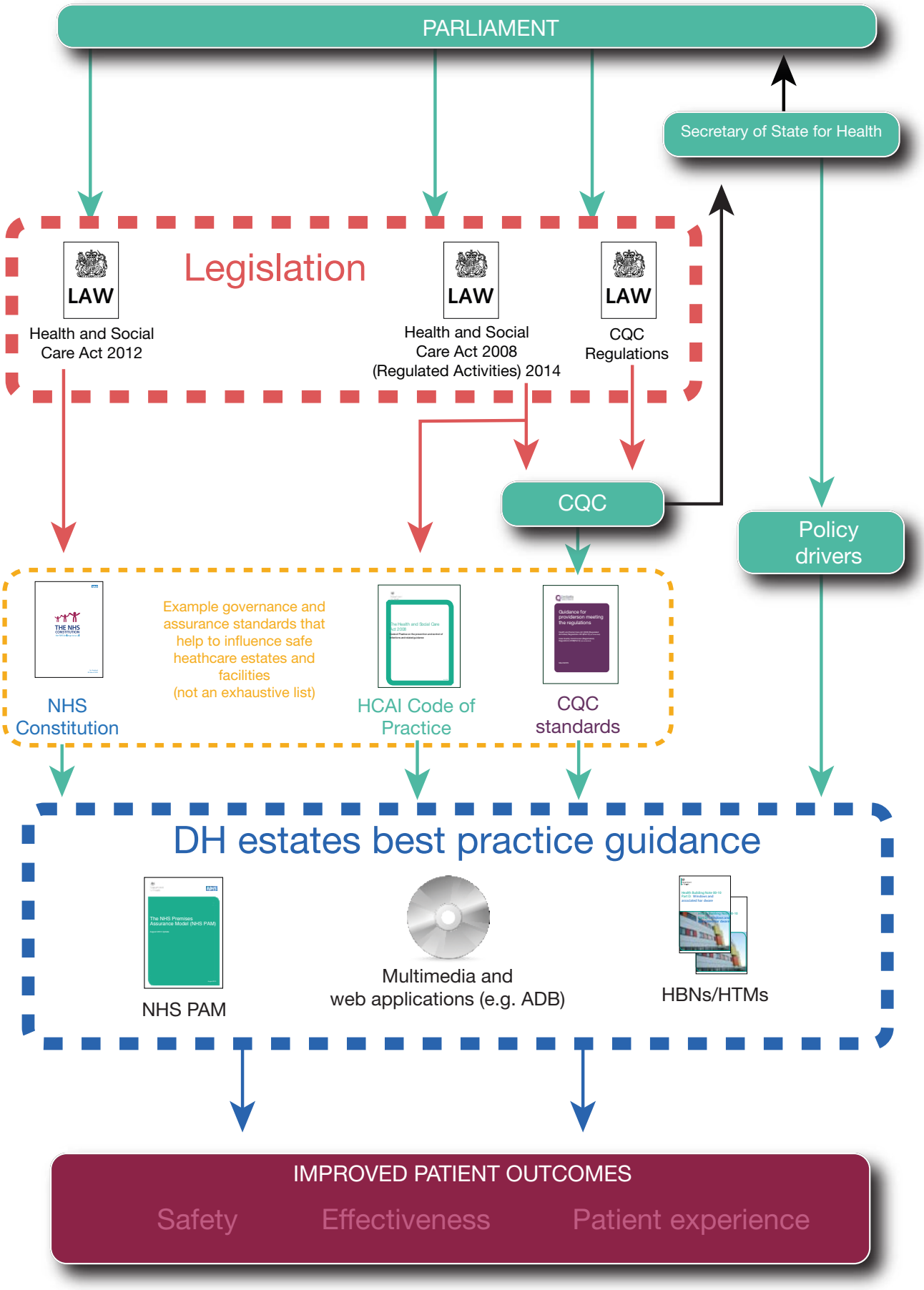

Figure 1 How best practice guidance on the safety and quality of healthcare estates and facilities fits in with the legislative and policy framework. (The statutes and mandatory requirements shown in this figure are not exhaustive. See Note after paragraph 0.6.)

1 Introduction to HTM 04-01

1.1 This edition of HTM 04-01 – 'Safe water in healthcare premises' supersedes HTM 04-01 – 'The control of *Legionella*, hygiene, "safe" hot water, cold water and drinking water systems' published in 2006.

1.2 This guidance has been revised to take account of HSE's revision of its Approved Code of Practice and guidance on regulations 'Legionnaires' disease: The control of legionella bacteria in water systems (L8)' and its complementary technical guidance HSG274.

1.3 This HTM gives comprehensive advice and guidance to healthcare management, design engineers, WSGs, estates managers and operations managers on the legal requirements, design applications, maintenance and operation of hot and cold water supply, storage and distribution systems in all types of healthcare premises. It is equally applicable to both new and existing sites.

1.4 The document comprises three parts. This part (Part A) outlines the principles involved in the design, installation and testing of the hot and cold water supply, storage and distribution systems for healthcare premises. Some variation may be necessary to meet the differing requirements for the various water undertakers. Part B covers operational management. Part C covers water safety in augmented care settings. HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)' contains guidance on the on-site testing and commissioning of thermostatic mixing valves.

General

1.5 Current statutory legislation requires both "management" and "staff " to be aware of their individual and collective responsibility for the provision of wholesome, safe hot and cold water supplies, and storage and distribution systems in healthcare premises.

1.6 Healthcare premises are dependent upon water to maintain hygiene and a comfortable environment for patients and staff, and for clinical and surgical care.

1.7 The safe development, construction, installation, commissioning and maintenance of hot and cold water supply systems and associated systems and equipment are vital for public health.

1.8 Interruptions in water supply can increase the risk of microbial ingress especially if these result in depressurisation of the supply pipework. The design of systems should ensure that sufficient reserve water storage is available to minimise the consequence of disruption, while at the same time ensuring an adequate turnover of water to prevent stagnation in storage vessels and distribution systems.

1.9 Measures to control the spread of microorganisms in healthcare premises include hand-washing but also the regular use of antimicrobial hand-rubs, and this can result in a significant reduction in the use of wash-hand basins. Under-use of taps encourages colonisation and growth with *Pseudomonas aeruginosa*, *Legionella* and other waterborne organisms. The design team should be aware

of this and, accordingly, consider how local infection policies with regard to hand hygiene and the use of antimicrobial hand-rubs might impact on the frequency of use of wash-hand basins and the volume of water being distributed (see also paragraphs 5.7–5.12 in Part B on the extent of utilisation).

Legislation, standards and guidance

1.10 As well as complying with the recommendations outlined in this document, the design and installation of the hot and cold water services, new or extended, in any healthcare premises should also comply with:

a. the Water Supply (Water Fittings) Regulations 1999, Defra guidance and the recommendations of the water suppliers in the Water Regulations Advisory Scheme's (WRAS) 'Water Regulations Guide', and any other requirements of the local water undertaker;

b. the HSE's Approved Code of Practice and guidance on regulations 'Legionnaires' disease: The control of legionella bacteria in water systems (L8)', which requires that sources of risk are identified and assessed and a written scheme of control is put in place in respect of controlling *Legionella* in water systems.

Notes

1. The Water Supply (Water Fittings) Regulations 1999 are set out – along with Defra's (1999) guidance (see References) and with the water industry's interpretation of these provisions – in the WRAS 'Water Regulations Guide'.

2. Special measures may have to be introduced to remove chloramines from mains water supplied to renal dialysis units. See guidance produced by the Renal Association.

General requirements

1.11 Where new healthcare premises are planned or existing premises are to be altered or refurbished, the WSG should be consulted at the earliest possible opportunity and water risk assessments be completed for all projects. This will enable the total water hygiene requirements to be assessed in the planning stages, and appropriate action taken, including ensuring that any pressure testing, flushing and cleaning does not lead to stagnation or contamination before being placed into service. The risk assessment should be reviewed once the system is operational.

1.12 At all stages of the design, installation and commissioning of new or extended water systems, the design team should liaise and consult with the local WSG in a timely manner, give consideration to HTM 04-01 Parts B and C and incorporate all operational managements requirement into their design.

1.13 Design teams and installers of hot and cold water distribution systems are required by the Water Supply (Water Fittings) Regulations 1999 to notify the water undertaker of any proposed installation of water fittings and to have the water undertakers' consent before installation commences. It is a criminal offence to install or use water fittings without their prior consent. Liaison with the local water undertaker is strongly recommended at an early stage to avoid problems of compliance in the design. Further information can be found on the WRAS website. See also paragraphs 1.19–1.20.

1.14 All water fittings used in the construction of systems referred to in this HTM must comply with the requirements of the Water Supply (Water Fittings) Regulations 1999 (Regulation 4: "Requirements for water fittings") and if

The water industry

Water supply

Defra is the government department responsible for safeguarding the natural environment. This includes the protection of the aquatic environment, water resources and drinking water quality. It has established a number of regulatory authorities to oversee these areas. A company may be appointed to be a water undertaker by the Secretary of State under Section 6 of the Water Industry Act 1991, with a duty under this Act to develop and maintain an efficient and economical system of water supply (see Chapter 2).

Ten water and sewerage companies provide water and wastewater services for 99% of the population of England and Wales and drinking water to 75%. (A number of water supply companies provide drinking water to most of the remainder of the population.)

Regulatory authorities

The Water Act 1989 provided for the establishment of several regulatory bodies whose functions are now set out in the Water Industry Act 1991 and the Water Resources Act 1991 and are summarised below:

a. the Office of Water Services (OFWAT), which regulates the prices set by the water companies, oversees the standards of service provision and protects the interests of water consumers. OFWAT also has ten regional WaterVoice committees that identify customer concerns, pursue them with the companies and report to the director-general of water services. OFWAT are overseeing changes to competition in the retail water market through the Open Water programme. These changes will allow businesses and other non-household customers to choose their supplier of water and wastewater services from April 2017. Retail services include things like billing and customer services.

b. the Environment Agency (EA) regulates the quality and controls pollution of “controlled” waters (that is, most inland and coastal waters) and protects the water resources in England and Wales.

c. the Drinking Water Inspectorate (DWI) regulates the quality of the public supply of drinking water in England and Wales.

d. Local authorities regulate private water supplies.

required be in accordance with relevant British Standards and codes of practice appropriate to their use.

1.15 Where water is required to be wholesome, all non-metallic materials in contact with water should comply with the requirements of BS 6920. Many of the materials satisfactorily tested against this standard are listed by WRAS in the ‘Water Fittings and Materials Directory’ or by the DWI in their published ‘List of Approved Products’. Some of these approval schemes also validate compliance against the mechanical performance requirements and where appropriate installation requirements set out in the Water Supply (Water Fittings) Regulations 1999. These fittings are considered compliant provided they are installed according to any conditions given with the approval. Additionally, fittings approved by BuildCert, NSF-WRc, Kiwa Watertec (UK) and WRAS, unless they specifically state they are not approved for

use under the Water Supply (Water Fittings) Regulations 1999, are deemed to meet these requirements. Currently no criteria exist to verify metallic materials. Only materials and fittings shown to comply with these regulations should be used (for example, registered in one of the approval lists given above).

Note

Organic materials increase the risk of microbial colonisation; materials should be chosen to minimise the risk where possible (see Chapter 3). See also Estates and Facilities Alert DH (2010) 03 – 'Flexible water supply hoses'

1.16 The provision of wholesome water to premises is governed by either the Private Water Supplies Regulations 2009 or the Water Supply (Water Quality) Regulations 2000 depending on how the water is supplied. Preserving water quality within premises is governed by the Water Supply (Water Fittings) Regulations 1999 (where water is supplied by a water undertaker), building regulations, approved codes of practice and technical standards intended to safeguard quality.

1.17 Only installers with the appropriate qualifications, regulatory knowledge and competence should be used to install and maintain water installations. There are seven Approved Contractors' Schemes authorised through the Water Supply (Water Fittings) Regulations 1999. All seven schemes require appropriate qualifications and knowledge of the regulations as part of their membership criteria. The seven schemes are:

- the Association of Plumbing and Heating Contractors (APHC)
- Anglian Water's APLUS
- the Chartered Institute of Plumbing and Heating Engineering (CIPHE)
- the Scottish & Northern Ireland Plumbing Employers' Federation (SNIPEF)
- Thames Water Approved Plumbing Scheme (TAPS)
- WaterMark
- the Water Industry Approved Plumbers' Scheme (WIAPS)

1.18 In addition to plumbing installers, four schemes (APLUS, TAPS, WaterMark and WIAPS) operate sector memberships for specialist areas of work covering external water services (below ground pipework etc), catering equipment and point-of-use (chilled water) equipment.

1.19 The WaterSafe register holds details from all seven Approved Contractors' Schemes for businesses who have registered and qualified plumbing installers (see also Part B).

1.20 A recognised benefit to using an Approved Contractor (including sector installers) is they can carry out some work without the need to provide advanced notification to the water undertaker and their work will be certified upon completion. A "work completed" certificate issued by a WaterSafe-recognised plumber provides a defence for property owners who are challenged by a water undertaker enforcing the Water Supply (Water Fittings) Regulations 1999 or during legal proceedings.

Hygienic installation and storage practices

1.21 Installers should adopt practices that reduce the likelihood of cross-contamination from tools, clothing or the environment. Separate clothing and tools used for other non-wholesome services such as sewerage and drainage systems should be kept separate and not used when working on hot and cold water systems.

1.22 Good working practices should be developed:

- to ensure that flushing and disinfection processes remain effective;
- to reduce the risk of seeding a system with anything that could lead to physical, chemical or microbiological

contamination;

- to reduce any damaging effects of the disinfection process through the need for multiple disinfection treatments.

Note

Guidance to principle of water supply hygiene can be found on Water UK's website.

1.23 Only components and sub-assemblies that have been hygienically stored (for example, wrapped in bags and or original packaging) should be used (see also paragraphs 3.42–3.44). All components and sub-assemblies should be inspected before installation to ensure they are clean and free of defect. Efforts should be made to ensure debris and or contamination do not enter the hot and cold systems during construction and maintenance works.

1.24 Where contamination is suspected, measures should be undertaken to neutralise their effects (for example, flushing and disinfecting).

Storage

1.25 Items and components intended for installation in hot and cold water systems should be stored in clean, dry locations. Pipework valves and fittings etc should be stored on suitable racking and not on the floor. Larger items of plant that may have to be offloaded from delivery vehicles and delivered directly to their final location should be appropriately covered and protected from damage.

Water hygiene training

1.26 It is important that any person who works on hot and cold water systems or who is responsible for cleaning water outlets should have completed a water hygiene training course so that they understand:

- the need for good hygiene when working with water distribution systems and water outlets; and
- how they can prevent contamination of the water supply and outlets.

Note

For new builds and refurbishments, it will be the contractor's responsibility to ensure that all operatives intending to work on the water system have completed water hygiene training covering associated risks of working practices and local requirements.

1.27 To ensure the delivery of safe wholesome water at all outlets and prevent contamination which may lead to a healthcare-associated infection, it is recommended that healthcare organisations implement a water hygiene training scheme which utilises local content where possible and is information appropriate for the target audience.

1.28 Consideration should also be given to integrating a health screening element into the training to help ensure those undergoing the training are not carriers of any waterborne diseases on the date of training and are aware of their responsibilities towards the water supply. It is important that individuals are aware of their duty to protect the health of patients, staff and visitors and that they are responsible for ensuring they inform their line manager if they come into contact with any disease that has the potential to cause harm.

1.29 As an example, the course should encompass the following topics (not exhaustive):

- organisational governance arrangements in relation to water hygiene and safety;
- familiarisation with local policies/ procedures in relation to the management and provision of water hygiene and safety;
- information on prominent waterborne

pathogens and their consequences;

- the ways in which water distribution systems and water outlets can become contaminated;
- the responsibilities of individuals to prevent the contamination of the water distribution system and water outlets and assisting in ensuring control measures in place are effective;
- how the safety of water can be maintained by good hygiene practices;
- system design;
- components/accessories (taps, TMVs);
- disinfection and cleaning equipment/ materials;
- how to store and handle pipes;
- organisation-specific control measures;
- the impact of getting it wrong;
- role of persons being trained;
- personal hygiene along with dealing with clothing, footwear, cleaning equipment/ materials, tools and storage when considering water hygiene (as applicable to each role).

Areas this HTM does not cover

1.30 Although many of this HTM's recommendations will be applicable, it does not set out to cover water supply for fire-fighting services nor water supply for industrial or other specialist purposes, other than to indicate precautions that should be taken when these are used in association with domestic water services. The point at which a domestic activity becomes an industrial process has not been defined, and the applicability will need to be considered in each case.

1.31 This HTM does not cover wet cooling systems such as cooling towers. Guidance on these systems is given in HSE's Approved Code of Practice and guidance 'Legionnaires' disease: The control of legionella bacteria in water systems (L8)' and HSG274 technical guidance Part 1.

1.32 While some guidance on other water-service applications is included, it is not intended to cover them fully. For:

- process waters used for laundries, see HTM 01-04 – 'Decontamination of linen in health and social care';
- endoscopy units, see HTM 01-06 – 'Decontamination of flexible endoscopes';
- primary care dental premises, see HTM 01-05 – 'Decontamination in primary care dental practices';
- renal units, see HBN 07-01 and HBN 07-02, the Renal Association's guidelines and ISO 13959 and 11663;
- sterile services departments, see HBN 13 – 'Sterile services department';
- hydrotherapy pools, see the PWTAG's 'Swimming pool water: treatment and quality standards for pools and spas';
- spa pools, see HSE/PHE's 'Management of spa pools: controlling the risks of infection';

Note

This document is currently being revised and will become HSG274 Part 4 – 'The control of legionella and other infectious agents in spa pool systems'.

- birthing pools, see HBN 21 – 'Maternity' and PWTAG's 'Swimming pool water: treatment and quality standards for pools and spas'.

2 Source of supply

General

2.1 The source of water supply to healthcare premises is by one or more service-pipe connections from the mains of a water undertaker. If the quantity and rate of flow is inadequate, or if the cost of providing the service connection appears to be uneconomical, alternative sources of supply such as boreholes or wells may be investigated or other water undertakers may be contacted.

2.2 The healthcare building needs could be met by using a private supply (see the Private Water Supply Regulations 2009) as an additional source to the water undertaker's supply provided the risk to health is fully assessed and the supply deemed acceptable for its intended purpose. In such cases, the water undertaker's supply should be the priority supply for drinking, culinary and special hygienic services. By limiting the use of the private supply to services not requiring the highest level of hygiene, the extent of treatment of the private supply may be reduced. Supplies used in this way should convey water through an entirely separate pipework system that is clearly labelled. Outlets served by the supply system should also be appropriately labelled.

Note

With regard to private water supplies, further guidance is given on DWI's website.

2.3 Provision should be included for alternative water supply arrangements to meet an emergency, regardless of the source or sources of supply finally adopted (see HBN 00-07 – 'Planning for a resilient healthcare estate'). Alternative arrangements would include a second service connection from the water undertaker or a private supply. In either case the alternative supply should not be vulnerable to the cause of loss of the original supply. Direct physical interconnection of pipework and valves of a water undertaker's supply with any private supply without adequate backflow protection is prohibited by the Water Supply (Water Fittings) Regulations 1999. The water quality requirements applicable to the main supply apply also to any alternative supplies.

2.4 The water undertaker and local authority must be advised if it is proposed to use any private supply as well as the water undertaker's supply, and advice should be sought on the limitations imposed in respect of break cisterns/RPZ (reduced pressure zone) valves and interconnections thereafter as required by the Water Supply (Water Fittings) Regulations 1999.

2.5 In England all water intended for human consumption is required by legislation to comply with the quality standards laid down in the Water Supply (Water Quality) Regulations 2000 and the Private Water Supplies Regulations 2009 (see Appendix 2 for an overview of all UK water legislation). These regulations apply to water sampled at the point where the water is available for use and embrace not just drinking water and water used for washing, but also water used in the preparation of food and beverages.

2.6 The responsibility for enforcing the Water Supply (Water Quality) Regulations for public water supplies rests primarily with the DWI,

and for private supplies it rests with the local authority. In respect of public water supplies, the water undertaker has a duty to provide water that is wholesome at the point of supply to premises and to demonstrate – by monitoring – that the supplies meet the above standards. Water undertakers are also required to carry out risk assessments of each of its treatment works and connected supply in order to establish whether there is a significant risk of supplying water that would constitute a risk to human health. Using a risk-based approach, local authorities have a duty to monitor public health and in particular where affected by water supplies.

Supplies from a water undertaker

2.7 The following factors should be taken into consideration in the initial stages of the design:

a. the water undertaker's requirements;
b. water supply quality variations (see paragraphs 2.11–2.15);
c. the type of disinfectant residual and the variability in its concentration;
d. maintaining water supplies in an emergency (see paragraph 2.16);
e. the estimated daily consumption, and the maximum and average flows required, together with the estimated time of peak flow;
f. the location of the available supply;
g. the quality, quantity and pressure required;
h. the cold water storage capacity required;
i. the likelihood of ground subsidence due to mining activities or any other reason;
j. the likelihood of there being any contaminated land on site;
k. the proposed method of storage and probable number and purpose of direct connections to pressure mains;
l. the minimum and maximum pressures available at the service connection;
m. details of the physical, chemical (including radionuclides) and microbiological characteristics of the water supply and scope of any possible variations in such characteristics;
n. the possibility of an alternative service connection from some other part of the water undertaker's network, including pressure details;
o. the water undertaker's contingency plans, which may result in disruptions to the quality or quantity of water supplied;
p. the proximity of the supply pipework to wastewater pipes and the materials used for the supply pipework (plastic pipework is permeable to oil-based leaks such as diesels etc).

Note

Regulations require notification to the water undertaker of any proposed changes and additions to the water supply system in the premises. Prior to making any changes, a risk assessment should be carried out and audited as detailed in Part B. Further details can be found on the WRAS website.

2.8 These initial design feasibility studies should normally reveal the need for any further treatment, pressurisation and storage of the water undertaker's supply to meet healthcare building requirements and enable an estimate of costs to be made.

2.9 BS EN 805, BS EN 806-1-5 and BS 8558 give further guidance on the procedures that should be followed when carrying out preliminary investigations in relation to new water supplies.

2.10 During the design stage, close collaboration with the water undertaker should be maintained, and consent must be sought on the final arrangements before proceeding with

the installation. These arrangements should include:

- siting of service connections, access chambers, metering, bypassing, flushing out, physical security of service connections, installation and provisions for the fire-and-rescue service, proximity to wastewater pipework etc;
- compliance with the Water Supply (Water Fittings) Regulations 1999.

Water supply quality variations

2.11 At different times of year or if an emergency affects a regular source of supply, water suppliers may use different sources of water to maintain supplies to an area, either separately or in combination by blending. While all the supplies will be wholesome and will meet the water quality standards set out in the Water Supply (Water Quality) Regulations 2000, the chemical composition of the water may vary.

2.12 One of the more common variations is in the hardness of the water, which is determined by the geology of the area from which the supply originates. By altering the source of the supply to an area, it is possible for the water to change from soft (for example total hardness of 50 mg/L as calcium carbonate ($CaCO_3$)) to hard (greater than 200 mg/L) or vice versa, causing users to notice differences with scaling in heating appliances, central heating and steam-raising plant, and with soap or detergent usage.

2.13 Water undertakers often access a variety of sources, which means that other possible changes may include temperature, taste or odour where the source changes between surface waters (such as rivers or reservoirs) and underground waters (such as borehole supplies). Differences may arise in dissolved iron, manganese or aluminium concentration and in the chlorine residual maintained in different supplies.

2.14 Healthcare organisations should liaise with their water supplier to find out whether it is likely that the quality of the water supplied may vary, either due to planned operational changes to the water sources used at different times of year, or in the event of an emergency that prevents the use of the regular source of supply (see HBN 00-07 – 'Planning for a resilient healthcare estate'). Where changes in the nature of supplies may be expected regularly, provision of water treatment facilities may be required within the healthcare facility to prevent complications occurring, such as scaling in central heating equipment, interference with sensitive medical equipment or adverse reaction from users.

2.15 Healthcare organisations should be aware of the type of disinfectant residual in the supply to their premises as it can have implications for choice of additional treatment. Under certain conditions, some water companies may also alternate the residual levels and type of disinfectant used (for example, chlorine and chloramine) between winter and summer.

Maintaining water supplies in an emergency

2.16 The Security and Emergency Measures (Water and Sewerage Undertakers) Direction 1998 is a statutory document produced under the provisions of Section 208 of the Water Industry Act 1991. Water suppliers have a duty arising from it to make and revise plans for the provision of essential water supply and wastewater services at all times. The Direction includes the duty for water suppliers to give priority to the domestic needs of the sick, older people, disabled people, hospitals, schools, and other vulnerable sectors of the population.

2.17 Healthcare organisations should cooperate with water suppliers in developing plans to maintain essential supplies of water in the event of major incidents. These plans might include (see also HBN 00-07 – 'Planning for a resilient healthcare estate'):

- The routine provision of public water supplies to the site from more than one water main, preferably using mains that are fed from different water supply

zones. Where mains are fed from different zones, particular care should be taken within the site to prevent direct interconnection and backflow between plumbing systems fed from the different supply zones.

Note: any arrangements designed-in should ensure there are no areas where water can stagnate (for example, dead-legs).

- Adequate storage cistern capacity and distribution arrangements within the healthcare facility to provide minimum volumes of water at the outset of a major incident to maintain hygiene and health for an initial period until other temporary arrangements can be introduced.
- The provision of facilities to connect to, and distribute water from, temporary storage cisterns such as pillow tanks at key locations within the site. Ground-level storage cisterns will require provision of booster pumps to either lift the water to existing storage cisterns or to distribute it directly through existing or temporary-site water mains.
- The provision of connection points to existing storage cisterns at suitable locations for delivery of emergency supplies of water, for example from tankers.
- Suitable distribution points for bottled water should be considered in the design process. This should work in conjunction with plans for the supply and distribution of bottled water for drinking to vulnerable patients and those unable to collect supplies from distribution points within the healthcare facility.

Private supplies

2.18 Private supplies independent of the water undertaker are governed by the Private Water Supplies Regulations 2009 and the Water Industry Act 1991. It is recommended that the Environment Agency (EA) and British Geological Survey (BGS) are consulted before considering a private water supply. In some situations, a licence may be required from the (EA) if more than 20 m^3/day is to be abstracted. Any borehole more than 15 m deep should also be registered with the BGS. If, for reasons of back-up or security of supply, there is a connection to the public supply (regardless of whether, or how often, it is used), the installations must comply with the Water Supply (Water Fittings) Regulations 1999. Private supplies should be registered with the local authority, which has the responsibility to maintain a register, risk-assess and monitor the wholesomeness of the supplies where these are used for domestic or food-production purposes.

2.19 The quality of water from a private supply is governed by the Private Water Supplies Regulations 2009. The standards are derived from the Drinking Water Directive and so are almost identical to those for public supplies. Reference should be made to the DWI's private water supply guidance.

2.20 The EA keeps records and maps of sources of private water supply together with details of the geological strata and water-bearing characteristics of the area under its control.

2.21 The feasibility of such a private supply should be decided by comparing the capital costs (of the construction of works, including mains, pumping plant, treatment plant etc) and revenue costs (of electricity for pumping, water treatment chemicals, direct and indirect maintenance and associated management costs, regular water analysis tests etc) with the long-term cost of water supply from the water undertaker over the predicted life-cycle of the installation. Due consideration should be given to the long-term costs of a private supply, and account should be taken of potential deterioration in water quality and/or capacity of the private supply source.

2.22 Where consideration is being given to the use of a private supply in a healthcare

environment, specialist assistance should be sought to:

a. confirm the integrity of the aquifer – that is, whether subject to surface water ingress/industrial effluent/radon/ nitrates/pesticides etc;
b. confirm the long-term quality of water and define requirements for water treatment;
c. design and specify the works needed;
d. carry out a full evaluation of the costs and practicability of a private supply compared to a connection from the water undertaker.

Other sources of supply

2.23 Greywater and rainwater should not be collected for use on, or in, healthcare premises.

Also see HTM 07-04 – 'Water management and water efficiency', which covers the use of greywater, rainwater and unwholesome borehole water.

3 Materials of construction

3.1 Any materials that come into contact with the water in a hot and cold water installation must comply with the requirements of the Water Supply (Water Fittings) Regulations 1999. To demonstrate compliance with these regulations, all non-metallic materials in contact with water should comply with the requirements of BS 6920. Currently no criteria exist to verify metallic materials. A number of lists exist for products and materials that have been assessed for compliance with the requirements and regulations (see paragraph 1.15). Further information on the selection of materials can be found in BS EN 805, BS EN 806-2, BS 8558 and BS 6920-1.

3.2 Materials of construction should be selected to take account of water quality and its potential corrosive properties. The water undertaker should be asked to provide details of any specific requirements and variability from standard conditions.

Note

Consideration needs to be given to substances that may be present in many rubber compounds, and are also occasionally associated with non-metallic materials such as plasticised (softened) plastics, which can provide nutrients for *Pseudomonas aeruginosa* growth. Most non-metallic materials meeting the full requirements of BS 6920-1 should not enhance microbial growth. Where oxidising chemicals are used, they may exacerbate leaching.

3.3 Water supplied by the water undertaker, although remaining wholesome, will nevertheless differ chemically. Some waters are slightly acidic while others are slightly alkaline, and this affects the choice of materials for pipes, fittings and cisterns. The water undertaker also blends water and, accordingly, the character of the water supply may vary from time to time. It will therefore be necessary to consult the water undertaker for advice on what materials should be avoided.

3.4 The choice of materials for piping and fittings should also take into account the nature of the soil in which the piping is to be laid. The materials selected should, where necessary, resist possible corrosion both inside and outside. The extent, if any, of anti-corrosion treatment of the outside of the piping will depend on the analysis of the soil. The advice of the water undertaker should be sought on the protective measures usually adopted in the area.

3.5 Corrosion (or erosion) can be caused by the motion of water when it is in a turbulent state and thus subject to rapid changes in pressure. At moments of low pressure, minute vapour or gas bubbles may be released which collapse with implosive force the moment the pressure is increased. The collapse of such bubbles on a metallic or concrete surface will quickly cause deep pitting or erosion of that surface. The design team should therefore avoid high velocities, the sudden increase of pressures or pulsating pressures.

3.6 Metallic piping should not be installed in contact with corrosive building products and materials.

3.7 Corrosion may result from galvanic action where dissimilar metals are connected. Dissimilar metals should therefore be avoided as far as practicable, but if that is not possible, it should be determined that deterioration through galvanic action is unlikely to occur, or effective measures should be taken to avoid deterioration.

3.8 The materials generally used for the conveyance of water in healthcare premises are copper, steel, stainless steel and plastics. Lead is no longer allowed under the Water Supply (Water Fittings) Regulations 1999 for pipework or solders. Lead-free solders only should be used (see also paragraphs 3.33 and 5.1–5.5).

3.9 Substances leached from materials of construction of pipes, cisterns or other water fittings in contact with water must not adversely affect the quality of water stored or drawn for domestic or food-production purposes (Water Supply (Water Fittings) Regulations 1999).

3.10 Direct gas-fired water heaters are particularly prone to corrosion and scale formation, and the inside of these heaters should be provided with suitable linings to limit these effects.

Steel pipes and fittings

3.11 Where steel is used for bolts, nuts and slip-on couplings, adequate protection from corrosion should be provided. This usually takes the form of bitumen coating, but bitumen is not permitted in contact with water required to be wholesome (that is, to be used for normal domestic or food-production purposes).

3.12 Unless adequately protected, steel is liable to corrosion both internally and externally, and therefore should not be used untreated. Steel piping is usually supplied zinc-galvanised, but depending on the character of the water to be conveyed, it may be necessary to use piping with an internal coating of either epoxy resin or polyurethane lining, which requires special application techniques (see the Water Industry Specification WIS 4-02-01 – 'Operational requirements: in situ resin lining of water mains').

3.13 External protection for piping may consist of bitumen or tar-wrapping with reinforcing layers of fibreglass or a plastic cladding.

3.14 Where screwed steel piping is used, any threads exposed after jointing should be painted or, in the case of underground piping, thickly coated with a bituminous compound or other suitable composition to prevent corrosion.

3.15 If the soil is corrosive, the pipes should be additionally protected by means of bitumen and fibreglass cloth wrappings.

3.16 Screwed steel piping is jointed with screwed socket joints, using fittings of wrought iron, steel or malleable cast-iron. A jointing compound or tape, which may be one of the many proprietary makes, should be used according to the manufacturer's instructions. Compounds containing red lead must not be used because of the danger of contamination of the water. Jointing compounds should not support microbial growth, should comply with BS 6920-1, and should have been assessed and shown to be appropriate (for example, suitable approvals) for the intended application (see paragraph 1.15). Care should be taken to remove any burr from the ends of pipes and to prevent the entry of excess jointing material. Steel piping may also be jointed with screwed flanges of steel or cast-iron or with mechanical couplings.

Stainless steel

3.17 Stainless steel is being increasingly used in hot and cold water service systems. Reference should be made to Chapter 3 of SHTM 04-01 Part E – 'Alternative materials and filtration' and also the British Stainless Steel Association's (2002) 'Operational guidelines and code of practice for stainless steel products in drinking water supply'.

Copper pipes and copper/copper alloy fittings

3.18 Copper in general is resistant to corrosion. Unless resistant to dezincification, brass fittings should not be used where water conveyed is capable of dissolving undue amounts of zinc from the fitting. External protection from corrosion for buried pipework may be obtained by using copper tube with a factory-applied polythene sheath. Dezincification-resistant material should be used for fittings that are concealed or inaccessible, for backflow prevention devices, and for temperature and pressure-relief devices on heating systems. Copper piping should conform to BS EN 1057 as appropriate for underground or above-ground installations.

3.19 Fittings should comply with the requirements of BS EN 1254-1–6. Copper piping may be jointed by means of compression joints or capillary joints or push-to-connect fittings made from copper or copper alloys. Effective capillary joints in copper pipes can be achieved if care is taken in their construction. For underground use, when using fully annealed copper piping, the fittings should be a manipulative joint.

3.20 Lead-free materials should be used in the formation of all wholesome water pipe capillary joints.

Solder and flux

3.21 When soldering copper tube and fittings, refer to WRAS Information and Guidance Note 9-04-02 – 'Solder and fluxes' (see also paragraph 3.33 on the prohibition of lead solder). If wax-based soldering flux is used, it should be used sparingly and be compliant with BS 6920. It poses a risk of bacterial contamination to the system, which can be difficult to eradicate.

Plastics

3.22 Most water systems operate at modest pressures and at a maximum temperature of 70°C. Such operating conditions are within the specified performance of plastics being produced in a range of sizes and costs suitable for healthcare premises. Most ranges of plastic pipework are not suitable for renal dialysis applications, where water at a temperature of 95°C is regularly circulated for sanitation and there is an incompatibility with reverse osmosis-treated water used in renal dialysis.

3.23 Advantages of plastic include corrosion resistance, lightness of weight, ease of handling and fully weldable systems.

3.24 Disadvantages include poorer mechanical strengths than metals, greater thermal expansion (about seven times that of copper), low temperature and shorter distances between pipe supports. The latter can be alleviated by employing the manufacturer's longitudinal tray that extends the distance between supports.

3.25 Materials in common use for plastic pipework are medium-density and high-density polyethylene, the latter being stronger. Unplasticised polyvinyl chloride (uPVC) pipework has mainly been replaced by the stronger chlorinated polyvinyl chloride (PVC-C). PVC pipes to BS 3505, BS EN 1452 (parts 1–5) and BS 3506 are of a rigid material that has a greater tensile strength than polyethylene, but is less resistant to fracture. These materials are less susceptible to frost damage than metal pipes. Although freezing is unlikely to damage the pipe, it will result in interruption of supply, and subsequent leakage from joints may occur.

3.26 Polyethylene pipes are generally not susceptible to corrosion from either the water or the ground in which they are laid. However, they are not recommended in any soils contaminated with organic materials likely to permeate the plastics and taint the water such as coal gas, methane, oils, petrol or other organic solvents. However, plastics pipes incorporating a protective barrier (barrier pipes) may be used if tested to Water Industry Specification WIS 4-32-19 – 'Polyethylene pressure pipe systems with an aluminium

barrier layer for potable water supply in contaminated land'. Further advice is available from the report by UK Water Industry Research (UKWIR) "Guidance for the selection of water supply pipes to be used in brownfield sites" https://www.ukwir.org/site/web/content/reports/reports.

3.27 It is essential to consider the locality of exposed plastic pipes to ensure that there is no likelihood of mechanical damage and effects of UV light; otherwise suitable protection around the pipe will be necessary. Plastic piping should be adequately supported and incorporate adequate means of accommodating expansion, bearing in mind that plastic pipes have a much greater coefficient of thermal expansion than metal pipes.

3.28 Methods of jointing employed include compression joints with insert liners, flanged, screwed and fusion-welded joints, as well as joints of the spigot and socket type. The method of jointing employed is dependent on the bore of the pipe and the applied internal pressure, and should be in accordance with the manufacturer's recommendations. A competent fitter who has been trained under an approved scheme should make joints.

3.29 Manufacturers tailor plastics used in pipes and fittings for specific applications and factors such as pressure, temperature and life-cycle analysis, which take into account specific water qualities inclusive of oxidation levels. Manufacturers' recommendations should be strictly followed when selecting appropriate pipes and fittings for a particular application. Assumptions should not be made that a pipe/fitting suitable for one application can be used in any application. For example, pressure requirements or the suitability for cold water use may not mean it has been designed and tested for hot water use; equally suitability for one high temperature application may not be suitable for another high temperature application. Incorrect selection may not be covered by the manufacturer's warranties and may result in shortened service life.

Multi-layer pipes

3.30 Multi-layer pipes are becoming more available and may consist of plastics or metal layers, for example aluminium pipe with an external and internal sheath of plastic. BS EN ISO 21003 should be used to demonstrate the performance of such pipes and fittings before considering their selection.

Iron pipes and fittings

3.31 Ductile iron is little used nowadays, but it may be encountered in the course of a refurbishment project or in areas of hostile soil conditions. Cast-iron has good resistance to corrosion, and this is further enhanced if the casting skin on the metal is still intact. Although ductile iron pipes are thinner than grey iron pipes, their resistance to corrosion is at least as good, and there is evidence that they tend to be more resistant. In assessing the life expectancy of ductile iron pipelines, account should be taken of any intended higher operating pressures that may be used or permitted.

3.32 In made ground containing ashes and clinker, or in certain natural soils such as aggressive waterlogged clays, and saline and peat marshes, additional external protection may be required. This may be provided by the use of protective coatings such as bitumen or coal-tar sheathing, by protective tapes, by loose polythene sleeving or, in certain circumstances, by concrete. Water undertakers are using more composite materials in pipework to overcome the risks.

Lead

3.33 The Water Supply (Water Fittings) Regulations 1999 prohibits the use of lead in wholesome and domestic water systems. No new lead piping or lead solder should be installed or used in any building. In the unlikely event of any lead pipework being discovered in existing healthcare premises, it should be removed as soon as practicable.

Concrete

3.34 Protection of concrete pipes may be required against sulphate and acid attack. The minimum size available in concrete pipework is 150 mm diameter, and therefore its practical use for healthcare premises is very limited.

3.35 Standard concrete pipes may be used when not subjected to internal pressure. Pre-stressed concrete pipes are available as pressure pipes, but only in larger sizes.

Asbestos cement pipes and fittings

3.36 Asbestos cement pipes generally withstand corrosion but may have to be protected when laid in soil of high sulphate content. If iron fittings are used, both internal and external protection should be applied.

3.37 Specialist advice should be taken if work on materials containing, or suspected of containing, asbestos is to be carried out. See also the EA's regulatory position statement 008 – 'Leaving decommissioned pipes in excavations'.

Flexible water supply hoses

3.38 Flexible hoses (also known as tails) have become a convenient method of connection between hard pipework and sanitary fittings and/or equipment. They typically comprise a steel-braided outer sheath with a synthetic lining.

3.39 There have been reports of high counts of *Pseudomonas* and *Legionella* in water samples taken from outlets fed by flexible lined hoses due to colonisation of the lining. Materials such as ethylene propylene diene monomer (EPDM) rubber may be susceptible to microbial colonisation. Careful selection of materials for their suitability for wholesome water systems is needed and they should be verified before being used.

3.40 New lining materials are now available such as polyethylene (PE), cross-linked polyethylene (PEX), linear low-density polyethylene (LLDPE) and post-chlorinated PVC (PVC-C) and should be compliant with BS 6920 (see paragraph 3.1).

3.41 Flexible hoses should be used only for the following applications:

- to allow for vibration of equipment;
- to accommodate vertical displacement of high and low baths and sinks;
- to facilitate essential maintenance and access of bespoke equipment when no alternative is available.

Note

Where fitted, flexible hoses should be kept as short as possible and be kink-free so as to not affect flow.

Cleanliness and hygiene

3.42 All pipes, fittings, valves, sub-assemblies, calorifiers, cisterns etc intended to form part of the hot and cold water service installation should be supplied to site cleaned, free from waterborne pathogens, particulate matter and other residues. All items should be identified as intended for water supply services.

3.43 Pipes should be capped at both ends and suitably wrapped in bundles. Pipe joint fittings should be suitably bagged or capped and boxed. Larger items of plant such as pumps, calorifiers etc should have connections blanked with plugs or flanges.

3.44 Where possible, leak-testing should be carried out using nitrogen or medical quality compressed air or oil-free dry compressed air. This must be carried out by competent personnel (see HSE's GS4 – 'Safety requirements for pressure testing'). Where water is used for test purposes, it should be supplied from a wholesome source. For pre-assembled items such as bathroom pods, all moisture should be removed before being delivered to site and be packaged in such a way as to prevent contamination from waterborne pathogens during transport and storage.

4 Water treatment and control programmes for hot and cold water systems

General

4.1 When control of the microbiological safety of water systems cannot be achieved throughout the system by maintaining temperatures, additional control strategies should be considered to reduce the risk of waterborne infection. Commonly used strategies include the use of filtration, pasteurisation or the use of biocides. Any biocide added to a water system should be dosed at the lowest concentration required to protect patient safety and ensure no undue exposure of individuals to harmful concentrations (refer to the COSHH Regulations 2002).

4.2 Some biocides require the system to be taken off-line before treatment; in these cases, users should take great care to ensure water cannot be drawn for bathing, food preparation or drinking until the treatment chemical has been completely flushed from the system. The WSG should ensure that measures are taken to protect vulnerable patients such as those in renal dialysis units.

Note

When building a new haemodialysis unit, a separate mains water supply should be considered so that other areas of the healthcare facility may be dosed without affecting the RO plants. If this cannot be achieved and biocides are required, appropriately monitored control systems need to be in place.

4.3 A biocidal product for this purpose is defined as one which controls harmful or unwanted organisms within water systems. The EU Biocides Regulation 528/2012 regulates the use of biocidal products that have been proven to be safe and effective for their intended uses. Within the EU, suppliers of active substances must be registered and as such there is a list of registered products and suppliers (Article 95 list). Within the UK the HSE is the designated authority for overseeing implementation of biocides.

4.4 The Drinking Water Directive, transposed into law in the Water Supply (Water Quality) Regulations 2000 (as amended) for public water supplies, and in the Private Water Supplies Regulations 2009 for private supplies, sets out additional separate requirements for products and substances that may affect water intended for human consumption. This ensures that treatment chemicals suitable for use in the preparation and distribution of drinking water have no adverse effects on the quality of water intended for human consumption. The DWI publishes a list of products approved for treatment of drinking water supplies.

4.5 The WSG should be satisfied that the design, specification and commissioning will enable the water treatment systems to achieve the required biocide concentrations throughout the system at all times to minimise microbial risk. The impact of treated water on the materials and components of the existing system should be taken into account along with advice from the necessary suppliers and installers. Within a healthcare facility, the detrimental effects of biocidal treatment, such as corrosion of metal components and deterioration of plastics and elastomers, should be taken into consideration as biocide use may

shorten the lifespan of particular components. Complete flushing of the system should be carried out and verified before being brought into use. Concentrations and stand times should be assessed and controlled.

4.6 Where treatment systems produce a discharge as part of the process, an appropriate air break to the drain must be installed such as a type AA air gap or an air-break-to-drain device in accordance with BS EN 1717 clause 9.

Biocidal treatment

Note

Any biocidal treatment system should not adversely affect the materials of construction.

4.7 In addition to maintaining a temperature control regimen, there may be occasions where additional biocidal treatment is required for the effective control of *Legionella* and other opportunistic waterborne pathogens. However, the selection of suitable treatment is complex and depends on a number of parameters, and the chosen biocide should be properly managed. This is particularly the case with cold water services compared with hot water services where, with the benefit of circulation, water is returned to the calorifier/water heater and is then pasteurised. However, it should be taken into consideration that effective concentrations of some biocides are difficult to achieve in hot water systems due to gassing off. For water intended for consumption, the biocide concentrations must not exceed prescribed concentrations for drinking water.

4.8 Where biocides are used to control microbial growth in water systems, as with the temperature regimen, meticulous control and monitoring programmes should be in place if they are to be effective. However, careful consideration should be given to any equipment that is connected to the water system that may be affected by the application of a biocide (for example, renal departments, haemodialysis units and neonatal units).

4.9 To meet legislative requirements, biocides used for water treatment must:

- contain an active substance approved for that use (subject to product-type) under the Biocidal Products Regulations (or be under review for the relevant product-type) and the biocidal product consisting of or containing that substance must be authorised for the product-type (subject to the transitional arrangements while the active substance review is ongoing);
- be suitable for drinking-water use.

4.10 The WSG should be actively involved in the decision-making process and should involve consultation with the water undertaker to ensure the suitability of biocidal products for their intended application.

4.11 There is no single water treatment regimen that is effective and appropriate in every case, and each system has both merits and limitations. The implementation of a biocide regimen together with maintaining temperature control requires constant vigilance to ensure the safety of particularly vulnerable patients in healthcare premises. For example, dedicated treatment and supply arrangements may be required for renal and haemodialysis units or for making up infant feeds where concentrations of biocides in the water would be harmful to patients.

4.12 Each water treatment system should be validated and monitored to demonstrate that the correct biocide concentration is being achieved for controlling microbial growth and does not exceed prescribed or guideline values under differing flow rates and water demands.

4.13 The frequency of biocide monitoring and verification, in addition to temperature monitoring, will depend on the treatment regimen selected. Each treatment system should have a failsafe mechanism to prevent overdosing while also ensuring that effective concentrations are maintained throughout

the system. When a water system is being purged and is initially dosed, checks should be made at various system outlets to ensure that satisfactory concentrations of treatment chemicals are being achieved throughout the system. Automatic leak detection of the biocidal treatment system is also recommended for hazardous substances.

4.14 The effective concentrations of biocides used to treat water systems in highly colonised systems or during commissioning of new installations in healthcare domestic water systems may initially need to exceed the safe concentrations for human consumption as stated in water legislation and may make the water unwholesome. The biocidal treatment systems used should be selected with care and must comply with the requirements of the Water Supply (Water Quality) Regulations 2000 and COSHH Regulations. Where there has been a requirement to provide biocides at concentrations above those permitted for drinking water approval, agreement should be sought from the WSG. In such circumstances the water system should be off-line and drinking-water outlets should be clearly labelled as unsuitable for ingestion (this includes the making up of neonatal feeds).

Note

When water treatment and control programmes are effectively maintained to control *Legionella*, there will be less likelihood of colonisation of other opportunistic microorganisms. However, in the case of *P. aeruginosa*, where the origin may be due to retrograde contamination from patients and/or staff, other local management control measures and monitoring will be necessary.

Chlorine dioxide

4.15 Chlorine dioxide is an oxidising biocide that is capable of reacting with a wide range of organic substances and has been shown to be effective in the control of organisms in water systems. The safe in situ generation of chlorine dioxide as a chemical for drinking water treatment is subject to BS EN 12671. Chlorine-dioxide-generating equipment should be selected to ensure product efficacy of greater than 90% to provide the optimum performance for the minimisation of total oxidants.

4.16 The use of chlorine dioxide as a control measure will depend on the design of the systems in use and, in an existing system, its operational history. See also HSG274 Part 2.

Copper/silver ionisation

4.17 Ionisation is the term given to the continuous release, by electrolytic action, of copper and silver ions into water.

4.18 The Water Supply (Water Quality) Regulations 2000 set a standard for copper of 2 mg/L, and this must not be exceeded.

Note

Currently the Drinking Water Directive does not include a prescribed concentration for silver, as there is insufficient toxicological data. The World Health Organization (WHO) (2011) states: “there is no adequate data with which to derive a health-based guideline value for silver in drinking water”. It continues: “special situations exist where silver may be used to maintain the bacteriological quality of drinking water [and] higher concentrations of up to 0.1 mg/L … could be tolerated in such cases without risk to health”.

4.19 Copper/silver ionisation requires soft water to prevent scaling of the electrodes, as maintaining adequate silver ion concentrations in hard water systems can be difficult because of scale build-up on the silver electrodes, potentially leading to the obstruction of copper and silver ion release. In addition, there have been cases of staining to sanitaryware in hard water areas. See also HSG274 Part 2.

Ozone and ultraviolet treatment

4.20 Whereas biocides dosed into the water are intended to be dispersive and will result in a biocide agent within the water system, ozone and ultraviolet (with appropriate pre-filtration) are intended to be effective close to the point of application. UV will only be active in waters of low turbidity and at the point where the water passes through the UV lamp housing. Ozone will only be effective very close to the point of application. Ozone and ultraviolet treatment will not ensure system control downstream in the hot and cold water service systems.

Note

Consideration needs to be given to the risk of bromate formation when using UV and ozone to further treat chlorinated mains water, which may contain bromide ions (see the DWI's research paper on the formation of bromate during drinking water disinfection).

Other water treatments

4.21 A range of biocidal treatment systems are available, and further advice on their use is given in HSG274 Part 2.

Point-of-use filtration

4.22 For pathogenic waterborne organisms including multi-drug-resistant strains, at a minimum, and in accordance with the organisation's water safety plan, a risk assessment should be made in order to determine whether sterilising-grade point-ofuse filters should be installed or whether taps need to be changed (see American Standard Test Method (ASTM) F838-05 – 'Determining bacteria retention of membrane filters utilised for liquid filtration').

4.23 During design and installation, it is necessary to ensure that:

- they comply with all appropriate regulations and meet EU legislation on the preparation of foodstuffs for infants and young children;
- tap outlets have appropriate fittings for attachment of filters;
- sufficient activity space between the filter outlet and the basin is provided so that an effective backflow prevention air gap (AUK3) is maintained, and hands can be comfortably washed without contaminating the body of the filter;
- there is sufficient flow once a filter has been fitted to enable effective hand-washing;
- the filters are changed at the intervals specified by the manufacturer.

4.24 In systems with high particulates, a prefilter may be necessary to prevent shortened lifespan due to filters clogging.

5 Metal contamination

5.1 If plumbing systems were installed before 1987 (in which lead pipes or lead-based solders are likely to have been used), it may be necessary to consult the water undertaker (see also paragraphs 3.8, 3.20 and 3.33).

5.2 The Water Supply (Water Quality) Regulations 2000 and the Private Water Supplies Regulations 2009 set an upper concentration for lead in drinking water of 10 µg/L. This value is likely to be exceeded if lead pipes are present or if copper pipes have been joined with solder containing lead. If drinking water contains more than 10 µg/L of lead, remedial action should be taken. The use of lead solder is prohibited on all plumbing installations where water is required to be wholesome.

5.3 Copper concentrations above 1 mg/L may cause staining of laundry and sanitaryware and increase the corrosion of galvanised iron and steel fittings. While the maximum allowable copper concentration in drinking water is 2 mg/L, most supplies will give a concentration at the tap of less than 1 mg/L.

5.4 Water supplies to certain specialist units such as maternity, neonatal paediatric, general paediatric and renal dialysis units (see the Renal Association guidelines) should be monitored to ensure that water quality is within acceptable limits. The design team should seek toxicological advice to ascertain the exact water quality requirements for specialist units.

5.5 Where the water supply is known to dissolve metals (for example, water with very low total dissolved solids), regular sampling to determine the condition of the system should be carried out. Strategic sampling points should be selected to ascertain that the level of metal contamination (for example, the dissolution of lead solder in capillary joints) in the water distribution does not result in limits above the stated safe levels.

5.6 Elevated concentrations of copper can occur in copper pipework systems with:

- naturally acidic (low TDS) water supplies;
- base-exchange softening;
- poor control of oxidising biocides;
- poor control of copper/silver ionisation;
- localised stagnation.

5.7 In some areas with known aggressive water issues, it is normal to specify corrosion-resistant pipework materials such as stainless steel or plastic. These materials may also need to be considered for demineralised, fully deionised and high purity water.

6 Water softening

6.1 Hard waters are unsuitable for many industrial and domestic purposes. Treatment may therefore be necessary to remove or alter the constituents to render the water suitable for particular purposes and increase the effectiveness of control measures.

6.2 Hardness is due to calcium and magnesium salts in the water and is expressed in terms of milligrams per litre as $CaCO_3$. Temporary (carbonate) hardness is related to the bicarbonate salts of calcium and magnesium. Permanent (non-carbonate) hardness is related to the other salts of calcium and magnesium – chlorides, sulphates, nitrates etc. The generally accepted classification of waters is shown in Table 1.

Table 1 Classification of water hardness

Description	Milligrams per litre (mg/L as $CaCO_3$)
Soft	0 to 50
Moderately soft	50 to 100
Slightly hard	100 to 150
Moderately hard	150 to 200
Hard	200 to 300
Very hard	Over 300

6.3 When the temperature of water is raised, the hardness will be reduced by some of the bicarbonate dissolved salts (temporary hardness) coming out of solution and forming solids in suspension, some of which will be deposited on heating surfaces to form an adherent limescale, thus reducing the heat-transfer rate. The tendency to form scale, and the morphology of that scale, is not just dependent on the hardness but also on the other chemical constituents of the water. For this reason scaling indexes such as Ryznar and Langelier combine various additional factors to assess the likelihood of scaling.

Note

Primary heating circuits that are filled/topped up by water supplied directly from the water mains should be treated with a chemical corrosion inhibitor. Specialist advice is given in BSRIA's BG50 –'Water treatment for closed systems'. In exceptionally hard water areas, additional measures may be required.

6.4 The extent of treatment required to prevent scale formation will depend on the process for which the water is being heated; it may therefore be necessary to achieve one of the following conditions:

a. replacement of calcium and magnesium salts by their more soluble sodium equivalents;

b. removal of all salts (demineralisation);

c. where water of enhanced purity is required for specialised uses, it can be produced from softened water by reverse osmosis or by demineralisation.

6.5 The most common water-softening process used for the protection of hot water calorifiers is base-exchange softening. This process removes permanent and temporary hardness from water. The technique uses an ion-exchange process in which the calcium and magnesium ions in solution are removed and replaced by sodium ions.

Note

All water softeners must be installed with an appropriate backflow prevention device in accordance with the Water Supply (Water Fittings) Regulations 1999. Where softeners discharge to a drain, they must have an appropriate air break to the drain installed such as a type AA or an air-break-to-drain device in accordance with BS EN 1717 clause 9.

6.6 Epidemiological studies have shown that the incidence of cardiovascular disease tends to be higher in areas with soft water supplies than in areas with hard water supplies. The association is clearest where the soft water supplies contain hardness below about 150 mg/L (as $CaCO_3$). The explanation is not known, but it is considered prudent, where possible, not to drink water that has been artificially softened to concentrations lower than this. Softened water may also tend to dissolve metals from pipes. Water softeners containing ion-exchange resins may be subject to bacterial contamination if not adequately maintained in accordance with the manufacturer's instructions (these may also require periodic disinfection). Softeners using salt-regenerated ion-exchange resins increase the sodium content of the water during softening, and this may be undesirable for young children and infants (including the making up of babies' bottles) and anyone on strict salt-restricted diets. These concerns can be avoided if water intended for drinking and cooking is not softened. See also paragraph 9.11.

6.7 Waters having a hardness of up to 400 mg/L have been used for public supplies without preliminary softening. While it is accepted that supplies for domestic purposes need not be softened, some water undertakers carry out partial softening.

6.8 The need for softened water in healthcare facilities for domestic purposes other than drinking and cooking should be considered on the merits of each case; if treatment is considered essential, the extent of softening should be the minimum to achieve an acceptable level. A generally acceptable range is between 80 and 150 mg/L, and not less than 60 mg/L, but this should not be taken as a requirement for healthcare facilities as it may be impracticable to achieve. The cost and difficulties of treatment may be prohibitive for certain waters if the hardness value is particularly high and the content of magnesium is appreciable.

6.9 Scale deposition is a significant problem in pipework, reducing flow, efficiency and increasing the surface area for biofilm formation. In hard water areas, softening may well be needed to reduce risk. Generally, within healthcare premises, softening of a hard water supply may be required on feeds to the following:

a. steam boilers – to prevent sludge and limescale building up (see BS 2486);
b. hot water services where outlets (particularly showers) are affected by limescale;
c. laundries – high maintenance costs and the uneconomic uses of soap or detergents are caused by the presence of hardness.

6.10 Other water-softening methods include physical water conditioning and magnetic water conditioning.

7 Filtration

7.1 Water delivered to premises may have been derived from various river and groundwater sources, the quality of which can be different, thus requiring a number of treatment processes. Filtration to an appropriate standard will normally have been carried out by the water undertaker or private water supply operator. Some treatment works use additional methods to remove minute and fine suspended particles from water. These include microfiltration, ozone, carbon and ion-exchange systems.

7.2 In exceptional circumstances or where a private water supply is used on site, additional on-site filtration may be required as part of a multi-barrier point-of-entry treatment system. Advice should be sought from the appropriate undertaker on the need and form of such treatment. For a list of approved products for private water supplies, visit the DWI's website.

Note

POU filtration is covered elsewhere in this publication.

8 Water storage

Note

Refer also to HSG274 Part 2.

8.1 Water is stored in healthcare premises for the following reasons:

a. to provide backflow protection;
b. to provide a reserve supply during disruption of the incoming cold water supply (this encompasses water quality as well as water quantity issues);
c. to reduce the maximum demand on the cold water supply;
d. to reduce the pressure from that of the distribution system.

Note

Separate arrangements are required to provide accommodation for the expansion of any water subjected to heat, that is, hot water and heating services.

8.2 The purpose for which the storage is used can vary, but has only a minor effect on its design. The following generally covers a typical range of uses:

a. cold water services, domestic, laundry etc;
b. cold water feed to hot water services;
c. pathology;
d. endoscopy units;
e. dental premises;
f. treated cold water for laundries, heating etc when local supplies are unsuitable;
g. break-tanks on cold water supplies serving points of use where backflow is, or is likely to be, harmful to health due to a substance representing a serious hazard (for example, supplies to pathology laboratories (see paragraphs 12.18–12.24 on the prevention of contamination));
h. fire-fighting.

Note

Feed and expansion cisterns for heating services, chilled water etc are excluded.

8.3 All water storage for domestic purposes should meet drinking-water quality requirements.

Note

Separate drinking water systems are not recommended for new buildings.

Water supplies

8.4 Where there is private supply in addition to the water undertaker's, it needs to be introduced separately into the healthcare facility's water storage system. See paragraphs 2.3–2.4 (connection arrangements and notification requirements) and 12.18–12.24 (backflow prevention requirements) for compliance with the Water Supply (Water Fittings) Regulations 1999.

Note

It is best practice to include the operation of valves in a regular maintenance and operation schedule to ensure they do not become seized. The flushing of the supply could be integrated into this programme to aid the prevention of stagnation of water at dead-ends. Additionally, advice from the water undertaker on an appropriate time period to undertake flushing cycles on supplies should be sought.

Extent of storage

8.5 Storage should be designed to minimise residence time in the cistern and maximise turnover of water to avoid stagnation and deterioration of water quality. Storage volume should be calculated on the basis of peak demand and the rate of make-up from source of supply. There may be more than one peak period during each day. The interval between peak periods is important as it affects the storage capacity based on the make-up flow. It also determines the available time for maintenance if twin cisterns are not installed. Unless the water supply is particularly vulnerable to disruption and subject to risk assessment (balancing the microbiological risk and contingency supplies), a nominal 12 hours' on-site storage is recommended. Reference should also be made to paragraphs 5.18–5.34 in HBN 00-07 – 'Planning for a resilient healthcare estate', which provides guidance on continuous water supplies and bulk storage.

8.6 There is currently no reliable guidance on the capacity of water storage. Design teams should therefore liaise with the commissioning trust to ascertain their recent records the quantity of water used over the previous five years. There is evidence that the historical values given for district general teaching hospitals that amounted to 900 L per day per bed are in excess of actual usage. If the proposed healthcare building development includes significant changes in function that introduce greater specialisms and more intensive treatment programmes, then the design team is advised to seek guidance from other healthcare organisations that are responsible for buildings of a similar nature.

Note

Useful data may be derived from the following:

- Water figures per unit of healthcare activity are published by the NHS Sustainable Development Unit. See Appendix 1.
- The Estates Returns Information Collection (ERIC) collects data from all NHS trusts on the consumption and associated costs of water.

8.7 The design team should ensure that the local water supplier is involved in proposals for planning the water storage values. CIBSE's Guide G – 'Public health engineering' gives further guidance on sizing cold water storage.

8.8 A summation of the average daily consumption for each ward unit contained in a building should be made. From the requirements of each building, the policy of water storage for the whole complex should be decided. It does not always follow that peak demands for each building will coincide, and therefore there may be scope for applying a diversity factor to the whole site.

8.9 Where the water requirement is to be met from a private supply, the summation for each building may require assessment on the basis of storing and using water according to the minimum treatment of the water for each particular use. Likewise, where the water is hard enough to require softening for certain domestic and/or laundry purposes, separate storage will be required, and this should be taken into account when assessing the total stored water.

8.10 Staff quarters and industrial areas may be remote from the main building and supporting departments. The laundry may serve a number of healthcare buildings as well as the premises

at which it is located. The storage requirement for such accommodation should therefore be calculated separately and integrated with the accommodation whenever this is practical.

8.11 Where new healthcare premises are to be built in separate phases, the water storage, supply and distribution service for the whole premises should, as far as possible, be planned and evaluated at the design stage. This will enable the total water supply requirement to be assessed in the planning stages, and appropriate areas of accommodation (but not tank storage) to be allocated. It is important that, where it is anticipated that a building will be occupied in phases, the storage levels can be raised as required. Commissioning should be carried out also as a planned process related to occupation following a risk assessment (see BS 8550). The design should facilitate compartmentalisation.

Location and form of storage

8.12 The location of storage will depend on the total volume required, the topography and layout of the site proposed for development, and the sources and adequacy of the water supply. A limited site footprint may call for much higher buildings to achieve the required accommodation. Depending on the supply water pressure, it may be necessary to install pressurisation equipment to boost the incoming supply. The cost of the supporting structure will have an important bearing on the solution adopted.

8.13 Where storage is located in individual buildings and an adequate supply is available from the water undertaker, a connection in accordance with the Water Supply (Water Fittings) Regulations 1999 to each point of storage may be the most economical arrangement. In such cases, interconnections between selected points of storage should be provided to deal with emergency and maintenance requirements, always providing that such interconnections do not contravene the Water Supply (Water Fittings) Regulations 1999 and do not result in water stagnating within the storage or distribution system.

8.14 Cisterns should not be located where there is any likelihood of flooding, excessive heat gain or any other factor that could affect the contents of the cisterns. They should not be installed in any location where access for general inspection or maintenance is restricted. Where refurbishment is undertaken, an assessment is carried out to ensure no adverse impacts occur on the temperature and quality of the water stored. As part of this assessment, climate change mitigation measures should be considered.

8.15 Separate systems are required to segregate domestic services (WC, hand-washing etc) from high-risk applications and equipment within such facilities as laboratory, pathology and mortuary departments.

8.16 An assessment of atmospheric and environmental (including feral wildlife such as birds etc) risks should be undertaken to ensure they do not affect the temperature or quality of stored water. If concrete water cisterns are to be considered, they should be designed to form an integral part of the building structure. The materials of construction, however, must comply with the Water Supply (Water Fittings) Regulations 1999.

8.17 As in the case of external storage, cisterns should be installed in positions where they can be readily inspected and maintained and where they will not be affected by frost or high temperatures.

8.18 To determine access requirements to cisterns, it is essential that a suitable and sufficiently comprehensive assessment of the risks for all work associated with cisterns is carried out to determine what measures and parameters are required under Regulation 3 of the Management of Health and Safety at Work Regulations 1999. For work in confined spaces this means identifying the hazards present, assessing the risks and determining what precautions to take.

8.19 Roof spaces in which cisterns are to be installed should have adequate trap doors or

other means of access and adequate lighting to facilitate inspection and maintenance.

8.20 Where storage is in ground, as distinct from being housed within a building, it is essential to ensure that there is no risk of contamination. Investigations of such risk require careful consideration of site conditions and should include such aspects as:

- flooding;
- subsidence;
- the location of sewers and drains and other buried services;
- the maximum and minimum height of the water table in the area;
- the natural drainage of surface water;
- ingress of contaminants such as dust, debris etc; and
- in the event of storage below a car-parking area or roads, the danger of oil/fuel seepage.

The future development of the healthcare building and probable extensions should also be taken into account in this respect. Accessibility should be limited to those who need access (see also NHS Protect's (2012) 'Guidance on the security and management of NHS assets').

8.21 Storage below ground should be adopted only as a last resort, and cisterns should be installed within a watertight bund allowing sufficient space all around and beneath the storage vessel to permit inspection and maintenance. Any underground construction arrangement, concrete or otherwise, not directly against earth will reduce the risk of contamination. The tank chamber should include provision for a sump to collect drainage water and any piping necessary to pump out tanks to the site drainage. The Water Supply (Water Fittings) Regulations 1999 require any buried concrete reservoir to be designed, constructed and tested in accordance with BS EN 1992-3.

Note

Refer also to the HSE's guidance on confined spaces.

Construction of cisterns

8.22 All storage cisterns:

- should be constructed in accordance with manufacturers' recommendations and should comply with the Water Supply (Water Fittings) Regulations 1999;
- should have been assessed and shown they are appropriate (for example, suitable approvals) for the intended purpose (see also paragraph 1.15); and
- should comply with BS EN 805, BS EN 806 (Part 1 to 5) and BS 8558. Glass-reinforced plastic (GRP) cisterns should comply with BS EN 13280.

8.23 The WRAS Information and Guidance Note 9-04-04 – 'Cold water storage systems – design recommendation for mains supply inlets' provides useful advice regarding the design of the inlet arrangements to ensure compliance with the Water Supply (Water Fittings) Regulations 1999. Of particular importance is the need to ensure cistern roof supports are of a type that do not retain water within the support, cause pockets of stagnation, or prevent the free flow of water throughout the cistern, as these have been shown to cause degradation of the stored water quality (see also Estates and Facilities Alert EFA/2013/004 – 'Cold water storage tanks'). Any other internal structures should be designed so as not to retain water, cause pockets of stagnation, or prevent the free flow of water throughout the cistern.

8.24 Depending on size and/or capacity, water storage should be divided into convenient compartments suitably interconnected and valved to facilitate cleaning, disinfection, repair, modification and inspection, without seriously disturbing the cold water service. Cistern strengthening should be by means of

stainless steel tie bars and not baffle plates. Where multiple cisterns are provided, they should be connected in such a way that there is equal flow to prevent stagnation. Pipework connections should also be arranged to ensure cross-flow across the cistern. A water meter should be considered at each inlet pipe to verify equal volumes are being used.

8.25 Separate cisterns should also be provided for storage of different water supplies, for example central-heating header cisterns, cold water storage, softened water, fire-fighting water, and high-risk areas (for example, laboratories, pathology and mortuary). Precautions should be taken to ensure that mixing does not take place between such supplies, and it should be noted that isolation by means of shut-off valves between them is not acceptable.

8.26 The materials used for storage cisterns serving healthcare should be appropriate for the intended use. The material selected should comply fully with the Water Supply (Water Fittings) Regulations 1999 (see paragraph 1.15). Pre-insulated sections are recommended where practicable.

8.27 Sectional cisterns fabricated from GRP or pressed steel provide a convenient means of bulk storage of water at atmospheric pressure. The components can be readily transported to site and, subject to unit multiples, they can be erected to give varying proportions of length to breadth and depth. It is also possible to make provision for future extension in capacity by an increase in available base area or, within limits, depth. If sectional cisterns are selected, designs with external assembly flanges and self-draining profiles should be used, since this arrangement facilitates easy cleaning of internal surfaces of the cisterns.

8.28 The Water Supply (Water Fittings) Regulations 1999 laid down the minimum requirements for wholesome water storage cisterns. Recommendations to comply with these are given in the WRAS 'Water Regulations Guide'. The requirements are indicated in Figure 2.

Note

Cisterns should be sited away from heat sources and be protected from heat gains by insulation. Adequate and safe access should be provided for inspection and maintenance (both internally and externally).

8.29 Each storage cistern or its compartment should also be provided with the following:

a. internal and external access ladders as necessary to comply with current health and safety requirements;

b. a full-way servicing valve at each inlet and outlet connection, except for cisterns providing water to primary circuits or heating circuits, overflow pipes, and warning pipes. Where practicable, all outlets should be taken from the base of the system and be sited opposite to the inlet;

c. a suitably-sized drain connection complete with isolating valve. The invert of the drain connection should be positioned so as to provide maximum drainage of the cistern.

8.30 Cisterns in roof spaces should be adequately supported on bearers placed under the longitudinal or lateral cistern section joints. To avoid distortion, a flat section of marine ply or equivalent should be sited between the support structure and the cistern. Final siting should be in accordance with the manufacturers' recommendations. See also the Note after paragraph 8.28.

8.31 The design may incorporate a watertight drip tray under the cistern to contain condensed water or leakage so as to avoid damage to accommodation below. The necessity of a drip tray or watertight bund with drainage will depend on individual case requirements. The floor of the drip tray or bund should be graded to a drainage sump complete with drain pipe. A single pipe should drain off any overflow water from the sump and lead to a discharge point at ground level where any water flow would be readily noticed.

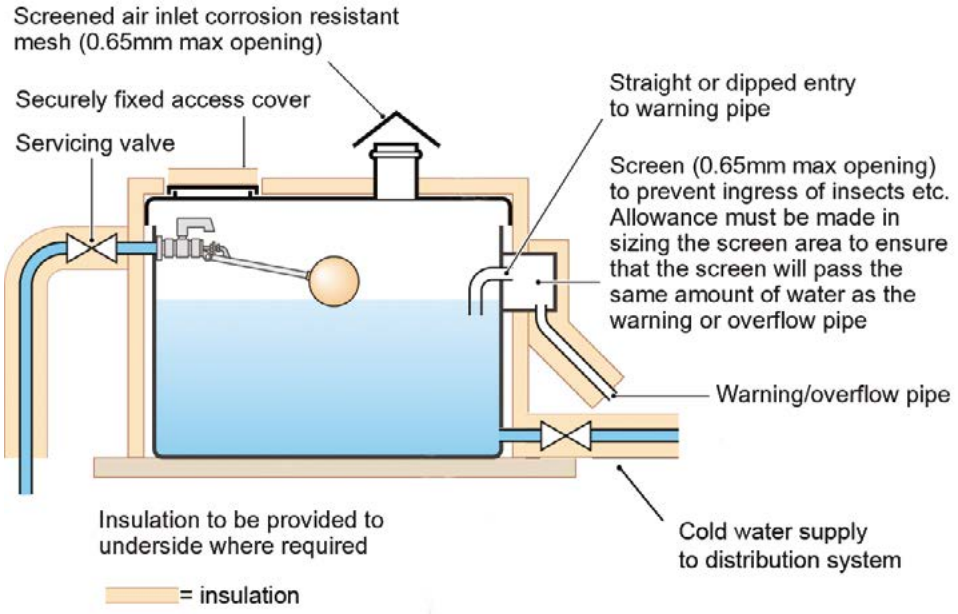

Figure 2 General wholesome water storage cistern arrangements

If it is not possible to terminate the discharge pipe from the sump so that any discharge of water can be seen, an audible alarm should be installed to warn of overflow conditions. Cistern support levels should be constructed to keep the valves clear of the water level in the drip tray or bund in the event of cistern leakage. Special requirements apply to the supporting of GRP sectional cisterns on bearers, and manufacturers' recommendations should be observed. The cistern should be provided with a warning pipe or a no less effective device to indicate leakage through the inlet control valve if this should occur.

8.32 On no account should a sectional cistern be installed on a concrete plinth (directly or on steel beams) that is protected by an asphalt membrane. Subsequent irregular settlement into the asphalt may lead to cistern distortion and leakage.

8.33 A consideration in deciding cistern shape and layout is the location of the services duct. Whereas the cistern room may be positioned aesthetically in relation to the building elevation, the duct serving it will be located to suit the internal layout. The pipe route from the system to the service duct will require access for inspection and protection from frost and heat gain.

8.34 Typical piping and valve arrangements for dual-cistern installations are shown in Figure 3. Supply and draw-off connections should be arranged to facilitate good through-flow and turnover of stored water without stagnation. Advice on interconnection is given in the WRAS leaflet "Interlinked cold water storage cisterns".

8.35 General space lighting should be provided in cistern rooms together with suitable power points for low voltage small tools and inspection lamps.

8.36 The contents and capacity of all cisterns should be clearly labelled as per the Water Supply (Water Fittings) Regulations 1999. The operational water level should be indicated.

Ancillary pipework, valves and fittings

8.37 The arrangement of the cisterns in the room should be such that the pipework runs are as short as possible, but accessibility and walkway clearances are ensured. Flanges on parallel runs should be staggered.

8.38 All cistern-room pipework and valves should be insulated and clearly labelled to identify their purpose.

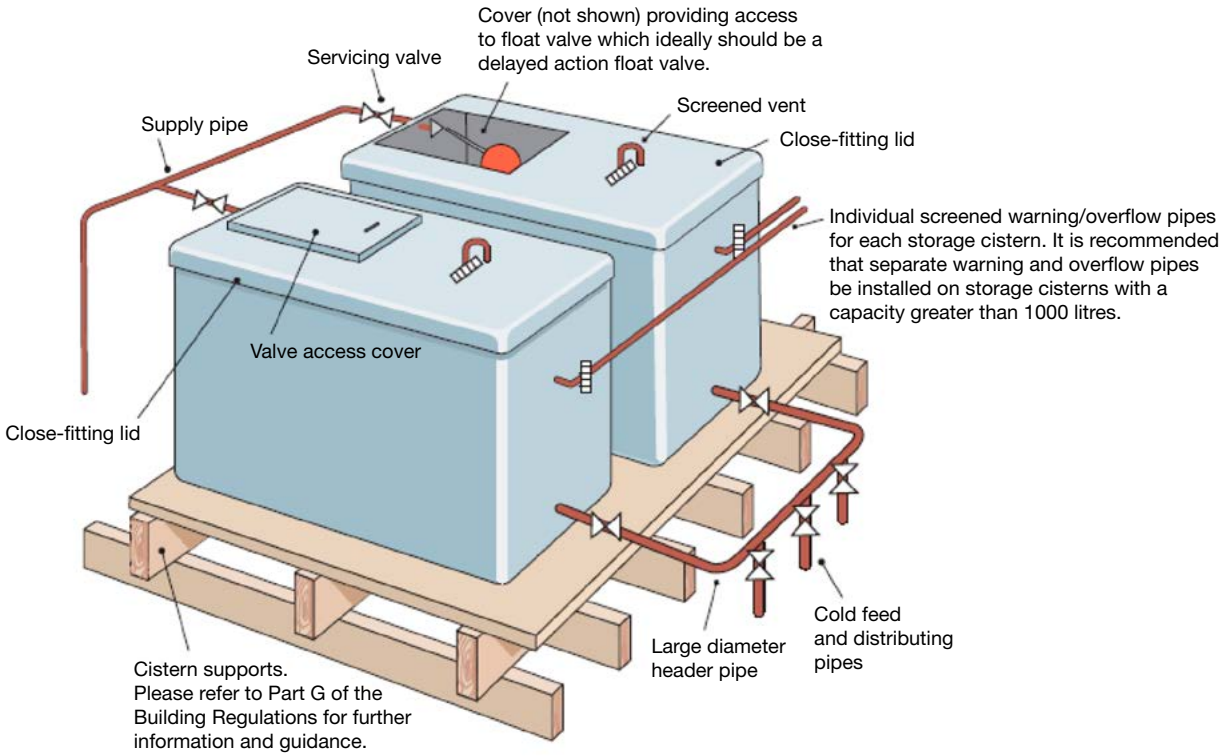

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Figure 3 Piping and valve arrangements for dual-cistern installations

8.39 The use of delayed-action float-operated valves on water storage cisterns should be considered, since these help avoid stagnation of water in the cistern. They may not be suitable when the supply is pumped.

8.40 Strainers should be fitted within the water pipework system if not supplied integral to the valves, and are used to protect vulnerable valves and fittings against ingress of particulate matter. The installation of these fittings should allow adequate access for maintenance/ replacement, and they should be provided with means of upstream and downstream isolation as appropriate. Strainers can be a source of *Legionella* bacteria and should be included in routine cleaning, maintenance and disinfection procedures (see Chapter 7, Part B).

8.41 Service isolation valves should be fitted to all pipework preceding sanitary tapware, WCs etc for servicing, repair or replacement. Drain-valve provision may also be appropriate for certain installations, for example, service pipework to en-suite facilities. Suitable backflow prevention devices or arrangements should be provided appropriate to the fluid risk category (see the Water Supply (Water Fittings) Regulations 1999).

Expansion vessels

Further advice can be found on the WRAS website.

8.42 Expansion vessels (see Figure 4) are typically vertical in orientation and have a bladder or diaphragm either with nitrogen or with air fill in the upper space. They introduce a potential problem of microbial colonisation, including *Legionella*, as plantroom temperatures usually exceed that of the incoming water.

8.43 Such vessels should be appropriately sized, installed and operated in a manner that prevents the accumulation of debris, pockets of stagnating water and increases in temperature

within the vessels. They should preferably be of a design in which water passes through the vessel entering at low level and exiting at high level. (For smaller vessels it may be appropriate to utilise specialist valves which promote the flow of water within the vessels or allow sufficient pressure movement so that water is changed over during normal operation.) When larger vessels are installed, adequate flushing and drain-down connections should be provided at the top and bottom of flow-through vessels or at the bottom only of diaphragm or bladder types.

8.44 All materials in contact with water should have been assessed and shown they are appropriate (for example, suitable approvals) for the intended purpose (see paragraph 1.15). It is important that the expansion vessel is located on the cold feed rather than on the hot water side of the system.

8.45 Interconnecting pipework should be kept to a minimum, and the vessel should be sited and insulated to minimise heat gain. Where diaphragm or bladder-type vessels are used, they should be designed to ensure an adequate turnover of water within the vessel.

Note

Vessels where the diaphragm or bladder is designed to be replaceable may be considered as they will facilitate routine checking and/or replacement when contaminated.

Water meters

8.46 BS EN 806-2 Section 11 gives guidance on the design and installation of water meters.

8.47 Revenue meters are normally supplied and installed by the water undertaker to their specification, whereas the consumer may install sub-meters within their system.

8.48 Adequate sub-metering of water supplies should be provided so that supplies can be monitored for individual heavy-use departments. Such monitoring will assist in the detection of leaks or abnormal water demands. Water meters can be connected to an automatic monitoring system such as a BMS, which can identify anomalous consumption and lead to the early detection of leaks.

There are several types of vessel available including diaphragm or bladder type, with fixed and interchangeable (replaceable) bladders, as shown below. These internal bladders are often made of synthetic rubber such as EPDM and may support the growth of microorganisms including *Legionella*, so check to see if these are approved against BS 6920. Vessels with a 'flow through' design should provide less opportunity for water to stagnate and become contaminated (as in the latter design).

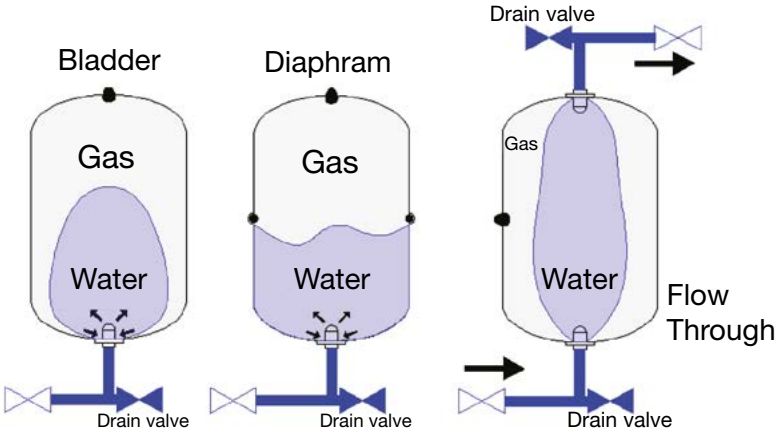

Figure 4 Expansion vessels

8.49 Only where downstream applications are considered critical and in need of a continuous supply of water should bypass arrangements be considered for consumer sub-meters. Where this is the case, sub-meters should have an upstream and downstream isolation valve. In case of servicing or replacement of the water meter, a replacement pipework insert should be stored in clean sterile conditions adjacent to the meter.

8.50 Meters should also be considered on the cold supplies to cisterns and hot water storage vessels where multiple units are installed supplying the same system. This will allow equal usage and turnover to be verified.

9 Cold water distribution system

9.1 The design and installation of the cold water distribution system should comply with the Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 806-2 and BS 8558. A simple cold (and hot) water system is shown in Figure 5.

Note:

All pipework should be insulated. All drains should discharge to waste via an appropriate air break to the drain such as a type AA air gap or an air-break-to-drain device in accordance with BS EN 1717 clause 9.

9.2 Cold water pipework runs should be designed and installed to reduce the risk of heat gain and should not be above or near heat sources including ceiling-mounted radiant heating panels. Cold water service mains should be run within a different ceiling space, riser or zone from other heat sources. This includes the hot water service distribution system and steam supply. If not possible, the cold water service and hot water services should be run apart as far as practicable and preferably with the hot water at a higher level than the cold.

9.3 All pipework and valves should be insulated, except for any exposed final connections to sanitary appliances. Insulation should be of an optimum performance (relating to thermal values and thickness) to minimise heat gains in order to achieve the temperature regime. The installation of insulated pipe supports and a vapour seal is recommended for all areas susceptible to heat gain and/or condensation. All insulation should be installed in accordance with BS 5970. All pipework should be arranged to eliminate or minimise dead-legs. Additional guidance can be found in BS 8558 Tables 6 and 7.

9.4 Currently there is no upper limit standard for drinking water temperature in European or domestic legislation. In normal circumstances temperatures should be delivered below 20°C but there is growing evidence that supply temperatures may rise above 25°C in summer months. Coupled with improvements in building thermal performance and climate change, rising cold water supply temperature is likely to become more problematical. The design aim should be to ensure that cold water temperature draw-off is as close to the supply temperature as possible (see also paragraphs 2.55–2.56 from HSG274 Part 2).

9.5 Government guidance to the Water Supply (Water Fittings) Regulations recommends that as far as is reasonably practical cold water temperatures should not exceed 20°C. As far as possible, the objective should be to design the cold water systems to ensure that the inlet, outlet and surface water temperatures of cisterns and cold water feed/header tanks for the hot water calorifiers are not greater than 2°C above that measured of the incoming water supply at the property boundary. Also, at cold water draw-off points, a temperature of no greater than 2°C above the temperature measured in cistern and cold water header tanks should be reached within two minutes (see also paragraph 11.1).

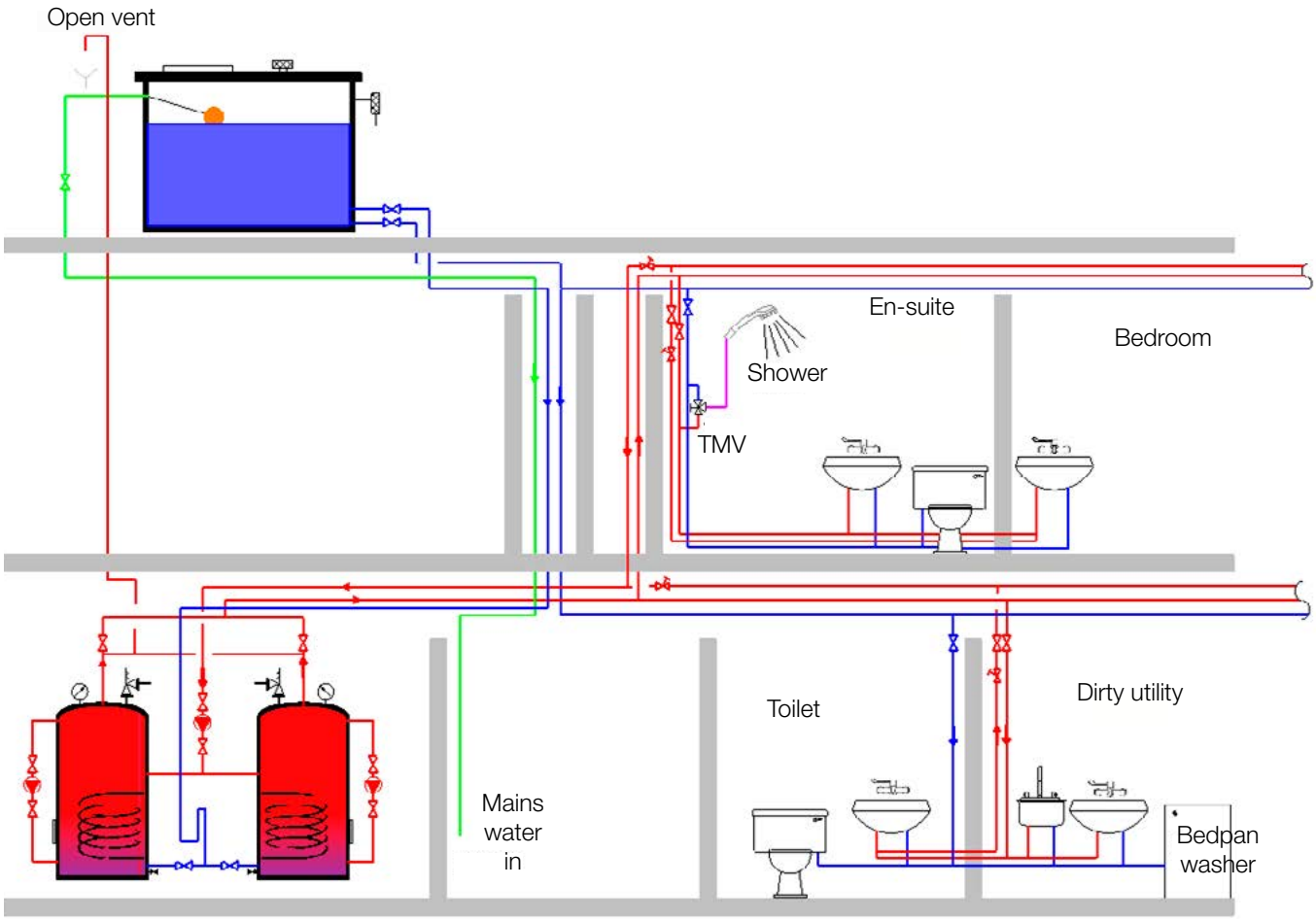

Figure 5 Schematic layout of a cold (and hot) water service system (note: this is a schematic only, and therefore all isolation valves and specific pipework configuration recommendations may not be included)

Note

For the control of *Legionella* and other waterborne organisms, 20°C is the quoted upper value above which multiplication of *Legionella* in particular begins to take place (see Part B, Chapter 4). It should be noted that during extremes of weather, environmental factors can influence the incoming water temperatures, particularly where water is provided from surface water sources.

9.6 Historical data should be sought on incoming cold water temperatures, especially during the summer and autumn, to allow these factors to be built into the design of the cold water system. If temperatures exceed 20°C, then further control measures should be implemented based on the risk assessment.

9.7 The control of water temperature in the cold water service, however, will essentially rely on good insulation and water turnover. Cold water services should be sized to provide sufficient flow at draw-off points and prevent stagnation.

9.8 Further cooling of cold water should only be considered in specialist units where people are at particular risk as a result of immunological deficiency, for example bone marrow transplant units. For other accommodation, the aim should be to promote turnover of cold water by means of the design of the distribution circuitry.

Note

Cooling water that may have been previously at a temperature conducive to *Legionella* growth will not reduce the risk of infection.

9.9 In all areas (particularly those with en-suite facilities), the aim should be to supply sanitary assemblies in series, with the WC connected as the final element to promote regular throughput of cold water. Elsewhere, pipeline routings should be run so that other outlets are connected in such a way that a WC or flushing device (for example, sluice hopper or pantry sink) provides the final element at the distal end of the branch – this may require pipe routing reversal (see Figure 6).

9.10 The cold water distribution system should be designed so that the pressure is the same as that for the hot water service at draw-off points. This may require the inclusion of pressure-reducing valves in the distribution pipework. If unequal pressures exist in the hot and cold water supplies to combination taps where water mixes in the body of the tap, a single check valve is required on each feed pipe to the tap to prevent backflow of water from one to the other.

Note

See also paragraph 10.53 on the fitting of in-line strainers.

Drinking water

9.11 When separate drinking-water systems have been provided, the policy has normally been to distribute directly from the mains without storage (nor softening), with stored cold water (down service) being used solely for supplies to WCs, wash-hand basins etc. Providing drinking water without storage may not be appropriate in healthcare premises because of the need to have some security of supply. The advantage of separate drinking and cold water services chiefly lies in the possibility of treating the latter (softening or other forms of treatment) without adulterating the drinking, cooking and food preparation supplies. The WSG should define a strategy either for separate supplies or for restricting the extent of softening (see also Chapter 6 on water softening).

9.12 Low water flow is often experienced in dedicated drinking water systems leading to stagnation, and the likelihood of temperatures exceeding 20°C. The preferred option may be to decommission dedicated drinking water systems totally. A risk assessment should show the optimum solution for patient safety.

9.13 A possible strategy, therefore, is to have a drinking water system that also provides WC flushing and, to some extent, this will assist water turnover and the maintenance of water quality. The disadvantage of the concept, apart from installation cost, is that the use of WCs, particularly in en-suite facilities, as the mechanism for achieving good utilisation in the cold water service no longer becomes possible (see paragraph 9.9). The concept for water turnover in en-suite facilities could still be achieved, however, if the cold water service were run in series to en-suite facilities with minimum dead-legs to draw-offs, with the final connection on the system being a highly utilised outlet, for example a sink. Automatic flushing WCs should be considered to assist with maintaining water turnover in cold water systems.

Note

Sensor-activated delivery devices that have a duty cycle feature to prevent stagnation should be considered. This feature should be activated only where required. Note that automatic flushing devices should not be located in accommodation used by patients who may become distressed by the noise.

Pumped systems

9.14 Where the pressure of the water undertaker's supply is inadequate, it will be necessary to use pressurisation plant. Similarly, pumping or pressurisation may be required for fire-fighting purposes.

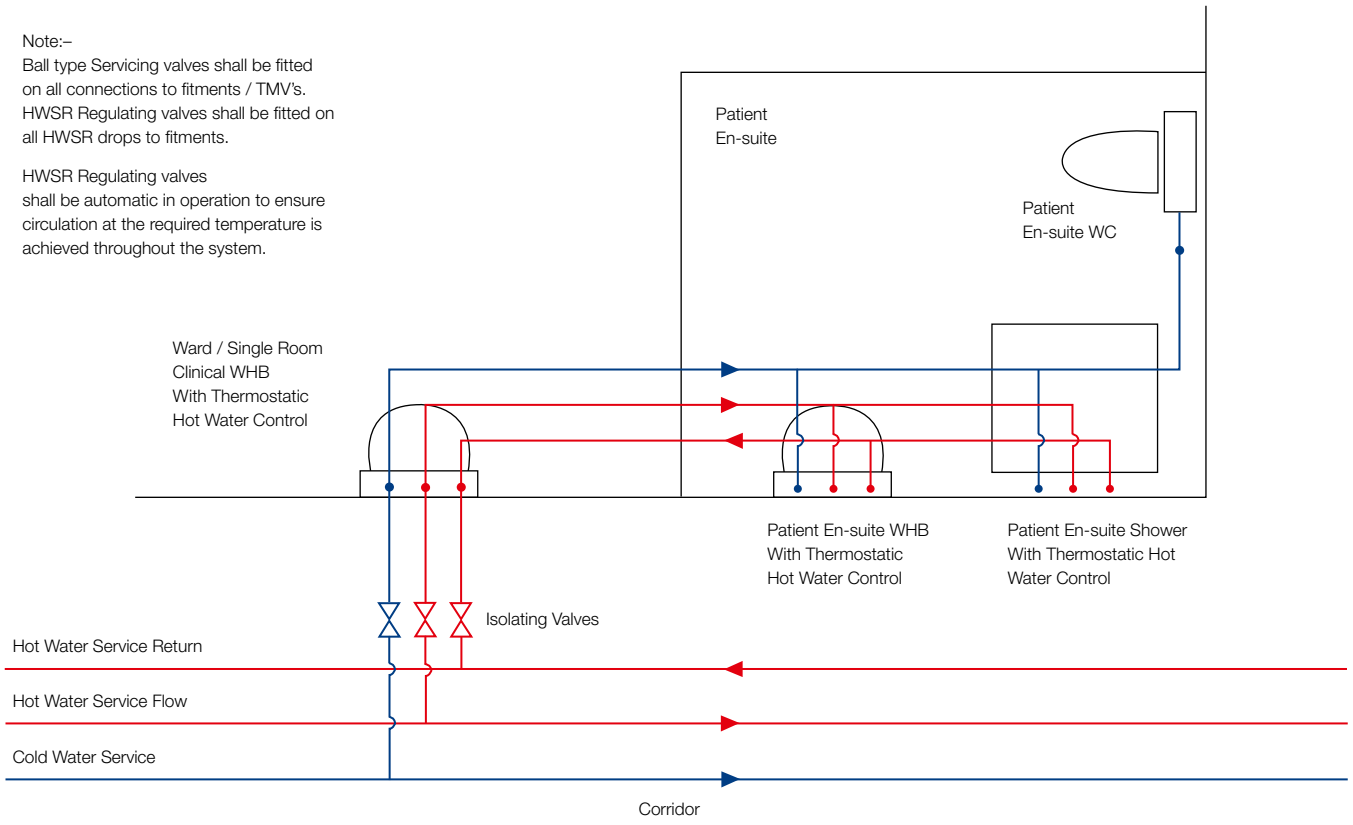

Figure 6 Piping arrangements for an en-suite facility

9.15 Various arrangements of pumping system are indicated in BS EN 806-1 Part 5 and BS 8558. Where booster pumps are to be installed, a break cistern will be required between the mains supply pipe and the pumps. This is required to comply with the Water Supply (Water Fittings) Regulations 1999 with regard to prevention of backflow. Any pump delivering more than 12 L/min must be notified to the water undertaker whose consent is required.

9.16 Control of the pump(s) should be fully automatic in operation and controlled by pressure sensors for the following reasons:

a. to reduce energy consumption;
b. to prevent heat gain from the pump to the water, which could become significant if large pumps are used;
c. to reduce wear on the pumps and hence reduce maintenance.

9.17 Factors to be considered when selecting pumps are:

a. quantity and pressure of water to be pumped;
b. the number of units required to obtain the necessary output and to provide adequate standby capacity;
c. the desirability of speed variation;
d. the degree of automatic sequence control required (but with manual override);
e. the characteristics of the system on both the delivery and suction sides, and in pumping efficiency and priming requirements;
f. the type of materials used in manufacturing the pumps relative to the chemical analysis of the water to be pumped;
g. ferrous materials should not be incorporated into wetted surfaces.

9.18 Depending on the circumstances, the operation and shutdown of pumps may be controlled by various methods (for example,

water-level float switches, pressure switches, flow switches, electrode probes or pneumatic systems). Certain services may also require the pumping equipment to be energised from the emergency electrical service as recommended in HTM 06-01 – 'Electrical services supply and distribution'.

9.19 Where two or more pumps are installed, automatic control should be provided to prevent stagnation. The design should ensure that appropriate resilience is considered.

9.20 The pumping sets for lifting to higher-level storage should be controlled from the level in the high-level tanks by transmitting sensors, level switches or other suitable devices. A low-level alarm should be arranged to give a warning when the storage volume of water falls to a predetermined low level.

9.21 The plantroom should be constructed with a waterproof and non-dusting floor, and non-dusting walls and ceiling. The floor should be constructed with a slight fall to a drainage trench that should terminate in a trapped gully. The trapped gully should incorporate provisions to either avoid or replenish any trap-water-seal loss. The plantroom will require adequate lighting, ventilation and heating (to prevent freezing or condensation), with electric power points and/or provision for low-voltage supplies for portable lighting and tools.

9.22 If heavy plant is to be installed that may need to be removed for testing, maintenance or replacement, fixed lifting beams of suitable capacity should be provided.

Specialist systems

9.23 Where water supplies are required for specialist systems such as endoscope cleaning installations, dialysis units etc (see also HBN 07-01 – 'Satellite dialysis unit'), the design team should consult the infection prevention and control team and equipment manufacturer to establish any specific water treatment requirements for the process. Additionally such systems also require backflow prevention arrangements to protect the source water supplies from contamination. The local water undertaker should be consulted:

- to clarify the backflow prevention arrangements or devices; and
- on any possible variation in the quality of its water supply or possible changes to the source of the supply.

Vending, chilled water and ice-making machines

Note

These should not be installed in augmented care areas.

9.24 The design, installation, location and risk assessment of all equipment should be approved by the WSG (see also HBN 00-09 – 'Infection control in the built environment'). The risk assessment should consider:

- carbon filtration in these devices, which are a high nutrient source for bacteria;
- cleanability and maintenance of the machine.

9.25 The water supply to this equipment should be taken from a wholesome supply via a double-check valve to prevent backflow and be upstream of a regularly used outlet with the minimum of intervening pipe-run, that is, less than 3 m. The supply should not be softened. Additionally, it should be established that the usage is sufficient to avoid deterioration in water quality, for example that the inlet water temperature does not exceed 20°C. The equipment should be positioned so that the warm air exhaust does not impinge directly on taps or hoses supplying cold water and to provide access for maintenance.

9.26 Design considerations include for example:

- no drinking fountain or vending machine should be installed at the end of the line (potential dead-leg);

- the pipework should be as short as possible from take-off point (mains water tee);
- the cold water supply pipework should be copper and fitted with a local isolation valve and drain valve;
- the flexible pipe connector should be kept as short as possible (see paragraphs 3.39–3.41).

Note

Flexible EPDM should not be used (see Estates and Facilities Alert DH (2010) 03 – 'Flexible water supply hoses').

9.27 Reference should also be made to the Food Safety (Temperature Control) Regulations 1995 and Food Safety (General Food Hygiene) Regulations 1995. The Automatic Vending Association of Britain (AV) 'Guide to good hygiene practice in the vending industry' should be followed regarding hygiene and water quality and hygienic operation of vending machines.

Vending machines dispensing carbonated drinks require special materials of construction which should have been assessed and shown to be appropriate (for example, suitable approvals) for the intended application (see also paragraph 1.15).

Other water uses

9.28 The guidance contained in this HTM is principally concerned with hot and cold water services used for domestic purposes within the interior of the healthcare premises.

9.29 This will include vending equipment, as above, and additional devices such as dishwashers and non-industrial clothes washing machines.

9.30 The cold water distribution system may also be used for non-domestic purposes outside the building but within the curtilage of the healthcare premises. This use may introduce potential hazards and reference should be made to HTM 04-01 Part B, Chapter 8.

10 Hot water services

10.1 Hot water services should be designed and installed in accordance with the Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 805, BS EN 806 (Parts 1–5) and BS 8558. The hot water system may be of either the vented or the unvented type.

10.2 The basic components of a hot and cold service system as used within healthcare facilities are shown in Figure 5; most installations will have additional features and components.

10.3 A vented system usually consists of a cold water storage cistern situated above the highest outlets, which feeds a hot water storage vessel (for example a calorifier or direct-fired boiler).

10.4 An unvented system usually has the hot water storage vessel connected to the mains water supply via a backflow prevention device and a pressure-reducing valve, or supplied via a break-tank or booster pump set.

See also HSE's:

- 'Managing the risks from hot water and surfaces';
- chapter 10 on hot water and surfaces in 'Health and safety in care homes'.

Hot water temperatures

10.5 Hot water is required in healthcare premises at various delivery temperatures for particular needs. The highest temperature, 55°C, is required typically in main kitchens, laundries, dirty utilities and food preparation areas. Elsewhere the delivery temperature for personal hygiene will depend on individual preference for comfort and safety of patients who require assistance. In circulating hot water systems, the highest temperature will be required at all draw-offs on a loop. To achieve this, see Note 2 below, the flow from the calorifier/water heater is required to be at least 60°C at its outlet with a minimum return temperature to it at 50°C.

Notes

1. Requirements in the Water Supply (Water Fittings) Regulations 1999 and ACoPL8 are prescribed to ensure efficient use and appropriate control of microbial elements in hot water systems. Guidance to these dual requirements recommends that, to comply, hot water should be delivered to the outlet 50°C within 30 seconds and 55°C within 1 minute of an outlet being opened.
2. The flow temperature of hot water out of the calorifier should be a minimum of 60°C. It should be a minimum of 55°C on flow and returns to all outlets and at the start of the hot water return. It should be a minimum of 50°C at the final connection to the calorifier.
3. Water system design should negate the need for trace heating in healthcare premises.

10.6 The individual outlets, taps, mixing valves or other outlet devices will be served from the circulating distribution system; this should be designed such that the minimum temperature

on all return loops should be at a minimum of 55°C. This will ensure a temperature of 55°C at all outlets within a maximum of 1 minute.

Note

Where the hot water return is local to the outlet, a much quicker response should be achieved: less than 30 seconds (typically less than 10–20 seconds). Failure to achieve this may indicate a hot water return fault and should be noted during subordinate loop monitoring. See also paragraph 10.59 on scalding risks.

10.7 A small number of localised hot water distribution systems can have advantages over one large centralised system. With smaller systems, hot water heaters are located closer to points of use, and it is therefore easier to maintain hot water distribution temperatures within recommended values. Balancing water flow rates in the hot water secondary distribution system becomes less of a problem, and distribution losses are reduced. A small localised hot water distribution system may comprise a gas-fired water heater or a storage calorifier. The adoption of localised hot water distribution systems will require the provision of local plantrooms.

10.8 With large centralised hot water systems, it is more difficult to maintain secondary distribution temperatures within recommended values; also, water flow rates in large secondary distribution systems can prove difficult to balance. This can be simplified by not overcomplicating the design for circulating systems (for example, by installing just one return to low level to accommodate the supply for two wash-hand basins back-to-back). See also paragraphs 8.42–8.44.

10.9 There are also maintenance factors to be considered. With a central hot water system, plant maintenance can be focused in one location, whereas with localised systems there will be a number of plantrooms at remote locations.

Hot water heater types

10.10 In most healthcare premises, hot water storage vessels include the heating source, which can be steam, high- or medium-pressure hot water, or electric immersion heating elements. The flow to the pipeline distribution system is normally taken from the top of the vessel, as too is the open vent, which may or may not be combined. The cold feed and return pipe should be connected to the vessel at the lowest point. Instantaneous water heaters for distribution systems have similar pipeline connections. They should be designed to have minimum water storage below the elements and should not be run to feed further outlets downstream. All water heaters should have been assessed and shown to be mechanically and materially appropriate (for example, suitable approvals) for the intended purpose (see paragraph 1.15).

10.11 Traditional design practice is for the use of vented hot water systems, which rely on a cold stored water feed and vent pipe to maintain its safe operation (that is, preventing water exceeding 100°C and maintaining fixed system pressures). Upon heating, expanded water can travel along both the cold feed and open vent pipework.

10.12 Means should be taken to prevent thermal circulation of hot water from the water heater to the cold-feed cistern occurring, leading to conditions conducive to microbial colonisation (for example, *Legionella*). It is common for an anti-thermal cycling loop to be installed on the cold water feed pipe between the water heater and the vertical cold water feed pipe, such as a U- or S-bend pipe configuration. When this arrangement is used, no heated water should extend upstream of the bend into the vertical cold water feed pipe.

10.13 To preserve the quality of stored water within the cistern, the practice of terminating the vent pipe (air vent) over the cistern is no longer permitted. The vent should be arranged to discharge over a separate air-break-to-drain arrangement (in accordance with BS EN 1717) or a visible Type AA air gap, and sited at a level

that takes account of the hydrostatic head of the system (see G20.3 in the WRAS 'Water Regulations Guide' or Figure 7 of BS 8558) to prevent unwanted discharges. The tundish should discharge to drain.

10.14 The vent pipe is critical to the safe operation of vented hot water systems and should be situated in a hygienic environment to prevent blockages of the vent. The ingress of small animals, insects, debris or dust should be prevented. Vents should be designed to prevent unnecessary discharges of hot water and should be sized in accordance with G20.3 in the WRAS 'Water Regulations Guide' and to Figure 7 of BS 8558. Where screens are used on the vent outlet, additional measures should be included to ensure the continuing operation of the vent. Suitable maintenance should be specified to ensure the vent outlet is kept free from the collection of dust or biofilms etc.

10.15 The calorifier or water heater should be provided with a suitable safety valve of appropriate size and vacuum release arrangement.

10.16 Most vessels have some means of access for inspection, either via a special panel or by removing the heating coils/elements. When new calorifiers are required, it should be specified that they have separate and adequately sized access panels.

10.17 The combined storage capacity and heater output should be sufficient to ensure that the outflow temperature, at continuous design flow from calorifiers or other heaters, is not less than 60°C under maximum design flow. This applies to both circulating and non-circulating hot water systems. The positioning of the control and high-limit thermostats, cold feed and return water connections should ensure that these temperatures are achieved.

10.18 There are three types of water heater:

a. instantaneous heater;
b. storage calorifier;
c. semi-storage calorifier.

Instantaneous water heaters

10.19 This type of heater can be further subdivided into:

a. instantaneous water heaters for single- or multi-point outlets: these devices usually serve one draw-off only and are either electrically or gas-heated. The general principles and limitations of instantaneous water heaters are in essence:

   i. the hot water flow rate is limited and is dependent on the heater's power rating;

   ii. the water in instantaneous water heaters is usually heated to about 55°C at its lowest rate, and its temperature will rise and fall inversely to its flow rate. Where constant flow temperature is important, the heater should be fitted with a water governor at its inflow. Close control of temperature is of particular importance for showers. To attain constant temperatures on delivery, water flow and pressure should also be controlled. Variations in pressure can cause flow and temperature problems when the heater is in use and when setting up or adjusting flow controls;

   iii. they are susceptible to scale formation in hard water areas, where they will require frequent maintenance;

   iv. this form of hot water heating should be considered only for smaller premises or where it is not economically viable to run a hot water circulation to a remote outlet;

   v. for electrical instantaneous point-of-use showering, consideration should be given to selecting an appropriate thermostatically controlled product (for example, those with BEAB Care Mark approval).

Note

Some designs of combination water heater are arranged to have the cold-feed tank above the hot closed water tank. These are not recommended for healthcare premises, but where they are used, careful consideration should be given to managing the risks from these types of systems, and this should be reflected in the risk assessment. See also Table 1 in HTM 04-01 Part B.

b. instantaneous-type water heaters for distribution systems: these devices, which normally use steam or high- or medium-pressure hot water as the primary heating medium, are designed to heat their rated throughput of water rapidly from cold to the design outlet temperature. They can be used either to feed directly into a hot water distribution system or in conjunction with a separate storage vessel (often referred to as a buffer vessel), which reduces the load on the heater during periods of peak demand. This type of heater includes:

   i. hot water generators: these are vertical instantaneous water heaters that contain modular helical primary coils normally served by steam, medium temperature hot water (MTHW) or high temperature hot water (HTHW). The unit incorporates a temperature control device, which varies the rate of primary energy input so as to maintain a constant hot water flow temperature over a range of secondary flow rates through the heater;

   ii. plate heat exchangers: plate heat exchangers consist of a number of rectangular plates sandwiched between two flat endplates and held together by tie bolts. The plates have ports in all four corners that allow entry and discharge of the primary and secondary liquids. Primary liquid is directed through alternate pairs of plates while the domestic hot water is normally fed in a counter flow direction through the remaining pairs of plates. Each plate is sealed round the edges by a gasketing system, the design of which should ensure that fluids cannot, under normal operating conditions, either leak to atmosphere or mix. This type of heat exchanger can be extended easily, or shortened, to suit changes in hot water demand.

Storage calorifiers

10.20 Storage calorifiers are usually cylindrical vessels mounted either vertically or horizontally; the base of a vertical calorifier can be concave or convex, with the vessel being supported on feet. The latter design is preferred, as it avoids the annular space where the base joins the cylinder wall. Heater batteries are usually located near the bottom of the cylinder, which can give rise to an area of water beneath the battery significantly below the storage temperature. This “dead” area can provide an ideal breeding ground for bacteria. Galvanised cylinders are particularly susceptible to scale formation, which can also provide a source of nutrition and shelter for bacteria.

Note:

Galvanised cylinders are not recommended in new healthcare installations or for replacement.

10.21 The following points should be considered during the design process (see also paragraphs 10.27–10.28):

a. the entire storage volume should be capable of being heated to 60°C without permanent pockets of lukewarm water;

b. the shell lining should be resistant to bacterial growth;

c. sufficient access to ensure adequate cleaning of the shell should be provided;

d. a suitably-sized drain should be connected to the base of the calorifier.

Semi-storage calorifiers

10.22 These calorifiers can either have an independent heating facility such as oil or gas burners or electric elements, or use primary water/steam from a boiler to heat the water via a heat exchanger. The equipment is available in a range of storage capacities and recovery flow rates. This type of equipment is particularly suitable where systems are being decentralised and water heaters are required close to the point of use.

Sizing of hot water storage vessels

10.23 Storage should be calculated on the requirements of peak demand and the rate of heat input. There may be more than one peak period in each 24 hours. The interval between peak periods is important, as it affects the recovery time. See CIBSE Guide G: 'Public health engineering', which gives guidance on sizing hot water storage.

10.24 The installed hot water capacity should be sized for current needs and should not be designed with built-in capacity for future extensions.

10.25 Some devices are optimistically rated so that, at a continuous demand equal to their design rating, the flow temperature can fall below 60°C. Semi-storage or high-efficiency minimum storage calorifiers and instantaneous heaters are especially prone to this if under-sized.

Stratification in storage vessels, calorifiers, water heaters and associated buffer vessels

10.26 Stratification will occur in any storage calorifier, heater or associated buffer vessel; the temperature gradient will depend on the rate of draw-off and heat input. In some calorifier designs, stratification is significantly more pronounced and is a feature of their design. There will always be a volume of water in the temperature range that encourages maximum growth of *Legionella*.

Note

Stratification: in a storage calorifier, the upper level above the heating element will be at operating temperature (60°C) during normal periods of demand. Below this level there will be a volume of water at a temperature between that of the return feed water and the operating temperature. A higher return temperature is preferable to ensure all water in the calorifier can be stored above 60°C. This level will vary as draw-off takes place according to the thermal input and rate of demand.

10.27 Storage and semi-storage calorifiers should be provided with independently pumped circulation from the top to the base of the calorifier; this is referred to as a "shunt pump". The pump should be run for long enough to ensure that the entire contents of the calorifier are raised to 60°C for an hour a day, often performed during periods of minimum demand. During periods of low draw-off, the temperature will readily achieve 60°C to effect disinfection. Control should be by a timing device that can be adjusted when the profile of demand has been established.

10.28 Some semi-storage/high-efficiency calorifiers are supplied with an integral pump that circulates water in the calorifier; in this case a second shunt pump is not required. A similar arrangement is included in some plate heat exchanger/storage vessel packaged systems where there is primary DHW circulation which

provides pasteurisation without the requirement of a further shunt pump on the vessel.

Unvented hot water systems

10.29 Hot water storage systems have traditionally been provided with an open vent pipe that relieves any steam generated in the event of failure of temperature controls. The open vent pipe also protects against rupture of the cylinder by expansion of water.

10.30 The use of unvented heating systems is covered in section G3 in Approved document G – 'Sanitation, hot water safety and water efficiency', which specifies the minimum safety precautions that should be taken when adopting such systems.

10.31 Where an unvented hot water system is connected directly to the water mains, no back-up will exist in the event of a water supply failure. Such an arrangement may also be unacceptable to the local water undertaker, since they will be required to meet the maximum demand at any time over a 24-hour period.

10.32 The design and installation of unvented hot water systems should comply fully with the Building Regulations and the Water Supply (Water Fittings) Regulations 1999.

10.33 The key requirements are that the temperature of stored water should be prevented at any time from exceeding 100°C and that discharges from safety devices should be conveyed to a safe and visible place and protected to prevent blockage and the ingress of birds, rodents or insects etc.

10.34 The discharge pipes from the temperature-relief valve and expansion valve should be carefully located so that they are readily visible – but do not present a risk to people – and protected to prevent blockage and the ingress of birds, rodents or insects etc.

10.35 Where the hot water is heated directly, for example by a steam or LTHW primary coil, a non-self-resetting thermal cut-out wired to a motorised valve on the primary coil should be provided for control of excessive temperature. This should further be protected by a direct-acting protection device.

Connection arrangements for calorifiers and water heaters

10.36 Where more than one calorifier or heating device is used, they should be connected in parallel, taking care to ensure that the flow can be balanced so that the water temperature from all the calorifiers exceeds 60°C at all times.

10.37 Installations should not include for series operation of calorifiers/heaters.

Provisions for maintenance

10.38 There should be adequate access to calorifiers for inspection and cleaning, removal and replacement of tube bundles and removal and replacement of the entire calorifier.

10.39 All calorifiers and water heaters should be fitted with a drain valve located in an accessible position at the lowest point on the vessel so that accumulated sludge may be removed safely and effectively from the lowest point. The drain should be of sufficient size to empty the vessel in a reasonable time.

10.40 Drain valves should be of the ball type to avoid clogging, and a drainage gully should be provided of sufficient size to accommodate the flow from the calorifier drain.

Sealed expansion tanks for unvented hot water systems

10.41 It is essential that the expansion vessel is located on the cold feed rather than on the hot water side of the system in an area not subject to heat gain. All expansion vessels should have a means of isolation and drain provision to allow periodic flushing.

10.42 These vessels should be appropriately sized to take account of the thermal expansion of the hot water service system. Otherwise, they should comply with the recommendations in paragraphs 10.45.–10.48.

Hot water distribution system

10.43 To achieve the required circulating temperatures, it will be necessary to provide some form of regulation to balance the flow to individual pipe branches serving groups of draw-off points, for example each washroom/toilet and en-suite facility.

10.44 The means of balancing the hot water circulation can be achieved by either manual or thermostatic regulating valves installed in the return loops to all outlets. There should be means of isolation, both upstream and downstream. Adequate access for servicing is also essential. Lock-shield valves should not be used for balancing.

10.45 In in-patient accommodation where en-suite facilities are provided, it is recommended that the hot water circulation be extended to draw-off points in series; for example, the supply to a basin, bath and/or shower should be run as one circuit (see Figure 6).

10.46 The operating pressures for both hot and cold water at draw-off points should be the same.

10.47 The domestic hot water system should not be used for heating purposes. This includes all radiators, towel rails, heated bedpan racks etc, whatever the pipework configuration.

10.48 Particular attention should be given to ensuring that pipework containing blended water should be kept to a minimum. Generally, the downstream supply from the mixing device should not exceed 2 m.

Note

The same restriction applies to communal blending, that is, where more than one outlet is served by one device. Central blending systems should not be used, since the length of distribution pipework containing water in the temperature range that supports *Legionella* growth would far exceed these maximum permissible lengths.

Hot water circulating pump

10.49 Hot water circulating pumps have traditionally been installed on either the flow or return. When installed on the flow, the arrangement comprises a full-bore non-return valve with a bypass into which the pump is installed (a non-return valve is also required in the return at the calorifier/water heater); appropriate valves and drain points are also required for servicing. The size of flow pipe is selected, with larger bores to begin with to ensure the maximum diversified simultaneous flow demand is achieved: the circulating pump bypass pipe size is selected to take account of the flow required to offset the heat losses in the flow and return pipework.

10.50 When installed in the return, the installation will require a downstream non-return valve, upstream and downstream isolation valves and drain valve. Installing the circulating pump in the return simplifies the valving arrangements and may improve circulation when draw-off is taking place. (During high demands, the flow from the calorifier may exceed that of the circulating pump in the flow resulting in the non-return valve at the calorifier closing.)

10.51 Duplex pumps should not be installed. A spare, clean, dry and ready-for-service replacement pump assembly with drain valve and non-return value should be available in the event of failure of the service pump.

Note

If a permanently installed standby pump is considered to be essential, the control system should automatically change over operation every 3 hours.

10.52 The electrical connection should be by means of a lockable socket outlet for either single- or three-phase as required.

In-line strainers

10.53 Where not integral to the fitting, in-line strainers should be fitted within the water pipework system to protect vulnerable valves and fittings (pressure-reducing, safety-relief and stop valves, terminal fittings including thermostats and solenoid valves etc) against ingress of particulate matter. The installation of these fittings should allow adequate access for maintenance/replacement and they should be provided with means of upstream (and downstream where appropriate) isolation. Strainers can be a source of microbial contamination including *Legionella* and *Pseudomonas aeruginosa* and should be included in routine cleaning, maintenance and disinfection procedures (see Chapter 7 of HTM 04-01 Part B).

Cold-feed cisterns

10.54 When separate cold-feed cisterns are provided for hot water service installations, they should comply with the requirements for cold water systems.

Note

Hot water cylinders with an integral feed and expansion tank should not be used.

10.55 Some instantaneous water heaters include a flow-limiting device to ensure that the rate of draw-off does not exceed the capacity of the heater to achieve the required temperature. Such devices are not recommended for larger systems as air can be introduced into the circulating system if taps are opened on upper floors during excess draw-off.

Service isolation valves

10.56 Service isolation valves should be fitted to all pipework preceding sanitary tapware and WCs etc for servicing, repair and replacement. These valves should not be used for balancing the hot water system. Drain-valve provision may also be appropriate for certain installations, for example service pipework to en-suite facilities. Care should be taken to ensure these do not constitute dead-legs. The design needs to ensure that there are enough isolation valves for maintenance and repair to allow essential services to continue (for example, on all riser connections and pipework entries to specific areas).

Water temperatures and delivery devices

10.57 With the change in focus towards improving the patient environment and minimising the risk of healthcare-associated infections, there has been an increase in the provision of single-bed rooms with en-suite facilities. Additionally, to promote good hand hygiene, wash-hand basin provision has increased significantly in all clinical areas. Effective hand-washing is best performed under running water at a safe, stable and comfortable temperature over basins/sinks (for clarification and design specifications, see HBN 00-10 Part C – ‘Sanitary assemblies’ and HBN 00-09 – ‘Infection control in the built environment’).

Note

The Water Supply (Water Fittings) Regulations 1999 place limits on the flow of water to draw-offs where plugs are not provided. Spray-type mixer taps are not recommended in healthcare premises; therefore, the type of tap should be carefully selected to minimise the formation of water droplets and aerosols. Water flow profile should be compatible with the shape of the wash-hand basin to avoid splashing. The fitting and basin combination should be such that the water stream never discharges directly into the basin's waste outlet (see HBN 00-10 Part C – 'Sanitary assemblies').

10.58 Components should be selected for their ability to minimise the accumulation of debris and splashing (including devices that deliver a smooth non-splashing/spraying flow), and to facilitate disinfection. See also paragraph 3.1.

Note

When selecting new taps, HBN 00-09 advises against using aerators, strainers and flow restrictors at the point of discharge.

10.59 The risk of scalding for vulnerable patients (young children and older people, disabled people and those with neuropathy) is of particular concern in healthcare premises caring for such individuals, and therefore thermostatic mixing devices could be needed for hot water outlets. A risk assessment for scalding risk versus the risk of infection from waterborne pathogens should be undertaken by the WSG. (Thermostatic mixing valves should comply with HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'.) See Table 2 for recommended devices and outlets.

Table 2 Recommended devices and outlets

Activity/area	Maximum recommended set delivery temperature (°C)	Type of device
Areas where TMV type 3 valves should be fitted		Type 3 thermostatic mixing valve
Showers and hair-wash facilities	41	
Unassisted baths	44	
Baths for assisted bathing	46 – to allow for the cold mass of the bath. NB – prior to patient immersion, water should be checked with a thermometer.	
Bidets	38	
Note: Bath fill temperatures of more than 44°C should only be considered in exceptional circumstances where there are particular difficulties in achieving an adequate bathing temperature. If a temperature of more than 44ºC is to be used, then a safe means of preventing access to the hot water should be devised to protect vulnerable patients.		

Wash-hand basins and sinks

Wherever wash-hand basins are installed, a mixed water temperature outlet is required: a risk assessment should be undertaken, which is overseen by the WSG, that considers the needs of patients and service-users to determine whether there is a scalding risk and whether additional protection is required (e.g. a type 1 with temperature stop, type 2 or type 3 mixing valve – see options below). **Hazard warning signs for scalding risk should be displayed if appropriate.**

For outlets not intended for hand-washing (e.g. sinks in kitchens, dirty utilities or cleaners' rooms), TMVs should not be installed. **All installations require a hot water hazard warning sign.** (The temperature could equate to the maximum temperature available from the calorifier.)

Note: Microbiological risks should also be considered for all installations.

Options:

1. Separate hot and cold taps

2. Mixed temperature outlet:

- Type 1 – a mechanical mixing valve with or without temperature stop (i.e. manually blended)
- Type 2 – a thermostatic mixing valve: BS EN 1111 and or BS EN 1287
- Type 3 – a thermostatic mixing valve with enhanced performance: HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'

Type 3 TMVs should have undergone third-party testing and certification to the requirements of HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'.

Notes:

1. Where installed, it is preferable that thermostatic mixing devices are fitted directly to the mixed temperature outlet or be integral with it, and be the method of temperature and flow control, i.e. the mixing device should not be separate nor supply water via a second tap or manual mixer since there will be many cases where draw-off of cold water will not occur. If a separate thermostatic device is used, it should be fitted as close to the outlet as possible, which should be a flow-only control. Where "T" type mixing valves are installed, they should be readily accessible for maintenance.
2. In the case of bidets with ascending sprays or a handle douche, which may be accidentally immersed, the water supply should be independently fed from storage with no draw-offs at a lower level (i.e. a break-tank arrangement). Appropriate backflow protection must be provided (see paragraphs 12.18–12.24).

3. Automatic taps (timed flow) can be considered as a result of a risk assessment and should be specified as appropriate for the conditions of use, either type 2 or 3. If the temperature is non-user adjustable, they should be supplied via a type 2 or 3 TMV set to 39–40ºC. The sensors should include a timer that can be adjusted to take account of the optimum washing time: this is particularly for scrub sinks. Sensors should be offset or positioned such as to reduce the risk of accidental contamination of the outlet and be positioned so that POU filters can be used. Facilities for overriding the sensors will be necessary. When a duty cycle setting exists, it should be activated to avoid stagnation. (If there is more than one tap/outlet, e.g. in the case of scrub sinks, then all should deliver water to avoid stagnation.)
4. In the case of dual-function delivery devices, i.e. bath/shower diverter, type 3 valves should deliver the temperature appropriate to each outlet e.g. bath max 44ºC or 46ºC, shower 41ºC. (Refer also to the commissioning procedure section in HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'.)
5. Taps, components and fittings should be removable and easily dismantled for cleaning and disinfection.
6. Where manual mixing devices with a temperature stop are installed, it is important to ensure that the normal maximum delivery temperature is controlled to safe limits. Installation, commissioning and maintenance should take account of the system's dynamic pressure and temperature changes, and the seasonal changes in incoming cold water temperatures.
7. This table does not cover birthing pools. (See "Areas this HTM does not cover" in Chapter 1.)

Showers

10.60 Showers with fixed heads are preferred for prevention of backflow (see paragraphs 12.18–12.24) and minimisation of contamination. Where flexible hoses and moveable shower outlets are provided, the outlet should not be capable of being accidentally immersed into a drain, WC or other potential source of contamination. They should be selected based on ease of cleaning, descaling and disinfection.

10.61 The flow of some showerheads can be adjusted by selecting different sets of nozzles (fine spray, pulsating flow etc); as this will exacerbate possible stagnation problems, they should not be installed in healthcare premises.

10.62 Thermostatic mixing valves should be accessible in such a way as to prevent damage to the shower installation, the supply pipework or the removable panels themselves in wet areas. Access for safe inspection and removal should be given high priority.

11 Building management systems

11.1 The continued safe operation of domestic hot and cold water systems requires a number of routine checks to be made by physical means using separate thermometric equipment. A number of the control parameters can, however, be continuously monitored by building management systems (BMS) even though routine checks will still be required for calibration purposes. Parameters that should be monitored are as follows:

a. incoming mains temperature (at the water meter), inlet, outlet, and surface water temperatures of cisterns and cold water feed tanks for hot water calorifiers;

b. temperatures for calorifier flow and returns, and the returns on each loop;

c. hot water service return temperatures on all primary loops;

Note

In non-recirculating hot and cold systems, at least two points, including the furthermost from the entry of the pipe into the department, should be monitored. In other departments, monitoring should be provided on a similar basis.

d. cold water service at furthermost point and in a location that demonstrates general temperature representation within the ward/department;

e. consideration should be given to the frequency, timing and monitoring of taps etc with automatic flushing devices.

11.2 In addition to temperature, the BMS should monitor pressurisation and circulating pumps and water treatment systems for fault conditions or change of status likely to result in a fault. There should be a documented procedure for regularly checking the BMS for such alarms.

12 Pipework installations

12.1 All hot and cold water pipework should be designed and installed in full accordance with the Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 805, BS EN 806-2 and BS 8558.

12.2 Within the system, it is essential to include facilities for measuring, regulating, isolating, venting, draining and controlling the flow of water. Regulating valves with built-in pressure tappings or orifice plates with manometer tappings will be required for the measurement of pressure drop, which enables the volume rates of flow to be determined. Care should be taken to ensure that regulating valves or orifice plates are sited well away from bends or fittings.

Sizing

12.3 Pipes should be capable of a rate of flow to satisfy the combined maximum demand of all the services to be supplied. All the maximum demands of the separate services may not occur simultaneously, and the actual combined maximum demand may be a proportion of the sum of the separate maximum demands, which will be determined by the number and character of the services.

12.4 Hot and cold water pipework should be sized using the procedure outlined in CIBSE's Guide G – 'Public health engineering'.

Pipe branches

12.5 Pipe branches should be designed with the aim of avoiding stagnation. As far as practical, the maximum length of any pipework between a terminal device and a recirculating system or a cold water distribution mains should be kept to a minimum; generally, the complete length of the spur should not exceed 3 m. The length is measured from the centre line of the circulation pipework to the point of discharge along the centre line of the pipework. These pipes should be insulated.

Routing of pipework

12.6 Pipework in buildings should be designed and routed:

- so that minimal heat transfer will occur between hot and cold or the surrounding environment (see also paragraph 9.2); and
- in a manner that will promote good turnover of water, particularly in cold water service systems.

12.7 All pipework should be accessible for inspection, maintenance and repair as far as is practicable. Ducts, trenches and chases containing pipework should be large enough to facilitate repairs.

Buried pipelines

12.8 Pipelines made of plastics are susceptible to hydrocarbons such as fuels and oils. These chemicals can travel through plastic pipes if they are nearby and contaminate the water supply, and it may take days, weeks or even months before a noticeable taste can be detected in the water supply. Whenever spills are reported, an assessment of services within the area should be undertaken (see WIS 4-32-19 and UKWIR's 'Guidance for the selection of water supply pipes to be used in brownfield sites').

12.9 Pipework distribution networks should be divided into sections by the provision of isolating valves in accessible locations to facilitate isolation for repairs, maintenance and flushing.

12.10 Underground mains need not be laid at unvarying gradients but may follow the general contour of the ground. As far as possible, however, they should fall continuously towards drain points and rise continuously towards the air vent. They should not rise above the hydraulic gradient; that is, there should always be a positive pressure, greater than atmospheric, at every point under working conditions. The gradient between air release and drainage valves should be not less than 1:500 rising in the direction of flow and not less than 1:200 falling in the direction of flow.

12.11 Underground pipes entering a building should do so with a cover of not less than 0.75 m below the external ground surface and should pass through the wall within a watertight built-in sleeve. The sleeve should be filled in around the pipe with a suitable material for a minimum length of 152 mm at both ends to prevent the ingress of water or vermin. External underground pipes should be at a depth, or otherwise sufficiently protected, to prevent damage by traffic and any consequent vibrations. A minimum depth under roadways of 1 m measured from the top of the pipe to the surface of the roadway is necessary. In other underground locations the depth should not be less than 0.75 m, subject to this depth being sufficient protection against frost (frost penetration depends on the nature of the subsoil and the ground surface). Freezing can occur at depths of up to 1.1 m (see paragraph 13.26). Local information on the prevalence of frost should be sought (see also paragraphs 13.25–13.29).

12.12 Marker tapes should be laid over the whole length of all underground water services pipework (see also the requirements in BS 1710). The tapes should be clearly marked with the description of the service and should be coloured blue and red for fire mains.

Vents and drains

12.13 Air-release valves should be provided at summits and drainage valves at low points between summits unless adequate provision is made for the discharge of air and water by the presence of service connections. Large-orifice air valves will discharge displaced air when mains are being charged with water. When air is liable to collect at summits under ordinary conditions of flow, small orifice air valves, which discharge air under pressure, may be required. “Double-acting” air valves having both large and small orifices should be provided where necessary. Air-valve chambers should be adequately drained to avoid the possibility of contamination.

12.14 Automatic air-release valves should be installed where accessible for maintenance. Installation in ceiling voids is not recommended.

12.15 Drain points should not discharge directly into a drain or sewer or into a manhole or chamber connected thereto without an appropriate air gap between the water system and the drain. This can be achieved with a Type AA air gap or an air-break-to-drain device in accordance with BS EN 1717 clause 9. Where a wash-out discharges into a natural watercourse, the discharge should at all times be well above the highest possible water level in the watercourse. Consent for this discharge may be required from the Environment Agency. In some cases it may be necessary for the wash-out to discharge into a watertight sump, which has to be emptied while in use by portable pumping equipment.

12.16 In order to minimise quantities of water that may collect in stub pipes at drain points, the length of such stub pipes should be kept to an absolute minimum. This relates in particular to drains from hot water calorifiers, storage cisterns and distribution pipework.

Valves

12.17 A clear indication should be given on all valves of the direction of rotation needed

to close the valve. Normal practice is to have clockwise closing when looking down on the valve.

12.18 Where blending valves have been installed at the end of a run of hot water pipework, consideration should be given to the inclusion of a drain valve adjacent to the mixer. This should be located upstream of the mixing valve so as to facilitate flushing out and routine temperature testing of the hot water without having to dismantle the blending valve.

Backflow protection and the prevention of contamination

12.19 In all cold water installations it is important that adequate protection be provided to all supplies against backflow. In a healthcare facility, there should be a high degree of protection not only to the water in the undertaker's mains, but also within the facility's installations to protect the patients and staff. Instances of water use in healthcare facilities where backflow is likely to be harmful to health include bidets, bedpan washers, dental spittoons and equipment, mortuary equipment, and water outlets located in laboratories.

12.20 In addition to backflow protection at all points of use, the whole installation protection should be provided as required by the Water Supply (Water Fittings) Regulations 1999.

12.21 Healthcare buildings and medical premises have been identified as involving Fluid Category 5 backflow risks (see Schedule 1 "Fluid Categories" from the Water Supply (Water Fittings) Regulations 1999), which are defined as points of use or delivery of water where backflow is likely to involve fluids contaminated with human waste or pathogens. Within healthcare facilities, water usage covers a wide range of applications, from domestic use by patients and staff to specialised use in operating departments and pathology laboratories, and with equipment such as bedpan washers and haemodialysis machines. Even within high-risk specialist areas such as pathology laboratories (see paragraph 13.22), further separation of water supplies may be required to protect water used for domestic uses from those high-risk applications. In addition, many apparently "commercial" usages may be classed as high-risk because they are for healthcare purposes (such as centralised laundries).

12.22 Where any doubt exists with regard to the level of protection required against water supply contamination, reference should be made to the Water Supply (Water Fittings) Regulations 1999 and guidance contained in the WRAS 'Water Regulations Guide', or to the water undertaker.

12.23 The Water Supply (Water Fittings) Regulations 1999 require the identification, by colour-coding or labelling, of all pipework carrying fluids other than wholesome water. This includes plumbed-in equipment used for diagnostic and treatment purposes (see BS 1710).

12.24 New systems should not include legs of pipework for potential future extensions to the system as these will create stagnant legs. Any alterations to existing systems – or where redundant pipework is found – should be cut back to the connection point; this includes replacing the branch 'T' with a straight coupling.

12.25 Certain departments such as pathology laboratories present particular risks of water contamination. Attention is drawn to section G15.24 in the WRAS 'Water Regulations Guide' on supplementing point-of-use protection by zone protection, where the pipes supplying a high-risk area can be given additional protection by installation of a secondary backflow protection device.

Frost protection

12.26 The Water Supply (Water Fittings) Regulations 1999 require that all cold water pipework and fittings be adequately protected against damage from freezing.

12.27 In the case of external pipework that is run underground, the Regulations require that consent be sought from the water supplier if pipes are to be run at depths of less than 0.75 m or greater than 1.35 m. Permission from the water undertaker should be sought if any deviation is required.

12.28 Particular care is required when routing pipework externally above ground or through unheated areas within buildings. The WRAS 'Water Regulations Guide' gives guidance on the minimum thickness of thermal insulating materials that should be applied in such cases (see the online calculation tool on the WRAS website).

12.29 Adequate provisions for isolating and draining sections of cold water distribution pipework will ensure that disruption caused by frost damage can be minimised.

12.30 For further guidance on frost protection, refer to the WRAS 'Water Regulations Guide'.

Maintaining cleanliness and hygiene

12.31 To prevent the risk of seeding the water system during construction and installation, care should be taken to prevent the ingress of contaminants and particulate matter into pipework, fittings and cylinders etc. Blanking flanges, plugs and caps should not be removed until connections need to be made. Open ends of pipes should be recapped when work ceases. Particular attention should be taken when pipes are passed through partitions etc and when transporting items from storage to installation locations.

13 Noise and vibration

Pump noise

13.1 Noise generated by centrifugal pumps will not cause problems if water velocity in the pipes and the speed of the pumps are low, for example about 1 m/s and 960 rpm respectively.

13.2 Care should be taken in locating water-boosting pumps within healthcare buildings to ensure that they will not cause interference to in-patient accommodation and other quiet zones.

13.3 Such interference may result from break-out noise from the boosting equipment, or noise transmitted through the pipework system or through the building structure. Pump noise may also result from cavitation caused by low suction head.

13.4 Where pumps are located close to sensitive areas, provision for noise and vibration reduction should be incorporated in the design. Such provision will include selection of quiet-running motors, vibration isolation of boosting equipment from pipework and structure and, if required, acoustic lining to the booster plant enclosure.

13.5 Guidance on recommended noise levels for various locations is given in CIBSE's Guide A – 'Environmental design'. See also HTM 08-01 – 'Acoustics'.

Other forms of system noise

13.6 Other forms of nuisance noise that may be generated by hot and cold water distribution systems are listed below:

a. noise from pipework due to excessive water velocity;

b. water hammer caused by rapid closure of valves or taps;

c. oscillation of the float of a float-operated valve;

d. tap washer oscillation;

e. noise caused by water discharging from float-operated valves into cisterns;

f. noise caused by thermal movement of pipes;

g. noise due to trapping of air within pipework, particularly on hot water systems.

13.7 Further details on the above sources of noise, including guidance on avoiding such noise problems, are given in the WRAS 'Water Regulations Guide'.

14 Water economy and energy conservation

Water

14.1 Hot and cold water distribution systems for healthcare buildings should be designed so as to minimise the use of water. The cold water distribution systems should incorporate an adequate number of water meters to allow for close monitoring of water consumption. Where practicable, consideration should be given to linking water meters to a building management system or an automatic metering system.

14.2 Measures to optimise water consumption – and yet avoid risk of stagnation – that should be considered at design stage include:

a. provision of automatic systems to control flushing of urinals;

b. use of showers rather than baths wherever practicable;

c. WC pans and flushing cisterns that use more than 6 L per flush are prohibited by Water Supply (Water Fittings) Regulations 1999;

d. control of water pressure to a level that is not excessive for the purpose required;

e. use of self-closing or non-concussive taps in appropriate circumstances;

f. locating warning pipes from cisterns and discharge pipes from relief valves in such a way that any discharge can be readily observed, and/or fitting alarms on such pipes.

14.3 Further guidance on the prevention of wastage of water is given in the WRAS 'Water Regulations Guide'. See also HTM 07-04 – 'Water management and water efficiency'.

Energy

14.4 Hot and cold water systems should be designed to operate by gravity as far as possible. Where water-boosting pumps are necessary, the pump motors should be selected to operate at maximum efficiency at the required duty.

14.5 The practice of pre-heating of the cold feed to calorifiers should not be carried out. The only time it is acceptable is when under all flow/demand conditions a temperature greater than 45°C can be guaranteed at the entry to the calorifier. Any pre-heater should have a low water capacity. Heat recovery/systems for pre-heating water for domestic purposes should not be installed in healthcare premises unless there are adequate fail-safe measures in place to ensure that the hot water distribution system is not compromised.

14.6 Further guidance on energy conservation in relation to hot and cold water systems is given in HTM 07-02 – 'Encode' (see also the Carbon Trust's website).

15 Installation, testing and commissioning

See also Chapter 11 on commissioning in HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'.

15.1 While testing and commissioning is regarded as a discrete activity, continuous monitoring is required throughout the installation to ensure that:

a. materials and equipment installed comply with the Water Supply (Water Fittings) Regulations 1999 and other British Standards, and are not otherwise unsuitable. For example, equipment has been assessed and shown mechanically and materially to be appropriate (for example, suitable approvals) for the intended purpose (see paragraph 1.15) and has been installed in accordance with any relevant conditions;

b. the work is done entirely within the specification for the scheme;

c. all the requirements of current legislation are met, both during construction of the installation and when it is completed, particularly with regard to the Health and Safety at Work etc. Act 1974.

Installation checks

15.2 The system should be regularly checked during installation to ensure that open pipes, valve ends, cylinder connections etc are sealed to prevent the ingress of dust/debris that could cause problems during commissioning and subsequent operation (see paragraph 12.30). Checks should also be made to ensure that lead solders are not being used.

Commissioning

15.3 Correct commissioning is vitally important for the satisfactory operation and long-term control of hot and cold water systems. Where buildings are to be occupied in stages, the systems should be commissioned in stages as occupation is anticipated. The design team should prepare a commissioning brief for use by the contractor's commissioning engineer which has been agreed as suitable for purpose by the WSG with input from specialist external advisers if necessary.

15.4 A water risk assessment and associated method statements should be prepared by the design team and contractors, and this should be agreed by the WSG before commissioning begins. As commissioning correctly is such an important step in ensuring the safety of the building for the rest of its life-cycle, estates departments should ensure competent staff are available for witnessing key stages.

15.5 The water system should be filled as late in the commissioning process as possible. The design team should consider using inert gas for pressure-testing when possible. The system should be flushed to remove all flux and debris before being filled with water as soon as possible, but within a maximum of 48 hours, to prevent biofilm development and corrosion. Wholesome water should be used for flushing. Treated water should be available for top-up as necessary during the commissioning process.

All strainers should be cleaned after flushing and before final filling.

15.6 After water has been introduced into the system, a flushing regime should be introduced to maintain the wholesomeness of the water content.

15.7 It is essential that the results of all checks and measurements are recorded in writing at the time they are made. Breaks in the continuity of commissioning operations are likely, and proper records will show the state of progress at any stage. It is most important that commissioning records are provided as part of the handover information.

15.8 The commissioning brief should specify fully and clearly the extent of the commissioning, the competencies of the personnel to be carrying out the process and the objectives which should be achieved, and should include:

a. full design data on temperatures, water flow rates and pressures;

b. plant and equipment data;

c. number of commissioning procedures for thermostatic mixing valves in accordance with Chapter 11 of HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)';

d. drawings and schematics;

e. a list of test certificates to be provided.

15.9 The design team's attention is drawn to CIBSE's Commissioning Code W – 'Water distribution', which provides guidance on information that will be required by the commissioning engineers.

15.10 In the preparation of commissioning instructions for domestic hot and cold water services, design teams should ensure that their work is in accordance with up-to-date guidance.

15.11 The design team should prepare for inclusion in the contract documents a list of tests and measurements that are to be taken by the contractor and recorded by him/her. These should be witnessed by the contract supervising officer or project engineer on his/her behalf and he/she, if approved, will circulate the results, in accordance with the client's instructions.

15.12 The installation, on completion, should be operated by the contractor as a whole, and subjected to functional or performance tests as specified by the design team.

15.13 The commissioning manual should be prepared by the contractor and submitted to the client's commissioning adviser and the WSG (with support from their specialist adviser if required) for review before being issued in final form.

15.14 Typical schedules of checks and performance tests should be included in the commissioning manual together with record sheets. These should be amended and supplemented as the design team considers necessary.

15.15 Once the client's commissioning adviser is satisfied that the system meets the design intent, the final accordance record sheets should be completed. If performance is not acceptable, the matter should be dealt with in accordance with the contract requirements.

15.16 The supervising officer or project engineer, who should countersign any relevant test record documents, should witness commissioning and testing.

15.17 "As installed" record drawings, schematic diagrams, and operating and maintenance instructions should be supplied at the time of handover. Certified records of pressure-testing and disinfection should also be made available.

15.18 The whole commissioning procedure should be carried out under the guidance of a single authority, although the involvement of specialists or manufacturers may be required for specific items of plant.

15.19 Valid calibration certificates should be submitted and checked for all measuring equipment to be used by the commissioning engineers before the start of the commissioning phase.

15.20 The commissioning should be carried out in a logical and methodical manner.

15.21 The installation, on completion, should be operated by the contractor as a whole, and subjected to specified functional or performance tests.

15.22 Once the system meets the design intent, the final completion record sheet(s) should be completed. In the event of performance not being acceptable, the matter should be dealt with in accordance with the contract requirements.

Commissioning and testing checklists

15.23 The following is a summary of the key activities associated with pre-commissioning and commissioning of hot and cold water storage and distribution systems. The list is not intended to be comprehensive.

Note

Before water is introduced into any part of the system, checks should be carried out to determine that appropriate backflow prevention measures have been implemented (see paragraph 9.10).

Cold water installations

15.24 Pre-commissioning checks can be carried out on completion of the system installation, filling and pressure-testing.

15.25 Pre-commissioning checks and tests to be applied are as follows. Check that:

a. systems have been provided and installed in accordance with specification and drawings, and that the systems are charged with water, vented and free from leaks;

b. water storage cisterns are free from distortion and leaks, are properly supported and secured, are provided with correctly fitting covers, and are in accordance with the Water Supply (Water Fittings) Regulations 1999;

c. distribution pipework is rigidly supported, insulated, and incorporates adequate provisions for venting, draining, expansion, isolation and measurement of flow, temperature and pressure;

d. pipework systems have been pressure-tested;

Note

Except where otherwise specified, testing of underground pipelines should be carried out in accordance with the requirements of the Water Supply (Water Fittings) Regulations 1999.

e. pipework systems and storage/break-tanks are correctly identified and marked;

f. regulating valves and flow control devices operate freely;

g. water meter(s) is/are fitted correctly;

h. electrical isolation, cross-bonding and wiring of system components are installed in accordance with the current edition of BS 7671.

Hot water installations

15.26 Pre-commissioning checks can be carried out upon completion of system installation, filling and pressure-testing.

Note

Before water is introduced into any part of the system, checks should be carried out to determine that appropriate backflow prevention measures have been implemented (see paragraph 10.10).

15.27 Pre-commissioning checks and tests to be applied are as follows. Check that:

a. systems have been provided and installed in accordance with the specification and drawings;

b. the system is charged with cold water, vented, and free from leaks;

c. hot water storage vessels are free from leaks and are properly supported and secured;

d. distribution pipework is rigidly supported, insulated, and incorporates adequate provision for venting, drainage, expansion, isolation, and measurement of flow, temperature and pressure;

e. pipework systems, storage cylinders etc have been pressure-tested;

f. pipework systems, calorifiers and cisterns are correctly identified and marked;

g. regulating valves and flow control devices operate freely;

h. all control and regulating valves are labelled or marked to correspond with reference numbers on contract drawings;

i. electrical isolation, cross-bonding and wiring of system components is installed in accordance with the current edition of BS 7671;

j. up-to-date system schematics are displayed in a frame in the relevant plantroom.

Commissioning checks of hot and cold water systems

15.28 Upon satisfactory completion of the pre-commissioning checks, the systems should be cleaned, disinfected and flushed before refilling. Advice on cleaning and disinfection is given in HSG274 Part 2. (See also paragraphs 16.42–16.44 for guidance on the safe discharge of wastewater used during disinfection procedures.)

15.29 Commissioning checks and tests to be applied are as follows. Check that:

a. drain-down points flow when released and are free from leaks when shut, that air vents and release valves open correctly and are airtight when shut off, and that overflows run freely and discharged water does not cause flooding or damage;

b. float-operated valves function satisfactorily and are adjusted to achieve the correct water level;

c. all temperature and other controls are adjusted and calibrated to agreed design limits of system performance;

d. all electrical circuits are tested and the pump motor direction of rotation is correct, and that electrical controls and alarms function correctly;

e. operation of any safety or anti-flood device is satisfactory;

f. circulating and pressurisation pumps are free from excessive noise, vibration and leaks and that pressurisation vessels are filled to the correct water level;

g. expansion vessels where installed are filled to the correct water level;

h. control valves operate correctly and shut-off valves close tightly;

i. heat exchangers operate satisfactorily;

j. primary heating circuits are adjusted and regulated, and thermostatic settings are correct; and that bypass circuits and automatic control valves operate correctly;

k. remote and automatic control of pumps (if appropriate) is satisfactory, and there are no leaks at joints under maximum flow conditions;

l. the cold water and hot water circulating and distribution systems are vented and regulated;

m. thermostatic mixing devices and regulating valves are adjusted and set to desired values (TMVs require hot and cold water for testing and commissioning, and should be commissioned in accordance with Chapter 11 of HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)');

n. all taps, mixers and outlets operate satisfactorily and that all strainers and shower outlets have been cleaned and are free from contamination;

o. water flow quantities at all plant items, regulating valves and flow-measuring valves are recorded;

p. mass flow rates from taps, main and other outlets in positions shown on contract drawings are satisfactory;

q. pressure drop at heat exchangers at full design demand flow is tested and recorded;

r. hydraulic balancing of hot water secondary circulation system is carried out to ensure that minimum temperatures are achieved in all parts of the circuit;

s. full load current of components does not exceed the recommended values;

t. the running current of components does not exceed the recommended values;

u. pump thermal overload trips are set.

Temperature testing

15.30 These tests should be performed and validated prior to contractual handover and bringing the system into use and when in occupation. Separate thermostatic measuring and recording equipment should be used, that is, independent of any building management system. It will be necessary to have systems fully operational and to simulate typical draw-off of water.

15.31 Tests should include:

a. measuring the incoming water temperature at the main water meter;

b. testing the inlet, outlet and surface water temperatures of cisterns and cold water feed/header tanks for the hot water calorifiers and water heaters. The temperature should be close to the water temperature of the incoming water;

c. testing the flow and return temperatures at connections to calorifiers and water heaters. These should not be less than 60°C and 50°C respectively;

d. testing the temperature in branches of hot water circulating systems installed in all departments to ensure that the system has been balanced, and that under "no draw-off" conditions 55°C is achieved in the circulating system at all outlets;

e. testing single cold water outlets and the cold water inlet to mixing valves to ensure temperature equilibrium below 20°C.

Sampling

15.32 The WSG should discuss and agree a sampling regime and appropriate parameters (physical, chemical and microbiological) depending on the intended use of the system and vulnerability of the patients. This should be agreed prior to tender.

15.33 Sampling should be carried out prior to any construction/refurbishment works and immediately prior to handover, but no sooner than 48 hours after disinfection. It is recommended that sampling is undertaken by an accredited organisation independent of the contractor.

Chlorine dioxide systems

15.34 Allowance should be made for the type of residual present in the incoming supply as part of the strategy for additional disinfection within premises. The dosage of chlorine dioxide should be proportional to the water flow and the dosing system should incorporate safeguards to prevent inadvertent overdosing. In the case of hot water distribution systems with calorifiers/water heaters operating conventionally (that is, at 60°C), there will be a tendency for chlorine dioxide to be lost by gassing off, especially if the retention time in a vented calorifier/water heater is long. In most cases, however, some concentration of total oxidant should be found in the hot water, although this should be far less than the 0.5 mg/L injected.

15.35 The required dose should be determined by incremental addition until it reaches a concentration that provides adequate preservation of water quality but should not exceed 0.5mg/L. The failsafe operation of the system plus any leak detection installed should be witnessed and verified.

Note 1

Initially during the commissioning process it may be necessary to maintain a higher dosage rate than 0.5mg/L.

Note 2

Excessive values of chlorine dioxide should be avoided subsequently since it can corrode copper and steel pipework and can also damage non-metallic pipework and component parts, particularly at higher temperatures.

15.36 Guidance on chlorine dioxide systems is given in HSG274 Part 2.

Copper and silver ionisation

15.37 Values of more than 0.2 mg/L copper and more than 0.02 mg/L silver are recommended at outlets to ensure effective control of *Legionella*. Analysis for silver and copper should be carried out by a UKAS-accredited laboratory.

15.38 Maintaining adequate silver/copper ion concentration in hard water systems can be difficult as a result of the build-up of scale on the electrodes potentially obstructing copper and ion release. Consequently more frequent checks will be necessary to ensure that the system is delivering adequate ion values. (The ionisation process of pH sensitive and dosing may need to be increased for pH values greater than 7.6.)

Note

The Water Supply (Water Quality) Regulations 2000 set a standard for copper of 2 mg/L, which must not be exceeded. However, there is currently no standard for silver used for domestic purposes. At the time of publication the European Union and WHO do not dictate any standards for silver as there is currently insufficient data for recommending a concentration limit. Equipment manufacturers generally recommend copper (0.2–0.8 mg/L) and silver (0.02–0.08 mg/L) ion concentrations to control *Legionella* effectively. See also paragraphs 5.18–5.20.

15.39 Where some of the outlets on the treated water systems are to be used for domestic purposes, regular water testing needs to be maintained to ensure that the copper concentration does not exceed 2.0 mg/L as Cu and the silver level does not exceed 0.1 mg/L as Ag at these outlets.

15.40 Guidance on silver and copper ionisation systems is given in HSG274 Part 2.

Other water treatment systems

15.41 A range of biocidal treatment systems are available, and further advice on their use is given in HSG274 Part 2.

Maintaining control of systems

15.42 After commissioning and prior to occupancy, it is essential to put in place measures to ensure that wholesome water and hot and cold water temperatures are maintained throughout the system. This will require regular flushing as determined by the WSG and associated risk assessments (see also clauses 2.40–2.52 in HSG274 Part 2).

Note

Once filled, systems should not be drained unless full disinfection is to be carried out prior to building occupancy and use. However, allowing water in newly installed capillary-jointed copper plumbing to stagnate can result in serious corrosion of the copper.

Discharge of wastewater used during disinfection procedures within buildings

15.43 Contaminated water from disinfection procedures that is run to waste, be it to a natural watercourse or sewer, can potentially result in a pollution hazard or affect the efficacy of a sewage treatment plant. It is therefore important that the appropriate consent to discharge is obtained prior to disposing of the contaminated water.

15.44 Where water from disinfection procedures is to be discharged into a watercourse or into a drain leading to a watercourse, consent to discharge should be obtained from the appropriate authority (the Environment Agency in England and Wales, the Scottish Environmental Protection Agency in Scotland, and the Department of the Environment for Northern Ireland in Northern Ireland).

15.45 Water from disinfection procedures may be classed as trade effluent and, prior to any discharge into a sewer or sewerage system, consent to discharge should be sought from the local water company that provides the sewage and trade effluent services to the site (sewerage undertaker). (See also page 33 of HBN 00-07 and also guidance from Water UK.)

Note

Dependent on the geographical location of the site with respect to the operational boundaries of water companies, the duties of the water undertaker and sewerage undertaker may be provided by two different companies.

16 Documentation

16.1 It is essential that a full report of all commissioning and testing activities is compiled and handed over to be incorporated within the operation and maintenance manuals.

16.2 These commissioning and testing records will be required so that subsequent maintenance and periodic checks can be made to ensure that the installation continues to operate as intended. Such information will include:

- results of temperature checks on the cold water supply;
- results of temperature checks on hot water circulating systems and trace-heated hot water installations; and
- commissioning and in-service test data for type 3 and type 2 TMVs (see Chapter 11 of HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)').

The information should also include identification of, and test results for, sentinel taps.

16.3 Where continuous water treatment is installed, the commissioning records should include details of settings of the equipment, dosing rates and requirements for testing.

16.4 Operation and maintenance manuals should be in accordance with BSRIA's Building Applications Guide BG1/2007 – 'Handover O&M manuals and project feedback'.

16.5 As a minimum, for new installations or major refurbishment, the contract should require the following documents and drawings to be supplied:

a. full manufacturing details, including batch numbers of all pipes and fittings;

b. (in accordance with BS 8580) a risk assessment for the control of *Legionella*;

c. full records and certificates of pressure tests for all sections of pipework;

d. settings of all balancing valves, with readings of flow rates where applicable;

e. full details of each item of plant, including arrangement drawings and appropriate test certificates;

f. as-fitted drawings showing clearly the location of balancing valves, flows and settings, isolation valves, drain valves;

g. schematic drawings for installation in plantrooms showing all valves and items of plant;

h. full details of water treatment parameters and operating modes and settings;

i. full details of maintenance requirements;

j. detailed confirmation of disinfection procedures to BS EN 806 series (parts 1–5) and BS 8558 and results of post-disinfection microbiological analysis;

k. full records confirming that all materials and fittings hold WRAS or equivalent accreditation;

l. expected operational conditions of the water system (that is, expected pressures, expected temperatures, expected flow rate throughout the system as pressure drops will occur etc).

Appendix 1 Impact of water consumption on care pathways

Water figures per unit of healthcare activity are published by the NHS Sustainable Development Unit (NHS SDU):

http://www.sduhealth.org.uk/areas-of-focus/carbon-hotspots/pharmaceuticals/cspm/sustainable-care-pathways-guidance.aspx

Estates Return Information Collection (ERIC) data on total water consumption (litres per day per floor area) for each type of healthcare organisation can be found at: http://hefs.hscic.gov.uk/

Appendix 2 Overview of UK water legislation and regulation

The table below shows the principal acts and secondary legislation created to regulate the provision of public and private water supplies in the UK.

Legislation

Legislation	England	Northern Ireland	Scotland	Wales
Primary Legislation – Principal Acts	Water Industry Act 1991 (as amended)	The Water and Sewerage Services (Northern Ireland) Order 2006 (as amended)	Water (Scotland) Act 1980 (as amended) and Water Industry (Scotland) Act 2002	Water Industry Act 1991 (as amended)
Powers and Offences provided by the Principal Acts which can be used in relation Water Fittings Regulations enforcement	Water Industry Act 1991; Section 73 & 75	The Water and Sewerage Services (Northern Ireland) Order 2006; Articles 113 & 115	Water (Scotland) Act 1980; Schedule 4; Section 30(1)(a)& (b),& 30(2), & 31(1)	Water Industry Act 1991; Section 73 & 75
Secondary legislation – Public Water Supplies – Drinking Water Quality	The Water Supply (Water Quality) Regulations 2000 (as amended)	The Water Supply (Water Quality) Regulations (Northern Ireland) 2007 (as amended)	The Public Water Supplies (Scotland) Regulations 2014	The Water Supply (Water Quality) Regulations 2010
Secondary legislation – Public Water Supplies – Water Fittings	The Water Supply (Water Fittings) Regulations 1999 (as amended)	The Water Supply (Water Fittings) Regulations (Northern Ireland) 2009	The Water Supply (Water Fittings) (Scotland) Byelaws 2014	The Water Supply (Water Fittings) Regulations 1999 (as amended)
Secondary legislation – Private Water Supplies	The Private Water Supplies Regulations 2009 (as amended)	The Private Water Supplies Regulations (Northern Ireland) 2009 (as amended)	The Private Water Supplies (Scotland) Regulations 2006 and amended by The Water Quality (Scotland) Regulations 2010	The Private Water Supplies (Wales) Regulations 2010 (as amended)

The secondary legislation transposes into law the European Union Council Directive 98/83/EC on the quality of water intended for human consumption. Compliance with this Directive is the responsibility of the UK legislators (as a member state) with Defra providing representation as the regulatory authority.

Water Supply (Water Fittings) Regulations 1999 have been amended by:

- The Water Supply (Water Fittings) (Amendment) Regulations 1999 – SI 1506/1999
- The Water Act 2003 (Consequential and Supplementary Provisions) Regulations 2005 – SI 2035/2005
- The Construction Products Regulations 2013 – SI 1387/2013

Regulators' specifications and Defra guidance

Defra Guidance (previously DETR)	Guidance
WC Suite Performance Specifications	Specification
Regulator's specification for backflow prevention arrangements and devices	Specification
Specification for the performance of water fittings	Specifications (WRAS website)
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Part B: Operational management

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Executive summary

Preamble

This current review and update of HTM 04-01 is intended to move users of the document towards a holistic management of water systems via Water Safety Groups (WSGs), Water Safety Plans (WSPs) and other initiatives.

This version draws together and updates the previous guidance and includes recommendations for the safe management of water systems, via the integration of the principle of WSGs and WSPs (first introduced in the HTM 04-01 *P. aeruginosa* addendum – published in March 2013), and how to manage and minimise the risks to health from various aspects, ranging from clinical risks, microbial and chemical contamination, changes to the water system, resilience of the water supply etc. It also introduces a stronger emphasis on staff competencies and the implementation of water hygiene awareness training.

Introduction

The development, construction, installation, commissioning and maintenance of hot and cold water supply systems are vital for public health. Healthcare premises are dependent upon water to maintain hygiene and a comfortable environment for patients and staff, and for treatment and diagnostic purposes.

Interruptions in water supply can disrupt healthcare activities. The design of systems should ensure that sufficient reserve water storage is available to minimise the consequence of disruption, while at the same time ensuring an adequate turnover of water to prevent stagnation in storage vessels and distribution systems.

This Health Technical Memorandum (HTM) gives comprehensive advice and guidance to healthcare management, design engineers, estate managers, operations managers, contractors and the supply chain on the legal requirements, design applications, maintenance and operation of hot and cold water supply, storage and distribution systems in all types of healthcare premises. It is equally applicable to both new and existing sites.

Aims of this guidance

The current review and update of HTM 04-01 is intended to move users of the document towards a holistic management of water systems via WSGs, WSPs and other initiatives.

It has been written to promote good practice for those responsible for the design, installation, commissioning, operation and maintenance of water services in healthcare premises, by:

- highlighting the need for robust governance and management;
- outlining the remit of the WSG and how this relates to the provision of safe water in healthcare premises;
- outlining key criteria and system arrangements to help stop the ingress of chemical and microbial contaminants and microbial colonisation and bacteria proliferation;
- illustrating temperature regimes for sanitary outlets to maintain water hygiene;
- ensuring the safe delivery of hot water;
- outlining how the correct selection of system components and correct use by occupants can help preserve the quality and hygiene of water supplies;

- providing a point of reference to legislation, standards and other guidance pertaining to water systems;
- providing a basic overview of possible potential waterborne pathogens;
- giving an overview of some of the different water systems (including components) and their safe installation, commissioning and operation and maintenance;
- providing typical system layouts and individual component location;
- providing information on thermostatic mixing valve configurations, appropriate usage and maintenance requirements;
- identifying key commissioning, testing and maintenance requirements for referral by designers, installers, commissioners, operators and management.

Controlling waterborne pathogens

The guidance gives comprehensive guidance on measures to control waterborne pathogens. While *Legionella* control is, in the main, associated with poor engineering configuration and maintenance, with no evidence of patient-to-patient or patient-to-outlet transfer, *P. aeruginosa* may be transferred to and from outlets and the water from both patients and staff. Suspected *P. aeruginosa* waterborne infections require additional investigations to determine the source and interventions from infection control specialists and microbiologists. Therefore, a temperature control regime is the traditional strategy for reducing the risk from *Legionella* and for reducing the growth and colonisation of other waterborne organisms within water systems. To prevent growth of *P. aeruginosa* and other waterborne pathogens, controls are necessary to manage the water system before and after the outlet.

As with all control measures, temperatures should be monitored at regular intervals to verify effective control.

Because of the complexity of hot and cold water distribution systems and the difficulty of maintaining a temperature control regime in some healthcare facilities, this guidance suggests that additional chemical, physical and other water control methods that have been shown to be capable of controlling microbial colonisation and growth may also be considered.

Main changes from the 2006 edition of HTM 04-01

- This 2016 edition of HTM 04-01 provides comprehensive guidance on measures to control waterborne pathogens such as *Pseudomonas aeruginosa Stenotrophomonas maltophilia, Mycobacteria* as well as *Legionella*.
- This edition has been updated to align with the Health and Safety Executive's (HSE's) recently revised Approved Code of Practice for *Legionella* (L8) and its associated HSG274 guidance documents.
- The Addendum to HTM 04-01 published in 2013 (and now Part C of HTM 04-01) introduced the concept of WSGs and WSPs. Part B of the HTM now includes updated guidance on the remit and aims of the WSG and WSP.
- New guidance has been included on the hygienic storing and installation of fittings and components and on the competency of installers/plumbers working on healthcare water systems. The guidance also outlines that any person working on water distribution systems or cleaning water outlets needs to have completed a water hygiene awareness training course. An example course outline is included.
- Guidance on sampling techniques for, testing for, and the microbiological examination of *Pseudomonas aeruginosa* samples – originally in the HTM 04-01 Addendum – is now included in Part B to complement similar guidance on *Legionella*.

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Glossary and abbreviations

In addition to the definitions listed below, other definitions can be found in the Water Supply (Water Fittings) Regulations 1999; BS 6100; BS 8558; and BS EN 806.

Alert organisms: Alert organisms are microorganisms that have the potential to cause harm and disease in individuals and which can cause an outbreak of infection in a hospital environment. An alert organism is identified by the microbiology laboratory and referred to the infection prevention and control (IPC) team for assessment of possible healthcare-associated acquisition and to identify any possible environmental/equipment sources.

Augmented care units/settings – There is no fixed definition of "augmented care"; individual providers may wish to designate a particular service as one where water quality must be of a higher microbiological standard than that provided by the supplier. While this document provides broad guidance, the water quality required will be dependent on both the type of patient and its intended use. Most care that is designated as augmented will be that where medical/nursing procedures render the patients susceptible to invasive disease from environmental and opportunistic pathogens such as *Pseudomonas aeruginosa* and other alert organisms. In broad terms, these patient groups will include:

a. those patients who are severely immunosuppressed because of disease or treatment: this will include transplant patients and similar heavily immunosuppressed patients during high-risk periods in their therapy;

b. those cared for in units where organ support is necessary, for example critical care (adult paediatric and neonatal), renal, respiratory (may include cystic fibrosis units) or other intensive care situations;

c. those patients who have extensive breaches in their dermal integrity and require contact with water as part of their continuing care, such as in those units caring for burns.

Backflow – Flow upstream, that is in a direction contrary to the intended normal direction of flow, within or from a water fitting.

Biofilm: A biofilm is a complex layer of microorganisms that have attached and grown on a surface. This form of growth provides a niche environment for a wide range of microorganisms to interact and where the secretion of exopolysaccharides by bacteria will form an extracellular matrix for both bacteria and other unicellular organisms such as amoebae and flagellates to remain in a protected state.

Colony forming unit: Unit that gives rise to a bacterial colony when grown on a solid medium; this may be a single bacterial cell or a clump of cells.

Dead-leg – a length of water system pipework leading to a fitting through which water only passes infrequently when there is draw-off from the fitting, providing the potential for stagnation.

Flow straightener: A device inserted into the spout outlet of a tap to modify flow, take out turbulence and create an even stream of water.

Healthcare-associated infections (HCAI): encompasses any infection by any infectious agent acquired as a consequence of a person's treatment or which is acquired by a healthcare worker in the course of their duties.

Healthcare facility/building – all buildings: infrastructure, equipment, plant, embedded systems and related items that support the delivery of healthcare and services of all types, irrespective of their ownership or operation by third parties.

Healthcare organisations: organisations that provide or intend to provide healthcare services for the purposes of the NHS.

Legionellosis: a collective term for diseases caused by *Legionella* bacteria including the most serious Legionnaires' disease, as well as the similar but less serious conditions of Pontiac fever and Lochgoilhead fever.

Manual mixing tap – a tap that controls both the flow and temperature of water delivered.

Point-of-use (POU) filter: a filter with a maximal pore size of 0.2 µm applied at the outlet, which removes bacteria from the water flow.

Redundant pipework (also known as blind end): a length of pipe closed at one end through which no water passes.

Remediation: Any process that reduces the risk from harmful agents such as microorganisms.

Thermostatic mixing tap: a tap that controls the flow and by thermostatic means the temperature of water delivered.

Thermostatic mixing valve: valve with one outlet, which mixes hot and cold water and automatically controls the mixed water to a user-selected or pre-set temperature.

Transmission: Any mechanism by which an infectious agent is spread from a person or environmental source to a susceptible person.

Waterborne pathogen: microorganism capable of causing disease that may be transmitted via water and acquired through ingestion, bathing, or by other means.

Water outlet: (In this document) refers mainly to taps and showerheads, but other outlets, as indicated by risk assessments, may be considered important.

Water Safety Group (WSG): A multidisciplinary group formed to undertake the commissioning and development and ongoing management of the water safety plan (WSP). It also advises on the remedial action required when water systems or outlets are found to be contaminated and the risk to susceptible patients is increased.

Water safety plan (WSP): A risk-management approach to the safety of water that establishes good practices in local water distribution and supply. It will identify potential hazards, consider practical aspects, and detail appropriate control measures.

Water supply [to the healthcare facility]: The water supplied can be via:

- the mains water supply from the local water undertaker;
- a borehole (operated by the healthcare organisation as a private water supply);
- a combination of mains water and borehole supply;
- emergency water provision (bulk tankered water or bottled drinking water).

Water undertaker – the role of a water undertaker is defined in a number of sections of the Water Industry Act 1991.

Wholesomeness: standards of wholesomeness are defined in section 67 of the Water Industry Act 1991. Separate legislation for public and private supplies sets out the prescribed concentrations and values for water and are detailed in the following legislation: the Water Supply (Water Quality) Regulations 2000 for water from a public supply; or the Private Water Supplies Regulations 2009 for water from a private supply.

List of abbreviations

cfu – colony forming units

COSHH – Control of Substances Hazardous to Health [Regulations]

CQC – Care Quality Commission

DWI – Drinking Water Inspectorate

EA – Environment Agency

EPDM – ethylene propylene diene monomer

HBN – Health Building Note

HSE – Health & Safety Executive

HSG274 Part 2 – The Health & Safety Executive's technical guidance on the control of Legionnaires' disease in hot and cold water systems.

HTM – Health Technical Memorandum

MCA: milk cetrimide agar

MRD: maximum recovery diluent

POU – point-of-use

PWTAG – Pool Water Treatment Advisory Group

SHTM – Scottish Health Technical Memorandum

WRAS – Water Regulations Advisory Scheme

WSG – Water Safety Group

WSP – Water safety plan

0 Policy and regulatory overview: water safety and the healthcare estate

Introduction

0.1 The National Health Service (NHS) has a corporate responsibility to account for the stewardship of its publicly funded assets. This includes the provision, management and operation of an efficient, safe estate that supports clinical services and strategy.

0.2 This corporate responsibility is carried by all accountable officers, directors with responsibility for estates and facilities and their equivalents, chairs, chief executive officers and non-executive board members. Together they have a responsibility to enact the principles set out in this document, provide leadership and work together to implement the necessary changes to provide a safe, efficient high quality healthcare estate.

0.3 To achieve this, quality and fitness-for-purpose of the healthcare estate is vital. Health Technical Memorandum (HTM) 04-01 seeks to set out the quality of, and standards for, water safety in the healthcare estate.

0.4 A healthcare organisation's Water Safety Group (WSG) (see Part B) is pivotal in ensuring that decisions affecting the safety and integrity of the water systems and associated equipment do not go ahead without being agreed by them. This includes consultations relating to decisions on the procurement, design, installation and commissioning of water services, equipment and associated treatment processes.

0.5 The quality and fitness-for-purpose of the estate are assessed against a set of legal requirements and governance standards. Adhering to the guidance outlined in this HTM will be taken into account as evidence towards compliance with these legal requirements and governance standards.

Compliance of the healthcare estate

0.6 Principles related to the safety of healthcare estates and facilities are enshrined in the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014, specifically Regulation 12(2)(h) and Regulation 15 of the Act.

Note

There are numerous other statutes and legal requirements that NHS organisations, supporting professionals, contractors and suppliers must comply with. These are covered in the respective Health Building Notes (HBNs), Health Technical Memoranda (HTMs) and the NHS Premises Assurance Model (NHS PAM).

Health and Social Care Act 2008 (Regulated Activities) Regulations 2014

0.7 Regulation 12(2)(h) decrees that registered providers must assess:

- the risk of, and prevent, detect and control the spread of, infections, including those that are health care associated.

0.8 Appropriate standards of cleanliness and hygiene should be maintained in premises used for the regulated activity. DH (2015) issued 'The Health and Social Care Act 2008 Code of Practice on the prevention and control of infections and related guidance' (the HCAI Code of Practice), which contains statutory guidance about compliance with regulation 12(2)(h) (see paragraphs 0.15–0.17).

0.9 Regulation 15 of the Act states that:

(1) All premises and equipment used by the service provider must be –

a. clean,

b. secure,

c. suitable for the purpose for which they are being used,

d. properly used,

e. properly maintained, and

f. appropriately located for the purpose for which they are being used.

(2) The registered person must, in relation to such premises and equipment, maintain standards of hygiene appropriate for the purposes for which they are being used.

Note

The "registered person" means, in respect of a regulated activity, the person who is the service provider or a registered manager in respect of that activity.

A "service provider" means a person registered with the CQC under Chapter 2 of Part 1 of the Health and Social Care Act 2008 as a service provider in respect of that regulated activity.

Regulator requirements

0.10 The CQC independently regulates all providers of regulated health and adult social care activities in England. The CQC's (2015) 'Guidance for providers on meeting the regulations' explains how to meet regulations 12(2)(h) and 15 outlined above.

0.11 Failure to comply with the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014 and the Care Quality Commission (Registration) Regulations (2009) is an offence, and the CQC has a wide range of enforcement powers that it can use if a provider is not compliant. These include the issue of a warning notice that requires improvement within a specified time, prosecution, and the power to cancel a provider's registration, removing its ability to provide regulated activities.

Examples of governance and assurance mechanisms arising from primary legislation (not exhaustive)

NHS Constitution

0.12 The Health Act 2009 places a duty on bodies providing and commissioning NHS services to have regard to the NHS Constitution. The Health and Social Care Act 2012 further applied this duty to the new bodies created by that Act or by amendments to the 2012 Act.

0.13 The NHS Constitution "sets out rights to which patients, public and staff are entitled". It also outlines "the pledges which the NHS is committed to achieve, together with responsibilities that the public, patients and staff owe to one another to ensure that the NHS operates fairly and effectively".

0.14 It commits the NHS to ensuring "**that services are provided in a clean and safe environment that is fit for purpose, based on national best practice (pledge)".** In order to deliver on this pledge, the NHS should take account of:

- the NHS Premises Assurance Model (NHS PAM) – the NHS PAM identifies where the NHS Constitution needs to be considered and where assurance is required;

- national best practice guidance for the design and operation of NHS healthcare facilities (such as HBNs and HTMs).

The HCAI Code of Practice

0.15 A complex range of issues distinguishes healthcare environments from most other building types. One of the most important of these relates to the control of infection. Infection prevention and control teams should be consulted on any design decisions and a risk analysis conducted on the many issues of design involving water systems (see HBN 00-09 – 'Infection control in the built environment'). To manage and monitor the prevention and control of infections effectively, the HCAI Code of Practice requires a WSG and a water safety plan (WSP) to be in place.

0.16 The information outlined in HBN 00-09 follows the general principles given in the HCAI Code of Practice, which sets out criteria against which a registered provider will be judged on how it complies with Regulation 12(2)(h) of the Health and Social Care Act 2008 (Regulated Activities) Regulations 2014 on the prevention, detection and control of infections, particularly waterborne infections in relation to this document.

0.17 The law states that the HCAI Code of Practice must be taken into account by the CQC when it makes decisions about registration against the cleanliness and infection control requirement. The regulations also say that providers must have regard to the Code when deciding how they will comply with registration requirements. Therefore, by following the Code, registered providers will be able to show that they meet the requirement set out in the regulations. However, the Code is not mandatory. A registered provider may be able to demonstrate that it meets the regulations in a different way (equivalent or better) from that described in this document. The Code aims to exemplify what providers need to do in order to comply with the regulations.

Figure 1 illustrates how DH estates and facilities best practice guidance (such as the NHS PAM and HTMs) aligns with the statutory and policy framework. This guidance is fundamental to ensuring that NHS trusts are able to deliver on their commitments under the NHS Constitution and to comply with the CQC's fundamental standards.

Never events

0.18 NHS England's never events policy framework defines "never events" as serious, largely preventable patient safety incidents that should not occur if the available preventive measures have been implemented by healthcare providers. On the list of never events is scalding of patients. The risk of scalding for vulnerable patients (children and young people, older people, and disabled people) is a particular problem in healthcare premises. HTM 04-01 provides guidance on reducing the risk of scalding.

Health and safety legislation

0.19 In the UK, the control of *Legionella*e falls within the requirements of the Health and Safety at Work etc. Act. This Act also places duties on design teams, suppliers and installers to ensure that articles or substances for use at work are safe and without risks to health and that any information related to the article or substance is provided. The Management of Health and Safety at Work Regulations 1999 provide a broad framework for controlling health and safety at work. The Control of Substances Hazardous to Health Regulations 2002 (COSHH) provide a framework of actions designed to assess, prevent or control the risk from bacteria like *Legionella* and take suitable precautions.

0.20 The Health & Safety Executive's (HSE) (2013) Approved Code of Practice 'Legionnaires' disease: The control of *Legionella* bacteria in water systems (L8)' contains practical guidance on how to manage

and control the risks in water systems. The HSE has published complementary technical guidance in HSG274, which is split into three specific areas:

- Part 1 – evaporative cooling systems;
- Part 2 – hot and cold water systems; and
- Part 3 – other risk systems.

0.21 In addition, under the Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (RIDDOR), there is a duty for employers to report any cases of legionellosis in an employee who has worked on hot and cold water systems that are likely to be contaminated with *Legionella*. Cases of legionellosis are reportable under RIDDOR if:

a. a doctor notifies the employer; and

b. the employee's current job involves work on or near cooling systems that are located in the workplace and use water; or work on water-service systems located in the workplace which are likely to be a source of contamination.

0.22 With regard to enforcement responsibilities, the HSE will take the lead with regard to incidents involving *Legionella*. See the Health and Safety Executive/Care Quality Commission/Local Government Association's (2015) 'Memorandum of understanding between the Care Quality Commission, the Health and Safety Executive and local authorities in England'.

Security

0.23 Accessibility to all plant and equipment should be limited to authorised personnel only (see NHS Protect's (2012) 'Guidance on the security and management of NHS assets').

Water regulations

0.24 As well as complying with the recommendations outlined in this document, the design and installation of the hot and cold water services, new or extended, in any healthcare premises should also comply with:

- the Water Supply (Water Fittings) Regulations 1999;
- Defra's guidance to the Water Supply (Water Fittings) Regulations;
- recommendations of the water suppliers in the Water Regulations Advisory Scheme's (WRAS) 'Water Regulations Guide'; and
- any other requirements of the local water undertaker.

Water Supply (Water Fittings) Regulations 1999

0.25 These Regulations set legal requirements for the design, installation, operation and maintenance of plumbing systems, water fittings and water-using appliances. They have a specific purpose to prevent misuse, waste, undue consumption or erroneous measurement of water and, most importantly, to prevent contamination of drinking water.

0.26 These Regulations apply in all types of premises supplied, or to be supplied, with water from a water undertaker. They apply from the point where water enters the property's underground pipe, to where the water is used in plumbing systems, water fittings and water-using appliances. However they do not apply in premises that have no provision of water from the public mains supply.

Water Supply (Water Quality) Regulations 2000

0.27 These Regulations cover the quality of water supplied by water undertakers for public distribution which is intended for domestic purposes; these purposes include drinking, cooking, food preparation, washing and sanitation. Water supplied meeting these quality requirements is referred to as wholesome water.

Private Water Supplies Regulations 2009

0.28 These Regulations cover private sources of water intended for human consumption including drinking, cooking, food preparation or other domestic purposes, such as boreholes and wells. Water meeting these quality requirements is referred to as wholesome water. These Regulations also place duties for monitoring and control of the quality of public water supplies where these are then further distributed to other users on separate premises by the water company's bill payer (this arrangement is often referred to as onward distribution).

0.29 Local authorities are the regulators for private water supplies and have a number of statutory duties under the Private Water Supplies Regulations.

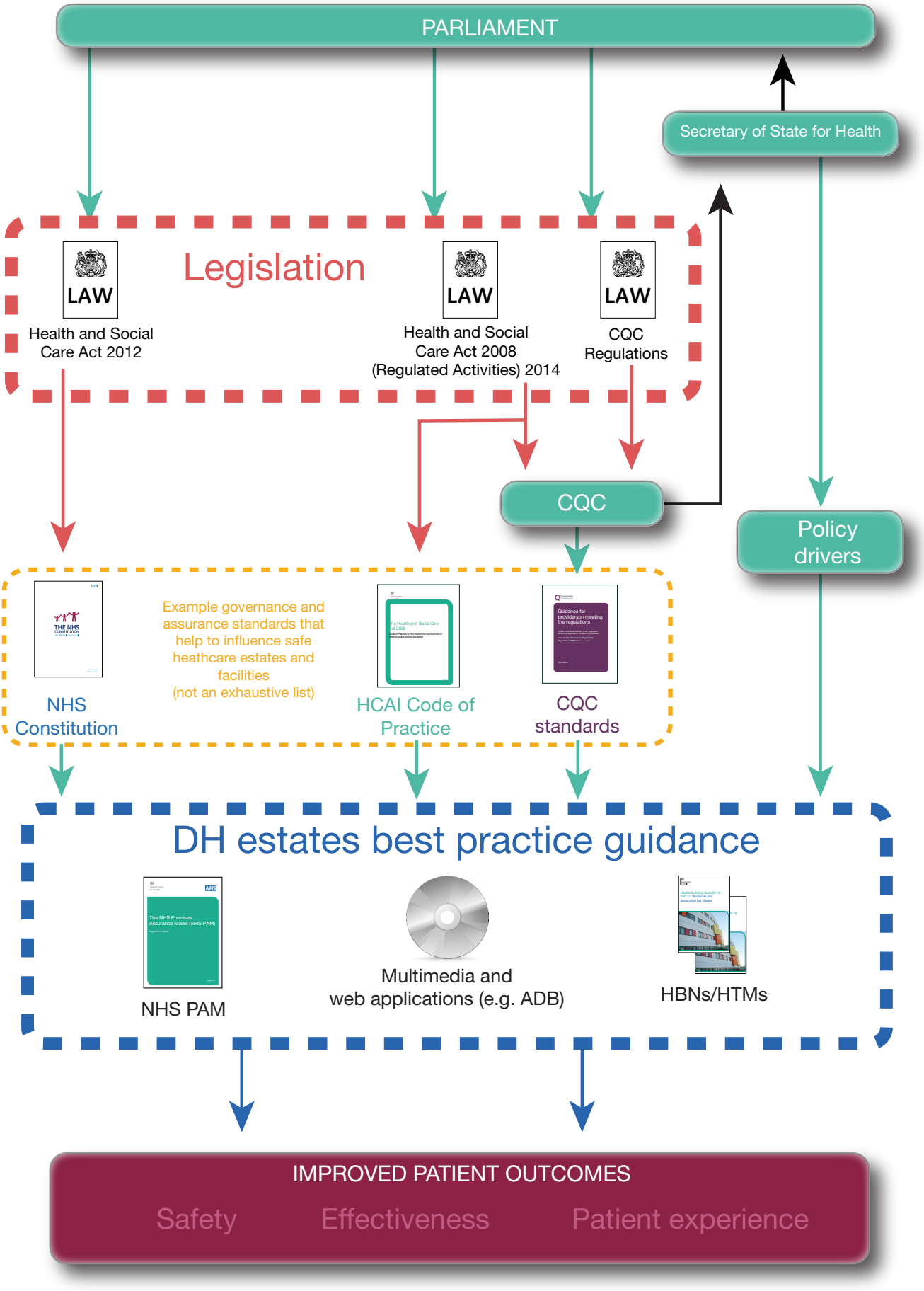


Figure 1 How best practice guidance on the safety and quality of healthcare estates and facilities fits in with the legislative and policy framework. (The statutes and mandatory requirements shown in this figure are not exhaustive. See Note after paragraph 0.6.)

1 Introduction

1.1 This edition of HTM 04-01 – 'Safe water in healthcare premises' supersedes HTM 04-01 – 'The control of *Legionella*, hygiene, "safe" hot water, cold water and drinking water systems' published in 2006. It is also based on the guidance given in HTM 04-01: Addendum '*Pseudomonas aeruginosa* – advice for augmented care units'. It has been revised to take account of revisions to the HSE's (2013) Approved Code of Practice L8 and technical guidance HSG274.

1.2 This HTM gives comprehensive advice and guidance to healthcare management, design engineers, estate managers, operations managers and infection control specialists on the legal requirements, design applications, installation, commissioning, maintenance and operation of hot and cold water supply, storage and distribution systems and associated equipment and systems in all types of healthcare premises. It is equally applicable to both new and existing sites.

1.3 In its new form, the document is divided in three parts. This part (Part B) covers operational management, including the control of *Legionella, P. aeruginosa* and other important waterborne pathogens. This document should be read in conjunction with Part A which outlines the principles involved in the design, installation, commissioning and testing of the hot and cold water supply, and storage and distribution systems for healthcare premises. Some variation may be necessary to meet the differing provision arrangements of the various water undertakers and complexity of distribution systems. Part C covers water safety in augmented care settings.

General

1.4 Current statutory legislation requires both "employers" and "employees" to be aware of their individual and collective responsibility for the provision of safe wholesome hot and cold water supplies, and storage and distribution systems in healthcare premises. Ultimate responsibility for management will fall to the statutory duty holder of the premises (see paragraph 1.8 for a definition of the statutory duty holder).

1.5 Healthcare premises are dependent on water to maintain hygiene and a comfortable environment for patients, visitors and staff, and for clinical and surgical care.

1.6 Intelligent design, construction, installation, commissioning and maintenance of hot and cold water supply systems to minimise the risks of waterborne illness are vital for public health.

1.7 Interruptions in water supply can disrupt healthcare activities. Procedures should be in place to minimise the consequence of disruption, while at the same time ensuring an adequate turnover of water to prevent stagnation in storage vessels and distribution systems (see paragraphs 2.16–2.17 in HTM 04-01 Part A and also HBN 00-07 – 'Planning for a resilient healthcare estate').

Duty holder

1.8 In this document, the statutory duty holder is either the employer or the person in control of the healthcare premises. Typically these could be the owner, chief executive, board of

directors, or other person who is ultimately accountable, and on whom the duty falls, for the safe operation of healthcare premises.

Shared premises

1.9 In estate management, it is increasingly common for there to be several duty holders in one building. In such cases, duties may arise where persons or organisations have clear responsibility through an explicit agreement such as a contract or tenancy agreement.

1.10 The extent of the duty will depend on the nature of that agreement. For example, in a building occupied by one leaseholder, the agreement may be for the owner or leaseholder to take on the full duty for the whole building or to share the duty. In a multi-occupancy building, the agreement may be that the owner takes on the full duty for the whole building. Alternatively, it might be that the duty is shared where, for example, the owner takes responsibility for the common parts while the leaseholders take responsibility for the parts they occupy. In other cases, there may be an agreement to pass the responsibilities to a managing agent. Where a managing agent is used, the management contract should clearly specify who has responsibility for maintenance and safety checks, including managing the risk from waterborne hazards.

1.11 Where there is no contract or tenancy agreement in place or it does not specify who has responsibility, the duty is placed on whoever has control of the premises or part of the premises.

Areas this HTM does not cover

1.12 Although many of this HTM's recommendations will be applicable, it does not set out to cover water supply for fire-fighting services nor water supply for technical, industrial or other specialist purposes, other than to indicate precautions that should be taken when these are used in association with domestic water services. The point at which a domestic activity becomes an industrial process has not been defined, and the applicability will need to be considered in each case.

1.13 This HTM does not cover wet cooling systems such as cooling towers. Guidance on these systems is given in HSE's Approved Code of Practice and guidance 'Legionnaires' disease: The control of *Legionella* bacteria in water systems (L8)' and HSG274 technical guidance Part 1.

1.14 While some guidance on other water-service applications is included, it is not intended to cover them fully. For:

- process waters used for laundries, see HTM 01-04 – 'Decontamination of linen in health and social care';
- endoscopy units, see HTM 01-06 – 'Decontamination of flexible endoscopes';
- primary care dental premises, see HTM 01-05 – 'Decontamination in dental practices';
- renal units, see HBNs 07-01 and 07-02, the Renal Association's guidelines, BS EN ISO 13959 and BS EN ISO 11663;
- sterile services departments, see HBN 13 – 'Sterile services department';
- hydrotherapy pools, see the PWTAG's 'Swimming pool water: treatment and quality standards for pools and spas';
- spa pools, see HSE/PHE's 'Management of spa pools: controlling the risks of infection';

Note

This document is currently being revised and will become HSG274 Part 4 – 'The control of *Legionella* and other infectious agents in spa pool systems'.

- birthing pools, see HBN 09-02 – 'Maternity care facilities' and PWTAG's 'Swimming pool water: treatment and quality standards for pools and spas'.

2 Governance and management responsibility

Note

Governance is concerned with how an organisation directs, manages and monitors its activities to ensure compliance with statutory and legislative requirements while ensuring the safety of patients, visitors and staff is not compromised.

To help achieve this, healthcare organisations need to ensure that sound policies are approved by the board of directors. These should:

- ensure safe processes, working practices and risk-management strategies are in place to safeguard all their stakeholders and assets in order to prevent and reduce harm or loss; and
- be backed up with adequate resources and suitably qualified, competent and trained staff.

2.1 This guidance should be applied to all healthcare premises, however small, where there is a duty of care under the Health and Safety at Work etc. Act 1974.

2.2 To ensure governance with regard to water safety, the duty holder will:

- identify and assess sources of risk;
- if appropriate, prepare a written scheme for preventing or controlling the risk;
- implement, manage and monitor precautions;
- keep records of the precautions;
- appoint a competent person with sufficient authority and knowledge of the installation to help take the measures needed to comply with the law.

2.3 To implement the above legal duties in a healthcare organisation, the duty holder should appoint a WSG to undertake the commissioning, development, implementation and review of a WSP. The aim of the WSG is to ensure the safety of all water used by patients/residents, staff and visitors, and to minimise the risk of infection associated with waterborne pathogens. This is supported by the HCAI Code of Practice (see paragraphs 0.15–0.17), which recommends that management and monitoring arrangements should ensure that a WSG and WSP are in place.

2.4 The WSP should demonstrate that any person on whom the statutory duty falls has fully appreciated the requirement to provide an adequate supply of hot and cold water of suitable quality. Though compliance with this guidance may be delegated to staff or undertaken by contractors, accountability cannot be delegated. The WSG should ensure that appropriate expertise and competence is available to ensure the delivery of safe water for all uses throughout the organisation. This group should have clearly identified lines of accountability up to the CEO and board. For membership of the group, see paragraphs 6.5–6.6.

2.5 The WSG should include persons who are fully conversant with the design principles and requirements of water systems and should be fully briefed in respect of the cause and effect all waterborne hazards.

2.6 The WSP is a holistic approach to manage water for all uses (including diagnostic and treatment purposes) so that it is safe for all users including those most at risk of waterborne infections as a consequence of their illness or treatment (see paragraphs 6.16–6.22).

2.7 All regular tests and checks set out in the WSP should be carried out even if they cause minor disruption to healthcare services, and comprehensive records should be maintained in accordance with the healthcare organisation's management policy.

2.8 A risk assessment forms an integral component of the WSP and is a legal requirement to identify potential hazards (which may be microbial, chemical or physical) in the system, risks of infection to patients, staff and visitors, and other indicators of water quality (for example, taste, odour, flavour and appearance if intended for drinking). See paragraphs 6.23–6.26 for typical examples of issues to consider in the risk assessment.

2.9 The risk assessment should be carried out by a competent person or persons. If the provision of risk assessments is contracted to an external organisation, it is recommended that those engaged to carry out any risk assessments associated with water safety should be able to demonstrate to the WSG their experience and competence in assessing specific risks from microbiological, chemical and physical hazards on the specific healthcare population. They should also be able to give advice on how to manage the systems/equipment to minimise the risks etc. It is the responsibility of the WSG to determine the method of demonstrating this competence. Core requirements including accredited training and personal examples of recent water safety risk assessments in the healthcare sector presented orally and/or by interview should be considered options. Detailed knowledge and expertise requirements of the risk assessor(s) are provided in the World Health Organization's (WHO) (2011) 'Water safety in buildings'.

2.10 The risk assessor(s) should be given access to competent assistance from the client. This may be in the form of:

- engineering and building expertise;
- as-fitted drawings and schematic diagrams;
- clinical expertise;
- knowledge of building occupancy and use including vulnerability of patient groups;
- bespoke equipment plus policies, procedures and any protocols (for example cleaning of wash-hand basins and disposal of clinical effluent etc).

2.11 In addition access should be made available to all required areas (and associated systems and equipment) unless deemed inaccessible by legislation (for example areas that contain asbestos).

2.12 For *Legionella* risk assessments, contractors should be able to demonstrate a full understanding of, and work to, BS 8580. In addition to guidance provided above, the documents below should also be referenced in relation to the specification, procedures and general requirements for completing robust and fit-for-purpose water safety risk assessments:

- HSG274 Part 2 (2014) – 'The control of *Legionella* bacteria in hot and cold water systems'.
- BS 8580 – 'Water quality: risk assessments for *Legionella* control – Code of Practice'.
- BSRIA's (1999) FMS 4/99 – 'Guidance and the standard specification for water services risk assessment'.
- BSRIA's (2015) BG 57/2015 – 'Legionnaires' disease – risk assessment'.

2.13 Management procedures should ensure that compliance is continuing and not notional. The prime purpose of the assessment is to be able to demonstrate that the WSG is aware of

all the relevant factors that may pose a risk of waterborne infection, that effective corrective or preventive action has been implemented, and that monitoring is in place to ensure the plans are effective in controlling the risk.

2.14 Healthcare organisations should be aware of the legal duty to notify the water undertaker when it is proposed to carry out works on any systems conveying water from the public water supply (see the WRAS website).

Note

Water undertakers can carry out a fittings inspection to ensure that work has been properly carried out and that the public water supply remains protected for work carried out.

3 Statutory requirements

3.1 It is the responsibility of the duty holder to ensure that their premises comply with all statutes.

3.2 Duty holders have an overriding general duty of care under the Health and Safety at Work etc. Act 1974. Therefore, they should ensure that the water supply, storage and distribution services are installed and operated within the terms of the following legislation.

Health and Safety at Work etc. Act 1974

3.3 Employers have a general duty under the Health and Safety at Work etc. Act 1974 to ensure, so far as is reasonably practicable, the health, safety and welfare of patients, visitors and staff and the public who may be affected by workplace activities.

3.4 These duties are legally enforceable, and the HSE has successfully prosecuted employers including health organisations under this statute. It falls upon owners and occupiers of premises to ensure that there is a management regime for the proper design, installation and maintenance of plant, equipment and systems. Failure to have a proper system of working and adequate control measures can also be an offence even if an outbreak of, for example, Legionnaires' disease or other such incident has not occurred.

The Management of Health and Safety at Work Regulations 1999

3.5 These regulations provide a broad framework for controlling health and safety at work. They require every employer to make a suitable and sufficient assessment of all risks to health and safety of employees and the public caused by work activities, and require employers to have access to competent help in applying the provisions of health and safety law. In addition to *Legionella* and other waterborne pathogens, other risks from a hot and cold water distribution system include deterioration of water quality, scalding at hot water outlets and danger due to pipe bursts at excessive pressures.

Control of Substances Hazardous to Health (COSHH) Regulations 2002

3.6 These regulations provide a framework of actions designed to control the risk from a range of harmful substances including waterborne pathogens such as *Legionella* and the chemicals that may be used to control the growth of microorganisms in water supplies. Employers have a duty to assess the risks from exposure to these substances to ensure that they are adequately controlled.

Public Health (Infectious Diseases) Regulations 1988

3.7 The Public Health (Infectious Diseases) Regulations 1988 require that a properly appointed officer inform the chief medical officer for England or for Wales, as the case may be, of any serious outbreak of any disease that to his/her knowledge has occurred in the district.

Note

Appendix 2.3 of HSE's *Legionella* technical guidance HSG274 Part 2 (2013) contains further advice and guidance on communication and cooperation with the consultant in communicable disease control (CCDC), and on arrangements for support of the CCDC and for this person to have access to provider units including healthcare organisations.

Water Supply (Water Quality) Regulations 2000

3.8 The Water Supply (Water Quality) Regulations 2000 apply to water supplied by a water undertaker to any premises that are used for domestic purposes such as drinking, cooking, personal hygiene, washing or food production.

Note

Two additional sources of advice on drinking water quality are:

a. the director of public health;

b. WHO's 'Guidelines for drinking water quality'.

Private Water Supplies Regulations 2009

3.9 These regulations cover private sources of water such as boreholes and wells intended for human consumption including drinking, cooking, food preparation or other domestic purposes. These regulations also place duties for monitoring and control of the quality of public water supplies where these are then further distributed to separate premises by the bill payer other than the water undertaker or licensed water supplier (often referred to as onward distribution).

Food Safety Act 1990

3.10 The Food Safety Act 1990 covers water used for food preparation or food manufacture and also includes water used for drinking. The Food Safety and Hygiene (England) Regulations 2013 are also relevant.

The Health and Safety Executive's (4th edition) Approved Code of Practice L8 2013

3.11 The HSE's (2013) Approved Code of Practice L8 (4th edition) came into effect on 7 November 2013 and is supported by the technical guidance (HSG274 Parts 1–3). It replaced the earlier publication entitled 'Legionnaires' disease: The control of *Legionella* bacteria in water systems – Approved Code of Practice and guidance' (L8 3rd edition). The onus is on the duty holder to demonstrate that procedures in place are as good as, or better than, those required by L8.

3.12 The Approved Code of Practice L8 has a special legal status. If a person or organisation is prosecuted for a breach of health and safety law and it is proved that they did not follow the provisions of the Code, they will need to show that they have complied with the law in some other equally effective way or a court will find them at fault. Health and safety inspectors seek to secure compliance with the law and may refer to this guidance.

3.13 Compliance with HSG274 will satisfy the Approved Code of Practice L8.

3.14 The health service, with responsibility for the wider aspects of public health and the operation of healthcare premises, is expected to be particularly vigilant.

3.15 The incidence of healthcare-associated waterborne illness including Legionnaires' disease is relatively low, but cases and outbreaks are considered to be avoidable. Management, operators and contractors should be aware that incidents or outbreaks cause widespread concern, especially if associated with healthcare premises. Investigation of these outbreaks has shown that they are generally related to poor training, flaws in system design, poor commissioning and risk assessments, defects and breakdowns. However, by far the greatest contributors to outbreaks of Legionnaires' disease are poor or inappropriate maintenance and control procedures and ineffective communication and systems management.

Water Supply (Water Fittings) Regulations 1999

3.16 These regulations set legal requirements for the design, installation, operation and maintenance of plumbing systems, water fittings and water-using appliances. They have a specific purpose to prevent misuse, waste, undue consumption or erroneous measurement of water and, most importantly, to prevent contamination of drinking water. These regulations are enforced by the local water undertaker.

3.17 These regulations apply in all types of premises supplied, or to be supplied, with water from a water undertaker. They apply from the point where water enters the property's underground pipe, to where the water is used in plumbing systems, water fittings and water-using appliances. However they do not apply in premises that have no provision of water from the public mains supply.

3.18 These regulations are set out – along with the Department for Environment, Food and Rural Affairs' (Defra) guidance on the regulations and the water industry's recommendations for fulfilling these provisions – in the 'Water Regulations Guide' published by the Water Regulations Advisory Scheme (WRAS).

British and European Standards

- The BS EN 805 series covers design and installation requirements for systems and components outside buildings.
- The BS EN 806 series covers installations inside buildings conveying water for human consumption.
- BS 8558 provides UK-specific complementary guidance to the BS EN 806 series of standards, replacing BS 6700.
- BS 1710 is the British Standard specification for identification of pipelines and services.
- PD 855468 – 'Guide to the flushing and disinfection of services supplying water for domestic use within buildings and their curtilages'.
- BS 8551 – 'Provision and management of temporary water supplies and distribution networks (not including provisions for statutory emergencies). Code of practice'.
- BS 8554 – 'Code of practice for the sampling and monitoring of hot and cold water services in buildings'.
- BS 7592 – 'Sampling for *Legionella* bacteria in water systems. Code of practice'.

4 *Legionella*: overview

Source of the bacteria

4.1 *Legionella* bacteria are ubiquitous in both the natural and constructed aquatic environment and are widespread in natural freshwater including rivers, lakes, streams and ponds and may also be found in damp soil.

4.2 Airborne dispersal may occur when aerosols or droplet nuclei are created. There is a strong likelihood of low concentrations of *Legionella* existing in all open water systems including those of building services; therefore, the main emphasis should be on preventing *Legionella* from multiplying in water systems in healthcare premises.

Ecology

4.3 The following factors have been found to influence the colonisation and growth of *Legionella*:

a. Water temperature between 20°C and 45°C will promote growth.

b. Areas of poor flow, stagnation or inappropriate components.

c. Biofilms play an important role in harbouring and providing favourable conditions in which *Legionella* can grow. Biofilms in water systems are heterogeneous and will consist of bacteria, fungi, algae, protozoa, debris and corrosion products. Nutrients can be provided to the biofilm from the incoming water, particularly where there is increased turbidity and also from scale, sediment, corrosion products, trapped organic and inorganic molecules supplied by the flowing water and a range of surface materials.

d. *Legionella* are unable to grow in sterile water as they require other microorganisms for growth. *Legionella* have also been shown to proliferate rapidly in association with some waterborne protozoa including amoebae.

e. Water fittings, pipework and materials used in the construction of water systems can encourage the growth of waterborne pathogens. Water quality can deteriorate within the system and in components such as terminal fittings, particularly when utilisation is low. Pipework downstream of thermostatic mixing valves may pose a particular problem as the lower temperature can encourage the growth of waterborne pathogens.

Epidemiology

4.4 Legionnaires' disease is often described as an atypical acute pneumonia of rapid onset often with gastrointestinal symptoms, which can confuse the diagnosis. Legionnaires' disease is predominantly caused by inhalation of *L. pneumophila* serogroup 1, which is the commonest cause of Legionnaires' disease accounting for around 85% of cases within the EU (see the European Centre for Disease Prevention and Control's (ECDC) website). However, other non-*pneumophila* species have also been shown to cause disease in

hospitals. Pontiac fever is a self-limiting form of legionellosis which usually requires no treatment. Outbreaks of Pontiac fever have been associated with patients showering.

4.5 The risk of healthcare-associated legionellosis depends on a number of factors such as:

- the presence of *Legionella* in sufficient numbers (see also paragraph 4.15);
- conditions suitable for multiplication of the organisms (for example temperatures between 20°C and 45°C);
- source of nutrients (for example scale, sludge, rust, protozoa, and other available organic carbon, bacteria and biofilms);
- a means of creating and disseminating aerosols (a typical droplet size of <5 µm can be inhaled deep into the lungs) that contain viable *Legionella* (potential sources, for example, include showers and most other water draw-offs that are capable of creating an aerosol);

Note

Some cases of Legionnaires' disease in healthcare premises have been associated with aspiration of contaminated ingested water rather than inhalation of aerosols.

- the presence of people who may have compromised immune or respiratory systems or have other risk factors that increase their susceptibility to *Legionella* infection.

4.6 Many, if not all, of these factors are likely to be encountered in healthcare premises.

Control measures

See also:

- paragraphs 5.11–5.16 on management of control;
- Chapter 4 in Part A on water treatment and control programmes for hot and cold water systems.

4.7 A temperature control regime is the traditional means of controlling *Legionella* in hot and cold water services. Hot and cold water systems should be maintained to keep cold water, where possible, at a temperature below 20°C, and to keep hot water stored at 60°C and distributed so that it reaches the outlets at 55°C within one minute. Owing to the complexity of hot and cold water systems found in healthcare facilities and the difficulty of maintaining a temperature control regime at all times, in all areas, chemical and other water treatments that have been shown to be capable of controlling microbial colonisation and growth may need to be considered as an additional control measure.

4.8 Validation of treatment systems is important to ensure that the design and operation of a new or modified system is effective. Ongoing verification by operational monitoring is essential to ensure that the control measures remain effective. The monitoring of treatment programmes should demonstrate that they are working within legislation and the established guidelines, and are effective in controlling the target waterborne pathogens. The frequency of monitoring and test procedures will vary according to the method selected.

4.9 The use of biocides requires meticulous monitoring and their use can have an effect on water quality including taste and odour.

4.10 Where biocides are used, risk assessments (which have been agreed with the WSG) should identify at-risk patient groups (and/or equipment) to ensure there are no unforeseen consequences for patients (for example, patients in neonatal units and renal units).

Route of infection

4.11 The principal route of infection for Legionnaires' disease is through inhalation of the bacteria into the lungs via aerosols. The risk rises with increasing numbers of inhaled bacteria. Aspiration of contaminated drinking water into the airways has also been described as a mode of transmission of Legionnaires' disease. Aspiration can be a significant source of legionellosis in certain vulnerable patients including those using nasogastric tubes, stroke patients, those taking sedatives and narcotics, and those with motor neurone disease. Sucking ice made from contaminated water has been linked to some nosocomial cases of Legionnaires' disease.

Aerosol generation

4.12 Contaminated water presents a risk when dispersed into the air as an aerosol with smaller aerosols (<5 µm diameter) remaining airborne for longer, penetrating deeply into the lungs (alveoli). However, larger droplets can evaporate and still contain several organisms. Amoebic vacuoles, typically 3 µm, may contain many *Legionella* and potentially provide an infectious dose.

4.13 Water services are capable of generating aerosols from the process of water splashing on to wash-hand basins, sinks, and baths, in shower cubicles and when flushing toilets. Equipment that uses or is cleaned by water should be assessed for its potential to produce aerosols both in normal and abnormal (for example during maintenance) operating conditions.

Number of infectious bacteria

4.14 The number of organisms that cause infection has not been reliably determined and is likely to vary from person to person.

4.15 Two factors determine the number of bacteria deeply inhaled:

a. The concentration of bacteria in the air is determined both by the concentration of bacteria in the water and by the amount of contaminated water dispersed into a given air volume. The concentration of live bacteria in the air reduces rapidly with distance from the source; however this depends on the humidity and temperature.

b. The duration of exposure to the contaminated air:

   i. exposure in a shower is usually limited to a few minutes, while exposure in a bath is much longer;

   ii. the risk increases with the number of *Legionella* in the air, the respiratory rate of the individual and the length of time the person is exposed. The chances of *Legionella* infections occurring increase with the number and susceptibility of people exposed.

Susceptibility of individuals

4.16 While previously healthy people may develop Legionnaires' disease, there are a number of factors that increase susceptibility:

a. increasing age, particularly over the age of 50 (children are rarely infected);

b. men are approximately three times more likely to be infected than women (this may change with altered smoking habits);

c. existing respiratory disease that makes the lungs more vulnerable to infection;

d. illnesses and conditions such as cancer, diabetes, kidney disease or alcoholism, which weaken the natural defences;

e. smoking, particularly heavy cigarette smoking, because of the probability of impaired lung function;

f. patients who are immunocompromised as a result of illness or treatment (for example, those on immunosuppressant drugs that inhibit the body's natural defences against infection).

5 *Pseudomonas aeruginosa* and other waterborne pathogens: overview

Ecology

5.1 *P. aeruginosa* is a Gram-negative bacterium, commonly found in wet or moist environments. It is commonly associated with disease in humans with the potential to cause infections in almost any organ or tissue, especially in patients compromised by underlying disease, age or immune deficiency. As a pathogen the significance of *P. aeruginosa* is exacerbated by its resistance to antibiotics, virulence factors and its ability to adapt to a wide range of environments and nutrients.

5.2 *P. aeruginosa* thrives in relatively nutrient-poor environments such as water systems at a range of different temperatures and can exist as planktonic cells in water or as biofilms where mixed populations of bacteria are bound to surfaces. Biofilms can become detached to contaminate the water phase or flow.

Transmission

5.3 *P. aeruginosa* is an opportunistic pathogen that can colonise and cause infection in patients who are immunocompromised or whose defences have been breached (for example, via a surgical site, tracheostomy or indwelling medical device such as a vascular catheter). In most cases, colonisation will precede infection. Some colonised patients will remain well but can act as sources for colonisation and infection of other patients. As a microorganism that is often found in water, the more frequent the direct or indirect contact between a susceptible patient and contaminated water, and the greater the microbial contamination of the water, then the higher the potential for patient colonisation or infection.

5.4 Contaminated water in a healthcare setting can transmit *P. aeruginosa* to patients through the following ways:

- direct contact with the water through:
  - ingesting;
  - bathing;
  - contact with mucous membranes or surgical site; or
  - through splashing from water outlets or basins (where the flow from the outlet causes splashback from the surface);
- inhalation of aerosols from respiratory equipment, devices that produce an aerosol or open suctioning of wound irrigations;
- medical devices/equipment rinsed with contaminated water;
- indirect contact via healthcare workers' hands following washing hands in contaminated water, from surfaces contaminated with water or from contaminated equipment such as reusable wash-bowls or refillable spray cleaning bottles.

Source

5.5 It is generally accepted in the case of *Legionella* that the source of bacteria in hot and cold water systems is the incoming water supply and that it becomes a problem where there is a failure of the recommended control measures (for example, maintenance of

temperatures or water treatment regimes). In contrast to *Legionella*, the origin of *P. aeruginosa* is less certain and its presence is particularly evident within the last two metres before the point of discharge.

5.6 Devices fitted to, or close to, the tap outlet (for example mixing valves, solenoids or outlet fittings) may exacerbate the problem by providing the conditions and nutrients that support microbial growth (for example, appropriate temperatures, a high surface-area-to-volume ratio or a high surface area for oxygenation of water, and leaching of nutrients from materials such as ethylene propylene diene monomer (EPDM)). The source, therefore, could be:

- the incoming water supply from the water provider;
- the water supply within the building (both from the storage and distribution system), usually within biofilms;
- the wastewater system (see Breathnach et al. 2012); or
- via external retrograde contamination from:
  - clinical areas due to the discarding of patient secretions or where medical equipment may have been washed in the wash-hand basin;
  - outlet users where hands may have been contaminated by *P. aeruginosa*;
  - poor hygiene or processes during cleaning, resulting in contamination from the drain or surrounding environment to the outlet fitting;
  - splashback from contaminated drains;
  - contaminated cloths/mops etc.

5.7 Given this variety, the challenge for the WSG is to risk-assess operational practices in an attempt to minimise contamination from any of these sources.

Mycobacteria

5.8 Non-tuberculous mycobacteria have been associated with healthcare outbreaks worldwide. These outbreaks usually involve sternal wound infections, plastic-surgery wound infections or post-injection abscesses.

5.9 Mycobacterial infections in patients undergoing dialysis treatment have also been reported. Other infections have been attributed to the transmission of *Mycobacterium chimaera* from contaminated heater cooler units used in theatre during cardiothoracic surgery (see also paragraph 8.23).

Stenotrophomonas

5.10 There are at least 14 species of *Stenotrophomonas*; the most important waterborne pathogen is *Stenotrophomonas maltophilia.* This is an emerging opportunistic environmental pathogen that causes healthcare-associated infections and is found in aqueous habitats including water sources. *S. maltophilia* is an organism with various molecular mechanisms for colonisation and infection, and can be recovered most notably from the respiratory tract of cystic fibrosis patients with *P. aeruginosa*. Its habits within the healthcare environment are very similar to *P. aeruginosa;* however, it is more heat-sensitive and will not grow above 40°C. Good temperature management should reduce the risk of colonisation. It has been associated with the colonisation of taps/tap water, sinks/sink traps, showers, hydrotherapy pools, ice-makers, disinfectant solutions, haemodialysers, nebuliser chambers, humidifier reservoirs, bronchoscopes and ventilator circuits. *S. maltophilia* isolated from tap water has been shown to be responsible for the colonisation/infection of five neonates in a neonatal intensive-care unit. Where clinical results indicate water may be a vector in the transmission of *Stenotrophomonas* spp., then water sampling should be carried out as per *P. aeruginosa* (see Appendix E).

Other emerging pathogens of concern in healthcare

Other organisms may have particular pathogenicity in certain circumstances. Specialist microbiological advice should be sought until their management and control within heathcare can be documented more fully.

Management of control

5.11 Management of water systems to reduce the risk of waterborne pathogens is vital to patient safety. It requires surveillance and maintenance of control measures including temperature control, usage, cleaning and disinfection measures as identified within the risk assessment and WSP for both hot and cold water systems.

5.12 To prevent the growth of waterborne pathogens, controls are necessary to manage the water system before and after the outlet.

5.13 The WSG should ensure that estates and facilities staff have up-to-date accurate records and drawings/diagrams showing the layout and operational manuals of the whole water system. Estates and facilities staff should have received adequate hygiene training (see paragraphs 6.29–6.30) and be fully aware of the extent of their responsibilities to prevent microbial contamination of plumbing components (see paragraphs 1.21–1.26 in HTM 04-01 Part A). There should be strict adherence to the recommendations in HSG274 Part 2.

5.14 The WSG should also ensure that infection prevention and control (IPC) teams have received adequate training and that there is compliance with national evidence-based guidelines for preventing healthcare-associated infections. Best practice advice relating to clinical wash-hand basins should be followed to minimise the risk of contamination of surfaces, outlets and drains due to waterborne pathogens (see Chapter 3 in HTM 04-01 Part C).

5.15 IPC teams and WSGs should continue to monitor clinical isolates of waterborne pathogens including the presence of *P. aeruginosa* in risk-assessed augmented care units as an alert organism and be aware of possible outbreaks or clusters of infection with this microorganism.

5.16 The HCAI Code of Practice sets out the criteria against which a registered provider's compliance with the requirements relating to cleanliness and infection control will be assessed by the Care Quality Commission. It also provides guidance on how the provider can interpret and meet the registration requirement and comply with the law. Regulations 12 and 15 state that providers should provide and maintain a clean and appropriate environment in managed premises that facilitates the prevention and control of infections (see paragraphs 0.15–0.17).

6 Operational management

Introduction

6.1 Healthcare organisations have an explicit duty under the Health and Safety at Work Act etc. 1974 to assess and manage the risks posed by water systems on their premises. In accordance with the HCAI Code of Practice (see paragraphs 0.15–0.17), the healthcare organisation's chief executive is responsible for having systems in place to manage and monitor the prevention and control of infection. These systems should make use of WSPs and risk assessments to consider how susceptible patients are at risk from their environment and from others. Ensuring these elements are in place will assist the organisation to fulfil its duties in relation to the provision of safe water systems. A programme of audit should be in place to ensure that key policies and practices are being implemented appropriately. This will inform the organisation's assurance framework.

6.2 Each healthcare organisation, through its WSG and WSP, should be able to demonstrate that they have suitable governance, competence and accountability arrangements in place to deliver safe water in healthcare premises.

The Water Safety Group (WSG)

6.3 The WSG is a multidisciplinary group formed to oversee the commissioning, development, implementation and review of the WSP. The aim of the WSG is to ensure the safety of all water used by patients/residents, staff and visitors, to minimise the risk of infection associated with waterborne pathogens. It provides a forum in which people with a range of competencies can be brought together to share responsibility and take collective ownership for ensuring it identifies water-related hazards, assesses risks, identifies and monitors control measures and develops incident protocols.

Note

Where estates and facilities provider services are part of a contract (including PFI), it is essential that these providers participate fully in all those aspects of estate and facilities management that can affect patients. This includes responding to specific requests from the IPC team and WSG, which may be in addition to relevant guidance and documentation.

6.4 The WSG should have clearly identified lines of accountability up to the duty holder. The roles, responsibility and accountability of the WSG should be defined (see Chapter 2).

6.5 The group should ensure that there is the appropriate expertise available to ensure that all elements of the WSP are fully implemented. This will require assurance from installers, maintainers and users with regard to the safety of all water used by patients/residents, staff and visitors, to minimise the risk of infection associated with waterborne pathogens. The WSG may typically comprise personnel who:

- are familiar with all water systems and associated equipment in the building(s) and the factors that may increase risk of

infection from *Legionella, P. aeruginosa* and other waterborne pathogens (that is, the materials and components, the types of use and modes of exposure, together with the susceptibility to infection of those likely to be exposed);

- have knowledge of the particular vulnerabilities of the at-risk population within the facility; as part of its wider remit, the WSG should include representatives from areas where water may be used in therapies, medical treatments or decontamination processes where exposure to aerosols may take place.

6.6 This would normally involve representation from estates (operations and projects), infection control, medical microbiology, nursing, augmented care, housekeeping/ support services, an Authorising Engineer/ independent adviser, medical technical officers, specialist users of water (such as renal units and departments offering aquatic therapy), and sterile services departments (SSDs). See Figure 2 for an example structure and see also the Note under paragraph 6.3.

6.7 As and when required, the local security management specialist (LSMS) should provide a security management input. The LSMS should be consulted in any vulnerability and risk assessments being undertaken, to ensure that the security of the water systems is fully considered and proportionate security measures are implemented.

6.8 The WSG should be led and chaired by a person who has appropriate management responsibility, knowledge, competence and experience. Where required, it may appoint in writing an independent professional adviser/ Authorising Engineer (Water) with a brief to provide services in accordance with this HTM and the HSE's technical guidance HSG274 Part 2.

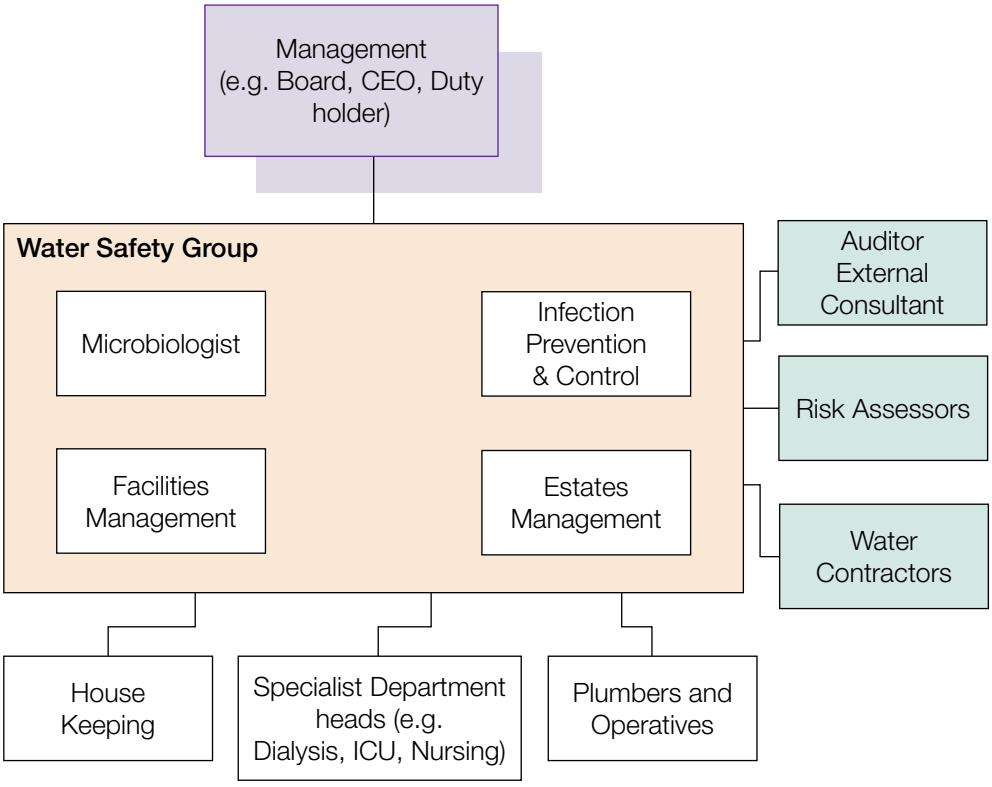


Figure 2 Example WSG structure

Remit of the WSG

6.9 The following is a typical list of tasks assigned to the WSG:

- to work with and support the IPC team;
- to ensure effective ownership of water quality management for all uses;
- to determine the particular vulnerabilities of the at-risk population;
- to review the risk assessments;
- to ensure the WSP is kept under review including risk assessments and other associated documentation;
- to ensure all tasks indicated by the risk assessments have been allocated and accepted;
- to ensure new builds, refurbishments, modifications and equipment are designed, installed, commissioned and maintained to the required water standards;
- to ensure maintenance and monitoring procedures are in place;
- to review clinical and environmental monitoring data;
- to agree and review remedial measures and actions, and ensure an action plan is in place, with agreed deadlines, to ensure any health risks pertaining to water quality and safety are addressed;
- to determine best use of available resources;
- to be responsible for training and communication on water-related issues;
- to oversee water treatment with operational control monitoring and to provide an appropriate response to out-of-target parameters (that is, failure to dose or overdosing of the system);
- to oversee adequate supervision, training and competency of all staff;
- to ensure surveillance of both clinical and environmental monitoring.

6.10 Detailed minutes of the group meetings should be recorded, distributed promptly and retained in accordance with the management policy to demonstrate good management, appropriate and timely actions and good governance.

6.11 The WSG should always act in an appropriate and timely manner. Individual responsibilities should not be restricted by the need to hold formal meetings.

6.12 Episodes of colonisation or infection of patients that could be related to the water system should be referred by the IPC team to the chair of the WSG for any additional action to be determined.

6.13 The WSG should review any proposed or existing developments associated with the water supply and distribution system to ensure that they:

- minimise the risk to patients/service-users, especially those treated in augmented care settings;
- are compliant with all extant legislation and DH policy and guidance.

6.14 All systems and equipment that use water to which patients, staff and visitors could be exposed should be approved by the WSG. When buying equipment, assurance should be sought from the manufacturer regarding safety for patients and service-users.

6.15 The WSG will need to ensure that decisions affecting the safety and integrity of the water system do not proceed without its agreement.

Water safety plans (WSPs)

6.16 To assist with understanding and mitigating risks associated with waterborne hazards in distribution and supply systems and associated equipment, healthcare organisations should develop a WSP, which provides a risk-management approach to the safety of water and establishes good practices in local water usage, distribution and supply

(see Figure 3). It will identify potential water-related hazards, consider practical aspects and detail appropriate control measures. The content of a WSP will depend on the size and complexity of the healthcare organisation's water system. The plan will include governance arrangements related to the management of water safety. See paragraph 6.21 for a list of elements that the WSP should incorporate.

6.17 Those organisations with existing robust water management policies for waterborne hazards will already have in place much of the integral requirements for developing a WSP.

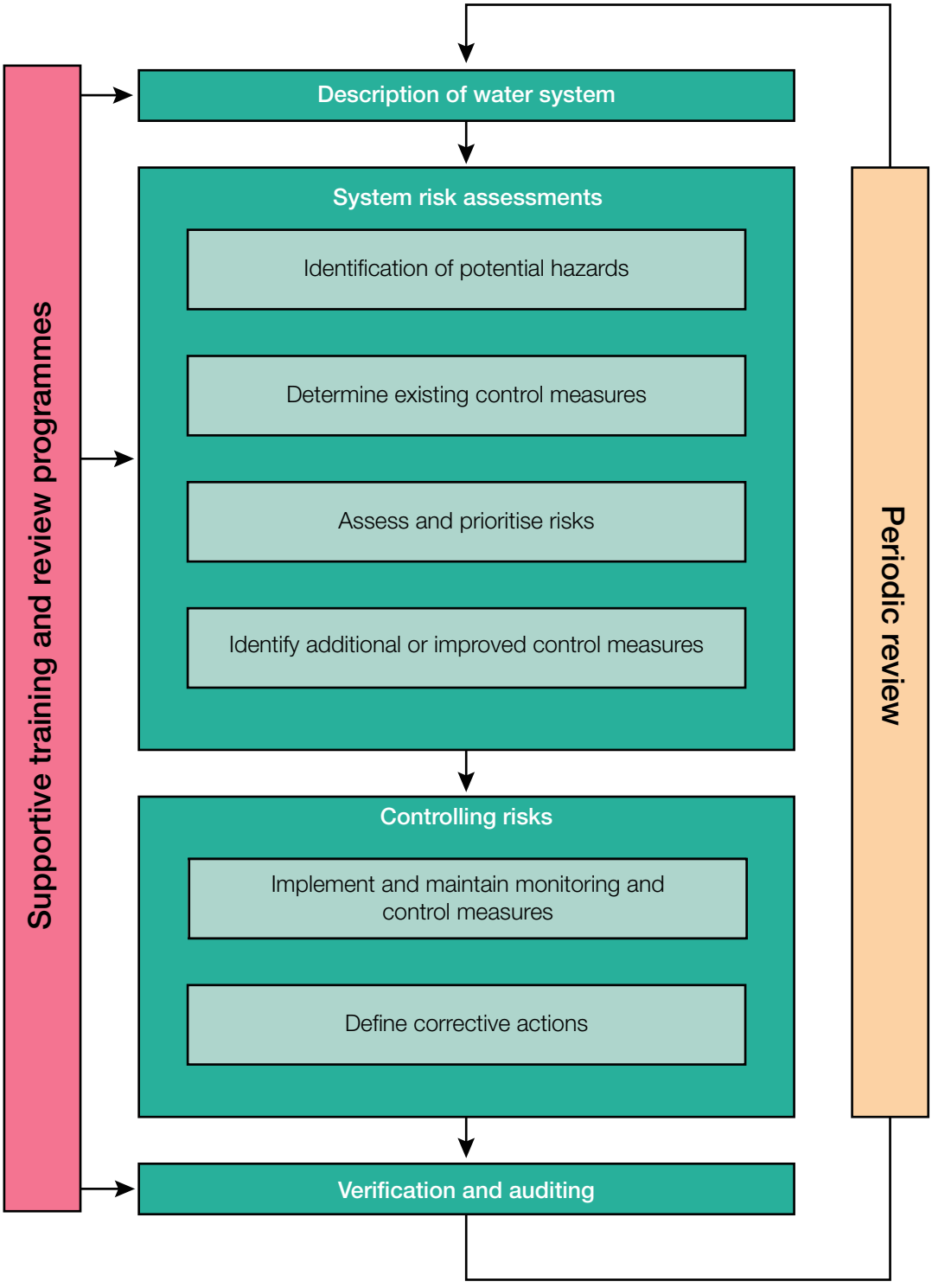


Figure 3 Documentation of management procedures (adapted from Figure 4.1 in WHO's 'Water safety in buildings')

6.18 The WSP is a living document. It should be kept under continual review and ensure the adequate assessment and control of the risks from a wide range of waterborne pathogens. It should be a standing agenda item at WSG meetings.

6.19 The first step in the development of a WSP is to gain a comprehensive understanding of the water system, including the range of potential hazards, hazardous events and risks that may arise during storage, delivery and use of water. It may require an understanding of the quality and management of the water as provided and how that water is used. Fundamental to this and any subsequent investigation or review is the provision and availability of accurate records. Schematic diagrams are useful for assisting understanding of how entire water systems operate; however, more advanced drawings (as-built) are often needed in complex buildings. The WSP should also be cross-referenced to the healthcare organisation's security management strategy and business continuity plan.

6.20 WSPs include the need to:

- assess the risks that may be posed to patients (including those with particular susceptibility), staff and visitors;
- put into place appropriate management systems to ensure the risks are adequately controlled;
- ensure there are supporting programmes, including communication, training and competency checks.

6.21 With respect to physical, chemical and microbiological hazards, the WSP should incorporate:

- a clinical risk assessment to identify those settings where patients are at significant risk from waterborne pathogens;
- an engineering and bacterial risk assessment of all water systems;
- operational monitoring of control measures and record-keeping methodology;
- information on the age and condition of the water distribution system and materials of construction (as different materials hold differing risks and react differently to chemicals);
- links to clinical surveillance;
- early warnings of poor or unsuitable water quality (for example, taste, odour, flavour and appearance if intended for drinking);
- plans for the sampling and microbiological testing of water in identified at-risk units (see Chapter 10 and Appendices E and F);

Note

Appendix F has been developed to provide technical guidance for a range of laboratories, including NHS, Public Health England (PHE) and commercial laboratories that have the capability and capacity to undertake water sampling and testing.

- adequate supervision, training and competency for all levels of staff including housekeeping staff and those employed by facilities management organisations;
- communication and documentation;
- appropriate design, installation, commissioning and maintenance of all components and equipment;
- remedial actions to be taken to remedy high counts for *P. aeruginosa* and *Legionella* and other relevant pathogens where appropriate;
- adjustments to clinical practice until remedial actions have been demonstrated to be effective;
- regular removal/cleaning/descaling or replacement of the water outlets, hoses and thermostatic mixing valves (TMVs) and other components where risk assessment necessitates;

- amendments when changes are carried out including new builds, refurbishments and recently decommissioned clinical departments or units;
- amendments made when changes are made at the annual review.

6.22 The WSP should identify potential alert organisms and microbiological hazards caused by *Legionella*, *P. aeruginosa* and other relevant pathogens, consider practical aspects and detail appropriate control measures.

Risk assessments

6.23 The risk assessments that inform the WSP should identify potential hazards caused by *Legionella*, *P. aeruginosa* and other relevant pathogens, chemicals, temperature and events that may arise during supply, storage, delivery, maintenance and use of water in healthcare facilities.

6.24 Once potential hazards and hazardous events have been identified, the severity of risk needs to be assessed so that priorities for risk management can be established. The risk assessment needs to consider the likelihood and severity of hazards and hazardous events in the context of exposure (type, extent and frequency) and the vulnerability of those exposed. Although many hazards may threaten water quality, not all will represent a high risk. The aim should be to distinguish between high and low risks so that attention can be focused on mitigating risks that are more likely to cause harm (see BS 8580 for guidance on *Legionella* risk assessments). Typical examples of issues to consider may include the following:

- governance and accountability;
- the susceptibility of all who may be exposed to water (including ice) used for diagnosis and treatment;
- scalding risk and the appropriate installation of TMVs;
- failure to maintain necessary hot and cold system supply temperatures;
- clinical practice where water may come into contact with patients and their invasive devices;
- the appropriate cleaning of the environment and equipment;
- the disposal of blood, body fluids and patients' wash-water;
- the maintenance and cleaning of wash-hand basins and associated taps, specialist baths and other water outlets;
- suitable siting and installation of wash-hand basins including the appropriate positioning of soap and antimicrobial hand-rub dispensers;
- change in use (for example, clinical area changed to office accommodation or vice-versa) due to refurbishment or operational necessity;
- other devices that increase/decrease the temperature of water (for example, ice-making machines, water chillers) which may not be appropriate where patients are at particular risk such as in augmented care settings;
- engineering assessment of water systems, including appropriate design, installation, commissioning, maintenance and verification of the effectiveness of control measures (see also the Water Supply (Water Fittings) Regulations 1999);
- infrequently used outlets;
- previous risk assessment, current control measures and documentation;
- policies and procedures;
- the unnecessary use of flexible hoses and any containing inappropriate lining materials;
- sampling, monitoring and testing programmes that needs to be put in place;
- backflow protection;
- safe access to equipment;

- prevention of unauthorised access to equipment;
- education and training.

6.25 Situations will arise where venous catheter sites and surgical wounds may become contaminated from water outlets such as showers. Similarly the practice of soaking leg ulcers or syringing ears may require consideration of the microbiological quality of water used and will require local assessment.

6.26 The likelihood of hazardous events is influenced by the size and complexity of the water system and can be exacerbated by poor or overcomplicated design, construction, commissioning, operation and maintenance.

Staff training and competence

6.27 The WSG should implement a programme of staff training to ensure that those appointed to devise strategies, carry out control measures and undertake associated monitoring are appropriately informed, instructed and trained. They should also be assessed as to their competency. It is also essential that they have an overall appreciation of the practices affecting water hygiene and safety, and that they can interpret the available guidance and perform their tasks in a safe and technically competent manner.

6.28 The WSG should review the competence of staff on a regular basis, and refresher training should be given; records of training attendance need to be maintained. Although training is an essential element of ensuring competence, it should be viewed within the context of experience, knowledge and other personal qualities that are needed to work safely. Competence is dependent on specific needs of individual installations and the nature of risks involved.

Water hygiene training

6.29 Individuals to whom tasks have been allocated (supervisors and managers as well as operatives) need to have received adequate training in respect of water hygiene and microbiological control appropriate to the task they are responsible for conducting. The training and competence assessment should be clearly defined and should include those responsible for simple housekeeping tasks such as outlet flushing and the cleaning of wash-hand basins, through to maintenance staff and up to individuals who define strategy and develop procedures.

6.30 It is important that any person working on water distribution systems or cleaning water outlets should have completed a water hygiene awareness training course so that they can gain an understanding of the need for good hygiene when working with water distribution systems and water outlets, and of how they can prevent contamination of the water supply and/or outlets.

Water hygiene training

As part of helping to ensure the delivery of safe wholesome water at all outlets and preventing contamination, which may lead to healthcare-associated infections, it is recommended that healthcare organisations implement a water hygiene training scheme.

Consideration should also be given to integrating a health screening element into the training to help ensure those undergoing the training are not carriers of any waterborne diseases on the date of training and are aware of their responsibilities towards the water supply. It is important that individuals are aware of their duty to protect the health of patients, staff and visitors and that they are responsible for ensuring that they inform their line manager if they come into contact with any disease that has the potential to cause harm.

The course should encompass the following topics (not exhaustive):

- organisational governance arrangements in relation to water hygiene and safety;
- familiarisation with local policies/ procedures in relation to the management and provision of water hygiene and safety;
- information on prominent waterborne pathogens and their consequences;
- the ways in which water distribution systems, water outlets, components and any associated equipment can become contaminated;
- the responsibilities of individuals to prevent the contamination of the water distribution system and water outlets and assisting in ensuring control measures in place are effective;
- how the safety of water can be maintained by good hygiene practices;
- when not to work with water intended for domestic purposes;
- system design;
- components/accessories (taps, TMVs);
- disinfection and cleaning equipment/ materials;
- how to store and handle pipes;
- organisation-specific control measures;
- the impact of getting it wrong;
- role of persons being trained;
- personal hygiene along with dealing with clothing, footwear, cleaning equipment/materials, tools and storage when considering water hygiene (as applicable to each role).

Management of water safety risks and issues

6.31 Identified water safety risks and issues should be assessed, prioritised and included on a risk register for discussion and management by the WSG and advice given on when these should be escalated to senior management/board level. Consideration should also be given to the potential and known threats from unauthorised access to the water supply for malicious purposes (see also NHS Protect's (2012) guidance on the security and management of NHS assets).

6.32 When the risks have been identified, an action plan needs to be developed with defined roles and responsibilities, and agreed

timescales to minimise these risks. The action plan should include:

- appropriate remedial actions, monitoring details and schedules for validation that show the remedial actions are effective and subject to ongoing verification (completion dates should be defined);
- any training and competency issues required to ensure compliance with this guidance.

Advice on exposure of augmented care patients to *P. aeruginosa* is covered in HTM 04-01 Part C.

Documentation

6.33 All records pertaining to the risk assessment and action plan should be held and managed by the WSG.

Safe hot water temperature

6.34 See paragraphs 10.57–10.59 in HTM 04-01 Part A for guidance on safe water temperatures and delivery devices. See also HSE's 'Managing the risks from hot water and surfaces'.

6.35 To reduce the risk of scalding, thermostatic mixing devices are required for specific hot water outlets (see Table 2 in HTM 04-01 Part A). A scalding risk assessment is necessary to establish the need and type of device to be installed.

6.36 As with any safety device, routine checks will be essential to ensure continued satisfactory operation (see Chapter 11 of HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'). Such devices, however, should not be a substitute for caution. For example, when performing assisted bathing, it is often necessary to set the delivery temperature to a higher level than that normally considered safe to allow for the cooling effect of large baths that are required.

6.37 Before lowering or assisting patients into the bath, the water temperature must be checked with a thermometer to ensure that it has fallen to a safe value.

Utilisation

6.38 One of the critical factors affecting the quality of water within hot and cold water distribution systems is the extent of utilisation.

6.39 Where stagnation occurs or utilisation is low, cold water temperature can increase significantly and approach the range that is conducive to the growth of a variety of waterborne pathogens such as *Legionella*. Where hot and cold water is mixed, further opportunities arise for deterioration in water quality.

6.40 TMVs should not be installed in series with mixing taps (thermostatic or manual) (see also paragraphs 6.52–6.58 on maintenance practice).

6.41 The WSG needs to ensure that there is good liaison between the estates officers/ maintenance providers and clinicians to ensure that the water services are sufficiently used.

6.42 HSG274 Part 2 recommends that generally, for infrequently used outlets, flushing is carried out once a week but that in healthcare facilities the risk assessment, as agreed by the WSG, may indicate a higher frequency, and water draw-off should form part of the daily cleaning process. The procedure for such practice should be fully documented and covered by written instructions.

6.43 Monitoring of water usage on a building-by-building basis can indicate when usage falls and the risk increases. Reductions in normal usage patterns should be investigated and remedial measures introduced following investigations by the WSG.

Note

Regular flushing applies to all infrequently used outlets. The use of portable temperature-monitoring devices may assist in identifying where additional flushing is necessary.

Temporary closure of wards/ departments

6.44 During the temporary closure of wards or departments, a flushing regime should be instigated to maintain system hygiene. Flushing should be continued until stable temperatures are achieved. Advice and guidance is also provided in BSI's PD855468.

6.45 Alternatively, when this is impracticable, or should the temporary closure become permanent, it should be disconnected from the rest of the system, ensuring there are no dead-legs. Before reconnecting, the system should be recommissioned and disinfected in accordance with Chapter 15 of HTM 04-01 Part A.

Leak detection/water conservation

6.46 It is essential to regularly check systems and all components for signs of leakage; for example, a tap left dripping can waste in excess of 14,000 L of water each year. Particular attention should be given to WCs as leakage appears as a dribble at the back of the pan, which often goes undetected.

6.47 Consumption should be monitored; if it increases for no apparent reason, this may indicate a leak. Wet or soggy patches of ground may identify underground leaks, for example areas of greenery that are more lush than their surroundings.

6.48 Where water conservation measures are to be considered, a risk assessment should be undertaken to ensure there is no detrimental impact that may cause stagnation or low water usage in the existing water or drainage systems.

6.49 For further guidance, see HTM 07-04 – 'Water management and water efficiency'.

Water treatment undertaken by the local water undertaker

6.50 Local water undertakers use different types of water treatment, which may include the use of chloramine, and add additional compounds such as fluoride. It is recommended that regular contact is maintained with the local water undertaker to keep up to date with changes that may affect water quality or other operational changes affecting the premises.

Energy management

6.51 Efforts should be taken to minimise energy consumption without compromising water safety management. An effective maintenance plan will also contribute to this. Further guidance is given in HTM 07-02 – 'EnCO_2de'.

Maintenance practice

Note

See also Chapter 11 in HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'.

6.52 Healthcare organisations should ensure that all personnel that work on water systems can demonstrate competence and have been inducted in local procedures, which should include safe water hygiene practices (see paragraphs 6.29–6.30).

6.53 There are legal, operational and economic reasons for introducing good maintenance practice. There is a legal requirement under the Water Supply (Water Fittings) Regulations 1999 to maintain water fittings to comply with the regulations. It sets a requirement to protect and preserve water quality for the safety of patients, staff and visitors. Complying with the law is

generally given the highest priority and is the minimum requirement that must be satisfied. Chapter 3 lists specific statutes that must be complied with. Additional advice is provided in BS EN 806-5 and BS 8558.

Note

BS EN 806-5 and complementary guidance in BS 8558 covers the operation and maintenance of water systems, in which a number of aspects are covered:

- operation;
- interruptions to operation and disconnection;
- resumption of supply;
- damage and faults (change in water quality, insufficient water supply, noise emission);
- alterations, extensions and refurbishment;
- accessibility of installation components;
- maintenance.

6.54 Regulations require notification to the water undertaker of any proposed changes and additions to the water supply system in the premises. Before making any changes, a risk assessment should be carried out and audited by an independent assessor. Further details can be found on the WRAS website.

6.55 Maintenance is required to achieve optimum economic life and maintain maximum operational efficiency of the plant.

6.56 There should be a risk assessment to decide the appropriate type of maintenance (for example scheduled, corrective or condition-based) for the different items of plant. The following should be considered:

a. Would a breakdown of a particular service during working, or outside normal, hours pose a risk to patient safety and wellbeing?

b. How long can a breakdown of particular plant be tolerated?

c. What cost can be justified to avoid breakdown of particular plant such as standby pumps, dosing pumps etc?

d. The availability of suitable spares.

6.57 If response to failure is critical for certain items of plant, the maintenance organisation will require a planned strategy of calling out skilled staff to achieve an agreed response time and to minimise the interval between breakdown and the diagnosis and repair of the plant.

6.58 The approach for healthcare premises should be based on that of planned preventive maintenance (PPM) as any failure in the water services would be seriously detrimental to the provision of healthcare. The PPM programme and any subsequent amendments should be agreed by the WSG (see also Chapter 5 on maintenance in HTM 00 – 'Policies and principles of healthcare engineering').

Maintenance responsibility

6.59 A maintenance manager should be given responsibility for implementation of a risk-based operational maintenance strategy. These responsibilities will include:

a. the provision of adequately trained and supervised labour (see also paragraph 6.30 on water hygiene training);

b. clear definitions of the equipment and services to be maintained, together with the procedures to be carried out on them;

c. monitoring of the quality of the work carried out to ensure that it is consistently acceptable;

d. the identification of appropriate resources and the implementation of financial control procedures.

Note

The maintenance manager should ensure that risk assessments have been carried out for all maintenance activities, and be particularly aware of any specific risks associated with hot and cold water installations (for example, hazards associated with gaining access to cisterns, the discharge of large volumes of hot water to enable the inspection of calorifiers and hot water storage vessels, and the possibility that these may have become colonised by *Legionella*).

Contract maintenance

6.60 The increasing complexity of building services equipment has resulted in a growing reliance on contractors for the provision of maintenance services. The decision to use either a contractor or in-house staff should be taken in the light of local circumstances.

6.61 Contracts between contractors and healthcare organisations should clearly define the responsibilities of both parties. See Chapter 2 of HBN 00-08 – '(Estatecode) Part B: Supplementary information for Part A' for further guidance. BSRIA's BG3: 'Maintenance for building services' also provides advice on aspects to be considered when obtaining contract maintenance.

6.62 All staff who work on or with water systems should have the necessary qualifications, regulatory knowledge, competence and experience needed to complete safely and effectively their specific tasks. Each individual should have a full understanding of their role and the impact of their actions on patient care (see also paragraph 6.30 on water hygiene training).

6.63 Only installers with the appropriate qualifications, regulatory knowledge and competence should be used to install and maintain water installations. There are seven Approved Contractors' Schemes (APHC, Aplus, CIPHE, Snipef, Taps, WaterMark and WIAPS) authorised through the Water Supply (Water Fittings) Regulations 1999. In addition to plumbing installers, four schemes (Aplus, Taps, WaterMark and WIAPS) operate sector memberships for specialist areas of work covering external water services (below-ground pipe etc), catering equipment and point-of-use (chilled-water) equipment.

6.64 The WaterSafe register holds details from all seven Approved Contractors' Schemes for businesses that have registered plumbing installers.

6.65 A recognised benefit to using an Approved Contractor (including sector installers) is they can carry out some work without the need to provide advanced notification to the water undertaker, and their work will be certified upon completion. A "work completed" certificate issued by a WaterSafe-recognised plumber provides a defence for property owners who are challenged by a water undertaker enforcing the Water Supply (Water Fittings) Regulations 1999 or during legal proceedings.

Maintenance brief

6.66 The maintenance manager requires a brief that is in line with all the requirements of the WSP and approved by the WSG. This will include (among others):

a. the scope of work;

b. budgeting – overall and single item limits;

c. level of reliability;

d. response time required to correct faults;

e. criteria for quality of service, works and equipment;

f. reporting procedure;

g. accountability and responsibility;

h. energy-saving policy;

j. health and safety policy;

k. environmental and sustainability factors.

6.67 The above requirements are necessary regardless of whether the work is carried out by contractors or in-house staff.

Performance monitoring

6.68 This involves the regular inspection of systems and records, which should be in such detail as to enable the WSG to form an opinion regarding compliance with the agreed criteria.

6.69 If a contractor is commissioned to carry out maintenance but in-house expertise is not available to monitor their performance, it may be necessary to seek advice from an independent professional adviser (this may be available from a neighbouring healthcare organisation). Using another maintenance contractor in a monitoring role could lead to a conflict of interest.

6.70 Performance monitoring should establish that:

a. the required level of service is met;

b. all the required plant is being maintained;

c. system performance is being maintained (that is, by the implementation of microbial sampling and temperature/biocide-level-monitoring regimes);

d. maintenance is being carried out to the agreed standard;

e. correct replacement parts are being used;

f. the agreed spares stocks are being held on site;

g. records are being correctly maintained;

h. the agreed standards, number of staff, and number of visits are being achieved;

i. plant is being operated to achieve optimum energy usage;

j. health and safety requirements are being complied with;

k. only agreed subcontractors with the appropriate knowledge and competence are being employed (see paragraphs 6.60–6.65);

l. the client and typical users of the building are satisfied;

m. invoices accurately reflect the work carried out, including materials expended;

n. breakdowns do not occur too often;

o. adequate consideration is being given to the potential environmental impact of contractors' actions, for example disposal of lubricants, chemicals, worn parts etc that cannot be recycled.

Monitoring systems

6.71 Where monitoring systems are provided to assist in the management of hot and cold water systems, they should be subject to a routine maintenance and calibration regime. Systems should be in place to ensure all set alarms should be responded to in a timely manner.

Emergency action

6.72 Contingency plans should be available in the event of the following:

a. A power or energy interruption or a plant failure resulting in the temperature control strategy or the delivery of effective control measures being maintained as designed.

b. A water supply failure that could last beyond the period for the designed storage capacity.

Note

The WSG should ensure that plans are in place for the supply and distribution of alternative safe water for drinking to vulnerable patients and those unable to collect supplies from distribution points within the healthcare facility. This will include the use of sterile water if appropriate.

c. Monitoring and sampling results that indicate patient safety may be compromised unless remedial action is undertaken with immediate effect.

d. Emergency action in the event of a case or an outbreak of a healthcare-associated waterborne infection (see also Appendix B for action to take on an outbreak of legionellosis).

e. Tampering and sabotage of the water supply which could impact on patient and staff safety.

6.73 Guidance on temporary supplies is provided in BS 8551.

Note

Under the Security and Emergency Measures Direction, water suppliers should liaise with healthcare authorities to develop emergency plans to maintain supplies for domestic purposes to healthcare premises.

These plans may include:

- Adequate storage cistern capacity and distribution arrangements within the healthcare facility to provide minimum volumes of water at the outset of a major incident to maintain hygiene and health for an initial period until other temporary arrangements can be introduced.
- The provision of facilities to connect to, and distribute water from, temporary storage cisterns such as pillow tanks at key locations within the site. Ground-level storage cisterns will require provision of booster pumps to either lift the water to existing storage cisterns or distribute it directly through existing or temporary site water mains.
- The provision of connection points to existing storage cisterns at suitable locations for delivery of emergency supplies of water, for example from tankers.
- Plans for the supply and distribution of alternative safe water for drinking to vulnerable patients and those unable to collect supplies from distribution points within the healthcare facility.

Data management and record-keeping

6.74 Given the amount of data that must be managed to facilitate the effective management of large and complex water systems, it is recommended that electronic data management tools be utilised to facilitate the intelligent use of data for the WSG to easily monitor trends and analyse chemical and microbiological parameters.

6.75 It is essential to have comprehensive operational manuals for all items of plant that include requirements for servicing, maintenance tasks and frequencies of inspection.

6.76 This information should be kept together with all commissioning data.

6.77 Documentation should also be drawn up as part of the health and safety file for the healthcare facility.

6.78 As a minimum, the following items should be recorded:

a. the names and positions of those responsible for conducting risk assessments, and managing and implementing the WSP;

b. the significant findings of risk assessments;

c. details of the procedures including sufficient detail to identify that the work was completed correctly and when the work was carried out; and

d. results of any monitoring inspection, test or checks carried out.

6.79 Records should be kept for at least five years unless they are required to be kept longer by the organisation's data management policy.

As-fitted drawings

6.80 The availability of accurate as-fitted drawings is essential for the safe operation of hot and cold water service systems and to inform the risk assessment. The drawings will be necessary to perform the temperature control checks on the systems and will assist in identifying any potential problems with poor hot water circulation and cold water dead-legs where flow to infrequently used outlets can be low. Such information should identify all key components in the installations, for example water meters, cisterns, filtration equipment (where fitted), calorifiers, and the location of isolating valves in the systems. Drawings should be kept up to date and amended when any changes are made to the system.

Schematic drawings

6.81 Separate schematic drawings should be prepared and displayed such that all plant items, control valves etc can be identified. A schematic diagram is an important tool to inform the risk assessment process. These are not formal technical drawings and are intended to be easy-to-read without specialised training or experience. While providing only an indication of the size and scale, they allow someone unfamiliar with the layout of a system to understand the positions and connections of the relevant components quickly. Drawings should be kept up to date and amended when any changes are made to the system.

Note

All drawings should be available to each person working on the systems and while conducting risk assessments.

6.82 In addition to drawings, there should be comprehensive schedules of outlets, lists of sentinel taps (outlets), other outlets to be tested (frequency as per the WSP) and other components in the system.

Asset register

6.83 The WSG should ensure that an accurate record of all assets relating to the hot and cold water distribution systems is set up and regularly maintained. They should also ensure that records of all maintenance, inspection and testing activities are kept up-to-date and properly stored.

6.84 The asset register should be designed to provide the following information:

a. an inventory of plant and water-associated equipment;

b. a basis for identifying plant details;

c. a basis for recording the maintenance requirements;

d. a basis for recording and accessing information associated with maintenance;

e. a basis for accounting to establish depreciation and the provision needed for plant replacement;

f. information for insurance purposes.

6.85 When completing records, it is essential that there is an audit trail in place indicating the individual concerned and dates/times where appropriate.

6.86 Further information on the monitoring of performance and effectiveness in carrying out maintenance tasks can be found in CIBSE's Guide M – 'Maintenance engineering and management'.

7 Description of systems, operational considerations and requirements

Source of supply

7.1 See Chapter 2 in HTM 04-01 Part A for comprehensive guidance and information on sources of water supply.

7.2 If supplies are taken from local boreholes or wells etc, the water should be tested to comply with the requirements of the Private Water Supplies Regulations 2009. The results of all analyses should be kept and recorded. Private supplies should be registered with the local authority.

Temperature control regime

7.3 A temperature control regime is the traditional strategy to control *Legionella* and other waterborne pathogens. This will require monitoring on a regular basis. Frequencies are listed in Table 1.

Note

The flow temperature of hot water out of the calorifier should be a minimum of 60°C. It should be a minimum of 55°C on flow and returns to all outlets and at the start of the hot water return. It should be a minimum of 50°C at the final connection to the calorifier.

7.4 Whereas many of the checks will, of necessity, require the use of separate thermometric equipment, some of the temperature checks can be carried out by continuous monitoring such as sensors and reporting systems (optionally linked to a building management system (BMS)).

Where a BMS is used, it will be essential to ensure that regular calibration and physical tests are performed in accordance with the manufacturer's instructions.

7.5 More extensive use of automatic sensors and reporting systems should be considered for surveillance of systemic temperature profiling. Sensors should be located throughout the whole hot and cold water systems to ensure they give representative temperature values.

Biocide regimes

Note

Any biocidal treatment is likely to adversely affect the lifecycle of the installation.

7.6 In addition to maintaining a temperature control regime, there may be occasions where additional biocidal treatment is required for the effective control of *Legionella* and other waterborne pathogens. However, the selection of a suitable treatment is complex and depends on a number of parameters. Moreover the chosen biocide needs to be properly managed. This is particularly the case with cold water services compared with hot water services where, with the benefit of circulation, water is returned to the calorifier/water heater and is then pasteurised. However, it should be taken into consideration that effective concentrations of some biocides are difficult to achieve in hot water systems due to gassing off. For water intended for consumption, the biocide concentrations must not exceed prescribed concentrations for drinking water.

7.7 The WSG should be actively involved in the decision-making process and should involve consultation with the water undertaker to ensure the suitability of biocidal products for their intended application.

For further information on biocidal treatment, see Chapter 4 of HTM 04-01 Part A. For ongoing checks, see HSG274 Part 2.

Metal contamination

7.8 See Chapter 5 in HTM 04-01 Part A.

Water softening

7.9 See Chapter 6 in HTM 04-01 Part A.

Filtration

7.10 See Chapter 7 in HTM 04-01 Part A for guidance on point-of-entry filtration. See paragraphs 7.45–7.49 and paragraph D22(k) in Appendix D and also Chapter 4 in HTM 04-01 Part A for guidance on POU filtration.

Metering

7.11 Metered data should be logged and recorded by an automatic metering system (see also HTM 07-04 – 'Water management and water efficiency'). Where water meters are installed in below-ground meter chambers, the chambers should be kept clean of debris and water; this will enable quick and accurate reading of the meters.

7.12 Meters should be periodically checked to ensure that they are operating and providing accurate readings.

7.13 Meters, other than the water undertaker's meter, should be removed at such intervals as recommended by the manufacturer for cleaning and renewal of worn parts and should be tested for accuracy and disinfected prior to replacement.

7.14 Meters should ideally provide a pulse output that can be monitored by a BMS, but if not should be read on a regular basis (monthly) and consumption monitored. Data from the meter should be reviewed over time for anomalies (typically low or high consumption rates). Graphical methods may be beneficial. Where it is desirable to connect to the water undertaker's meter, its authorisation must be gained in advance.

7.15 Consumption should be checked against the utility bill and any discrepancies investigated.

Water storage

7.16 For general information on water storage, see Chapter 8 in HTM 04-01 Part A.

7.17 The Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 806 and BS 8558 specify minimum standards for cold water storage cisterns to ensure that the stored water is retained at a wholesome standard suitable for domestic use. It is necessary to minimise stagnation and stratification of the stored water. A nominal 12 hours' total on-site storage capacity is recommended. The quantity of the water stored should be carefully assessed in relation to the daily requirement so that a reasonable rate of turnover is achieved. The storage capacity should be reduced where it is known or established that it is excessive and where it is practicable to do so.

7.18 All cold water storage cisterns and cold feed cisterns should be examined at least annually or more frequently if the risk assessment dictates, paying particular attention to the presence of foreign objects, biological material and excessive corrosion. On completion of the examinations, the cisterns should be cleaned, if required, and any remedial work carried out. Before the cisterns and system are put back into use, they should be disinfected in accordance with the procedure detailed in chapter 2 of HSG274 Part 2. Where consideration is being given to the removal of a storage cistern, a

full assessment of the downstream system is needed to ensure any consequential impacts are properly understood. These may include, but not be limited to, changes in pressure for pipes or equipment and any backflow prevention protection the cistern may have been providing.

7.19 Any chemicals or biocidal products used in the cleaning or maintenance of cisterns should be flushed from the cistern before it is put back into use. See Chapter 4 in HTM 04-01 Part A.

7.20 Cistern insulation should be checked to ensure that it is adequately positioned and in good condition.

7.21 Float-operated valves should be checked to ensure that they are securely fixed and set to achieve a correct water level in accordance with the Water Supply (Water Fittings) Regulations 1999.

7.22 Overflow/warning pipes should be checked to ensure that they maintain a steady fall (which ensures they will drain freely) and that they are clear and correctly routed to give an obvious visual alarm of an overflow condition. A weatherproof label fixed adjacent to the warning pipe identifying the tank and its location, together with the person/department to be contacted in the event of a discharge, will contribute to a quick and accurate defect report which could then be acted upon, so minimising water wastage (consideration to the provision of high- and low-level alarms linked to the BMS should also be given – see Chapter 8 in HTM 04-01 Part A). When connected to drain, they should comply with BS EN 1717.

7.23 A schematic drawing illustrating typical piping and valve arrangements for dual-cistern installations is shown in Figure 3 of HTM 04-01 Part A.

Pressurisation/supply pumps

7.24 Where two or more pumps are installed for pressurising systems, automatic control should be provided to prevent stagnation. The automatic control should also ensure all pumps are brought into operation at a regular frequency (for example, weekly) as determined by the risk assessment.

7.25 The maintenance carried out on this type of equipment should be in accordance with the manufacturer's recommendations.

Cold water distribution system

7.26 The design and installation of the cold water distribution system should comply with the Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 806-2 and BS 8558 (see Chapter 9 of HTM 04-01 Part A for further information).

7.27 The control of water temperature in the cold water service will essentially rely on good insulation and water turnover. Maintaining regular movement of cold water in sections prone to stagnation and guarding against excessive heat gain are effective control measures for *Legionella* and other waterborne pathogens. Special attention should be given to the maintenance and monitoring of these systems.

Notes

1. For the control of *Legionella* and other waterborne pathogens, 20°C is the quoted upper value above which multiplication of *Legionella* in particular begins to take place (see Chapter 4). It should be noted that during extremes of weather, environmental factors can influence the incoming water temperatures, particularly where water is provided from surface-water sources.

2. Where automatic flushing of urinals is used, their careful location and connection to the water system can be effectively used to assist in water turnover. When used, devices having a duty cycle should be set to flush as deemed necessary by the WSG. Information from water quality programs could be used to determine appropriate timeframes. Note that automatic flushing devices should not be located in accommodation used by patients who may become distressed by the noise.

7.28 Schematic diagrams (or as-built drawings) of the system with numbered and labelled valves reduce confusion and save time in trying to identify appropriate isolating valves and other system components.

7.29 Checks and actions should be carried out to show that:

a. the system components show no sign of leakage or corrosion or limescale;

b. the system insulation is in good condition;

c. the system filters have been changed and/or cleaned in accordance with manufacturers' recommendations. Regularly check and clean strainers;

d. all isolating valves have periodically been worked through their full range of travel;

e. every water outlet complies with the backflow protection requirements of the Water Supply (Water Fittings) Regulations 1999.

Drinking water

7.30 If separate drinking water supplies are provided, reference should be made to paragraphs 9.11–9.13 in HTM 04-01 Part A, and subject to a risk assessment consideration should be given to their removal.

Notes

1. Current guidance does not draw a distinction between drinking and general cold water services, and separate systems are no longer recommended. The installation of separate drinking water supplies has been standard policy. But in many cases where such systems have been installed, the quality of drinking water (particularly at infrequently used draw-offs, for example wash-rooms) has generally been inferior to that of the general cold water supply.

2. Softeners using salt-regenerated ion-exchange resins increase the sodium content of the water during softening, and this may be undesirable for children and infants (including the making up of babies' bottles) and anyone on strict salt-restricted diets.

Hot water storage and distribution

7.31 Hot water services should be designed and installed in accordance with the Water Supply (Water Fittings) Regulations 1999 and relevant parts of BS EN 806-2 and BS 8558. The hot water system may be of either the vented or the unvented type (see Chapter 10 of HTM 04-01 Part A for further information).

7.32 To control possible colonisation by waterborne pathogens including *Legionella*, it is essential to maintain the temperature within the hot water circulating system. To some extent, if properly maintained, the calorifier/water heater will provide a form of barrier to microbial growth. The minimum flow temperature of water leaving the calorifier/water heater should be 60°C.

7.33 The minimum water temperature at the connection of the return to the calorifier/water heater should be 50°C. To achieve the required circulating temperatures, it will be necessary to maintain the balance of flows to individual pipe branches and draw-off points.

7.34 Calorifiers should be subjected to regular procedures that include the following:

- Inspection, cleaning and maintenance at least annually, or as indicated by the rate of fouling.
- Quarterly drain flushing to minimise the accumulation of sludge. This may be extended to annual draining if, during inspection, it is found that there is little accumulation of debris.
- Whenever dismantled for statutory inspection, or every year in the case of indirect calorifiers, calorifiers should be thoroughly cleaned to remove sludge, loose debris and scale.
- Whenever a calorifier is taken out of service, it should be refilled, drained, refilled again and the entire contents brought up to, and held at, the nominal operating temperature of 60°C for at least an hour.
- The calorifier should remain isolated until the procedure is completed. When bringing calorifiers back on line, it is important that service valves are opened slowly to avoid any disturbance of sediment debris. Calorifiers that are to be taken out of service for more than a few days should be drained and should not be refilled until ready to return to service. The drain valve should be left open while the calorifier is out of use.
- Where it is known or established that gross over-capacity exists in a calorifier, and where it is practicable to do so, it should be replaced by a calorifier of the appropriate size.

Note

Full-flow (spherical/ball-type) isolating valves should be specified to avoid clogging. The drain from the gully should be of sufficient size to take the flow from the calorifier drain.

7.35 Hot water circulating pumps should be of adequate performance to ensure a minimum available temperature at draw-off points of 55°C and an absolute minimum of 50°C at the return connection to the calorifier. It is undesirable to have standby pumps owing to stagnation risks. If, however, an existing installation includes a standby pump and it is impracticable to remove it from service, it should be automatically controlled so that each is regularly brought into operation as determined by the risk assessment.

7.36 It is not permissible to shut down the pumped circulation. To do so will lead to the loss of the required system temperatures.

7.37 Electrical trace-heating is not recommended except for very small systems and those existing systems that would be difficult to rectify. Where it has been retained, it should be checked routinely (at least monthly) to ensure that it maintains the water temperature above 55°C. Care should be taken to ensure there are no cool spots. Consideration should be given to monitoring the temperatures by means of a BMS (sensors should be located at the most distal points).

Note

Dead-leg lengths should be as short as practicable; that is, the trace-heating should be taken up to the draw-off or mixing device. The continuity of the trace-heating should be monitored to avoid localised failure.

Instantaneous water heaters for single or multi-point outlets

7.38 These devices usually serve one draw-off only and are either electrically or gas-heated. In essence:

a. the flow rate is limited and is dependent on the heater's hot water power rating;

b. where restricted rates of delivery are acceptable, the heater can deliver continuous hot water without requiring time to reheat;

c. they are susceptible to scale formation in hard water areas where they will probably require frequent maintenance;

d. this form of hot water heating should only be considered for smaller premises or where it is not economically viable to run hot water distribution to a remote outlet;

e. they should be monitored to ensure they operate above 55°C (see Table 1).

Pressure and expansion vessels

7.39 Pressure and expansion vessels should be subject to routine inspection and maintenance as recommended by the manufacturer. Where practicable, these should be flushed through with wholesome water and purged to drain. If replaceable, bladders or diaphragms should be changed according to the manufacturer's guidelines or as indicated by the risk assessment.

Safe hot water delivery devices

7.40 Thermostatic mixing devices should only be installed where a risk assessment indicates their need. For guidance on applications and for a list of the types of mixing devices used, see Table 2 in HTM 04-01 Part A.

7.41 It is essential to check the temperature settings and operation of water mixing devices regularly (see manufacturers' instructions, the checklist at paragraph 7.53, and Chapter 11 in HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'). Other maintenance, where required, should be strictly in accordance with the manufacturer's instructions. Local water quality will influence the maintenance frequency for any installation.

7.42 All pipework supplying existing thermostatic mixing taps should be inspected to ensure that there is no preceding TMV supplying water to the hot port of the tap (see Figure D3 in Appendix D).

Notes

1. In existing installations, it may be necessary to install inline strainers. See the checklist at paragraph 7.53 for maintenance checks.

2. When bathing, or assisting patients, healthcare staff must always check the water temperature with a thermometer.

Showers

7.43 Disinfection of showerheads and angle-valve strainers has only a short-lived effect on microbial colonisation and growth. Manual cleaning to remove scale and other deposits or replacement of disposable showers should be carried out as based on the risk assessment. Traditionally this has been a quarterly task but the water quality and evidence base will influence the risk assessor's decision of the actual frequency implemented. Regular flushing of showers reduces microbial growth, but counts can significantly increase if regular flushing should cease. The most effective management of showers will be achieved by the removal of unnecessary ones and the regular use of others. Where showers are removed, it is important to cut back and remove all associated pipework (see paragraph 7.50 on the removal of redundant pipework).

7.44 It is important to note the distinction between self-purging and self-draining showers. Self-purging showers can be an effective Legionella control procedure, while self-draining showers can support the proliferation of *Legionella*.

Note

Adjustable showerheads are not recommended in healthcare facilities. See paragraph 10.61 in HTM 04-01 Part A.

Point-of-use filtration

7.45 Point-of-use (POU) filtration should be considered and agreed by the WSG only as an interim safeguard where control measures have been ineffective, prior to and during engineering remedial works, during periods of plumbing refurbishments and maintenance works, and where additional protection is required for vulnerable patients. Continuous long-term use of POU filters is not recommended, except where there is no effective alternative. The WSG should review their continued use and ensure an action plan is created and enacted to make certain they are changed at the intervals specified by the manufacturer.

7.46 Where POU filters are installed as a temporary measure while appropriate remedial work is carried out, they should be changed in accordance with the manufacturers' recommendations, typically at least once a month. Once removed for whatever reason, a replacement filter should be fitted. When changing filters, it is recommended that sampling of water quality takes place at outlets identified as sentinel points before refitting a replacement filter. It is essential to ensure that – where filters are to be used – they are constructed of the appropriate materials (see paragraph 3.1 in HTM 04-01 Part A).

7.47 Where POU filters are to be used, the backflow protection requirements need to be maintained in accordance with the Water Supply (Water Fittings) Regulations 1999. This may require additional backflow protection or modification of the system. In addition, sufficient activity space should be maintained to enable the outlet to be used without contaminating the filter.

7.48 Where filters are in place, follow manufacturers' instructions for cleaning, or they should be wiped clean as part of the basin/sink cleaning protocol as agreed by the WSG.

7.49 Where point-of-use filters are no longer required, the outlet connection should be flushed, cleaned and disinfected to remove any accumulated biofilm.

Removal of redundant pipework and services

7.50 In existing systems or during refurbishments, water systems should be inspected to identify redundant pipework (often referred to as blind ends) or services. In such cases, pipework should be cut back to the connection point including replacing the branch T with a straight coupling to ensure all redundant pipework is removed and to eliminate any opportunity for stagnation to occur.

Cleaning and disinfection

7.51 At some stage during the occupancy and use of a building, it is possible that the hot and cold water systems could become contaminated with waterborne pathogens. Should this be the case, the WSG may wish to consider cleaning and disinfection of part of the, or the entire, system. Advice may be found in HSG274 Part 2 and BSI's PD855468. See also Chapter 4 in HTM 04-01 Part A for guidance on the impact of treated water on materials and components.

Note

Whenever disinfection is planned, liaison with specialist departments (such as renal units and neonatal units) should take place.

Summary checklist for hot and cold system water systems

7.52 A summary checklist for hot and cold water systems is shown on the following pages (adapted from HSG274 Part 2 by kind permission of the HSE). For every operation that is undertaken, a method statement needs to be prepared and followed to ensure the stages are completed safely and effectively.

7.53 In Table 1 the suggested frequencies of inspecting and monitoring the hot and cold water systems will depend on their complexity and the susceptibility of those likely to use the water, and are for guidance only. The risk assessment should define the frequency of inspection and monitoring depending on the type of use and user and particularly where there are adjustments made by the assessor to take account of local needs. The water quality and evidence base will influence the risk assessor's decision.

Table 1 Checklist for hot and cold water systems (adapted from HSG274 Part 2)

Service	Action to take	Frequency (see paragraph 7.53)
Calorifiers	Inspect calorifier internally by removing the inspection hatch or using a borescope, and clean by draining the vessel. The frequency of inspection and cleaning should be subject to the findings and be increased or decreased based on conditions recorded	Annually, or as indicated by the rate of fouling
	Where there is no inspection hatch, purge any debris in the base of the calorifier to a suitable drain Collect the initial flush from the base of hot water heaters to inspect clarity, quantity of debris and temperature	Annually, but may be more frequent as indicated by the risk assessment or result of inspection findings
	Check calorifier flow temperatures (thermostat settings should modulate as close to 60°C as practicable without going below 60°C) Check calorifier return temperatures (not below 50°C).	Monthly
Hot water services	For non-circulating systems: take temperatures at sentinel points (nearest outlet, furthest outlet and long branches to outlets) to confirm they are at a minimum of 55°C within one minute	Monthly
	For circulating systems: take temperatures at return legs of principal loops (sentinel points) to confirm they are at a minimum of 55°C. Temperature measurements may be taken on the surface of metallic pipework	Monthly
	For circulating systems: take temperatures at return legs of subordinate loops; temperature measurements can be taken on the surface of pipes, but where this is not practicable, the temperature of water from the last outlet on each loop may be measured, and this should be greater than 55°C within one minute of running. If the temperature rise is slow, it should be confirmed that the outlet is on a long leg and not that the flow and return has failed in that local area	Quarterly (ideally on a rolling monthly rota)
	All HWS systems: take temperatures at a representative selection of other points (intermediate outlets of single pipe systems and tertiary loops in circulating systems) to confirm they are at a minimum of 55°C to create a temperature profile of the whole system over a defined time period	Representative selection of other sentinel outlets considered on a rotational basis to ensure the whole system is reaching satisfactory temperatures for *Legionella* control
POU water heaters (no greater than 15 litres)	Check water temperatures to confirm the heater operates at 55°C, or check the installation has a high turnover	Monthly–six monthly, or as indicated by the risk assessment
Combination water heaters	Inspect the integral cold water header tanks as part of the cold water storage tank inspection regime; clean and disinfect as necessary. If evidence shows that the unit regularly overflows hot water into the integral cold water header tank, instigate a temperature-monitoring regime to determine the frequency, and take precautionary measures as determined by the findings of this monitoring regime	Annually
	Check water temperatures at an outlet to confirm the heater operates at 55°C	Monthly

Service	Action to take	Frequency (see paragraph 7.53)
Cold water storage cisterns	Inspect cold water storage cisterns and carry out remedial work where necessary	Annually
	Check the cistern's water temperature remote from the ball valve and the incoming mains temperature. Record the maximum temperatures of the stored and supply water recorded by fixed maximum/minimum thermometers where fitted	Annually (summer) or as indicated by the temperature profiling
Cold water services	Check temperatures at sentinel taps (typically those nearest to and furthest from the cold cistern, but may also include other key locations on long branches to zones or floor levels). These outlets should be below 20°C within two minutes of running the cold tap. To identify any local heat gain, which might not be apparent after one minute, observe the thermometer reading during flushing	Monthly
	Take temperatures at a representative selection of other points to confirm they are below 20°C to create a temperature profile of the whole system over a defined time period. Peak temperatures or any temperatures that are slow to fall should be an indicator of a localised problem	Representative selection of other sentinel outlets considered on a rotational basis to ensure the whole system is reaching satisfactory temperatures for *Legionella* control
	Check thermal insulation to ensure it is intact, and consider weatherproofing where components are exposed to the outdoor environment	Annually
Showers and spray taps	Dismantle, clean, descale and disinfect removable parts, heads, inserts and hoses where fitted	Quarterly or as indicated by the rate of fouling or other risk factors, e.g. areas with high-risk patients
POU filters	Record the service start date and lifespan or end date and replace filters as recommended by the manufacturer (bacterial-retention filters should be used primarily as a temporary control measure while a permanent solution is developed, although long-term use of such filters may be needed in some healthcare applications)	According to manufacturer's guidelines
Base exchange softeners	Visually check the salt levels and top up salt, if required. Undertake a hardness check to confirm operation of the softener	Weekly, but depends on the size of the vessel and the rate of salt consumption
	Service and disinfect	Annually, or according to manufacturer's guidelines
Multiple-use filters	Backwash and regenerate as specified by the manufacturer	According to manufacturer's guidelines

Service	Action to take	Frequency (see paragraph 7.53)
Infrequently used outlets	Consideration should be given to removing infrequently used showers, taps and any associated equipment that uses water. If removed, any redundant supply pipework should be cut back as far as possible to a common supply (e.g. to the recirculating pipework or the pipework supplying a more frequently used upstream fitting) but preferably by removing the feeding 'T' Infrequently used equipment within a water system (i.e. not used for a period equal to or greater than seven days) should be included on the flushing regime Flush the outlets until the temperature at the outlet stabilises and is comparable to supply water and purge to drain Regularly use the outlets to minimise the risk from microbial growth in the peripheral parts of the water system, sustain and log this procedure once started	Weekly, or as indicated by the risk assessment
TMVs	Where integral, inspect, clean, descale and disinfect any strainers or filters associated with TMVs. To maintain protection against scald risk, TMVs require regular routine maintenance carried out by competent persons in accordance with the manufacturer's instructions. There is further information in paragraphs 2.152–2.168 of HSG274 Part 2.	Annually or on a frequency defined by the risk assessment, taking account of any manufacturer's recommendations
Inline strainers	Where fitted, inspect, clean, descale and disinfect any strainers or filters associated with TMVs or other sensitive equipment.	Annually or on a frequency defined by the risk assessment, taking account of any manufacturer's recommendations
Pressurisation and expansion vessels	Where practical, flush through and purge to drain. Where removable, bladders or diaphragms should be changed according to the manufacturer's guidelines or as indicated by the risk assessment	Monthly–six monthly, as indicated by the risk assessment
Biocidal treatment systems	Check the dosing and control system operation including alarms Measure the treatment parameters to establish the required values are being achieved at representative outlets including sentinel outlets **Note:** Consider 24-hour automatic monitoring for biocidal treatment on large or complex systems	Weekly Weekly Validation and calibration of the automatic monitoring system should be carried out at the frequencies recommended by the manufacturer

8 Other operational considerations

8.1 The WSG need to identify other waterborne hazards (the list below is not exhaustive but provides examples).

8.2 Waterborne pathogens including *Legionella* may colonise other areas where droplets of contaminated water of a size suitable for deep inhalation are generated. Such aerosol-generating plant and equipment should not be installed next to patient accommodation or air inlets. Some patients may be particularly susceptible to infection (see also HSG274 Part 3).

Vending, chilled-water and ice-making machines

8.3 See paragraphs 9.24–9.27 in HTM 04-01 Part A for guidance on installation of this equipment.

8.4 Where equipment is hand-filled, there should be clear instructions on the water used; it should be hygienically collected and decanted into the equipment from a clean vessel.

8.5 Chilled-water drinking fountains normally include a reservoir to assist in the cooling cycle; if machines are turned off, water quality can deteriorate. Provision of bottle dispensers should be approved only by the WSG. Where carbon filters and/or UV are fitted, these should be maintained as per the manufacturer's instructions. Additional cleaning to ensure adequate hygiene of nozzles etc should be put in place as recommended by the WSG.

Note

Proprietary water containers for water dispensing machines should be returned to the supplier.

8.6 Ice machines should not be placed in augmented care units. Where ice is needed for treatment purposes, it should be made using water obtained through a microbiological POU filter or boiled water in sterile ice trays or ice bags.

8.7 Ice should not be allowed to stagnate in an ice-making machine's storage bin, but should be changed frequently. Appropriate cleaning and hygienic procedures, agreed by the WSG, including the cleaning and disinfection of scoops etc should be put in place. For guidance on infection-control precautions with regard to ice-making machines, see HBN 00-09 – 'Infection control in the built environment'.

8.8 Maintenance for ice-making machines should be carried out in accordance with the manufacturer's recommendations. Care should be taken to ensure that the water supply to the ice-making machine is not subjected to heat gain.

Portable/room humidifiers

8.9 Designs should not include the use of "portable" or "room" self-contained humidifiers (having a water supply that is sprayed/atomised into the room space). In clinical/patient areas the decision to use this type of humidifier should rest with the IPC team. See also Safety

Notice NHSE SN(96)06 – 'Evaporative type cooling fan'.

Non-wholesome water storage

8.10 Non-wholesome water is sometimes stored for emergency use (for example for fire-fighting purposes). These systems should have an appropriate backflow prevention device in accordance with the Water Supply (Water Fittings) Regulations 1999. They should be considered by the risk assessment and the WSG.

Deluge showers

8.11 Deluge showers (sometimes called emergency showers) are intended for use in an emergency where a staff member or a patient has suffered external chemical contamination. Similarly, there may be other special outlets used for personal emergencies, for example eyebaths. These should not be installed on the end of lines. They should be dismantled, descaled and disinfected regularly in accordance with the risk assessment, and should be flushed in accordance with the recommendations in HSG274 Part 3.

Trolley wash procedures

8.12 High-pressure hoses will generate aerosols and have been associated with causing cases and outbreaks of Legionnaires' disease. The contamination of the aerosol can be reduced if the water supply is taken from a wholesome water system via a suitable air gap giving fluid category 5 protection – typically an air gap of Type AA or AB, which requires a break tank and booster pump to provide adequate pressure to the washing equipment. Procedures should be put in place to flush to drain before use, and to disinfect them if they have been out of use for a prolonged period.

Lawn sprinklers and garden (or similar) hoses

8.13 In certain conditions, lawn sprinklers may retain stagnant water in the pipework/hose supplying the sprinkler head; they may also produce an aerosol spray. The pipework may be installed underground or via a flexible hose over ground. In either case it is very unlikely that they can be completely drained down after use or when not required; at certain times in the year the retained water may be at temperatures suitable for the colonisation by, and multiplication of, *Legionella* and *P. aeruginosa*. There is evidence linking cases of Legionnaires' disease with permanently installed systems that use underground supply plumbing. Irrigation systems and hoses for these purposes should be supplied via backflow prevention devices giving fluid category 5 protection – typically an air gap of Type AA or AB, which requires a break tank and booster pump to provide adequate pressure to the irrigation nozzle or hose outlet. A risk assessment should be undertaken prior to use to minimise the risk by implementing suitable control measures.

8.14 Hoses used for filling of remote equipment or mobile units should be of materials suitable for use with wholesome water systems and should be drained, capped between uses and kept in cool conditions. They should be disinfected (by immersing in hypochlorite) before use.

Vehicle-washing plant

8.15 Vehicle washing is carried out either using a hand-held pressure spray or by a frame wash that consists of a bay containing a rectangular pipework frame fitted with several high-pressure sprays. In the latter case, this equipment should be flushed regularly. Pressure washers and frame washers should be supplied via backflow prevention devices giving fluid category 5 protection – typically an air gap of Type AA or AB, which requires a break tank and booster pump to provide adequate pressure to the outlets. See the

Water Management Society's 'Managing the risk of legionnaires' disease in vehicle-washing systems W046-5'.

8.16 Permanent hard-standing areas for vehicle-washing purposes should have an even surface to avoid ponding and have a slope or dish to a suitable drain.

Decorative internal and external water features

8.17 Internal ornamental water features (for example a water cascade in the main entrance hall) are susceptible to airborne contamination and are not recommended.

External water features

8.18 Ornamental fountains have been implicated in cases of legionellosis. External water features should not be situated under trees where fallen leaves or bird droppings may contaminate the water. Exposure to high winds should be avoided as they can disperse spray beyond the immediate confines of the basin/pond. The apex of the water column/jet should not exceed the distance to the nearest edge of the basin/pond for the same reason. An overflow/outlet to a suitable drain should be provided for easy emptying and cleaning. Where possible, a permanently installed freshwater supply pipe with topping-up device should be provided. Their provision should be subject to a risk assessment and approval by the WSG, and appropriate action is required to minimise the risk. Any top-up supply from a wholesome water supply should be supplied via backflow prevention devices giving fluid category 5 protection – typically an air gap of Type AA or AB.

8.19 A risk assessment should be undertaken prior to use to minimise the risk by implementing suitable control measures.

Wet fire and automatic sprinkler systems

8.20 Wet fire protection systems have been implicated in outbreaks of legionellosis. All hose reels, sprinkler systems and wet risers should be isolated from the potable water supply by a method permitted by the Water Supply (Water Fittings) Regulations 1999. Many fire authorities are not in favour of local fire-fighting, preferring early professional intervention. It may, therefore, be possible to remove hose reels, thus avoiding their hazards. (Any redundant pipework should be cut back to the connection point including replacing the branch 'T' with a straight coupling.)

8.21 Fire-fighting systems should be included within risk assessments. See FPA Technical Briefing Note – '*Legionella* and firefighting systems' for further guidance.

Patient contact equipment (for example respiratory nebulisers, humidifiers)

8.22 Patient contact equipment (such as respiratory nebulisers and humidifiers) should be used, drained, cleaned, rinsed, and dried strictly in accordance with the manufacturer's recommendations and agreed by the WSG. They should always be allowed to dry thoroughly before use. See also Chapter 3 in HTM 04-01 Part C, which should be applied to all healthcare settings.

Heater cooler units used in cardiac surgery

8.23 Heater coolers units (HCU) are used in cardiopulmonary bypass operations and for extracorporeal membrane oxygenation or extracorporeal life support, and their function is to regulate the temperature of the blood perfusing the patient using water in the HCU tanks to indirectly raise or lower the patient's body temperature. There have been a number of outbreaks and fatalities due to the presence

of *Mycobacterium chimaera* in the HCUs that has resulted in endocarditis, surgical site infection and disseminated infection possibly via the aerosol route. HCUs should be operated and decontaminated according to the manufacturer's instructions, and their use should be subject to a risk assessment that should be approved by the WSG.

Flowers and plants

8.24 Consideration should be given to providing specific facilities for regularly disposing of wastewater and compost outside in-patient accommodation. This should not be provided in dirty utilities.

Buried pipelines

8.25 Pipelines made of plastics are susceptible to hydrocarbons such as fuels and oils. These chemicals can permeate through plastic pipes if they are nearby and contaminate the water supply, and it may take days, weeks or even months before a noticeable taste can be detected in the water supply. Whenever spills are reported, an assessment of services within the area should be undertaken. See also paragraphs 12.8–12.16 in HTM 04-01 Part A.

8.26 Where there is a risk of hydrocarbon contamination of the supply, barrier pipe can be used instead. This is a double-layer plastic pipe with a barrier layer (usually aluminium) in between.

Other risk systems

8.27 Further guidance on other risk systems is given in Appendix 3.1 of HSG274 Part 3.

9 Microbiological monitoring

9.1 Where there are taste or odour problems, microbiological monitoring for total viable counts (TVCs) may be considered necessary. However, routine microbiological monitoring for TVCs is not recommended as there is no direct association with TVCs and the presence of waterborne pathogens.

9.2 If performed, TVCs may be used to analyse trends. Counts taken before and after disinfection (samples at least 48 hours post-disinfection) can give an indication of the efficacy of a disinfection procedure.

9.3 All microbiological measurements should be by approved methods and/or be carried out by United Kingdom Accreditation Service (UKAS)-accredited laboratories for the method being used. Dip slides are not acceptable on hot and cold water systems.

See also the Standing Committee of Analysts' 'Microbiology of drinking water – Part 7' for methods suitable for TVCs.

10 Testing for *Legionella*

10.1 *Legionella* monitoring should be carried out where there is doubt about the efficacy of the control regime or where the recommended temperatures, disinfectant concentrations or other precautions are not consistently achieved throughout the system. The WSG should use risk assessments to determine when and where to test, which may include the following circumstances:

a. When storage and distribution temperatures do not achieve those recommended and systems are treated with a biocide regime, testing should be carried out monthly, although that frequency may be altered depending on the results obtained.

b. In systems where the temperature or biocidal control regimes are not consistently achieved, weekly checks are recommended until the system is brought under control, after which the frequency of monitoring can be reviewed.

c. When a nosocomial outbreak is suspected or has been identified.

d. Where there are at-risk patients with increased susceptibility.

Legionella bacteria (cfu/L)	Action required
Not detected or up to 100	The primary concern is protecting susceptible patients, so any detection of *Legionella* should be investigated and, if necessary, the system resampled to aid interpretation of the results in line with the monitoring strategy and risk assessment.
>100 and up to 1000	Either: If the minority of samples are positive, the system should be resampled. If similar results are found again, a review of control measures and a risk assessment should be carried out to identify any remedial action necessary, or If the majority of samples are positive, the system may be colonised, albeit at a low level. An immediate review of control measures and a risk assessment should be carried out to identify any other remedial action required. Disinfection of the system should be considered.
>1000	The system should be resampled and an immediate review of the control measures and a risk assessment should be carried out to identify any remedial actions, including possible disinfection of the system. Retesting should take place three days following systemic chemical or thermal disinfection and at frequent intervals thereafter until a satisfactory level of control is achieved as agreed by the WSG.

10.2 As a minimum, samples should be taken as follows:

- from the cold water storage and the furthermost outlet from the tank;
- from the calorifier flow, or the closest tap to the calorifier, and the furthermost tap on the hot water service circulating system;
- additional samples should be taken from the base of the calorifier where drain valves have been fitted;
- additional random pre- and post-flush samples may also be considered appropriate where systems are known to be susceptible to colonisation in line with BS 7592 guidance;
- methods should be in accordance with BS 7592.

10.3 Analysis of water samples for *Legionella* should be performed in UKAS-accredited laboratories with the current ISO standard methods for the detection and enumeration of *Legionella* included within the scope of accreditation. These laboratories should take part in a water microbiology proficiency testing scheme (such as that run by PHE or an equivalent scheme accredited to BS EN ISO 17043). Alternative quantitative testing methods may be used as long as they have been validated using BS EN ISO 17994 and meet the required sensitivity and specificity. The laboratory should also apply a minimum theoretical mathematical detection limit of ≤100 *Legionella* bacteria/litre sample.

10.4 Action following *Legionella* sampling in hot and cold water systems is given in the following flowcharts (see Figures 4–6).

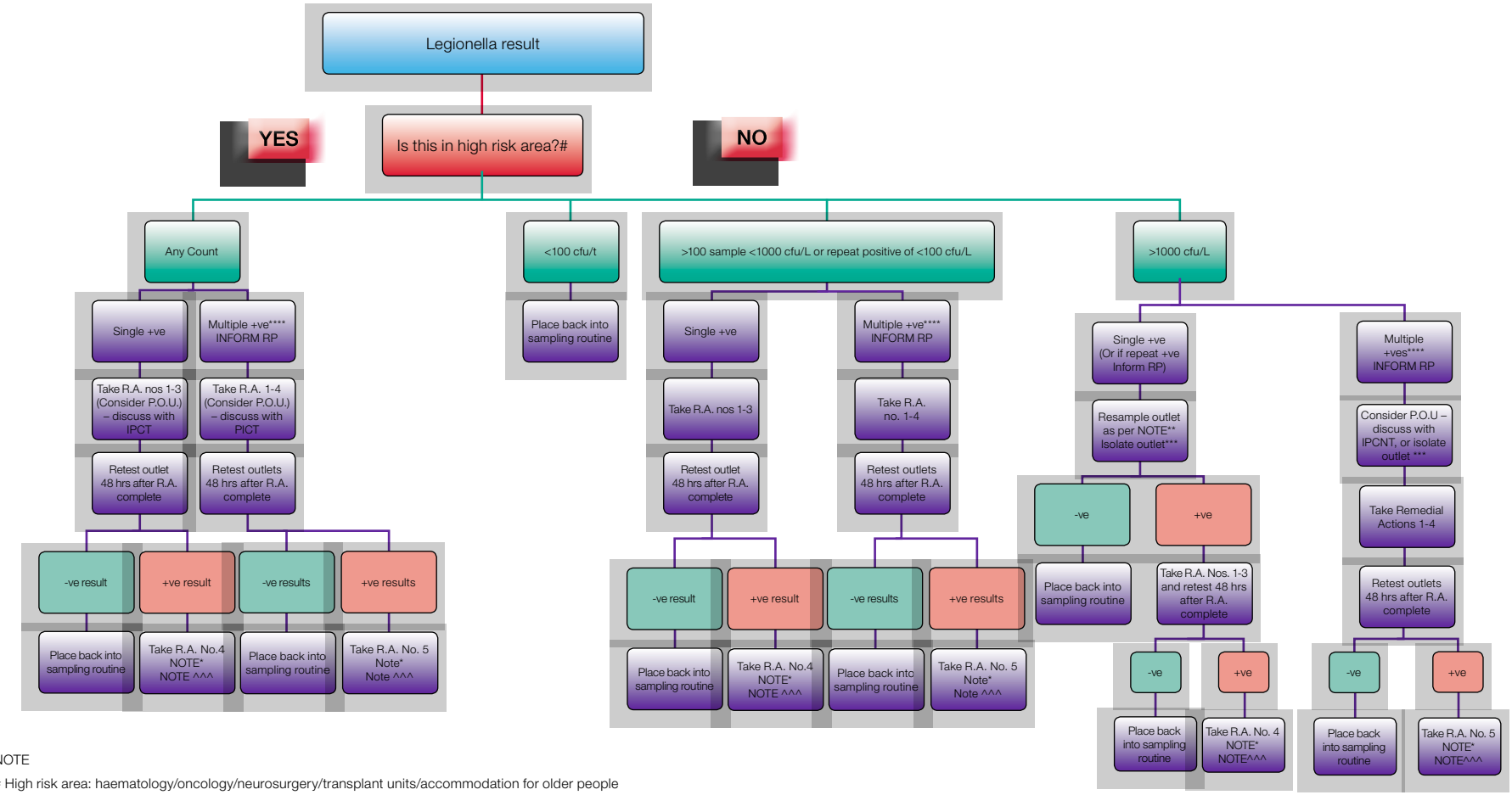
Note

Figures 4–6 are given as examples of a range of various methods that may be used. Individual conditions will dictate which procedure is appropriate and therefore the charts can be adapted accordingly.

Legionella bacteria (cfu/l)	Results from Pre-flush samples	Systemic results (Post-flush samples)
	Pre-flush samples tend to be an indicator of local conditions and if detected will often require Post-flush samples in order to determine that the contamination is local and not systemic.	Post-flush samples (or multiple positive samples) may be an indication that the whole water systems is contaminated and that controls are not effective.
Legionella not detected	Continue with current control scheme	
***Legionella* From detection to 100**	Action required • The detection limit for Legionella by culture methods was historically 100cfu/L, at present laboratories may be able to report to levels of 20cfu/L or less. This can cause confusion over what level should bring about corrective actions. The primary concern is protecting susceptible patients, so any detection of legionella should be investigated and, if necessary, the system resampled to aid interpretation of the results in line with the monitoring strategy and risk assessment.	
100- less than 1000	Action required • Identify remedial actions, Investigate:– o Usage frequency o Outlet for corrosion and scale o local heat gain, o Local Dead ends o Cross flow between hot and cold and vice versa, o Localised failure of the HWS return • It may be appropriate to immediately resample to indicate if initial remedial actions have been effective. The locations should then be resampled after 3 to 6 months to confirm any actions taken have remained effective. In addition to the above, and if the outlet is served by a TMV: • Review the need for the TMV taking into account the relative risks of scalding. Remove the TMV if considered appropriate • If the TMV is to remain, clean and disinfect the TMV, the outlet and the strainers on both cold and hot feeds. • Identify any flexible hoses (particularly after the TMV) and consider replacement, avoiding the use of flexible hoses where practicable.	Action required Whilst low numbers are unlikely to pose a risk to the general population, • Review immediately the system control measures and risk assessment • Identify remedial actions, Investigate: - o Check for any hot water backflow via the calorifier cold feed pipes o Calorifier discharge via open vents to the cold tank. o Failure of HWS to operate at target temperatures o Over capacity or under usage • Cleaning & Disinfection of the entire system should be considered • It may be appropriate to confirm effective disinfection, any required microbiological samples should be taken between two and seven days after the system is treated. (Samples taken immediately after a disinfection process might give false negative results). • The water system should then be resampled regularly to confirm any actions taken have remained effective.
1000-10,000	Action required • In addition to the above • Review immediately the local control measures and risk assessment to identify any required remedial action (dead ends etc) • Cleaning and Disinfection of the outlet should be undertaken – (especially showers and spray taps) • If a shower (spray outlet) cannot be taken out of use, consider installing point of use microbiological filters on all affected showers. • It is likely to be appropriate to resample, between two and seven days after, to indicate if initial remedial actions have been effective. • The locations should then be resampled (e.g. 1 to 3 months) to confirm any actions taken have remained effective.	Action required • In addition to the above • Cleaning & Disinfection of the entire system is likely to be required. • To confirm effective disinfection microbiological samples should be taken between two and seven days after the system is treated. (Samples taken immediately after a disinfection process might give false negative results).

Legionella bacteria (cfu/l)	Results from Pre-flush samples	Systemic results (Post-flush samples)
	Pre-flush samples tend to be an indicator of local conditions and if detected will often require Post-flush samples in order to determine that the contamination is local and not systemic.	Post-flush samples (or multiple positive samples) may be an indication that the whole water systems is contaminated and that controls are not effective.
>10,000	Action required • In addition to the above. • Take immediate measures to prevent exposure from this outlet until remedial measures are taken and shown to be effective. • If the outlet cannot be taken out of use, install a point of use microbiological filter on all affected outlets. • Resample, between two and seven days after, to indicate if initial remedial actions have been effective. • The locations should then be regularly resampled to confirm any actions taken have remained effective.	Action required • In addition to the above. • Take immediate measures to prevent exposure from all outlets fed by the system until remedial measures are taken. • Clean & Disinfect the entire system as soon as possible.

Figure 4 Action levels following *Legionella* sampling in hot and cold water systems. Example 1



NOTE

High risk area: haematology/oncology/neurosurgery/transplant units/accommodation for older people

* If all remedial actions are exhausted and there is no improvement in results, then take remedial action No. 6.

** Take additional samples pre and post from adjacent areas on same loop based on worst case scenario (Low usage outlets).

*** Remedial Action should occur 48 hours after isolation.

**** More than one positive on same loop in the system.

^^^ More frequent sampling is required

- Remedial Actions (R.A.)
  1. Review control measures e.g. temperatures flow and return and local, CLO_2 levels, investigate if outlets are being used and/or flushing regime in place
  2. Consider changing outlet i.e. shower head and hose or tap as appropriate
  3. WHB – TMV change/consider removal
  4. Full investigation of the plumbing system including dead leg invesitgation, flexible connections, has there been remedial works in the area, has anything changed
  5. Consider Installation of standalone ClO_2 Unit
  6. Consultation/inform WSG

RP = Responsible person

Figure 5 Action levels following *Legionella* sampling in hot and cold water systems. Example 2

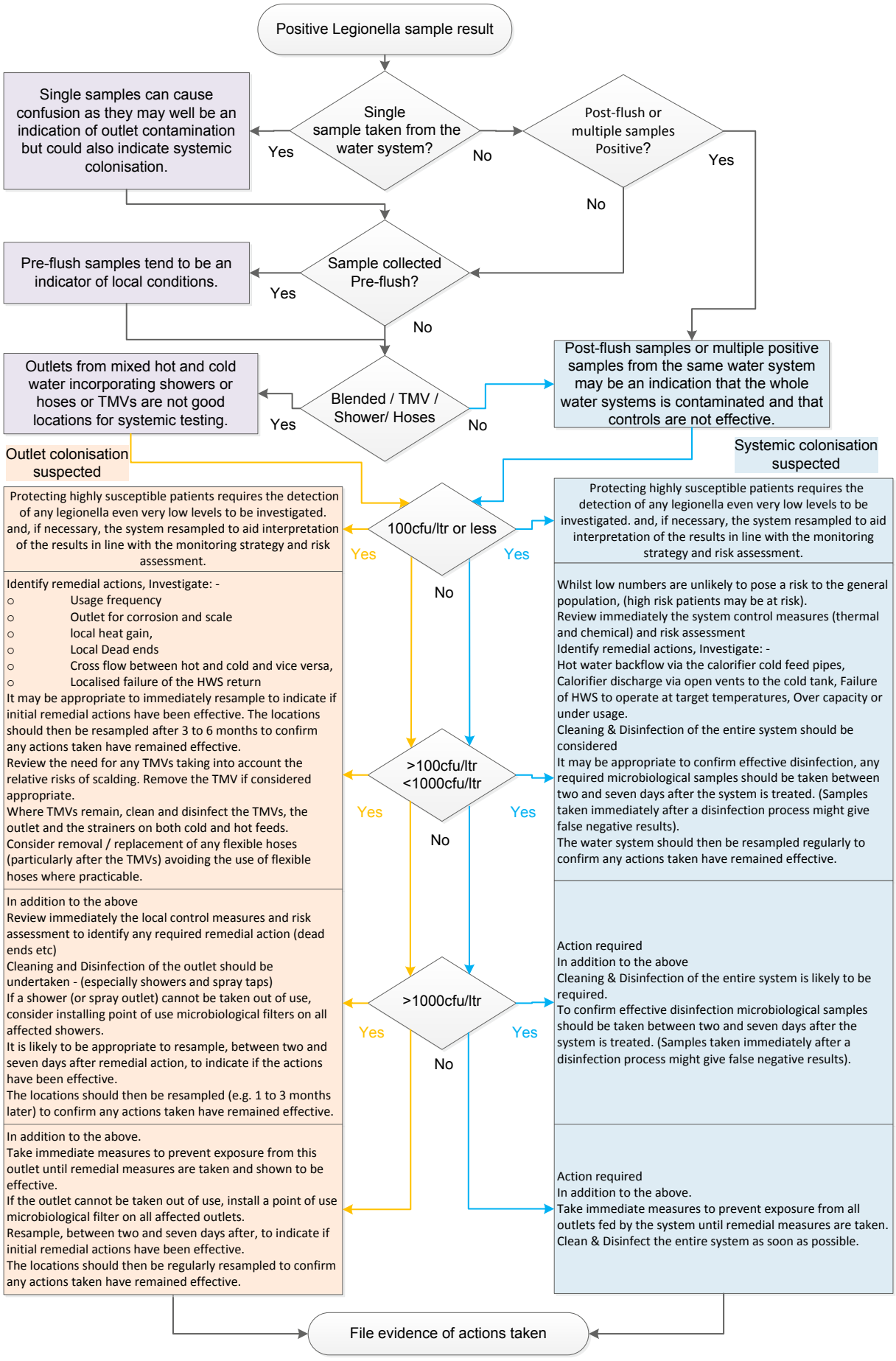


Figure 6 Action levels following positive result

Appendix A Examples of the use of water within a healthcare facility and water quality types

A1. All water and water systems in healthcare facilities should be risk-assessed according to their intended use and patient immune status taking into account any identified inherent hazards within the facility and the quality of the water supply to the systems being assessed. The assessment of risk should take account of the most vulnerable population likely to be exposed to each potential source.

A2. Examples of different categories of water for differing uses are shown in Table A1.

Note

The hazards and their occurrence will depend on the quality of source water. Therefore the associated risks should be calculated taking into account local conditions.

Table A1 Examples of water quality parameters for different applications in healthcare

Healthcare area	Application	Physical, chemical or microbial quality indicators	Water quality parameters given in:	Particular hazards (based on an assessment for each system)	See also
All settings	Wholesome water for drinking, cooking, food preparation and washing	Physical: Odour, colour, taste Chemical: Heavy metals, nitrates, orthophosphates, pesticides, surfactants Biological: Coliform bacteria, *E. coli, enterococci*	Water Supply (Water Quality) Regulations http://www.dwi.gov.uk/stakeholders/legislation/wqregs2007cons.pdf tables A and B. Where private water supplies are also used, see The Private Water Supplies Regulations 2009 http://www.dwi.gov.uk/stakeholders/legislation/pwsregs2009.pdf		DWI guidance and codes of practice Details of recommended sampling procedures are given in the Microbiology of Drinking Water https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/316769/MoDW-2-232.pdf and BS EN ISO 19458.
All settings	Circulated hot water systems and cold water systems	*Legionella* spp.: colony counts per litre *Pseudomonas aeruginosa* colony counts per 100 mL	HTM 04-01 Parts A, B and C BS 7592 Sampling for Legionella bacteria in water systems. Code of practice	*Legionella* spp., *Pseudomonas aeruginosa* and other waterborne pathogens	For *Legionella*, see Health and Safety Executive (2014). *Legionnaires' disease HSG 274 Part 2. The control of Legionella bacteria in hot and cold water systems.*
Flexible endoscope reprocessing units	Initial flush	Hardness	HTM 01-06 Decontamination of flexible endoscopes Part B – Design and installation HTM 01-06 Decontamination of flexible endoscopes Part E – Testing methods		
	Intermediate flush	Hardness			
	Water for diluting disinfectants and detergents	Hardness			
	Final rinse-water	• Hardness • Total organic carbon • Electrical conductivity • Appearance • pH • Total viable count • *P. aeruginosa* • Environmental mycobacteria			

Healthcare area	Application	Physical, chemical or microbial quality indicators	Water quality parameters given in:	Particular hazards (based on an assessment for each system)	See also
Renal units and satellite dialysis units	**Water for haemodialysis**	**Total viable counts and endotoxin concentrations**	• BS ISO 13959: Water for haemodialysis and related therapies. • BS ISO 11663: Quality of dialysis fluid for haemodialysis and related therapies. • BS ISO 13958: Concentrates for haemodialysis and related therapies.		• HBN 07-01 Satellite dialysis units • HBN 07-02 Main renal units
Augmented care units		**Pseudomonas aeruginosa: Colony counts per 100 mL**	HTM 04-01 Part B and Part C: *Pseudomonas aeruginosa* – advice for augmented care units		
Aquatic therapy pools		pH, free residual halogen, total and combined halogen and other treatment parameters. Coliforms, *E.coli*, *Pseudomonas aeruginosa* and TVCs			• See the PWTAG's 'Swimming pool water: treatment and quality standards for pools and spas';
Spa pools and whirlpools		pH, free residual halogen and other treatment parameters			• See HSE/PHE's 'Management of spa pools: controlling the risks of infection'; **Note** This document is currently being revised and will become HSG274 Part 4 – 'The control of Legionella and other infectious agents in spa pool systems'.

Healthcare area	Application	Physical, chemical or microbial quality indicators	Water quality parameters given in:	Particular hazards (based on an assessment for each system)	See also
Sterile services departments	Final rinse	Appearance: • pH • Conductivity at 25ºC • Total dissolved solids • Total hardness • Chloride, Cl • Heavy metals, determined as Lead, Pb • Iron, Fe • Phosphate, P2 O5 • Silicate, SiO2 • Total viable count at 22ºC • Total viable count at 37ºC • Bacterial endotoxins	HTM 01-01 Part D Washer disinfectors		
	Other stages	• Total hardness • Chloride, Cl • Silicate, SiO2			
Laundries	Final rinse	Hardness (total Ca2+/Mg2+) • pH • Turbidity • Colour • Iron • Manganese • Copper • Surfactant • Bioburden (TVC)	HTM 01-04 Decontamination of linen for health and social care: engineering, equipment and validation	*Clostridium difficile* *Bacillus cereus*	Textile Services Association's (2008) "Target specification for recycled water to meet final rinse quality".
Dental facilities	Dental unit water lines and water systems	*Legionella* spp.: colony counts per litre *Pseudomonas aeruginosa* colony counts per 100 mL	Chapter 19 in HTM 01-05 Decontamination in primary care dental facilities https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/170689/HTM_01-05_2013.pdf	*Legionella* spp. *Pseudomonas aeruginosa* and other waterborne pathogens	

Appendix B Action in the event of an outbreak of legionellosis

B1. In England and Wales, Legionnaires' disease is notifiable under the Health Protection (Notification) Regulations 2010. Under these regulations, registered medical practitioners must report cases of Legionnaires' disease to the Proper Officer. These regulations also require human diagnostic laboratories to notify PHE of cases of Legionnaires' disease identified by laboratory testing.

B2. An outbreak is defined as two or more cases where the onset of illness is closely linked in time (weeks rather than months) and location and where there is epidemiological evidence of a common source of infection, with or without microbiological evidence. An outbreak control team should always be convened to investigate outbreaks. It is the responsibility of the Proper Officer to declare an outbreak. The Proper Officer, appointed by the local authority, is often a consultant in communicable disease control (CCDC) within the local PHE centre health protection team.

B3. The local PHE centre health protection team has established incident plans to investigate major outbreaks of infectious diseases including legionellosis.

B4. The HSE or local environmental health officers (EHOs) may be involved in the investigation of outbreaks, their aim being to pursue compliance with health and safety legislation. The local authority, Proper Officer or EHO acting on their behalf will make a site visit to carry out a public health risk assessment, often with the relevant officer from the enforcing authorities (the HSE or the local authority) for health and safety reasons. Any infringements of relevant legislation may be subject to a formal investigation by the appropriate enforcing authority.

B5. There are published guidelines on the PHE website for the investigation and management of incidents, clusters and outbreaks of Legionnaires' disease.

B6. If the hot and cold water system is implicated in an outbreak of Legionnaires' disease, the WSG may wish to consider cleaning and disinfection of part of the, or the entire, system. Advice may be found in HSG274 Part 2.

Appendix C Exemplar temperature test sheets

SERVICE VISIT REPORT - Temperature

Client _____ **Visit Date** _____
Site _____ **Report Ref** _____

Equipment type	Location	Mains	Cold	Hot	Mixed	Fail	Comment
Limits	Tanks & Cold taps	<20	<20				(Within 2 minutes)
	Calorifier Flow			57-65			
	Calorifier Return			50-60			(& HWS return loops)
	HWS			>50			(Within 1 minute)
	Thermostatic valve (TMV)	<20	<20	>50	38-46		

Comments & Recommendations

Report by _____ **Signature** _____
Site Contact _____ **Site Signature** _____

C1. A unique identification is required for each outlet/mixing device as well as identification of its type.

C2. At commissioning, hot and cold water pressures also need to be measured and recorded for each mixing device together with all the test parameters from the in-service tests in HTM 04-01: Supplement – 'Performance specification D 08: thermostatic mixing valves (healthcare premises)'. This allows supply conditions to be reassessed in the future if outlet temperatures have changed.

Appendix D Testing for *P. aeruginosa*

D1. *P. aeruginosa* may be present within the water storage, distribution and delivery systems and also in the water supplied to the healthcare facility.

D2. The sampling protocol (Appendix E) is intended to help healthcare providers establish whether the water in augmented care units is contaminated with *P. aeruginosa* and, if it is, to help locate its origin and to monitor the efficacy of remedial measures.

D3. *P. aeruginosa* contamination is generally found in the last two metres of the point of water delivery; therefore pre-flush samples should be collected to assess the highest risk to outlet users and at-risk patients.

D4. Biofilm will constantly be released as clumps or free-floating individual cells (planktonic forms).The concentration of planktonic bacteria will build up over time when the water is stagnant, but will be diluted as water is used and flows through the pipework or tap containing the biofilm.

D5. Water outlets can give very different results and may be negative if water from the tap has been used before a sample is collected.

D6. The first water to be delivered from the outlet (pre-flush sample) should be collected to assess the microbial contamination in the last two metres of pipework.

D7. To maximise the recovery of these free-floating planktonic bacteria, water samples should be taken:

a. during a period of, preferably, no use (at least two hours or preferably longer); or

b. low use.

D8. If water flows over a biofilm containing *P. aeruginosa* located in the last two metres, planktonic bacteria arising from that biofilm will be diluted and a subsequent sample will give low bacterial counts. If contamination is upstream in the system, this will not affect bacterial counts, which may actually increase.

D9. The sample obtained after allowing water to flow from an outlet is referred to as a “post-flush” sample (see paragraphs E12 and E13 in Appendix E). Comparison of counts from pre-and post-flush samples can help locate the source of the *P. aeruginosa*. If a pre-flush sample gives a high count, subsequent paired pre- and post-flush samples should be tested to help locate the source of the contamination.

D10. In order to be able to carry out the appropriate microbiological examinations on a sample and provide a meaningful interpretation of test results, it is essential that samples are collected in the correct manner using the correct equipment and that the sampling protocol in Appendix E is adhered to.

D11. Protocols for microbiological examination of samples are provided in Appendix F.

Where to sample water outlets

D12. The water outlets to be sampled should be those that supply water which:

- has direct contact with patients;
- is used to wash staff hands; or
- is used to fill or clean equipment that will have contact with patients as determined by risk assessment.

When and how to sample water outlets

D13. The outlets identified above should be sampled to provide an initial assessment of contamination levels. There is no need to sample all taps that are due to be sampled on the same occasion; samples can be taken in batches on separate occasions. It may assist the receiving laboratory if the sampling schedule is agreed beforehand (see Figure D1 and also Appendix F).

Interpretation of *P. aeruginosa* test results

D14. If test results are satisfactory (not detected), there is no need to repeat sampling for a period of six months unless there are changes in the water distribution and delivery systems components or system configuration (for example, refurbishments that could lead to the creation of dead-legs) or occupancy.

D15. However, the WSG could indicate that water sampling is required within six months if there are clinical evidence-based suspicions that the water may be a source of patient colonisation or infection (that is, with *P. aeruginosa* or another potentially waterborne pathogen).

D16. If tests show counts of 1 to 10 cfu/100 mL, the WSG should risk-assess the use of water while simultaneously retesting the water outlet (see Figure D1 and Note below).

D17. If test results are not satisfactory (>10 cfu/100 mL), further sampling along with an engineering survey of the water system could be used to identify problem areas and modifications that may be implemented to improve water quality.

D18. After such interventions, the water should be resampled (see Figure D1 for suggested frequencies).

Note

Figure D1 gives an example of sampling frequencies. Sampling may be undertaken more frequently according to the risk assessment. It is important that samples are taken as described in Appendix E to avoid false negative results.

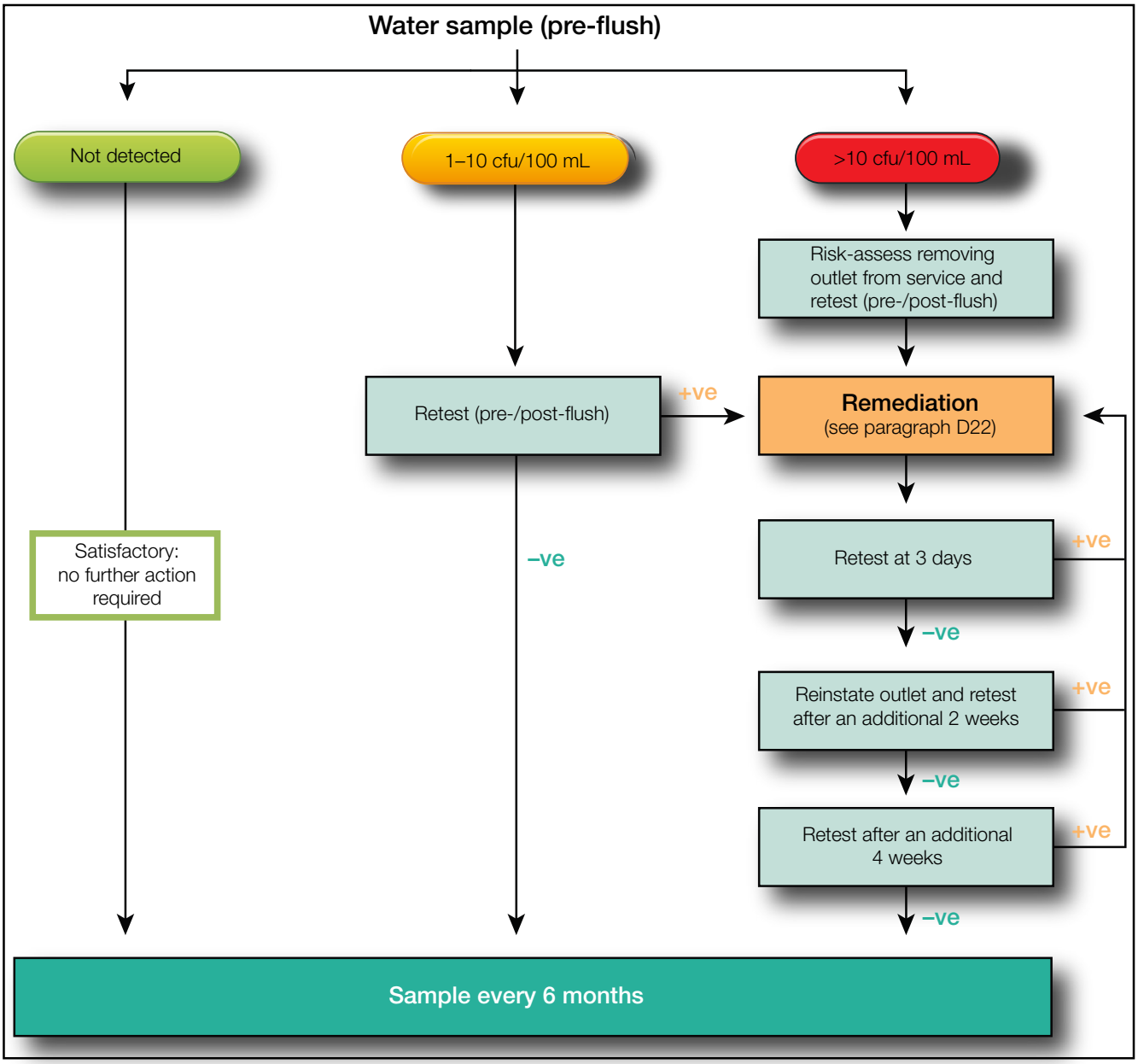


Figure D1 Summary of suggested water sampling and testing frequencies

Interpretation of pre- and post-flush counts

D19. High counts in pre-flush samples but with low counts or none detected at post-flush could indicate that areas/fittings at or near the outlets are the source of contamination (see Table D1).

- A few positive outlets, where the majority of outlets are negative, would also indicate that the source of contamination is at or close to the outlet.
- If both pre- and post-flush samples from a particular outlet are >100 cfu/100 mL and other nearby outlets have no or low counts, this shows that the single outlet is heavily contaminated, despite the high post-flush count. This could be explored by testing dilutions of pre- and post-flush water samples from this outlet or by using an extended flush such as for five minutes prior to post-flush sampling or by taking a post-flush sample after disinfection of the outlet as occurs with *Legionella* post-flush sampling.

Table D1 Interpretation of pre- and post-flush counts

High *P. aeruginosa* pre-flush count (>10 cfu/100 mL) and low post-flush count (<10 cfu/100 mL)	Suggestive of a local water outlet problem
High *P. aeruginosa* pre-flush count (>10 cfu/100 mL) and high post-flush count (>10 cfu/100 mL)	Suggestive of a problem not related to a local water outlet but to a wider problem within the water supply system

Note

Overlaying sample results onto schematic drawings of the system may help to identify the source of contamination and locations for additional sampling.

D20. If the sampling indicates that the water services are the problem, then most outlets would possibly be positive and other points in the water system could then be sampled to assess the extent of the problem (see Table D1).

D21. Figure D2 provides a summary of the sampling procedure and interpretation of results for *P. aeruginosa*.

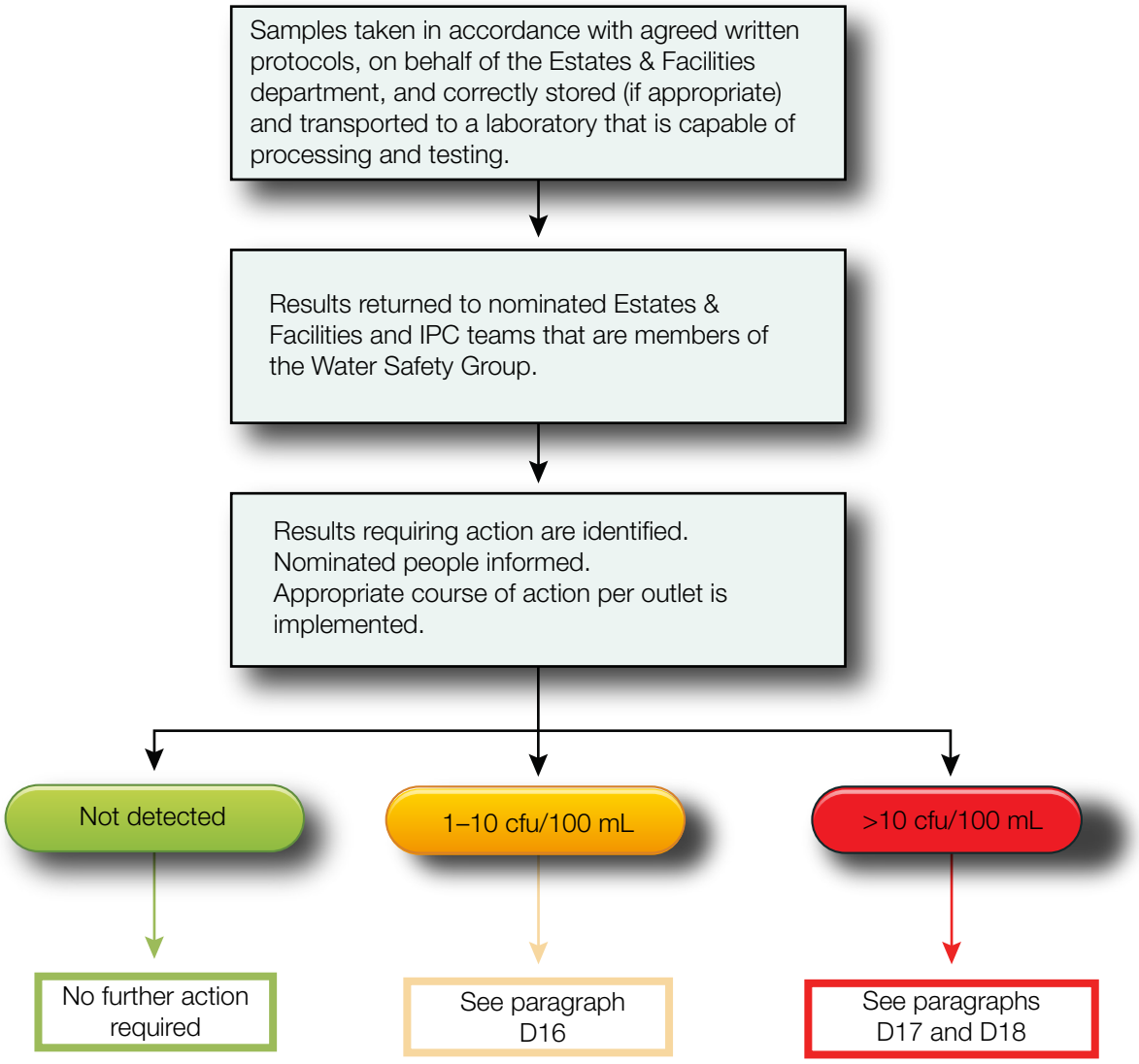


Figure D2 Summary of sampling procedure and interpretation of results for *P. aeruginosa*

What to do if a contamination problem is identified

D22. Should risk assessment or water testing identify contamination with *P. aeruginosa*, the following risk reduction and preventive measures should be considered.

a. Inform the WSG and hold a focused incident control meeting (for example, IPC team, estates and clinical staff) to ensure patient safety is prioritised and to formulate an action plan.

b. If a water outlet has been taken out of service because of contamination with *P. aeruginosa*, continue daily flushing while the outlet is out of normal use to prevent water stagnation and exacerbation of the contamination.

c. Where practical, consider removal of flow straighteners. However, the removal of flow straighteners may result in splashing and therefore additional remedial action may need to be taken. If they are seen to be needed, periodically remove them and either clean/disinfect or replace them. Replacement frequency should be verified by sampling/swabbing.

d. Splashing can promote dissemination of organisms, resulting in basin outlets becoming heavily contaminated. If splashing is found to be a problem, investigate the causes. Example causes include:

   i. the tap's designed flow profile is incompatible with the basin;

   ii. the tap discharges directly into the waste aperture;

   iii. incorrect height between tap outlet and surface of the basin;

   iv. excess water pressure;

   v. a blocked or malfunctioning flow straightener.

e. Hand-washing should be supplemented with the use of an antimicrobial hand-rub.

f. To prevent water stagnation, check for infrequently used outlets – assess frequency of usage and if necessary remove infrequently used outlet(s). For example, the provision of showers in areas where patients are predominantly confined to bed, and the resultant lack of use, could lead to stagnation.

g. Check connections to mixing taps to ensure that the supply to the hot connection is not supplied from an upstream TMV. In a hot water service, a dead-leg will exist between the circulating pipework and hot connection of a fitting such as a mixing tap. In the case of cold water services, sometimes there will be no draw-off from any part of the system and the entire service is in effect a dead-leg. To minimise the stagnation of water in a cold water system, it can be beneficial to arrange the pipework run so that it ends at a frequently used outlet. A dead-leg may also exist when a TMV is installed upstream of a mixing tap (see Figure D3). Depending on the activities of the room in which the tap is located, cold water may never be drawn through the pipe between the cold water connections of the mixing valve and mixing tap.

h. Risk-assess the water system for redundant pipework and dead-legs (for example, where water is supplied to both the cold water outlet and a TMV supplying an adjacent blended water outlet, as such cold water outlets in augmented care units may be infrequently used). When removing outlets, the branch hot and cold water pipes should also be cut back to the main distribution pipework in order to eliminate redundant pipework.

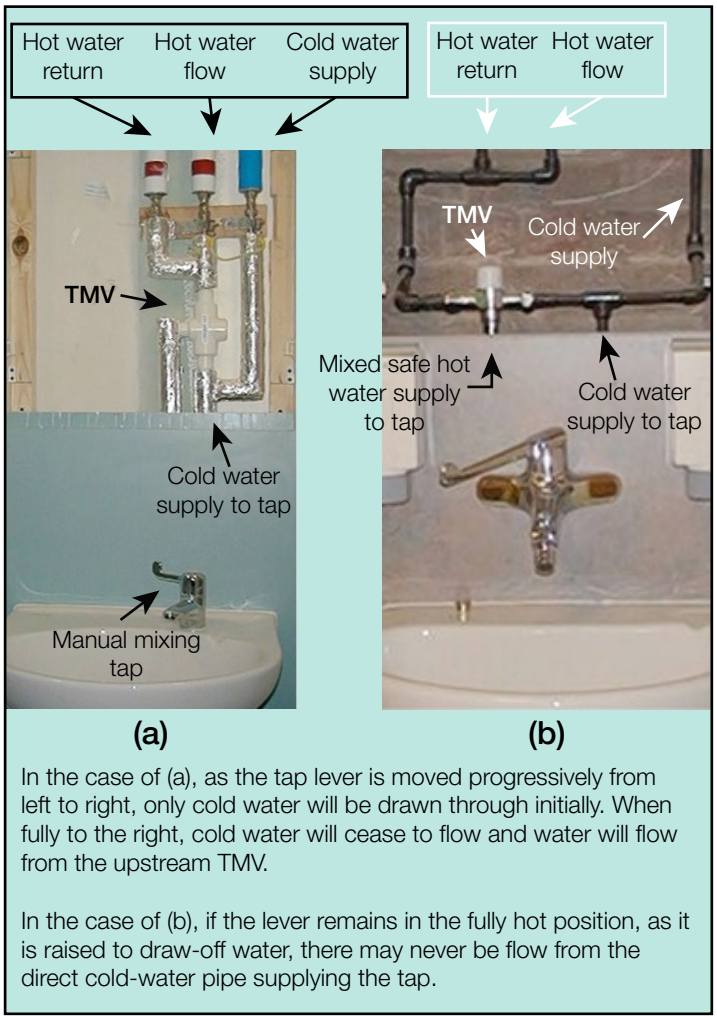


Figure D3 Dead-leg formed by the cold pipework when a TMV is installed upstream of a mixing tap

i. Assess the water distribution system for non-metallic materials that may be used in items such as inline valves, test points and flexible hoses. They should be replaced according to the guidance in safety alert DH (2010) 03 – 'Flexible water supply hoses'.

j. All materials in contact with water should have been assessed and shown they are appropriate for the intended purpose (see paragraph 1.15 in HTM 04-01 Part A) and should not leach chemicals that provide nutrients that support microbiological growth. Materials should also be compatible with the physical and chemical characteristics of water supplied to the building. Flexible pipes should only be used in exceptional circumstances (for example, where height adjustment is necessary as in installations such as rise-and-fall baths and hand-held showers).

k. POU filters, where they can be fitted, may be used to provide water free of *P. aeruginosa*. Where fitted, regard filters primarily as a temporary control measure until a permanent solution is developed, although long-term use of such filters may be required in some healthcare applications. Where POU filters are fitted to taps, follow the manufacturer's recommendations for renewal and replacement and note that the outer casing of a POU filter and the inner surface can become contaminated (see also paragraphs 7.45–7.49). There should be sufficient activity space once a POU filter has been fitted.

l. In certain circumstances, the WSG may decide it is necessary to carry out a disinfection of the hot and cold water distribution systems that supply the unit to ensure that contaminated outlets are treated. See chapter 2 of HSG274 Part 2 for guidance on how to carry out the disinfection procedure. Note that with respect to *P. aeruginosa*, hyperchlorination is not effective against established biofilms. Consider replacing contaminated taps with new taps; however, there is currently a lack of scientific evidence to suggest that this will provide a long-term solution. When replacing taps, consider fitting:

   i. removable taps;

   ii. taps that are easy to use;

   iii. taps that can be readily dismantled for cleaning and disinfection;

   iv. taps to which a filter can be attached to the spout outlet. Note: Such taps can be used for supplying water for cleaning incubators and other clinical equipment.

Note

In the event of an outbreak or incident, further advice on the management of *P. aeruginosa* contamination in water systems can be sought from PHE.

Appendix E Water sampling for *P. aeruginosa*

Note

These sampling methods will also be applicable if sampling for *Stenotrophomonas maltophilia* and *Mycobacterium* spp.

Click here for an online video showing methods for obtaining water samples.

E1. Sampling should be undertaken by staff trained in the appropriate technique for taking water samples including the use of aseptic technique to minimise extraneous contamination. The method used in this guidance may differ from the collection of water samples for other purposes (for example, for sampling *Legionella*, which should be carried out to BS 7592).

E2. Carefully label samples with the type of samples (pre- or post-flush) and outlet such that the outlet can be clearly identified; system schematics indicating each numbered outlet to be sampled can be helpful in this respect.

E3. The main strategy for sampling is to take the first sample of water (pre-flush) delivered from a tap at a time of no use (at least two hours or preferably longer) or, if that is not possible, during a time of its lowest usage. This will normally mean sampling in the early morning, although a variety of use patterns may need to be taken into account.

E4. Disinfectants in the water, such as chlorine or chlorine dioxide, will have residual activity after taking the sample and may inactivate bacteria in the sample prior to its processing and therefore the WSG should take advice from the receiving laboratory. To preserve the microbial content of the sample, neutralise oxidising biocides by dosing the sample bottle with 18 mg of sodium thiosulphate (equating to 18 mg/L in the final sample, which will neutralise up to 5 mg/L of free chlorine). It should be further noted that dosing a 1 L sample bottle with 180 mg of sodium thiosulphate (equating to 180 mg/L in the final sample) will neutralise up to 50 mg/L free chlorine or will neutralise up to at least 80 µg/L silver and 600 µg/L copper). Sterile bottles are normally purchased containing the neutraliser. However, EDTA has been found to be totally ineffective as a neutraliser for silver and therefore should not be used as such in any bacterial test including *P. aeruginosa* and *Legionella* spp. Where other disinfectants are being applied to the water system, take advice on the appropriate neutralisers to use.

E5. The tap should not be disinfected by heat or chemicals before pre-flush sampling (see paragraph E9) nor should it be deliberately cleaned or disinfected immediately before sampling.

E6. Label a sterile collection vessel (200–1000 mL volume) containing a suitable neutraliser for any biocide the water may contain. The labelling information should contain details of the tap location, sender's reference, pre- or post-flush (see paragraph E12), person sampling, date and time of sampling.

E7. If *P. aeruginosa* has been found in a preflush sample, take a second paired set of samples. The first would be a pre-flush sample as before. Run the tap for two minutes and take a second identical post-flush sample. Bacteria in this second sample (termed postflush) are more likely to originate further back in the water system. A substantially higher bacterial count in the pre-flush sample, compared with the post-flush, should direct remedial measures towards the tap and associated pipework and fittings near to that outlet. A similar bacterial count in pre-

flush and post-flush samples indicates that attention should focus on the whole water supply, storage and distribution system. A more extensive sampling regime should be considered throughout the water distribution system, particularly if that result is obtained from a number of outlets.

E8. Although water sampling is the principal means of sampling, there may be occasions when water samples cannot be obtained immediately for analysis. In the event of a suspected outbreak, swabbing water outlets (as per section 5.4 of the Standing Committee of Analysts' (SCA) 'Microbiology of drinking water – Part 2: practices and procedures for sampling') to obtain strains for typing may provide a means of assessing a water outlet, but this does not replace water sampling (see paragraph E15 on swabbing).

Procedure for obtaining the samples

E9. Pre-flush sample: Aseptically (that is, without touching the screw thread, inside of the cap or inside of the collection vessel) collect at least 200 mL water in a sterile collection vessel containing neutraliser. Replace the cap and invert or shake to mix the neutraliser with the collected water.

E10. Depending on the water distribution system design and the type of water outlet, the water feed to the outlet may be provided by:

- a separate cold water supply and hot water supply to separate outlets;
- a separate cold water supply and hot water supply, which may have its final temperature controlled by the use of an integral TMV within the outlet; or
- a separate cold water and a pre-blended hot water supply that has had its temperature reduced by a TMV prior to delivery to the outlet.

Collect at least 200 mL water in a sterile collection vessel

E11. For separate hot and cold water outlets, each outlet is individually tested with its own collection vessel and outlet identifier. For blended outlets (that is, where both hot and cold water come out of the same outlet):

- sample water with the mixing tap set to the fully cold position using an individual collection vessel and outlet identifier, and note the temperature setting;
- sample the blended outlet set to the maximum available hot water temperature using an individual collection vessel and outlet identifier, and note the temperature setting.

E12. Post-flush sample: where this is required, allow the water to flow from the tap for two minutes (see above) before collecting at least 200 mL water in a sterile collection vessel with neutraliser. Replace the cap and invert or

shake to mix the neutraliser with the collected water. This sample, when taken together with the pre-flush sample, will indicate whether the tap outlet and its associated components are contaminated or if the contamination is remote from the point of delivery (see Table D1 in Appendix D).

E13. If a sample from a shower is required, then place a sterile bag over the outlet. Using sterile scissors, cut a small section off the corner and collect the sample in a sampling container (see PHE's (2013) 'Guidelines for the collection, microbiological examination and interpretation of results from food, water and environmental samples taken from the healthcare environment'). Appropriate precautions should be taken to minimise aerosol production as described in BS 7592.

E14. The collected water should be processed within two hours. If that is not possible, then it should be refrigerated within two hours, kept at 2–8ºC and processed within 24 hours.

E15. To take a swab sample, remove a sterile swab from its container and insert the tip into the nozzle of the tap or other designated area. Care should be taken to ensure no other surfaces come into contact with the tip of the swab. Rub the swab around – that is, move it backwards and forwards and up and down, as much as possible, on the inside surface of the tap outlet or flow straightener (see photograph). Replace the swab carefully in its container, again ensuring no other surfaces come into contact with the tip of the swab. Place the swab in a transport medium or maximum recovery diluent (MRD) and send to the laboratory.

A sterile swab should be rubbed on the inside surface of the tap outlet or flow straightener

Appendix F Microbiological examination of water samples for *P. aeruginosa*

Notes

This appendix has been developed to provide technical guidance for a range of laboratories (including NHS, PHE and commercial laboratories) that have the capability and capacity to undertake water sampling and testing.

Alternative water-testing methods other than filtration methods may be used as long as they have been validated using BS EN ISO 17994 and meet the required sensitivity and specificity such as those identified in BS EN ISO 16266.

An oxidase test alone is not sufficiently specific to identify *P. aeruginosa*.

Methods approved by the Standing Committee of Analysts and listed in the 'Microbiology of drinking water – Part 8: the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa'* are allowed to be used.

Definition

F1. *P. aeruginosa* are Gram-negative, oxidase-positive bacteria that, in the context of this method, grow on selective media containing cetrimide (cetyl trimethylammonium bromide), usually produce pyocyanin, fluoresce under ultraviolet light 360 ± 20 nm, and hydrolyse casein. *P. aeruginosa* needs to be identified by the following methods – identification by a positive oxidase test alone is insufficient.

Testing principle

F2. A measured volume of the sample or a dilution of the sample is filtered through a membrane filter (≤0.45 µm) to retain bacteria and the filter is then placed on a solid selective and differential medium.

F3. CN agar contains cetyl trimethylammonium bromide and nalidixic acid at concentrations that will inhibit the growth of bacteria other than *P. aeruginosa*. Other selective and differential agars are available and acceptable if validated.

F4. The membrane is incubated on a selective/differential agar and characteristic colonies are counted. Confirmatory tests are carried out where necessary (see paragraph F15) and the result is calculated as the colony count per 100 mL of water.

F5. *P. aeruginosa* usually produces characteristic blue-green or brown colonies when incubated at 37°C for up to 48 hours. Confirmation of isolates is by subculture to milk agar supplemented with cetyl trimethylammonium bromide (commercially available) to demonstrate hydrolysis of casein.

Sample preparation and dilutions

F6. Water samples should be received and handled as described in the SCAs' 'The microbiology of drinking water Part 8' (currently under review). For example, samples should be examined as soon as is practicable on the day of collection. In exceptional circumstances, if there is a delay, store at 2–8°C and do not exceed 24 hours before the commencement of analysis.

Filtration and incubation

F7. Aseptically measure and dispense 100 mL of water sample into the sterile filter-holder funnel. If the funnel is graduated to indicate volume, this can also serve to measure the volume.

F8. If high bacterial numbers are present in water samples, it may be impossible to count individual colonies accurately on the filter membrane. Therefore, if high counts are expected, a range of dilutions made in sterile diluent (water, MRD or similar) can be processed in parallel with the undiluted sample. An example of this would be a 1-in-10 and a 1-in-100 dilution processed as well as an undiluted sample. Filtration of 10 mL rather than 100 mL is an alternative to filtering 100 mL of a 1-in-10 solution.

F9. Draw the water sample through the filter.

F10. Aseptically place the membrane onto the *P. aeruginosa* selective and differential agar (see paragraph F3) and incubate aerobically at 37°C.

Counting of colonies

F11. Examine plates after 22 hours ± 4 hours and 44 hours ± 4 hours of incubation.

F12. Count all colonies that produce a green-blue (demonstrating pyocyanin production) or reddish-brown pigment and those which fluoresce under ultraviolet light (optional). Exposure of colonies to daylight for two-to-four hours enhances pigment production. When there is a moderately heavy growth of *P. aeruginosa* and other organisms on the membrane, colonies adjacent to pyocyanin-producing colonies of *P. aeruginosa* can also appear green after 44 hours ± 4 hours of incubation, making the interpretation of the count difficult. Observing the plates after 22 hours ± 4 hours assists in the interpretation in these instances.

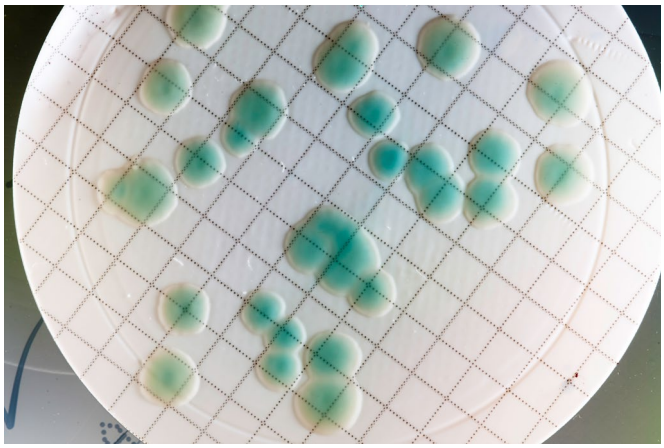


Plate showing raised counts of pyocyanin-producing colonies of *P. aeruginosa*

Processing of swabs

F13. Swabs can show presence of *P. aeruginosa* but will not provide equivalent quantitative results as water sampling. They can be used to show the presence or absence of *P. aeruginosa* at the outlet.

F14. In the laboratory, use the swab to inoculate a portion of an agar plate that is selective and differential for *P. aeruginosa* (see paragraphs F2 and F3). Streak the inoculum on the plate as for a clinical sample. Incubate as described for filter samples above. Alternatively, after sampling, place the swab in 10 mL MRD, vortex, then plate out (using serial dilution) on the appropriate media and incubate as above.

Confirmatory tests

F15. Colonies that clearly produce pyocyanin (green-blue pigmented) on the membrane are considered to be *P. aeruginosa* and require no further testing. Other colonies that fluoresce or are reddish-brown require confirmation. If more than one volume or dilution has been filtered, proceed if possible with the membrane yielding 20–80 colonies to enable optimum identification and accurate enumeration of colonies. Where there is doubt, perform additional tests to yield reliable species identification.

F16. To confirm other colonies, subculture from the membrane onto a milk cetrimide agar (MCA) plate and incubate at 37°C for 22 hours ± 4 hours. Examine the plates for growth, pigment, fluorescence and casein hydrolysis (clearing medium's opacity around the colonies). If pigment production is poor, expose the MCA to daylight at room temperature for two-to-four hours to enhance pigment production and re-examine.

F17. *P. aeruginosa* is oxidase-positive, hydrolyses casein and produces pyocyanin and/or fluorescence. Occasionally atypical non-pigmented variants of *P. aeruginosa* occur. A pyocyanin-negative, casein-hydrolysis-positive, fluorescence-positive culture should be regarded as *P. aeruginosa*. Additional tests may be necessary to differentiate non-pigmented *P. aeruginosa* from *P. fluorescens* (such as growth at 42°C or resistance to C-390, 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan or phenanthroline or more extensive biochemical tests). See Table F1.

Retention of *P. aeruginosa* isolates

F18. Where a clinical investigation is underway, inform the testing laboratory that the *P. aeruginosa* isolates and associated sampling location information should be retained for a minimum of three months as they may be required for typing at a later date.

F19. It will then be the responsibility of the testing laboratory to ensure that these isolates are supplied to the typing laboratory (for example, PHE at Colindale) when requested, and this should be written into the contract for testing.

Calculation of results

F20. Express the results as colonies of *P. aeruginosa* per 100 mL of the undiluted sample, for example:

- for 100 mL sample – the count on the membrane;
- for 10 mL of sample – the count on the membrane multiplied by 10;
- for 1 mL of sample – the count on the membrane multiplied by 100.

Reporting

F21. If *P. aeruginosa* is not detected, report as "Not detected in 100 mL".

F22. If the test organism is present, report as the number of *P. aeruginosa* per 100 mL. Reports should be specific to *P. aeruginosa*, and not generic *Pseudomonas* species.

F23. The sample reference originally submitted should be reported with each result.

Table F1

Colony on CN agar	Oxidase test	Fluorescing on MCA	Caseinolytic on MCA	Confirmed *P. aeruginosa*
Blue or green	+	NT	NT	Yes
Fluorescing and not pigmented	+	+	+	Yes
Reddish-brown non-fluorescing	+	+/–	+	Yes
NT = No testing necessary				

Microbiological typing

F24. Water and/or tap-swab isolates being sent to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for molecular analysis of *P. aeruginosa* should only be referred if the isolates have been confirmed to be *P. aeruginosa* and if there is a possible link to the outbreak strain under investigation.

F25. Referrals of *P. aeruginosa* isolates for typing should only be sent after consultation with the typing laboratory.

F26. Where many taps are positive for *P. aeruginosa*, send one colony of the *P. aeruginosa* from each water sample. Save the primary isolation plate for possible further examination once the results of typing are known and have been discussed with the typing laboratory. Analysis of results to date has consistently shown that multiple picks have been representatives of the same strain; since multiple taps are being sampled, an idea of the extent of homogeneity or otherwise will still be gained where only one colony is sent from each water sample.

F27. If only two or three taps are positive for *P. aeruginosa*, then send two separate colony picks of confirmed *P. aeruginosa* from the primary plate per water sample to AMRHAI (taking the stipulations in paragraph F25 into account). Label these clearly as being from the same water sample (so that AMRHAI can accumulate data on how common mixed strains are seen in the same tap water).

F28. It is important that the request forms have information about the links between tap water and cases as illustrated in the following examples:

a. water from tap in room "A" ref patient "X";

b. water from taps in dirty utilities;

c. tap water from room "C" with no cases.

F29. It is important to recognise that there are some types of *P. aeruginosa* that are relatively commonly found in the environment and among patient samples globally. These include the PA14 clone and clone C; a match between patient and water samples with these strains is not necessarily evidence of transmission between the two.

F30. If a contamination problem has been identified, the remediation measures in paragraph D22 of Appendix D should be followed.

Flowchart showing the processing and enumeration of *P. aeruginosa* by membrane filtration

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Guidelines

Prevention and control of multi-drug-resistant Gram-negative bacteria: recommendations from a Joint Working Party

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NICE has accredited the process used by the Healthcare Infection Society to produce its 'Prevention and control of multi-drug-resistant Gram-negative bacteria: recommendations from a Joint Working Party' guidelines. Accreditation is valid for 5 years from March 2015. More information on accreditation can be viewed at http://www.nice.org.uk/about/what-we-do/accreditation

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1. Executive summary

Multi-drug-resistant (MDR) Gram-negative bacterial infections have become prevalent in some European countries. Moreover, increased use of broad-spectrum antimicrobial agents selects organisms with resistance and, by increasing their numbers, increases their chance of spread. This report describes measures that are clinically effective for preventing transmission when used by healthcare workers in acute and primary healthcare premises. Methods for systematic review 1946—2014 were in accordance with SIGN 50[1] and the Cochrane Collaboration;[2] critical appraisal was applied using AGREEII.[3] Accepted guidelines were used as part of the evidence base and to support expert consensus. Questions for review were derived from the Working Party Group, which included patient representatives in accordance with the Patient Intervention Comparison Outcome (PICO) process. Recommendations are made in the following areas: screening, diagnosis and infection control precautions including hand hygiene, single-room accommodation, and environmental screening and cleaning. Recommendations for specific organisms are given where there are species differences. Antibiotic stewardship is covered in a separate publication.

2. Lay summary

MDR Gram-negative bacteria are bacteria (or germs) that are resistant to at least three different antibiotics. These bacteria are commonly found in the gut, where they do no harm; however, they can cause infection at other body sites, mainly in patients who are vulnerable due to other underlying diseases, injury or hospitalization. Infection often happens when the bacteria enter the body through an open wound or via a medical device such as a catheter. Infections caused by MDR Gram-negative bacteria are difficult to treat, and can cause additional pain to patients with slow wound healing and other complications such as pneumonia or infection in the blood. This can prolong the length of stay in hospital and, in some cases, can cause death.

Some types of resistant Gram-negative bacteria can be carried on the skin rather than the gut, again with no obvious signs or symptoms. 'Colonization' describes this carriage of bacteria in the gut, on the skin or in the nose, throat or elsewhere on the body. Although the patients lack symptoms of infection, they may still need to be isolated/segregated and/or other contact precautions may be necessary in order to stop their resistant bacteria spreading to others.

3. Introduction

This guidance has been prepared by the Working Party to provide advice on screening (testing), treatment and precautions needed to prevent the spread of MDR Gram-negative bacteria. The guidance describes appropriate infection prevention and control precautions to include hand hygiene, equipment and environmental cleaning and guidance on screening for MDR Gram-negative bacteria. There is an accompanying guideline describing best practice in antimicrobial prescribing and stewardship which should be used in conjunction with this report.

The Working Party comprises a group of medical microbiologists and scientists, infectious disease physicians, infection control practitioners, epidemiologists and patient representatives. The patient representatives are lay members and have direct experience of the treatment of healthcare-associated infections through personal experience and/or through membership of the Healthcare-acquired Infection Service Users Research Forum, patient charities and/or through involvement in the development of National Institute for Health and Care Excellence (NICE) guidelines.

4. Guideline Development Team

4.1. Guideline Advisory Group

- Martin Kiernan, Nurse Consultant, Prevention and Control of Infection, Southport and Ormskirk NHS Trust, Southport, UK
- Phil Wiffen, Cochrane Pain, Palliative and Supportive Care Group Pain Research, Churchill Hospital Oxford, Nuffield Department of Clinical Neurosciences, Oxford, UK
- Karla Soares-Wieser, Enhance Reviews Ltd, Wantage, UK

4.2. Acknowledgements

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4.3. Source of funding

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4.4. Disclosure of potential conflict of interest

- APRW: Consultant on Drug Safety Monitoring Boards for Roche and Genentech. Advisory panel for 3M.
- DML: Advisory boards or consultancy for Achaogen, Adenium, Alere, Allecra, AstraZeneca, Basilea, Bayer, BioVersys, Cubist, Curetis, Cycle, Discuva, Forest, GSK, Longitude, Meiji, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx and Wockhardt. Paid lectures for AOP Orphan, AstraZeneca, Bruker, Curetis, Merck, Pfizer and Leo. Relevant shareholdings in Dechra, GSK, Merck, Perkin Elmer and Pfizer (collectively amounting to $<$10% of portfolio value).
- JAO: Part-time employment at Bioquell during the production of these guidelines. Paid lectures for 3M. Research funding from Pfizer and the Guy's and St Thomas' Charity.
- PJ: Advisory board for Baxter.
- DAE: ECCMID conference attendance funded by Astellas and Eumedica.
- BO: Advisory board for Astellas and Forest. Lecture for Alere.
- CM: Travel expenses funded by Mérieux Diagnostics.
- MC: IPS conference attendance funded by corporate sponsorship from Mölnlycke Healthcare.
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- All other authors declared no conflict of interest.

4.5. Relationship of authors with sponsors

BSAC, BIA and HIS commissioned the authors to undertake the Working Party Report. IPS provided additional panel members and financial support for them to attend meetings. The authors are members of these societies.

4.6. Responsibility for guidelines

The views expressed in this publication are those of the authors, and have been endorsed by the sponsoring societies following consultation.

5. Working Party Report

Date of publication: January 2016 (published online 16th November 2015)

5.1. What is the Working Party Report?

This report is a set of recommendations covering prevention of transmission of MDR Gram-negative bacteria (i.e. resistant to at least three different antibiotics).

Antimicrobial chemotherapy and stewardship are covered in a separate publication.

The Working Party recommendations have been developed systematically through a multi-professional group based on published evidence. They should be used in the development of local protocols for all acute and long-term healthcare settings.

5.2. Why do we need a Working Party Report for these infections?

Colonization and infection by MDR Gram-negative bacteria have become prevalent in some European countries. Heavy use of broad-spectrum agents selects for organisms with resistance, and increases their chance of spread. National antibiotic consumption is increasing in the UK (6% increase in 2013 compared with 2010).[4] The spread of these infections risks increasing the length of hospital stay, and has an adverse effect on the quality of care of patients. Public awareness of resistance and healthcare-associated infections is increasing, and the paucity of new antimicrobial agents to treat these infections has resulted in the formulation of the five-year Antimicrobial Resistance Strategy by the Department of Health for England to address the problem. When outbreaks of infection involving MDR strains occur, there is a considerable financial, physical and psychological cost. Unless controlled, outbreaks are likely to become more common and MDR strains will become endemic. Evidence-based infection prevention and associated quality improvement methods are effective in reducing the number of infections with these organisms.

5.3. What is the purpose of the Working Party Report's recommendations?

This report describes measures that are clinically effective for preventing infections when used by healthcare workers in acute and long-term health care.

5.4. What is the scope of the guidelines?

Two sets of guidelines have been developed. This report includes appropriate infection prevention and control precautions. The other report describes best-practice antimicrobial prescribing and stewardship.[5]

5.5. What is the evidence for these guidelines?

In the preparation of these recommendations, systematic reviews of peer-reviewed research were undertaken. Expert opinion was also derived from published guidelines subjected to validated appraisal.[3] Evidence was assessed for methodological quality and clinical applicability according to the protocols of the Scottish Intercollegiate Guidelines Network (SIGN).

5.6. Who developed these guidelines?

A group of medical microbiologists, scientists, infectious disease physicians, infection control practitioners, epidemiologists and patient representatives.

5.7. Who are these guidelines for?

Any healthcare practitioner can use these guidelines and adapt them for local use. Users are anticipated to include clinical staff (i.e. medical, nursing and paramedical staff) as well as healthcare infection prevention and control teams. The guidelines should be used to improve practice of infection prevention, and to help patients and their carers to understand the methods available to prevent acquisition of antibiotic-resistant bacteria.

5.8. How are the guidelines structured?

Each section comprises an introduction, a summary of the evidence base with levels, and a recommendation graded according to the available evidence.

5.9. How frequently are the guidelines reviewed and updated?

The guidelines will be reviewed at least every four years and updated if change(s) in the evidence are sufficient to require a change in practice.

5.10. Aim

The primary aim of this report was to assess the current evidence for prevention and control of MDR Gram-negative infections.

6. Summary of guidelines

The guidelines relate to MDR Gram-negative bacteria and have been derived from current best peer-reviewed publications and expert opinion. Table IV contains expert opinion. Each recommendation is associated with a class of supporting evidence, as follows.

6.1. Surveillance

1,2. The minimum susceptibility tests performed on all significant Gram-negative isolates should include meropenem; in addition, cefpodoxime should be tested for Enterobacteriaceae, and ceftazidime should be tested for *Pseudomonas* spp. Strong

3. Travel history (i.e. countries or known endemic areas visited within previous year) should be collected for all patients with carbapenemase-producing Gram-negative bacteria. Strong

4. Each healthcare organization should have access to robust microbiological arrangements for detecting and reporting all MDR Gram-negative organisms in routine clinical samples and for screening using highly-sensitive tests with a diagnostic turnaround time of $<$48 h. Conditional

6.2. Screening

5. Active screening rather than passive surveillance is recommended for high-risk specialties. Conditional
6. Patients at high risk of colonization or infection with carbapenem-resistant organisms include those admitted to intensive care units (ICUs) and from long-term care facilities (e.g. care homes). Conditional
7. Screening for rectal and wound carriage of carbapenemase-producing Enterobacteriaceae should be undertaken in patients at risk. Strong
8. All patients transferred from, or with a history of admission to, healthcare facilities with known endemic carbapenemase-producing Enterobacteriaceae in the preceding year should be screened. Strong
9. Screening for carbapenem-resistant *Acinetobacter baumannii* and MDR *Pseudomonas aeruginosa* is required in the management of outbreaks. Strong
10. A rectal swab (with visible material) or stool sample (and urine sample if catheter present) should be used for screening for MDR Enterobacteriaceae and *P. aeruginosa*. For *Acinetobacter* spp., skin sites should be sampled, or, if a catheter or endotracheal tube is present, urine or respiratory secretions should be sampled. Conditional
11. In the event of secondary cases of carbapenem-resistant Enterobacteriaceae, standard infection control precautions (SICPs) and contact precautions should be monitored and re-inforced among clinical staff. Screening of patients not identified as carriers should be repeated weekly and on discharge from affected units until no new cases are identified for more than seven days. Strong
12. Patients with previous samples with carbapenem-resistant or other MDR Gram-negative bacteria should be screened at the time of admission. Conditional

6.3. Prevention of transmission

13. In addition to SICPs, apply contact precautions for those patients who present an infection risk. Strong
14. Where possible, single-room isolation should be provided for patients with MDR Gram-negative bacterial infection/colonization, and contact precautions should be continued for the duration of their stay. Conditional
15. Use disposable gloves and gowns or aprons to care for patients with MDR Gram-negative bacteria: *A. baumannii*, carbapenem-resistant and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, *P. aeruginosa*. Strong
16. Identify and place infected and colonized patients in single rooms where available in this order of priority: carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *A. baumannii*, ESBL-producing *Klebsiella* spp., carbapenemase-producing *P. aeruginosa*, ESBL-producing *Escherichia coli* and other Enterobacteriaceae, AmpC Enterobacteriaceae. Strong
17. If insufficient rooms are available, cohort patients following local risk assessment. Conditional
18. Hand hygiene is required before and after direct patient contact; after contact with body fluids, mucous membranes and non-intact skin; after contact with the immediate patient environment; and immediately after the removal of gloves. Strong

6.4. Cleaning and environment

19. Environmental screening should be considered where there is unexplained transmission of MDR Gram-negative organisms or a possible common source for an outbreak. Strong
20. Respiratory and other contaminated equipment should be decontaminated (or respiratory secretions discarded) away from the immediate bed area in designated cleaning sinks and not in handwash sinks. Strong
21. For *P. aeruginosa*, including MDR strains, at a minimum, in accordance with the organization's water safety plan, a risk assessment should be made when levels of patient colonization or infection rise in order to determine if point-of-use filters should be installed or if taps need to be changed. Strong
22. Terminal disinfection of vacated areas with hypochlorite should be used in the control of outbreaks of infection due to MDR Gram-negative bacteria. Conditional
23. Hydrogen peroxide vapour should be considered as an adjunctive measure following cleaning of vacated isolation rooms/areas. Conditional
24. The routine use of selective decontamination of the mouth or digestive tract is not recommended for control of MDR Gram-negative bacteria. Conditional

6.5. Miscellaneous

25. Monitor hand hygiene of all staff when patient cohorting is being applied. Strong

7. Implementation of these guidelines

7.1. How can the guidelines be used to improve clinical effectiveness?

The guidelines can be used to inform local infection prevention and control guidance, and to direct clinical decision-making. They provide a framework for clinical audit tools aiming to achieve quality improvement.

7.2. How much will implementation of the guidelines cost?

In most areas, there are no anticipated additional costs unless existing practice falls well below currently-accepted best practice. Failure to implement the recommendations would result in greater costs in terms of economics and quality of life. Screening and isolation will result in significant cost pressures where they are not currently practised, but these costs are set against reduced transmission and fewer cases needing antibiotic treatment. Prolonged isolation can have adverse effects on a patient's psychological health, so may have additional unexpected costs.

7.3. Summary of audit measures

The following are expressed as percentage compliance:

- All Gram-negative isolates requiring antibiotic treatment are to be tested for susceptibility to meropenem (or all blood isolates should be tested).
- The microbiology laboratory reports all patients infected or colonized with carbapenemase-producing Gram-negative bacteria to Public Health England (PHE) or an equivalent body.
- All patients colonized or infected with carbapenem-resistant Enterobacteriaceae and *A. baumannii* are placed under contact precautions within 6 h of identification.
- All patients colonized or infected with carbapenem-resistant Enterobacteriaceae and *A. baumannii* are placed under contact precautions in a single room or cohort for the duration of their stay.
- Travel history is obtained at the time of admission for all acute hospital patients, and patients from endemic areas are screened.

7.4. E-learning tools

Continuing Professional Development questions and model answers are listed for self-assessment in Appendix 4.

8. Methodology

8.1. Evidence appraisal

Methods were in accordance with SIGN 50[1] and the Cochrane Collaboration,[2] and critical appraisal was applied using AGREEII.[3] Accepted guidelines were used as part of the evidence base and to support expert consensus. Questions for review were derived from the Working Party Group, which included patient representatives in accordance with the PICO process.[1]

K. Soares-Wiesner of Enhance Reviews Ltd and Dr P. Wiffen of Pain Research and Nuffield Department of Clinical Neurosciences, Oxford University used a systematic review process. Guidelines and research studies were identified for each search question. Systematic reviews, randomized controlled trials and observational studies were included and assessed by two reviewers. In context, observational studies included non-randomized controlled studies, controlled before–after studies and interrupted time series.

All languages were searched. Search strategies for each area are given in the sections below. MeSH headings and free-text terms were used in the Cochrane Library (Issue 11 2012), Medline (1946–2012), Embase (1980–2012) and Cumulated Index of Nursing and Allied Health Literature (CINAHL) (1984–2012). On 23rd May 2014, an update search was conducted on Medline alone using the same strategy for references after 1st January 2013. Reference lists of included studies were searched. Two review authors independently screened all citations and abstracts identified, and screened full reports of potentially eligible studies (those that addressed review questions in primary or systematic secondary research or a clinical or in-use study). Disagreements were resolved by discussion, and rationales for exclusion of studies were documented. Pretested data extraction forms were used, and study characteristics and results were collected. Data were extracted from observational studies for multiple-effect estimates: number of patients, adjusted and unadjusted effect estimates with standard error or 95% confidence interval (CI), confounding variables and methods used to adjust the analysis. If available, data were extracted from contingency tables. Risk of bias was assessed using SIGN critical appraisal checklists. Interrupted time series were assessed using the Cochrane Effective Practice and Organisation of Care (EPOC) Group.[1,6] Quality was judged by reported details of protection against secular changes (intervention independent of other changes) and detection bias (blinded assessment of primary outcomes and completeness of data). For outbreak patterns associated with particular pathogens, the Working Party made additional searches of descriptive studies to extract effective interventions.

Clinical outcomes were mortality, effectiveness of treatment and length of hospital stay. Microbial outcome measures were decreases in the prevalence of multi-drug resistance among Gram-negative bacteria, or decreases in colonization or infection by specific Gram-negative pathogens. Risk ratios (RR) were used for dichotomous variables, and mean differences with 95% CI were used for continuous outcomes.[7] Analyses were performed using Revman 5.2.[8] SIGN summary tables were used.

Evidence tables and judgement reports were presented and discussed by the Working Party, and guidelines were prepared according to the nature and applicability of the evidence, patient preference and acceptability, and likely costs. The strength of evidence was defined by SIGN (Table I), and the strength of recommendation was adopted from GRADE (Grading of Recommendations Assessment, Development and Evaluation) (Table II). The grading relates to the strength of the supporting evidence and predictive power of the study designs, rather than the importance of the recommendation. Any disagreements between members were resolved by discussion. For some areas, only expert opinion is available; in such cases, a good practice recommendation has been made.

8.2. Consultation process

On completion, these guidelines were opened to consultation with the stakeholders listed in Appendix 1. The draft report was placed on the HIS website for one month. Views were invited on format, content, local applicability, patient acceptability and recommendations. The Working Party considered and collated comments, and agreed revisions.

9. Rationale for recommendations

9.1. Epidemiology

9.1.1. What is the definition of multi-drug-resistant Gram-negative bacteria?

For the purposes of this guideline, MDR Gram-negative bacteria were defined as having three or more antimicrobial

Table I
Levels of evidence for intervention studies[1,6]

1++	High-quality meta-analyses, systematic reviews of RCTs or RCTs with a very low risk of bias.
1 +	Well-conducted meta-analyses, systematic reviews or RCTs with a low risk of bias.
1 −	Meta-analyses, systematic reviews or RCTs with a high risk of bias.[a]
2++	High-quality systematic reviews of case–control or cohort studies. High-quality case–control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal. Interrupted time series with a control group: (i) there is a clearly defined point in time when the intervention occurred; and (ii) at least three data points before and three data points after the intervention.
2+	Well-conducted case–control or cohort studies with a low risk of confounding or bias, and a moderate probability that the relationship is causal. Controlled before–after studies with two or more intervention and control sites.
2−	Case–control or cohort studies with a high risk of confounding or bias and a significant risk that the relationship is not causal. Interrupted time series without a parallel control group: (i) there is a clearly defined point in time when the intervention occurred; and (ii) at least three data points before and three data points after the intervention. Controlled before–after studies with one intervention and one control site.
3	Non-analytic studies (e.g. uncontrolled before–after studies, case reports, case series).
4	Expert opinion. Legislation.

RCT, randomized controlled trial.
[a] Studies with an evidence level of '1−' and '2−' should not be used as a basis for making a recommendation.

Table II
Recommendation grading[1]

	Recommendation
Undesirable consequences clearly outweigh desirable consequences	Strong recommendation against
Undesirable consequences probably outweigh desirable consequences	Conditional recommendation against
Balance between desirable and undesirable consequences is closely balanced or uncertain	Recommendation for research and possibly conditional recommendation for use restricted to trials
Desirable consequences probably outweigh undesirable consequences	Conditional recommendation for
Desirable consequences clearly outweigh undesirable consequences	Strong recommendation for

resistance mechanisms affecting different antibiotic classes. For a full discussion of the definitions in use, please refer to the companion paper.[5]

9.1.2. Which Gram-negative bacteria cause infection control problems?

Opportunistic Gram-negative bacteria that present increasing resistance issues include Enterobacteriaceae (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., Proteeae) and the non-fermenters, *P. aeruginosa* and *A. baumannii*. *Stenotrophomonas maltophilia* is inherently MDR in most cases, but a less common cause of cross-infection. Gonococci are Gram-negative bacteria and are increasingly resistant, but were excluded because relevant public health control actions are substantially different.

In this report, emphasis is placed on strains resistant to β-lactams, including carbapenems, cephalosporins and β-lactamase inhibitor combinations, and strains resistant to fluoroquinolones insofar as these are the core components of most therapies for severe infections. Aminoglycosides are most often used as adjuncts to β-lactam therapy in severe infection, whereas polymyxins are mainly used in cases where β-lactams cannot be used due to resistance. Resistance to these latter groups of agents should nevertheless prompt concern, especially where it is coupled with resistance to multiple β-lactams, as is often the case. Means of infection control remain the same irrespective of the specific resistance.

9.1.3. What are the relative contributions of community and hospital acquisition?

The mechanisms and time course of resistance accumulation by Gram-negative opportunists, both internationally and in the UK, are reviewed in a companion paper.[5] This introduction, rather, is concerned with the distribution of these resistance types in hospitals, long-term care facilities and the community. The distinction between these sectors is increasingly blurred, with many elderly patients moving back and forth between hospital and care homes,[9] and with hospital stays becoming shorter, so that healthcare-associated infections often become apparent after hospital discharge[10] or on re-admission. Consequently, MDR Gram-negative bacteria — including those producing carbapenemases — are

increasingly seen in general practice specimens, principally urine samples. Careful enquiry often reveals that the patient recently received secondary care. The period of time that may elapse from acquisition in hospital, often in colonization sites, to the development of an obvious infection in the community is variable, and different papers use different intervals when classifying infection diagnosed in the community as 'healthcare-associated'. Intervals of one to three months are commonly used to distinguish community acquisition from that acquired during hospital admission, but the literature shows that carriage, and the potential for infection, can persist for much longer periods (commonly one year); it is recommended that this longer period should be used.[11]

9.1.4. What is the evidence for reservoirs and spread of multi-drug-resistant Gram-negative bacteria in care homes and secondary care?

Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp., whether resistant or not, can all be transferred among vulnerable patients by staff vectors and contaminated equipment, leading to well-defined local clonal outbreaks.[12] Both *A. baumannii* and *Klebsiella pneumoniae* (by virtue of their capsules) can survive on dry surfaces, including hands.[13,14] MDR Enterobacteriaceae can colonize the gut, providing — without any symptoms — a reservoir for transfer to other body sites where infection may ensue, or transfer to other patients. The risk of transmission is increased if the carrier experiences diarrhoea or incontinence.

In general, and excluding particular high-risk clones discussed below, there is no evidence that MDR strains are more likely to be associated with cross-infection than other strains. Enterobacteriaceae that owe carbapenem resistance to combinations of ESBLs or AmpC β-lactamase activity together with porin loss are often thought to have impaired fitness, and to be less likely to spread among patients than those with carbapenemases, but cross-infection by porin-deficient Enterobacteriaceae has been reported from Italy, Korea and Portugal.[15—17] In a nested case—control study in the USA, mechanical ventilation, pulmonary disease, days of antibiotic treatment and colonization pressure were associated with acquisition of carbapenem-resistant Enterobacteriaceae.[18] Typing of *K. pneumoniae* in this study suggested clonal transmission within and between hospitals.

9.1.4.1. High-risk clones. Bacterial typing has revealed the role of 'high-risk clones' in the international spread of resistance.[19] For example:

- The majority of fluoroquinolone-resistant ESBL-producing *E. coli* causing infection in hospitals and the community belong to sequence type (ST) 131-B2-O25b.[20,21]
- The growing prevalence of *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in hospitals (e.g. in Israel, Italy and the USA) substantially reflects the clonal expansion of ST258 variants with KPC-2 or -3 enzymes.[22]
- In Russia, Belarus and Kazakhstan, there is extensive nosocomial spread of ST235 *P. aeruginosa*, with VIM-2 carbapenemase only susceptible to colistin.[23]

Except in the case of ST131 *E. coli* (where infection may be preceded by a long period of innocuous gut carriage), UK hospitals are minimally affected by these lineages, although both ST258 *K. pneumoniae* and ST235 *P. aeruginosa* have been recorded.[22,24] National clones that have achieved considerable traction in the UK include *A. baumannii* OXA-23 clone 1, recorded at >60 hospitals.[19,25] It remains uncertain whether this prevalence reflects site-to-site transfer via colonized patients, or the selection, at multiple sites, of a pre-existing but previously rare subtype of this very clonal species. Focusing infection control on specific types rather than resistances has not been explored for ESBL-producing *E. coli*.

E. coli ST131 has spread globally, and is transmitted within hospitals, families, through pets and long-term care facilities whilst being very rare in food animals. It is often resistant to fluoroquinolones and multiple other antimicrobials, as well as producing CTX-M ESBLs.[26] The lineage can be distinguished by serotyping and polymerase chain reaction (PCR).[27] Among faeces sent for culture from international travellers returning to the UK, many of which were from the Indian subcontinent, 18% contained ESBL *E. coli*, mainly with CTX-M-15 enzymes, and 2.1% had ST131 strains with ESBL.[28]

9.1.4.2. Plasmid outbreaks. In this situation, a plasmid or family of related plasmids disseminate(s) among strains of one or more species in a locale.[29,30] This is the case, for example, in the current spread of pKpQIL plasmids encoding KPC carbapenemases in and around Manchester.[31] Unlike in a clonal outbreak, the isolates are diverse in terms of species, strain and in their antibiograms, which also reflect the host strain and any other plasmid(s) carried. Single-strain clusters occur within this overall diversity, but do not come to dominate the picture as in a classical single-strain outbreak. It is inferred (although rarely proven) that frequent plasmid transfer among gut bacteria leads to the diversity of strains involved.[32] As there is no single 'outbreak' organism to target this scenario, it is more challenging for infection control teams than a classical outbreak. Moreover, it presents a greater recognition challenge to the microbiology laboratory; consequently, reliable and consistent application of SICPs is extremely important.

9.1.4.3. Outbreaks due to Pseudomonas aeruginosa contamination of water systems. Classical clonal outbreaks of hospital infection have a clear train of transmission if carriage is taken into account, and, assuming consistent application of contact precautions, can be controlled in a relatively short time if the strain(s) are not re-introduced.[12] However, a different epidemiology is seen occasionally, particularly with *P. aeruginosa* (MDR or not), when a single clone or small number of clones causes infections in multiple patients in a unit or hospital, often without obvious links, over a prolonged period, sometimes extending over several years and with gaps of months between cases.[33,34] Such instances often reflect contamination of the hospital plumbing system by the pseudomonas clone(s), and control may require modification/assessment, including, for example, replacing sinks and toilets with easier-to-clean models less prone to splashback, educating staff to reduce blockages and inappropriate storage, reviewing cleaning protocols, and reducing shower flow rates to minimize flooding.[35,36]

9.1.4.4. Long-term care facilities and the spread of multi-drug-resistant Enterobacteriaceae. Long-term care facilities are increasingly identified as reservoirs of antibiotic resistance, particularly for colonization. The data to support this

view are considerable but are not based on systematic surveillance, except in France, where the incidence of ESBL-producing Enterobacteriaceae infection per 1000 days in long-term care facilities increased from 0.07 in 1996 to 0.28 in 2005. This largely reflected the proliferation of *E. coli* with CTX-M enzymes, which were later recognized as representatives of the international ST131 clone.[37,38]

Long-term care facilities range from establishments offering assisted living to largely independent residents through to those providing complex medical support.[39,40] This spectrum of care varies between countries, reflecting healthcare organization and cultural factors.[41–43]

The distribution of incontinent and catheterized residents is likely to influence the transmission of Gram-negative bacteria, including those with multi-drug resistance. Variation may be very local; March *et al.* found that gut carriage of resistant bacteria varied across five subunits in one long-term care facility in Bolzano, Italy.[44] Overall, carriage was higher than in the hospital's geriatric unit, which perhaps had more knowledge and reliable application of infection prevention and control precautions (75% of 111 in long-term care facility vs 22% of 45 in geriatric unit). In contrast, Gruber *et al.* in Germany found higher carriage rates of MDR bacteria in geriatric units (32.6%) than in nursing homes (18.5%) or ambulatory care (15.6%).[45]

While most resistance studies on long-term care facilities relate to those caring for the elderly, spread of carbapenemase producers as gut colonizers has also been recorded in a care home for children and young adults with neurodevelopmental problems.[46]

The literature supporting the view that long-term care facilities constitute a reservoir of multi-drug resistance comprises, firstly, numerous analyses showing that previous stay in a long-term care facility is a risk factor for later infections with MDR Gram-negative bacteria, including those with ESBLs and carbapenemases; and, secondly, multiple snapshot surveys showing frequent (although very variable) gut carriage of ESBL-producing *E. coli* and *Klebsiella* spp. among residents in long-term care facilities, including in Australia, Belgium, France, Germany, Israel, Japan, Malaysia, the UK, Italy and the USA where these enzymes have already proliferated in hospitals.[44,47–51] Accumulation of MDR Gram-negative bacteria in long-term care facilities probably reflects a combination of:

- the frequent transfer into long-term care facilities of patients/residents who were initially colonized or infected in hospitals;
- oro-faecal transfer within long-term care facilities, reflecting breakdowns of personal hygiene in populations with high rates of dementia and incontinence;
- frequent antibiotic use and its contingent selection pressure on the gut flora; and
- high rates of urinary tract catheterization.

Only one sizeable UK study of the carriage of MDR Gram-negative bacteria by nursing home residents has been published.[9] This was conducted in Belfast from 2004 to 2006, early in the national dissemination of *E. coli* with CTX-M ESBLs. This study included 16 long-term care facilities, and found *E. coli* that were both ciprofloxacin-resistant and produced ESBLs in faeces from 119 of 294 residents (40.5%). This was a 40-fold higher carriage rate than for diarrhoeal samples from community patients. Virtually all (99%) of these MDR isolates were ST131 variants; half belonged to the CTX-M-15-positive 'strain A' variant that is common elsewhere in the UK.[52,53] Two small (six- and 12-bed) long-term care facilities had no colonized residents, and others had up to 75% (18/24) colonized residents, with considerable diversity among the ST131 variants at many sites. Fluoroquinolone use and a history of urinary tract infection were independently associated with carriage in a multi-variate model.[9] Duration of nursing home residency did not correlate with increased likelihood of carriage, although it seems likely that carriers commonly acquire their organism within their long-term care facilities.

9.1.5. Multi-drug resistance in the community

Multi-drug resistance remains uncommon among true community-acquired infections in the UK, and few studies have correlated resistance in clinical infections and faecal carriage in these cases. Nevertheless, stool carriage of ESBL-producing faecal *E. coli* was found in 11.3% of patients in Birmingham, rising to 22.8% in those with surnames suggesting a Middle Eastern or South Asian patrimony compared with 8.1% among names suggesting European patrimony. This differential perhaps reflects frequent travel to parts of the world where ESBLs are common outside the hospital setting.[54] A few references specifically indicated travel to South or East Asia as a risk factor for acquisition of ESBL-producing *E. coli* in faeces. Carriage is often persistent and, in Canada, prior travel to a country with a high prevalence of ESBL-producing *E. coli* was identified as a risk factor for subsequent urinary infection with these organisms, typically with the particular ESBL type prevalent in the country visited.[55]

Most cases of infection or colonization by carbapenemase-producing Enterobacteriaceae and non-fermenters occur in hospital and healthcare settings, at least in Europe and North America.[22,56–58] However, in areas of high prevalence, particularly parts of the Indian subcontinent, it seems that a large reservoir of community carriers of carbapenemase-producing Enterobacteriaceae has been established, which likely eclipses the hospital-based reservoir in terms of numbers, but not risk.[59,60]

MDR *P. aeruginosa* and other non-fermenters are an important problem in patients with cystic fibrosis, who also span the hospital/community divide. There is a growing prevalence of high-risk clones, such as the Liverpool epidemic *P. aeruginosa* strain.[61] Cross-infection occurs[62] and can be interrupted by segregation of colonized and non-colonized patients with cystic fibrosis.[63] Resistance is often extensive but evolves very variably in the individual patient, and there is no specific resistance pattern associated with any of the successful cystic fibrosis lineages.[64]

9.1.6. What is the role of agricultural use of sewage and antibiotic treatment in veterinary practice in spreading extended-spectrum β-lactamases?

Gut *E. coli* are ubiquitous in mammals, and MDR strains are reported repeatedly in both food and companion animals.[65] Johnson *et al.* demonstrated that the same ESBL-producing *E. coli* strains can be shared among household members and their pet dog, although the direction of transmission is uncertain.[66] Transmission of resistant *E. coli* down the food chain can occur. At a population level, fluoroquinolone-resistant *E. coli* from chickens and humans were reportedly more similar than fluoroquinolone-resistant and -susceptible *E. coli* from

humans.[67] However, sequence typing needs to be examined. In the Netherlands, the same *E. coli* strains, plasmids and ESBL genes ($bla_{CTX-M-1}$ and bla_{TEM-52}) were found in humans, broilers and retail chicken meat.[68] However, the ESBLs in retail chicken meat in the UK are predominantly CTX-2 or -14-like,[69,70] and their host strains are non-clonal, whereas clonal ST131 *E. coli* with CTX-M-15 enzyme predominates among human ESBL isolates and is very rare in chicken meat.

Recently, a large UK, German and Dutch study found that only 1.2% of ESBL-producing *E. coli* from food animals resembled human ESBL-producing isolates. The authors concluded that human-to-human faecal-oral or plasmid transmission was considerably more important than food chain transmission, but noted that food animals represent a reservoir (and evolution site) for resistant strains that may pose future challenges in humans.[71]

9.1.7. What insights have national Escherichia coli bacteraemia surveillance provided?

Bacteraemias caused by *E. coli* result from a variety of aetiologies including pre-existing urinary tract infection, indwelling urinary catheters and biliary-related infection. In sentinel surveillance undertaken by PHE, most cases arose in elderly patients in the community who had visited their general practitioner at least once in the preceding weeks with urinary tract infection, suggesting that co-morbidity or treatment failure may be a significant factor.[72] One-third of patients with bacteraemia had received antibiotics for genitourinary infection in the preceding four weeks, but the adequacy of treatment was not known. There is a notable rise in incidence in the summer for all Gram-negative bacteraemias,[73–76] and a number of hypotheses are possible, including the role that hydration status in the elderly has to play in predisposition to infection. Reporting resistance data in *E. coli* bacteraemia helps in making local risk assessments on patients transferred from other hospitals.[77]

9.1.8. Is there evidence for high-/low-risk areas within a healthcare facility?

Sharing a room with a colonized patient and ICU admission are risk factors for acquisition of carbapenem-resistant organisms.[78–80] A German point prevalence study of 56 hospitals in 2011 showed that, overall, prevalence of resistance was highest in ICUs (ESBL-producing *E. coli* 2.5% on ICU) and higher on medical wards compared with surgical wards,[81] as also seen in a UK study.[82] A European survey of 19,888 patients, mainly in Belgium and France, showed the highest prevalence of healthcare-acquired infection in ICUs (28.1%).[83]

Long-term care facilities report high prevalence of colonization with MDR Gram-negative bacteria in residents compared with acute hospitals, associated with prolonged stay, antimicrobial treatment and faecal incontinence.[84,85] In one series of carbapenem-resistant *Acinetobacter* and *Klebsiella* isolates, over half were obtained from patients admitted from long-term acute care facilities.[86]

Evidence

ICUs in acute hospitals and any long-term care facilities have higher prevalence of MDR Gram-negative bacteria than general wards. 2+

Recommendation

Patients at high risk for colonization or infection with carbapenem-resistant organisms include those admitted to ICUs and from long-term care facilities (e.g. care homes). Conditional

9.2. Is there evidence of differences between organisms in respect of transmission, morbidity and mortality?

9.2.1. Resistant Enterobacteriaceae

Enterobacteriaceae are part of the gastrointestinal flora of humans and animals, and some are readily transmitted, particularly in the healthcare setting (Table III). It remains unclear why the *E. coli* ST131 lineage has been so successful compared with many other ESBL-producing strains.[87] Transmission from patient to patient is believed to be mainly via hands of staff, although common environmental sources have occasionally been described and should be sought where no other plausible vectors can be found (e.g. ventilator equipment or water supply).[12,35,88,89] Infection prevention and control relies on the consistent application of SICPs (e.g. hand hygiene, appropriate use of personal protective equipment, and ensuring a clean and well-maintained care environment). Patient screening, used as part of a bundle of infection prevention and control measures, is effective for identifying carriage of ESBLs by *E. coli*, *K. pneumoniae* and *Enterobacter* spp.[90–92]

For colonization or infection with ESBL-producing bacteria, the presence of a gastrostomy, urinary catheter or nasogastric tube were risk factors.[93–95] Antibiotic treatment has been shown to select for ESBL-producing *E. coli* in a variety of healthcare settings.[96] For some strains, piperacillin-tazobactam can select for quinolone-resistant bacteria that produce CTX-M,[97] and carbapenem use is associated with acquisition of carbapenem-resistant *E. coli*.[98]

Screening for carriers with subsequent isolation of those identified is effective in preventing transmission, and is important for early recognition.[99] Awareness of carriage is important and, therefore, communications regarding those identified to be infected or colonized with MDR strains is essential when transferring patients within and between institutions.

9.2.2. Acinetobacter baumannii

Infection control precautions against *A. baumannii* have been adapted following experience with outbreaks, and generally address the organism's major epidemic modes of transmission and the excessive use of broad-spectrum antibiotics (Table III). Control can sometimes be achieved when a common source is identified and eliminated.[12,100] A review of 51 hospital outbreaks showed that 25 had common sources. Of these, 13 outbreaks were predominantly respiratory tract infections, and 12 were predominantly bloodstream or other infections. They were controlled by removal or disinfection and sterilization of contaminated ventilator (or related) equipment or contaminated moist fomites.[12]

When neither common sources nor environmental reservoirs are identified, control has depended on surveillance and isolation of colonized and infected patients, along with promoting improvements in the hand hygiene practices of healthcare workers,[101] and ensuring the aseptic care of vascular catheters and endotracheal tubes.[12] Increased cleaning of the general care environment has been the next most common outbreak

intervention,[12] reflecting the concern that *Acinetobacter* spp. can survive for months on wet or dry surfaces, thereby facilitating nosocomial transmission.[102] Disinfection regimens used on surfaces include 0.1% hypochlorite[103,104] and, increasingly, hydrogen peroxide vapour.[105–109]

Patient screening has been suggested in a number of studies.[104,110,111] Several studies also advocate reduced prescribing of broad-spectrum antibiotics, such as fluoroquinolones or carbapenems.[12,112] Antibiotic exposure is often a risk factor for an outbreak; however, the use of multiple interventions and historical controls complicates interpretation of these studies. Patient decolonization by skin cleansing with chlorhexidine or the use of polymyxin on wounds, orally or by inhaled aerosol, has been an occasional adjunctive control measure but may be a risk for development of resistance.[113–115] Often, the use of a multi-factorial or 'bundle' approach is the most effective way of controlling this organism.[116]

9.2.3. *Pseudomonas aeruginosa*

Sources and mechanisms of transmission vary, and surveillance is complicated by the close association between patient and environmental isolates. Association with moist environmental sources is well documented, although significant persistence on dry surfaces, including hospital linen and floors, with a range of 6 h to 16 months is reported.[117] Water systems act as a source of infection, or indicate environmental contamination from other sources (e.g. staff hands or re-usable care equipment being cleaned in handwash sinks).[34] Levels of sink colonization are higher in critical care areas than general wards.[118]

Transmission occurs via the hands of healthcare workers, contaminated either from patients or from the environment, and has been reviewed systematically by Loveday.[34,119–124] Pseudomonal carriage on hands may be less persistent than for other Gram-negative bacteria, but other factors such as glove usage and artificial nails contribute.[13,125–127] Patient-to-patient transmission can occur via the air among patients with cystic fibrosis, with evidence of infectious droplet nuclei,[128] or via patient hand and environmental contamination.[129,130]

Sporadic and epidemic strains tend to co-exist and may be difficult to track without molecular typing.[124,131] There is no evidence for the effect of routine surveillance on the control of MDR *Pseudomonas* spp., but reports of outbreak interventions support the utility of screening.[132,133]

There is little evidence that isolating patients in single rooms reduces endemic MDR *Pseudomonas* spp. levels. In outbreak settings, use of isolation measures as part of a multi-faceted infection control regime is usual, but direct evidence for the impact of isolation alone is lacking.[132,134–138] There is a risk of bias in outbreak reports, and balance between desirable and undesirable effects of physical isolation should be considered. There is a poor level of specific evidence as to the effect of hand hygiene, but expert opinion extrapolated from other situations supports the use of this measure as part of a wider infection prevention strategy.[33,132,133,138,139] Care should be exercised with production, storage and turnover of cleaning products as the organism has a degree of tolerance to disinfectants, and there is evidence for pseudomonal contamination of detergent-type cleaning products.[140,141]

9.3. Surveillance

9.3.1. Selection of samples and antimicrobials to test

In order to support surveillance and infection control, national uniformity is needed in the testing of clinically significant isolates and in the detection of MDR strains. This may involve widespread testing of organisms with antibiotics that would not ordinarily be used in the individual patient.

In particular, testing of parenteral agents against urinary Gram-negative isolates from community patients is necessary. This may impose costs on diagnostic laboratories without matching benefits beyond earlier detection of spread of such infections. At present, the major requirement is detection of carbapenem-resistant organisms, although detection of quinolone-resistant and ESBL-producing organisms is important. Plasmid transmission of carbapenemases to a wide variety of Gram-negative species makes it difficult to be proscriptive. Validated, sensitive algorithms for testing need to be developed if universal testing is not applied. Testing cephalosporin-resistant isolates solely for carbapenem resistance may miss strains with OXA-48 carbepenemases, but this is a useful minimum standard for detection of other carbapenemases. Wider testing of temocillin may detect more OXA-48-producing strains.[142]

The basic phenotypic strategy to detect carbapenemase producers is to use a carbapenem as an indicator, and then to undertake supplementary tests to distinguish carbapenemase producers from those that have other carbapenem resistance mechanisms.[143] Some carbapenemases may not be associated with clinical resistance to carbapenems, and tests that detect hydrolytic capacity [e.g. the modified Hodge/clover leaf test, or synergy tests between carbapenems and boronates (to inhibit KPC enzymes) or EDTA (to inhibit metallo-carbapenemases)] are more useful in identifying these strains. European Committee on Antimicrobial Susceptibility Testing (EUCAST) advice is that Enterobacteriaceae with a minimum inhibitory concentration (MIC) for meropenem >0.12 mg/L should be treated with suspicion, not just those with MICs above the clinical breakpoint of 2 mg/L; the screening MIC of >0.12 mg/L equates to a zone with a diameter of <25 mm on Mueller-Hinton agar.[144] Ertapenem is a more sensitive indicator of carbapenemase production than meropenem or imipenem, but is less specific as it is affected more than other carbapenems by porin-mediated mechanisms. It is also less used and tested. Meropenem or imipenem have better specificity and are to be recommended for screening for national surveillance. EUCAST screening breakpoints should be used.[143]

Many laboratories do not test meropenem susceptibility routinely for all Gram-negative blood isolates. For national surveillance of carbapenem resistance to be effective, phenotypic meropenem resistance must be tested for all blood isolates, and resistance must be reported to central authorities. However, provision for reporting meropenem-resistant Gram-negative isolates from all body sites is important, and should not burden laboratories excessively; electronic and paper reporting systems should be made available. All secondary and tertiary care hospitals, as well as private hospitals, should be included. Monitoring by identifying specific carbapenemases would require reference laboratory reports; therefore, local confirmatory tests are encouraged. Automated PCR methods are being developed or available for specific carbapenemase gene detection, but are not yet widely used. Most meropenem resistance in *P. aeruginosa* is due to loss of

Table III
Dissecting the epidemiology of multi-drug-resistant (MDR) Gram-negative rods

	Resistant Enterobacteriaceae		MDR non-fermenters		
	AmpC, ESBL	CPE	*Acinetobacter baumannii*[a]	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*
Microbiology	Fermentative, oxidase-negative, motile or non-motile, facultatively anaerobic, rods		Non-fermentative, oxidase-negative, non-motile, obligate aerobic, coccobacilli[330]	Non-fermentative, oxidase-positive, motile, aerobic, rods[331]	Non-fermentative,[b,332] motile, oxidase +/−, obligate aerobic, rods[333]
Reservoirs	Human and animal gastrointestinal tract, water		Respiratory and gastrointestinal tract, dry surfaces[330,334]	Ubiquitous: plants, animals, moist environments[331]	Ubiquitous: plants, animals, humans, moist environments[333,335]
Sites of colonization	Gastrointestinal tract[22]		Skin, respiratory and gastrointestinal tract[187,334,336]	Gastrointestinal tract, moist body sites (throat, nasal mucosa, axillary skin, perineum)[337]	Respiratory and gastrointestinal tract[333,335,338]
Duration of colonization	Months to more than one year[339–341]		Days to weeks[334]	—	—
Clinical manifestation	Urinary tract (e.g. *E. coli*), pneumonia (e.g. *K. pneumoniae* and *Enterobacter* spp.), intra-abdominal infection[337,342]		Ventilator-associated pneumonia, catheter-related bloodstream and urinary tract infections, wound infections[330,334]	Pneumonia, urinary tract, surgical site, bloodstream infections, cystic fibrosis lung, burns[331]	Pneumonia, bloodstream infections; less commonly, urinary tract and wound infections[333,335]
Environmental survival	Hours to weeks on dry surfaces;[117] contaminated environment likely to play a minor role in transmission[252,263]		Weeks to months on dry surfaces;[117,251] difficult to remove from surfaces by cleaning and disinfection[103,106]	Contaminates moist hospital environments: tap aerators, respiratory therapy equipment[337]	Contaminates moist hospital environments; can form biofilms on surfaces; low biocide susceptibility[333,335]
Transmission routes	Hands (++), contaminated surfaces (+/−)[319]		Contaminated surfaces (++), hands (+), air (+/−)[31,252,334]	Hands (+), contaminated moist surfaces (+), air (+/−), water systems[337,343]	Hands (+), contaminated moist surfaces (+), air (+/−)[333,343]
Antimicrobial resistance — intrinsic	Ampicillin, first- and second-generation cephalosporins.[344] *Serratia* and *Proteeae* spp. are intrinsically resistant to polymyxins		Ampicillin, amoxicillin-clavulanate, cefazolin, cefotaxime, ceftriaxone, ertapenem, trimethoprim, fosfomycin[330]	Some β-lactams and fluoroquinolones, macrolides, tetracyclines, cotrimoxazole[345]	Most agents except cotrimoxazole[333,335]
Antimicrobial resistance — acquired	Penicillins (except temocillin), ESBLs, carbapenems (through mechanisms other than more common acquired carbapenemases), aminoglycosides, sulphonamides, quinolones[344,346]	Most or all β-lactams, carbapenems, polymyxins (rarely) (exact profile depends on particular carbapenemase and any co-produced ESBL)[31,347]	Quinolones, aminoglycosides, β-lactams (including carbapenems), polymyxins, tigecycline[330,348]	Aminoglycosides, β-lactams (including carbapenems), monobactams, fluoroquinolones, polymyxins[345]	Trimethoprim/sulfamethoxazole

Common acquired resistance enzymes	AmpC (intrinsic in Enterobacter), ESBLs (TEM, SHV, CTX-M), various aminoglycoside-modifying enzymes	Carbapenemases (KPC, VIM, IMP, NDM)[22]	Various aminoglycoside-modifying enzymes or ribosomal methyltransferase, class-D OXA type carbapenemases[330,349]	Metallo-β-lactamases (VIM and IMP)[345]	*sul* genes (resistance to sulphonamide)
Mortality (bacteraemia)	Moderate/substantial increase in attributable mortality[342,350]	Stark increase in attributable mortality[22,262,351]	Minimal increase in attributable mortality[350]	Moderate/substantial increase in attributable mortality depending on type of infection[350,352]	Minimal increase in attributable mortality[353]
Risk factors	Hospital: prolonged hospital stay, prior hospitalization, previous use of antibiotics, presence of indwelling catheters, mechanical ventilation Community: older age, recurrent urinary tract infections/prior invasive procedures (e.g. catheterization), known faecal carriage, contact with healthcare facilities, antimicrobial treatment[143]	Prior antimicrobial use, length of stay, severity of illness, mechanical ventilation, admission to ICU, high procedure score, presence of wounds, positive culture from a blood isolate, transfer between hospital units within the same hospital, prior surgery, prior hospital stay, proximity to other colonized/infected patients, presence of a biliary catheter and recent transplantation.[168] For NDM, prior hospitalization on Indian subcontinent; for OXA-48, prior hospitalization in Middle East	(i) Major trauma, particularly burns, surgery and battlefield injury; (ii) previous antimicrobial therapy; (iii) prolonged hospital and ICU stay; (iv) mechanical ventilation, drainage tubes and indwelling catheters; (v) high prevalence of MDR *Acinetobacter* spp. on the unit; (vi) proximity to other colonized/infected patients[330,349]	(i) Prior use of antibiotics; (ii) mechanical ventilation; (iii) prolonged hospital and ICU stay; (iv) co-morbidities (e.g. cystic fibrosis, burns units)[352,354]	Severely compromised health status, malignancy, indwelling devices (such as intravascular catheters and ventilation tubes), exposure to broad-spectrum antimicrobials, long hospital stay, ICU stay[333,335]
At-risk population	Patients in acute, long-term and community settings; patients travelling to areas of high prevalence[56]	Patients in acute settings, particularly those with recent travel to areas of high prevalence[22,355]	Immunocompromised patients in the ICU and burns units;[330] rare cause of community-acquired infection[334,356]	Immunocompromised patients in the ICU and burns units; patients with cystic fibrosis;[345] rare cause of community-acquired infection[337]	Immunocompromised patients in the ICU; patients with cancer and cystic fibrosis; rare cause of community-acquired infection[333,357]
Common international clones	*E. coli* ST131 with CTX-M ESBLs[19]	*K. pneumoniae* ST258 with KPC enzymes[19,22]	International clones I–III[19,330]	Clonal diversity.[19] A few international high-risk clones [e.g. ST111 (serotype O12)] acquire multi-drug resistance; spread of ST235 with VIM carbapenemase in Russia, Belarus and Kazakhstan	Clonal diversity[333,335]

KPC, *Klebsiella pneumoniae* carbapenemase; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; ESBL, extended-spectrum β-lactamase; CPE, carbapenem-producing Enterobacteriaceae; ICU, intensive care unit.

[a] From a taxonomic viewpoint, four species are virtually indistinguishable (*A. baumannii*, *Acinetobacter calcoaceticus*, genomic species 3 and genomic species 13TU) so are grouped together as '*A. calcoaceticus–A. baumannii* complex'; however, *A. baumannii* is by far the most important human pathogen in this group. However, as methods commonly used to speciate *Acinetobacter* spp. in the clinical laboratory are unable to distinguish these species, the relative contribution of each to the burden of human disease is difficult to establish.

[b] Recent data indicate that the 'non-fermentative' status of *S. maltophilia* should be re-assessed.

OprD porin and not a carbapenemase. Sensitivity to ceftazidime, piperacillin-tazobactam and carbenicillin despite meropenem resistance suggests this mechanism, and also suggests that neither high-level infection control action nor submission to a reference laboratory is needed.

To detect ESBLs, *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* should be screened for clavulanate-reversed resistance to ceftazidime and cefotaxime[143] or cefpodoxime. AmpC producers are resistant to cefotaxime (reversed by cloxacillin), but susceptible (or intermediate) to cefepime. For AmpC-inducible genera, such as *Enterobacter* spp. and *Citrobacter freundii*, comparison of cefepime and cefepime plus clavulanate discs can be used to detect additional presence of ESBLs. Confirmation of ESBL production is most easily accomplished by comparing inhibition zones for discs with cephalosporin alone with discs containing clavulanic acid. A zone expansion of >5 mm indicates ESBL production. Alternatively, an Etest strip is used to demonstrate at least eight-fold reduction in MIC.

Faster diagnostic methods may be considered, particularly during outbreaks, to allow more rapid isolation. Selective media or combinations of non-selective media and a chromogenic or genetic test achieve a result within 24 h. Detection within a few hours is possible if molecular tests are applied directly to the clinical specimen, although this approach is still new.

Various selective commercial media are available to seek ESBL, CTX-M ESBL or carbapenemase producers directly from clinical specimens or early growth in blood culture bottles. Media-seeking ESBL producers often have good sensitivity but poor specificity in distinguishing these organisms from strains that hyperproduce AmpC enzymes.[145–147] The sensitivity of media-seeking carbapenemases varies with the particular enzyme,[148] with OXA-48 being the most difficult to detect due to the low levels of resistance often conferred. The alternative approach is to seek ESBL or carbapenemase activity in colonies growing on non-selective agars. Colorimetric and biochemical approaches include the following:

- The chromogenic oxyimino-cephalosporin HMRZ-86 turns from yellow to red on hydrolysis.[149] If used in combination with inhibitors, it can be used to distinguish strains with AmpC, ESBLs or metallo-carbapenemases, although KPC enzymes may be confused with AmpC and it is unclear whether OXA-48 is detected.
- Acidimetric β-lactamase tests can be adapted to detect carbapenemase producers, as in the 'Carba-NP' test where, again, some authors report problems in detecting OXA-48.[150–152]
- MALDI-ToF assays for carbapenemase activity, exploiting the molecular mass change that occurs when the β-lactam molecules are hydrolysed.[153]

Molecular tests can be used to seek β-lactamase genes in overnight cultures. One PCR/array system (Check-MDR CT03) can rapidly detect a wide range of relevant acquired AmpC, ESBL and carbapenemase genes, distinguishing between those encoding classical and extended-spectrum TEM and SHV types.[154]

PCR may be used directly on rectal swabs, without culture, and can give results within 1h of the specimen being taken.[155] Sensitivity and specificity are good, although positive results are often obtained for patients from whom the laboratory fails to grow a carbapenemase-producing pathogen.[156] This is a wider issue with molecular diagnostics when used directly on specimens, and may either indicate a poor positive predictive value or that the culture is not the 'gold standard'.[157]

Recommendation

The minimum susceptibility tests performed on all significant Gram-negative isolates should include meropenem; in addition, cefpodoxime should be tested for Enterobacteriaceae, and ceftazidime should be tested for *Pseudomonas* spp. Strong

9.3.1.1. When to seek reference laboratory typing of isolates

- To inform cross-infection and outbreak investigations.
- To seek a particular type associated with specific clinical characteristic(s) (e.g. K1 capsular type of CC 23 of *K. pneumoniae* associated with hypermucoviscosity and liver abscesses).
- To provide national/international context (e.g. in tracking the spread of 'high-risk clones', such as ST258 *K. pneumonia*, with KPC carbapenemases).

Typing results can never stand alone, and need to be interpreted in the context of all available epidemiological, clinical and demographical data.[158] Typing of isolates is helpful to inform cross-infection and outbreak investigations among groups of patients with potential links. Comparison of isolates without epidemiological linkage information may result in patients being linked in error, simply because they share the same international high-risk clone, or both have representatives of a widespread cluster. Typing of environmental isolates may be helpful, especially where a piece of equipment common to all the affected patients is implicated. However, it may also be confusing and needs to be focused. All environmental samples should have a clear link to an affected patient; there is no point in typing environmental isolates on their own. Isolates from sink plug holes/drains may well match patient isolates, but this provides little information regarding the source as the isolate is likely to have come from the patient rather than the patient having acquired it from a drain. Large-scale environmental sampling is rarely helpful, and there should be a clear hypothesis as to a likely source and the link between that source and the patient(s).

9.3.2. What national surveillance is performed and how should it be developed?

National surveillance of antimicrobial resistance is essential in detecting the emergence of new strains and resistance mechanisms, providing information for formularies and assessing the effect of control strategies. Outputs must be timely and tailored to the needs of medical and nursing staff, healthcare organizations and commissioners of health care. The World Health Organization (WHO) provides WHONET database software, which is used for collecting data in some areas, while the European Antimicrobial Resistance Surveillance Network (EARS-NET) provides resistance information on blood and cerebrospinal fluid isolates across Europe.[159] EARS-NET identified the early accumulation of carbapenemases in *K. pneumoniae* in Greece,[160] although they are now also

proliferating in other countries such as Italy. As such, travel history on admission can be a useful indicator of risk of carriage of MDR organisms.

In the UK (except Scotland), PHE collects susceptibility data in a voluntary scheme for bloodstream isolates of all species. The Second Generation Surveillance System is a web-enabled database application that collects both communicable disease reports (which were previously collected by the UK CoSurv system and include bloodstream isolates) and antimicrobial resistance reports (which were previously collected by the UK AmSurv system).[159] Antimicrobial resistance reporting is voluntary and only applies to England, but now covers over 80% of laboratories. In May 2015, an enhanced surveillance system for the surveillance of carbapenemase-producing Gram-negative bacteria was launched. In addition, the BSAC Resistance Surveillance Project (http://www.bsacsurv.org) tracks the prevalence of antibiotic resistance for a range of species and antibiotics in bacteraemia and lower respiratory tract infection, based on collection and central testing of isolates from a panel of 40 laboratories across the UK and Ireland.

Evidence

There is a significant increasing trend of carbapenem-resistant *K. pneumoniae* and other Gram-negative bacteria in most European countries, with major proliferation in Greece and Italy, suggesting a risk of occurrence in the UK. 2+

Recommendations

Laboratories should test meropenem susceptibility in all clinically significant Gram-negative isolates. Strong

Travel history (i.e. countries or known endemic areas visited within previous year) should be collected for all patients with carbapenemase-producing Gram-negative bacteria. Strong

9.3.3. How should we undertake local screening, why is it important and how should it be interpreted?

Screening at hospital level is useful for infection control, and to track resistance types (e.g. carbapenemase producers) by rectal swab or stool on admission, weekly during hospital stay and at discharge (Table III). Rectal swabs have maximum sensitivity for MDR pathogens (other than *Acinetobacter* spp.), but it is critical to ensure the compliance of staff with guidance on how and when to take samples by means of audit and feedback, as well as the specific actions arising from a positive result.[161] When a carbapenem-resistant organism is identified (or an isolate with any other index resistance is sought), any epidemiologically-linked patients should be screened. Screening of other patients depends on an assessment of risk of shedding of the organism and duration of exposure, and is less likely to be required if the patient has been isolated from admission.[162]

The primary purpose of local screening is the detection of outbreaks of resistant colonizing or infecting organisms with minimum delay. Few hospitals have sufficient single rooms to allow segregation of all patients at risk when they are admitted. Therefore, local identification of carriers allows prioritization of single rooms, potentially limiting spread. Hospital-level surveillance provides faster notification of an emergent problem than awaiting results from the reference laboratory, particularly if a single clone and species is responsible. Passive surveillance of clinical infections alone will be too delayed to help to limit spread. In an outbreak, isolation and infection control precautions are only effective if combined with active surveillance.[161] A plasmid-based outbreak (e.g. carbapenemase producers) can be more difficult to recognize because multiple bacterial species may be involved.

9.3.4. At what point should passive surveillance switch to active surveillance (screening)?

Examination of routine diagnostic tests or discharge summaries requires few resources compared with screening an entire 'at-risk' group. However, screening quickly identifies patients colonized with MDR Gram-negative pathogens who require source isolation but who might otherwise be placed in a shared bay. Choosing to screen depends on available resources, outbreak progression and clinical characteristics. Current national advice to screen patients at risk for carbapenemase-producing Enterobacteriaceae is made despite a low prevalence of these organisms in most UK centres because the clinical risk of spread is thought to be high.[162]

Passive surveillance of routine cultures did not distinguish 12 (86%) of 14 patients later found to have faecal carriage of carbapenemase-producing *K. pneumoniae*.[163] MIC was highly dependent on the inoculum. On average, routine cultures identify patient carriage of ESBL Enterobacteriaceae three days later than active screening.[164] The virulence of the strain, host susceptibility and the sensitivity of the diagnostic method will affect the efficiency of passive identification of patients.[161] As such, no single recommendation can be made.

Evidence

Passive surveillance is less sensitive and slower in identifying outbreaks of MDR Gram-negative infections than active screening. 3

Recommendation

Active screening rather than passive surveillance is recommended for high-risk specialties. Conditional

9.4. What is the evidence that infection prevention and control precautions prevent transmission?

Trials of infection control strategies are difficult to mount with sufficient power to determine efficacy, and most trials use a package of measures so the effect of single interventions cannot be extracted. A systematic review of infection control precautions in care settings for patients receiving stem cells or treatment for cancer showed a combination of prophylactic antibiotics, control of air quality and isolation in a room was associated with a lower rate of mortality [odds ratio (OR) 0.60, 95% CI 0.50–0.72] at 30 days.[165] Gram-negative bacteraemia was reduced by the package of measures. Gram-negative infections were significantly less common in patients who were isolated, but there were insufficient data to assess the specific effect on MDR strains. Environmental cleaning and screening are discussed in other sections.

9.4.1. Are standard infection control precautions sufficient to stop transmission?

Existing national guidelines are unequivocal that SICPs should be used by all staff, in all care settings, at all times, for all patients (adults, children and infants), whether infection is known to be present or not, in order to ensure the safety of

those being cared for, staff and visitors in any environment where care is given. SICPs are the basic infection prevention and control measures necessary to reduce the risk of transmission of infectious agents from both recognized and unrecognized sources of infection.

Sources of (potential) infection include blood and other body fluid secretions or excretions (excluding sweat), non-intact skin, mucous membranes, and any equipment or items in the care environment that could have become contaminated.

To be effective in protecting against infection risks, SICPs must be used continuously by all staff. Patients who move frequently between the hospital, the community and long-term care facilities may render location-based screening inadequate as a means to identify outbreaks.

However, as underscored by recent systematic reviews,[166,167] there is a paucity of evidence directly testing infection prevention and control advice as related to Gram-negative organisms, particularly MDR strains. A similar lack of evidence was noted in the ESCMID guidelines on preventing transmission of MDR Gram-negative bacteria.[161] Nevertheless, the European Centre for Disease Prevention and Control (ECDC) risk assessment on carbapenemase-producing Enterobacteriaceae showed that there was agreement across Europe that SICPs are an essential integral part of any strategy to control MDR Gram-negative organisms.[168] The supporting European survey of carbapenemase-producing Enterobacteriaceae emphasized the importance of diagnosis, early containment through patient screening and SICPs.[169]

A number of authoritative bodies have produced detailed guidance on carbapenemase-producing Enterobacteriaceae in particular based on expert consensus; these emphasize the importance of continuous implementation of SICPs, with particular emphasis on hand hygiene.[143,162,170,171] PHE, the Centers for Disease Control and Prevention, and ESCMID all recommend contact precautions (patient isolation) in addition to SICPs for all colonized or infected patients with MDR Gram-negative bacteria, as well as those previously colonized and not known to be free of these bacteria.[161,162,170] All patients should be assessed for transmission risk on or before arrival at the care area, and reviewed for any changes in risk during their stay.

In an endemic setting (with constant challenge from admissions of colonized or infected patients), ESCMID does not recommend isolation for ESBL *E. coli*. Other guidance emphasizes basing isolation on a risk assessment while maintaining high levels of hand-hygiene compliance and environmental cleaning.[143] The ST131 clone of *E. coli* appears to be more readily transmissible, and further study is needed.

Other guidelines use general principles based on a range of pathogens. Both National Evidence-Based Guidelines (EPIC 3) and Health Protection Scotland's National Infection Prevention and Control Manual specify good-practice standards based predominantly on expert consensus or Health and Safety legislation, rather than evidence from controlled trials.[166,172]

SICPs include[166,173] the following elements:

- hand hygiene;
- environmental cleanliness, including the decontamination of patient care equipment, the safe management of linen and disposal of healthcare (clinical) waste;
- safe use and disposal of sharps;
- aseptic practice;
- respiratory hygiene; and
- assessment of infection risk, use of personal protective equipment and patient placement.

Contact precautions entail donning personal protective equipment on room entry, and discarding before exiting the patient room. A single room is preferred.[173] Hand hygiene is performed before touching the patient, and prior to wearing gloves for touching the patient and the patient's environment.

Strategies to minimize the transmission of pathogens, including MDR Gram-negative bacteria, will only be successful if there is a reliable high level of compliance with SICPs and contact precautions by all healthcare workers.[174,175] Training, education, audit and feedback are therefore important. Low levels of compliance with hand hygiene and inappropriate glove usage are commonly described.[174,176] Invasive medical devices breach the body's natural defence mechanisms and increase the likelihood of infection and colonization; therefore, device avoidance and minimization are important.

Evidence

Consistent application of SICPs with contact precautions for patients colonized or infected with MDR Gram-negative pathogens reduces transmission. 3

Recommendation

In addition to SICPs, apply contact precautions for those patients who present an infection risk. Strong

Good Practice Recommendation[166]

Apply and maintain SICPs in all care settings, at all times, for all patients.

9.4.2. Screening

9.4.2.1. What is the role of screening in patients and staff?

Early detection of patients colonized or infected with MDR Gram-negative organisms is important for managing their status effectively, and for implementing timely interventions to prevent subsequent spread. Screening of potential colonization sites of patients (e.g. faeces) is essential in limiting the spread of carbapenemase producers in hospitals. Identification of other MDR Enterobacteriaceae is useful to identify those patients who may need carbapenems if treated empirically. Although ESBL *E. coli* are often resistant to ciprofloxacin, the proportion varies widely by country.[26,177,178]

In a multi-centre German study of screening of patients with haematological malignancies, colonization rates with ESBL Enterobacteriaceae varied between 5.3% and 21.8% of patients.[179] In a Korean study of ICU patients, 28% of 347 were found to have ESBL Enterobacteriaceae faecal carriage on admission, and another 12% acquired these organisms during follow-up in ICU. As assessed by pulsed-field gel electrophoresis, none of the acquisitions were nosocomial transmissions, but the methods used would not have readily identified plasmid outbreaks.[180] Routine screening of urine for ESBL *E. coli* and *Klebsiella* spp. followed by single-room isolation of carriers did not result in any significant reduction in the number of ESBL producers isolated from non-urinary sites in hospital.[181] Against the background of a high prevalence of ESBL

Enterobacteriaceae in Korea, routine screening of carriage sites was not cost-effective in ICUs.[180] The risk of dissemination in the local community was high. In Europe, ST131 CTX-M-15 strains are common, but specific screening for that strain has not been studied.

A nationwide intervention in Israel against a clonal outbreak of ST258 *K. pneumoniae* (with a KPC carbapenemase) was successful because it depended on mandatory patient screening and isolation, and patient and staff cohorting.[77] Short- and long-term care facilities were involved, as the latter were a reservoir for re-introduction to acute units. Compliance with national guidelines was reinforced by visits to facilities, reporting of carrier and isolation status, and contact tracing. In high-intensity units such as ICUs, rectal swabs from all the patients on the ward were assessed. Two rectal swabs negative by culture and one rectal swab negative by PCR were required before screening was discontinued for an individual patient in any ward. The programme successfully reduced acquisition of carbapenem-resistant organisms from 55.5 to 4.8 instances per 100,000 patient-days. Screening for non-fermenters such as *Pseudomonas* spp. or *Acinetobacter* spp. is not supported by high-quality evidence, but may be performed in outbreaks (Tables III and IV).

There is usually no indication for screening faecal cultures from healthcare workers or family members, although good personal hygiene should be emphasized. Outbreak investigations that do not identify a single environmental source suggest that transmission is occurring via the hands of hospital staff, but hand cultures are usually negative, presumably because contamination is transient.[182] Gram-negative organisms isolated from nurses' hands are, in most cases, different from those causing significant infections in patients.[13,183] However, outbreak reports are selective and open to bias.

Evidence

Mandatory screening and full implementation of SICPs combined with contact precautions throughout the area of care is effective in controlling clonal outbreaks of carbapenemase-producing pathogens. 2++

Routine screening of carriage sites for ESBL-producing Enterobacteriaceae for infection control purposes may not be cost-effective if community transmission and carriage is frequent. Screening and isolation of carriers of ESBL *Klebsiella* spp. is more likely to be useful than that for ESBL *Enterobacter* spp., *Serratia* spp. or *E. coli*. However, specific screening for specific clones has not been studied. 3

Recommendation

Screening for rectal and wound carriage of carbapenemase-producing Enterobacteriaceae should be undertaken in patients at risk. Strong

Good Practice Recommendation

Routine screening of family contacts and staff is not recommended.

9.4.2.2. What organisms should screening include? Enterobacteriaceae and non-fermenters (i.e. *A. baumannii* and *P. aeruginosa*) constitute the majority of MDR Gram-negative pathogens causing healthcare-acquired infections. Carbapenem-resistant organisms should have priority, as meropenem is currently the most widely used broad-spectrum antibiotic of last resort. Some MDR strains are readily transmissible and require patient isolation. Most carbapenem resistance seen in Enterobacteriaceae, at least among reference laboratory submissions, is now associated with production of KPC, OXA-48, NDM and VIM carbapenemases; almost all carbapenem resistance in *A. baumannii* is associated with OXA-23-, OXA-40-, OXA-51- and OXA-58-related carbapenemases. Carbapenem resistance in *P. aeruginosa* may involve carbapenemase production, but is more commonly related to porin loss, which confers a narrow-spectrum carbapenem-specific resistance profile.

ESBL producers and plasmid or chromosomal AmpC Enterobacteriaceae are resistant to a number of antibiotics, and infection control precautions are used to prevent transmission. A major driver for the use of carbapenems is the suspicion of the presence of ESBL. Screening for ESBL producers may therefore be useful in guiding and thereby limiting empirical use of carbapenem, although no confirmatory reports are available. Where isolation facilities are limited, cephalosporin-resistant Enterobacteriaceae cases and carriers should have a lower priority than patients carrying carbapenem-resistant Enterobacteriaceae. Subject to local risk assessment, colonized patients with diarrhoea or discharging wounds would usually take precedence for single rooms over patients without those characteristics but the same MDR organism.

Recommendation

Screening for carbapenem-resistant *A. baumannii* and MDR *P. aeruginosa* is required in management of outbreaks. Strong

9.4.2.3. Who, how and when to screen patients for multidrug-resistant Gram-negative bacilli?

9.4.2.3.1. Who to screen. The potential risk factors identified for colonization or infection with MDR Gram-negative organisms are similar and wide ranging, and include recent antimicrobial treatment, presence of indwelling devices, severity of illness, admission to an ICU, transfer between hospital units, residence in long-term care facilities, previous surgery, hospital inpatient stay within the preceding year (particularly overseas in an endemic area), recent solid organ or stem cell transplantation, presence of wounds, presence of biliary catheter and mechanical ventilation.[38,161]

Although only limited data are available from studies on interhealthcare transmission of carbapenem-resistant Gram-negative bacteria within countries, a number of descriptive studies indicate that cross-border transfer of patients is associated with a risk of transmission of carbapenem-resistant organisms, particularly in respect of patients coming from the Middle East, India, Pakistan, Italy and Greece.[168] This applies to patients transferred from endemic areas to healthcare facilities in another country, and where patients have received medical care abroad in areas with high rates of carbapenem-resistant organisms. Based on this, a recent ECDC report recommended that all countries should develop guidance for active screening of faeces of all patients transferred from any healthcare facility in an endemic area.[161] Among the first 250 patients in the UK with an isolate producing the NDM carbapenemase, 100 had a travel history, with half of these having travelled to the Indian subcontinent.[184]

Table IV
Infection prevention and surveillance by organism: recommendations by organism/resistance

Recommendation	Application in respect of		
	Resistant Enterobacteriaceae (AmpC, ESBL and carbapenem-resistant organisms)	MDR non-fermenters	
		Acinetobacter baumannii	*Pseudomonas aeruginosa*
1,2. Laboratory test for susceptibility to meropenem for all significant Gram-negative isolates	Test susceptibility to meropenem + cefpodoxime	Test susceptibility to meropenem. Usually confined to ICU	Test susceptibility to meropenem + ceftazidime. Most carbapenem resistance is via loss of OprD, and is less important for infection control than carbapenemases. Carbenicillin and ceftazidime sensitivity indicates OprD loss
3. Request international travel history for patients with carbapenemase-producing Gram-negative bacteria	Find and record	Not usually required — local acquisition	Find and record
4. Diagnostics: detect and report all MDR Gram-negative organisms (at least three resistance mechanisms) in clinical samples and screens in <48 h	Early recognition and infection control intervention to reduce transmission	Early recognition and infection control intervention to reduce transmission	Early recognition and infection control intervention to reduce transmission
5. Active screening rather than monitoring of laboratory reports for high-risk specialities	Case finding/screening for carbapenemase for ICU and other high-risk patients but not routinely recommended for ESBL/AmpC	In outbreaks, case finding/screening for ICU and other high-risk patients. Otherwise, monitor laboratory reports	Poor evidence for screening — may be appropriate in high-risk units (e.g. cystic fibrosis units, burns units, haematology units) or in context of outbreak. Sporadic and epidemic strains co-exist, so tracking of outbreaks may be problematic without typing unless there is a clear phenotype (e.g. metallo-β-lactamase)
6. Risk assessment on admission to ICU and from long-term care facility	Risk assess on admission for carriage of carbapenem-resistant Enterobacteriaceae. ESBLs — not recommended but may be considered on admission from long-stay units	Not recommended on admission except when outbreak in referring ward or hospital	Not recommended on admission except when outbreak in referring ward or hospital
7,8. Screen patients at risk for carbapenem-resistant Enterobacteriaceae	Screen patients assessed as at risk/coming from endemic settings. Screening not recommended for family contacts or staff	Screen in outbreaks. Screening not recommended for family contacts or staff	Screen in outbreaks. Screening not recommended for family contacts or staff
9. Type of sample for screening	Rectal/stool	Skin sites or, if a catheter or endotracheal tube is present, urine, rectal or respiratory secretion	Rectal/stool

10. Screen patients with stay in healthcare facility with endemic carbapenemase-producing organisms in last year	Carbapenem-resistant — targeted screening of patients with history of health care in high-risk areas [i.e endemic, not just sporadic cases (e.g. India, Manchester in UK)]	Carbapenem-resistant — targeted screening of patients with history of health care in high-risk areas (i.e. based on local knowledge of referring centres)	Carbapenem-resistant — targeted screening of patients with history of health care in high-risk areas (i.e. based on local knowledge of referring centres)
11. Monitor SICPs and contact precautions during outbreaks; repeat screening of negative patients weekly and on discharge until no new cases for seven days	Carbapenem-resistant — during outbreak, screen all patient contacts in ward of a new case	Carbapenem-resistant — during outbreak, consider screening all patient contacts in bay/ward of non-isolated case. No evidence for regular admission, ongoing or discharge screening except in outbreaks	No high-quality evidence for patient contact screening, but consider screening all patient contacts in bay/ward in outbreak. No evidence for regular admission, ongoing or discharge screening except in outbreaks
12. At admission, screen patients with previous carbapenem-resistant or other MDR Gram-negative bacteria	Screen all with known previous carriage or infection	Screen all with known previous carriage or infection	Screen all with known previous carriage or infection
13. Contact precautions for patients who present an infection risk	Carbapenem- resistant — pre-emptive isolation of previous positive patients pending screening. Restrict unnecessary patient movements where possible	Carbapenem-resistant — pre-emptive isolation of high-risk patients pending screening. Restrict unnecessary patient movements where possible	No evidence for pre-emptive isolation. Extrapolation from evidence for other MDR organisms suggests that isolation or cohorting of known cases with contact precautions and restrictions on unnecessary movement are appropriate
14. Patients colonized with MDR Gram-negative organisms in single room for duration of stay	No recommendations for tests of clearance — assume long-term carriage during the same inpatient stay	No recommendation for test of clearance	No recommendation for test of clearance
15. Personal protective equipment: use disposable gloves and gown/apron in caring for patients	Apron/gown and gloves for all patient interactions. No requirement for facemasks or respirators	Use apron/gown and gloves for all patient interactions. No requirement for facemasks or respirators	Apron/gown and gloves for all patient interactions. No evidence for the effectiveness of respiratory precautions except for patient-to-patient spread by droplet nuclei in patients with cystic fibrosis
16. Prioritize single-room accommodation (local risk assessment to determine priority against other pathogens, but carbapenem-resistant Enterobacteriaceae usually higher than *Clostridium difficile* or meticillin-resistant *Staphylococcus aureus*)	First priority: carbapenem-resistant Enterobacteriaceae. Carbapenem-resistant Enterobacteriacae: single room, preferably with en-suite facilities, throughout admission. Other MDR Enterobacteriaceae: isolate where possible according to assessment of risk of spread; otherwise, contact precautions	Second priority: carbapenem-resistant *Acinetobacter* spp. These are usually confined to ICU. Carbapenem-resistant: single room, preferably with en-suite facilities, throughout admission. Other MDR *Acinetobacter* spp.: room if available	Third priority: carbapenemase-producing *P. aeruginosa*. Other MDR *Pseudomonas* spp. (e.g. permeability variant): use single room if available
17. Cohort isolate if there are insufficient single rooms	Consider cohorting patients whose isolates have the same phenotypic resistance pattern. Consider staff cohorting where feasible, and education of staff in infection control	Consider cohorting patients whose isolates have the same phenotypic resistance mechanism. Level of evidence for staff cohorting is low	Extrapolation from other infections suggests that staff cohorting may be useful in controlling outbreaks

(*continued on next page*)

Table IV (*continued*)

Recommendation	Application in respect of		
	Resistant Enterobacteriaceae (AmpC, ESBL and carbapenem-resistant organisms)	MDR non-fermenters	
		Acinetobacter baumannii	*Pseudomonas aeruginosa*
18. Hand hygiene before and after direct patient contact	Contact precautions; ensure high awareness of need for attention to hand hygiene, especially with soap and water	Contact precautions and high awareness of need for hand hygiene, especially with soap and water	Low-level evidence. Ensure high awareness of need for attention to hand hygiene, especially with soap and water
19. Environmental screening when unexplained transmission	Persistence in environment in wet areas and respiratory equipment	Environmental persistence, particularly in dust and dry surfaces. Bed rails, bedside tables, hygroscopic bandages, HEPA filters and extract vents implicated	Association with persistently colonized water systems (e.g. taps, filters, aerators, sink traps). Screening of tap outlets in augmented care areas according to local risk assessment. Outbreaks associated with contamination of ventilator equipment
20. Decontaminate equipment in designated cleaning sinks	Indirect evidence for transmission via equipment	Some evidence for transmission through equipment	Evidence for transmission through equipment contamination (e.g. ventilators) demonstrates importance of dedicated and single-use equipment, and decontamination of re-usable equipment by appropriate agents
21. Risk assess point-of-use filters for taps if *P. aeruginosa* colonization/ infection is increasing	Not applicable	Not applicable	Effective in reducing infection but need monthly replacement
22. Terminal disinfection with hypochlorite in outbreak control	Increase cleaning frequency to at least twice daily and every 4 h for high-contact surfaces	Increase cleaning frequency to at least twice daily and every 4 h for high-contact surfaces	Some evidence that cleaning protocols have a role for controlling spread in outbreak settings
23. Consider hydrogen peroxide vapour as adjunct to cleaning	Effective in reducing reservoirs	Effective in reducing reservoirs	Effective in reducing reservoirs on surfaces but not taps or sink traps
24. Routine use of selective digestive decontamination not recommended	Not normally recommended as contrary to antibiotic stewardship	Not recommended	Not normally recommended as contrary to antibiotic stewardship
25. Monitor hand hygiene of all shared staff in cohort isolation	Self-contained nursing unit is effective in control of spread	Self-contained nursing unit is effective in control of spread	No direct evidence

ESBL, extended-spectrum β-lactamase; ICU, intensive care unit; MDR, multi-drug-resistant; SICPs, standard infection control precautions; HEPA, high-efficiency particulate arrestor.

All patients with epidemiological links (same hospital unit or care home) to an index and secondary cases should be screened to determine the extent of secondary transmission. However, carriage may be prolonged in the community (especially in patients with urinary catheters), and the chronology can be difficult to determine. As such, isolation is started on re-admission. Admission screening by rectal swab (or axilla/groin swab for *Acinetobacter* spp.) is required for patients transferred from countries or institutions (including those in the UK) with a prevalence of epidemic or endemic carbapenem-resistant Gram-negative pathogens, as well as those patients with previous colonization or infection. However, that assumes the receiving area (infection control practitioner) has been informed appropriately of prior hospitalization and carriage data, and where endemic or epidemic problems are present.

Given reported prolonged gastrointestinal carriage of MDR Gram-negative organisms, clearance samples are not recommended. Patient isolation should continue for the duration of the inpatient stay unless there is extensive spread and large numbers of colonized or infected patients. Cohorting affected patients together may then be necessary. Colonized or infected patients should be isolated if re-admitted as emergency cases, and should then be screened. Previously colonized or infected patients should be screened as outpatients if admission is planned, and isolated on admission if screening yields the relevant organism. A method of flagging records is needed.[185]

9.4.2.3.2. How to screen. As the intestinal flora is the main source of MDR Gram-negative bacilli (except *Acinetobacter* spp.), a rectal swab (or stool) is preferred for ease of collection, handling and processing,[162,186] but faecal material must be visible on the swab before putting it into transport medium. A stool sample may be used if there is a risk of mucosal trauma. Rectal or perirectal swabs or stool samples have higher yield than testing of other body sites.[116] In patients with indwelling devices, specimens from the related site should be screened. Skin swabs, urine and sputum should be checked in those with chronic wounds, indwelling urinary catheters or endotracheal intubation. *Acinetobacter* spp. are best detected in axillal, groin or wound swabs.[187]

Screening tests should have a turnaround time of less than 48 h. Confirming the specific carbapenemases is important, but requires molecular methods that often limit availability to reference laboratories. Nevertheless, locally performed phenotypic tests can be extremely helpful as the report is available without delay. These tests are easy to implement for most laboratories, provided that the resources are available and laboratory staff have been trained.[162,168] If carbapenemase confirmation is not possible, isolates should be sent to reference laboratories, although infection control precautions should not be delayed. These tests can prove to be even more useful if they are interpreted in conjunction with data on the background prevalence of carbapenem-resistant organisms in a specific region. A rapid diagnostic turnaround time and timely communication of laboratory results to physicians, nurses and the infection control team are extremely important for infection prevention and control and clinical therapy.

Commercial media for detection of carbapenemase-producing Enterobacteriaceae are increasingly available. In a comparison of four chromogenic media used to detect carbapenemase-producing Enterobacteriaceae, chromID Carba had the best sensitivity and specificity, although this may not be adequate for OXA-48.[148] Disc or tablet diffusion synergy tests use meropenem combined with boronic acid to inhibit KPC carbapenemases, or EDTA to inhibit metallo-carbapenemases (IMP, NDM and VIM). Cloxacillin inhibits AmpC but not KPC, facilitating discrimination between isolates with these types of enzyme in an Etest.[143] High-level temocillin and piperacillin/tazobactam resistance without potentiation of meropenem by EDTA is a marker of OXA-48. Molecular confirmation tests show high sensitivity and specificity, and are used in reference laboratories but are expensive and will not detect novel genes. Although several pseudomonas-selective media are marketed, there are no specific data on screening methodology, frequency or duration with respect to *P. aeruginosa*.[161] *P. aeruginosa* resistant to carbapenems but susceptible to other β-lactams can be assumed not to have carbapenemases, and do not warrant reference investigation.

The accuracy of carbapenemase detection may be affected by the species and origin of the pathogen, type of carbapenemase and other resistance properties, such as porin loss or ESBL production. Phenotypic confirmatory tests such as the modified Hodge test are within the capability of most local laboratories, but depressed AmpC enzymes (and sometimes ESBLs) are associated with weak false-positive modified Hodge tests, especially with ertapenem. The test can be difficult to interpret. Colorimetric and MALDI-ToF methods can be used.[188] Some organisms with OXA-48-like carbapenemases only exhibit low-level carbapenem resistance without cephalosporin resistance, thus escaping the standard identification methods.[188]

High sensitivity and specificity in detecting ESBLs can be achieved using chromogenic selective media, despite mixed flora in catheter urine or faeces. However, competitive bacterial flora resistant to multiple antibiotics, especially cephalosporins, can reduce the specificity of selective media. The use of CTX-M Chromagar (CHROMagar, Paris, France) is superior to ESBL chromogenic agars if seeking cases with a CTX-M ESBL in an outbreak, but the medium is less suitable where, for example, TEM 10 ceftazidimase is present.

Screening for carriage of MDR *A. baumannii* has been described using a variety of media with samples from various body sites including axilla, groin, wounds, rectum or pharynx.[104,116,189–192] There is no consensus on site or method of screening for *Acinetobacter* spp., and sensitivity is poor.[193] Most carbapenem resistance involves OXA-23/40/51/58/143-like carbapenemases, whilst a few isolates have metallo-carbapenemase.[194,195]

9.4.2.3.3. When to screen. There is insufficient evidence to mandate routine screening of all patients for colonization by all MDR Gram-negative organisms. However, screening of high-risk patients is used in control efforts for carbapenem-resistant Enterobacteriaceae (e.g. patient transfers from hospitals where these organisms are prevalent). The contribution of this practice to decreasing transmission is unknown. Nevertheless, identifying patients who are at high risk of colonization or infection with MDR organisms (including carbapenem-resistant organisms) and performing screening by rectal swab (or skin for *Acinetobacter* spp.) on admission to healthcare facilities is recommended, and is now becoming more widespread in healthcare settings.[169] Patients at high risk include patients admitted to ICU and from long-term care facilities (e.g. care homes or endemic areas).

Screening for carriage of ESBL Enterobacteriaceae can identify patients in whom empirical treatment with meropenem may be justified, thus supporting antimicrobial stewardship. Discharge screening for ESBL or carbapenem-resistant organisms is appropriate when admission screening is practised, or if a colonized patient is likely to be re-admitted for further procedures or is going to a long-term care facility. Patients with carbapenem-resistant organisms should have notes flagged pending re-admission or if they transfer to long-term care facilities, where there is a risk of further spread. Long-term care facilities should be informed of positive results of discharge screening on their transferees, and may need to consider if their routine SICPs are sufficiently robust for caring for these patients.

Good Practice Recommendations

Effective communications between healthcare settings will help to facilitate efficient patient transfers, and are crucial in reducing spread.

Local screening policies should be developed to define those patients at high risk of carriage of, for example, carbapenemase producers.

9.4.2.4. What can be done in the case of patients unable or unwilling to consent to a rectal swab? On being admitted to hospital, a patient consents to receive those diagnostic and screening tests that are deemed necessary to the management of their presenting problem. In situations where a patient is incapacitated, those giving care may proceed with any interventions deemed necessary to provide medical treatment for the patient's well-being. This follows the principles of 'implied consent' (i.e. it is reasonable to assume that the person would consent if they were not incapacitated and unable to do so). Implied consent is already used for meticillin-resistant *Staphylococcus aureus* (MRSA) screening, even for patients who have the capacity to consent for themselves.

Screening as part of the ward/hospital policy to guide antimicrobial therapy and/or prevent disease transmission does not require specific written consent, but verbal agreement from the patient before sampling is conducted is required whenever possible. Individual, religious and societal concerns have to be respected. Patients should be informed, whenever possible, of the need and reason for screening (i.e. that it is for their benefit and that of other patients, and what it involves). They should be given the option of who carries it out, including self-screening, but only after assessment of their ability and willingness to comply, and safety of the procedure. The option of a same-sex healthcare practitioner should be provided. Some patients may be unwilling to accept a rectal swab but will provide a stool sample, although this may result in delay or absence of a sample. Ideally, patients should be placed pre-emptively in an isolation room while the screening results are awaited, but this is unlikely to be practicable in many high turnover wards. Patients having chemotherapy or with an underlying bowel condition (stoma, colon cancer, recent anal or rectal surgery) may be more easily screened using stool. Nevertheless, rectal swabs can be collected safely in haematology patients.[196]

9.4.2.5. How frequently does screening need to be performed? Extensive active screening during outbreaks due to carbapenem-resistant organisms is recommended[168] (e.g. follow-up screening of negative cases at weekly intervals and/or for all inpatient contacts with confirmed cases). Although such accounts must be interpreted with caution, experience from outbreaks of MDR Gram-negative organisms, including carbapenemase- and ESBL-producing Enterobacteriaceae, in acute healthcare settings suggests that the implementation of screening for early detection and isolation of colonized patients coupled with contact precautions can help to control transmission.[77,169,197] Screening of those not known to be carriers of carbapenem-resistant organisms in an endemic situation is advisable at least weekly and on discharge.

9.4.2.6. Is there evidence for effective interventions on positive patients (i.e. can carriage be cleared)? A number of studies have evaluated the duration of colonization with MDR Gram-negative bacteria. Studies of hospital inpatients suggest that they tend to remain colonized for the duration of their stay.[104,198–200] Most studies evaluating the duration of colonization outside of acute settings for a range of MDR Gram-negative bacteria have identified mean durations of colonization of months rather than days.[11,201–204] This duration is likely to reflect the particular strain, not its resistance.

Several studies have investigated the duration of colonization with carbapenem-resistant Enterobacteriaceae following discharge from acute care facilities. Risk factors for prolonged carriage of MDR Gram-negative bacteria tend to be associated with healthcare contact, underlying medical conditions and the presence of invasive devices.[11,47,199,205] For example, Lubbert *et al.* evaluated prolonged colonization following an outbreak of carbapenem-resistant *K. pneumoniae* following discharge from an acute hospital.[205] Although 26 (31%) of the 84 patients included tested negative for carbapenem-resistant *K. pneumoniae* at one month post discharge, 45% remained colonized at six months and one patient remained positive for almost 40 months. Two other studies found that approximately half of patients colonized at the time of hospital discharge were spontaneously free by six months.[11,47] However, a number of studies identified patients who retested positive after negative screens, suggesting that gastrointestinal colonization is suppressed rather than eliminated in many cases.[47,205] For this reason, the authors of these studies recommend at least three consecutive negative screens separated by at least 24 h before a patient can be considered 'decolonized'.[47,200,205] In practice, colonized inpatients should be considered 'carriers' during the rest of their hospital admission. The risk period from previous hospitalization exceeds one year.

There is no effective equivalent of the topical suppression used to reduce shedding of MRSA in the healthcare environment. Attempts at eradication of MDR Gram-negative organisms from the gastrointestinal tract have not been successful.[206–209] Selective decontamination of the digestive tract (SDD) can produce some temporary reduction in the number of organisms in faeces (see Section 9.4.6).

Evidence-based criteria for discontinuing contact precautions for carbapenem-resistant Gram-negative organisms in acute care settings have not been developed. Given the likelihood for prolonged gastrointestinal carriage by these organisms and risk of spread, organizations should be cautious in discontinuing contact precautions (patient isolation). In most cases, contact precautions should continue for the duration of the hospitalization

during which the organism was first found on culture. Patients re-admitted within 12 months of that hospitalization should be considered probably colonized, and managed with contact precautions until at least one negative screen is available.

Evidence

Early recognition of patients infected with MDR Gram-negative organisms and implementation of rigorous infection control interventions is usually associated with reduced secondary transmission. 3

Screening (except *Acinetobacter* spp.) is most sensitive when performed on rectal (or perirectal) swabs or stool specimens. 3

Patients transferred from, or who have received medical care in, a healthcare facility in an endemic area of the UK or abroad are at high risk of carriage of carbapenemase-resistant organisms. 2++

Recommendations

A rectal swab (with visible material) or stool sample (and urine sample if catheter present) should be used for screening for multi-resistant Enterobacteriaceae and *P. aeruginosa*. For *Acinetobacter* spp., sample skin sites or (if a catheter or endotracheal tube is present) urine or respiratory secretions. Conditional

Each healthcare organization should have access to robust microbiological arrangements for detecting and reporting MDR Gram-negative organisms in routine clinical samples, and for screening using highly-sensitive tests with a rapid diagnostic turnaround time of $<$48 h. Conditional

All patients transferred from, or with a history in the preceding year of admission to, healthcare facilities with known endemic carbapenemase-producing Enterobacteriaceae should be screened. Strong

In the event of secondary cases of carbapenem-resistant Enterobacteriaceae, SICPs and contact precautions should be monitored and re-inforced with clinical staff. Screening of patients not identified as carriers should be repeated weekly and on discharge from affected units until no new cases are identified for more than seven days. Strong

Where possible, single-room isolation should be provided for patients with MDR Gram-negative bacterial infection/colonization, and contact precautions should be continued for the duration of their stay. Conditional

Patients with previous samples with carbapenem-resistant or other MDR Gram-negative bacteria should be screened at the time of admission. Conditional

9.4.3. Isolation and segregation

A long-standing principle of infection prevention and control is to physically segregate those known to be infected or colonized with a pathogen of epidemiological importance from those who are not infected or colonized. However, critical appraisal is difficult as segregation is usually assessed as part of a package of measures. This physical separation can be achieved through placing patients with known/suspected infection/colonization in single rooms; and/or identifying and isolating patients with the same, confirmed pathogen in a cohort room/area. An additional cohort measure (nursing staff numbers permitting) is to identify staff to care for those patients placed in cohorts. The implementation of screening cultures at the time of admission or during a patient's stay on a particular ward is a way to improve the impact of physical segregation by identifying those who are colonized with the pathogen of concern.

Placing patients known to be infected or colonized with MDR Gram-negative bacteria in single rooms reduces transmission. Several studies have reported the impact of converting a unit from multi-occupancy to single rooms on rates of MDR Gram-negative infection/colonization.[210–214] A Canadian study reported the acquisition rate ratio of various Gram-negative bacteria, comparing an intervention unit that had been converted into single rooms with a control unit in a sister hospital that had not been converted into single rooms.[210] The number of *Acinetobacter* spp., *Klebsiella* spp. and *Enterobacter* spp. fell significantly, but the number of *Pseudomonas* spp. and *E. coli* did not. A 20-year before–after study from a US burns ICU that was converted into single rooms reported a significant reduction in Gram-negative bacteraemia, the time to a first positive Gram-negative culture and mortality.[212] Significant reductions were also reported in bloodstream infections due to *P. aeruginosa*, *K. pneumoniae*, *E. cloacae*, *E. coli* and *Providencia stuartii*. However, there was no control unit, and improvements in burns care during the study may have contributed to these reductions. A separate study from the same renovation reported an overall reduction in infection rates, mortality and non-enteric Gram-negative species.[213] One study from a US ICU reported no significant reduction in overall infection rates, or in rates of *E. coli*, *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp. or *Serratia* spp.[211] Again, this study did not include a control ward. Hand hygiene compliance was low and did not increase when the unit was converted into single rooms, in contrast to other studies.[215,216]

The impact of enhancing patient isolation aside from other interventions has been evaluated. A French study in 2001 found that introducing patient isolation to care for ESBL carriers resulted in a sequential reduction in ESBL incidence and hospital acquisition.[217] However, this preceded the emergence of CTX-M and *E. coli* as dominant factors. A study in a Vietnamese ICU found that improving patient isolation by re-inforcing hand hygiene, and limiting exchange of equipment, materials and staff between patients did not reduce exogenous transmission of various Gram-negative bacteria (gentamicin-resistant *K. pneumoniae*, ESBL Enterobacteriaceae, amikacin-resistant *Acinetobacter* spp. and *P. aeruginosa*), whereas rates of MRSA fell significantly. However, this study did not include physical segregation of patients or the use of gowns or aprons.[218]

Various studies have evaluated switching from a multi-occupancy ward to a single-occupancy ward. An Israeli study showed that there were significantly fewer acquisitions of resistant organisms when an ICU was converted from a multi-occupancy bay to single rooms.[215] The study included a control ward which had not been converted. Patients in the single-room ICU had a significantly lower acquisition rate of resistant organisms when compared with the control multi-occupancy ICU over the same period [3/62 (5%) vs 7/39 (18%), respectively, $P = 0.043$]. SICPs were applied throughout, but compliance with hand hygiene was better in the single-room ICU. Although MDR Gram-negative organisms were included, the significant changes related to Gram-positive pathogens. Another study found that the mean number of nosocomial infections and length of stay were reduced significantly when a multi-occupancy paediatric ICU

was converted to single occupancy.[219] However, no control ward was used, and no specific data on MDR Gram-negative pathogens were reported.

Although these studies suggest that converting multi-occupancy wards to single rooms reduces the spread of MDR Gram-negative pathogens, there are several limitations. First, most studies did not include a control ward (although both of the studies that included a control ward found a significant reduction in transmission).[210,215] Second, compliance with hand hygiene is higher in a single-room format compared with a multi-occupancy setting.[215,216] Thus, it could be that improved hand hygiene rather than improved physical segregation is the critical factor for reducing transmission. All of the studies were performed in an ICU setting, limiting their applicability to settings outside of critical care. Converting wards to single rooms often includes other changes, such as the size of each bedspace, and the location and number of hand hygiene facilities, which could influence transmission rates.[215] Single rooms are associated with a number of drawbacks, particularly an increased risk of adverse events due to reduced observation and psychological effects, so the requirement for patient observation is a key consideration when deciding on the optimal configuration of wards.[220] Finally, patients are increasingly moved around the hospital for procedures and investigations, challenging their segregation.

9.4.3.1. Cohorting staff. No studies have evaluated the impact of cohorting staff aside from other interventions, but several studies have reported cohorting staff as an element of a successful multi-faceted strategy.[77,104,221,222]

9.4.3.2. Disposable aprons and gloves. Data are limited in terms of the most appropriate personal protective equipment to use when caring for patients with MDR Gram-negative bacteria.

Studies have evaluated interventions that have included the use of gloves and gowns or aprons as an element of contact precautions.[137,217,223,224] Hands and uniforms (or gloves and gowns or aprons if worn) can become contaminated with MDR organisms.[126,127,225–230]

The use of gloves is an essential part of prevention of transmission of infection, and the evidence has been reviewed elsewhere.[176] While they protect hands from contamination with biological fluids and micro-organisms, gloves are not a substitute for hand hygiene, and unnecessary use can result in increased cross-contamination. Loveday *et al.* recommended that gloves should be removed immediately after the activity has been completed, and the hands then decontaminated to prevent transmission as they may be contaminated during glove use or during removal.[176]

Evidence

Units composed of single rooms have less transmission of MDR Gram-negative bacteria: *A. baumannii*, ESBL-producing (and, by inference, carbapenemase-producing) Enterobacteriaceae, *P. aeruginosa*. 3

Recommendation

Use disposable gloves and gowns or aprons to care for patients with MDR Gram-negative bacteria: *A. baumannii*, carbapenem-resistant and ESBL Enterobacteriaceae, *P. aeruginosa*. Strong

9.4.3.3. What is the role of isolation in care home/hospital settings? Current practices for the identification and isolation of patients with MDR Gram-negative bacteria (in either single rooms or cohorts) vary widely among healthcare facilities.[231,232] A survey of 66 hospitals in 26 US states and 15 other countries found that 74.2% isolated patients with ESBLs, 93.9% isolated patients with carbapenem-resistant organisms, 81.8% isolated patients with MDR *Pseudomonas* spp. and 84.9% isolated patients with MDR *Acinetobacter* spp. There was considerable variation in the duration of isolation, and few facilities performed screening. Isolation of patients in long-term care facility rooms may not be practicable for psychological reasons.

A number of studies have evaluated the impact of isolating patients with ESBL Enterobacteriaceae aside from other interventions.[217,233–236] A six-year Canadian study evaluated the impact of placing patients with ESBL Enterobacteriaceae in single rooms for the duration of their stay, and applying contact precautions for symptomatic patients.[233] There was an overall increase in ESBL Enterobacteriaceae colonization/infection, but a relative decrease in cases attributable to the hospital, suggesting that local transmission was reduced. A 2-year before–after study in France before the proliferation of CTX-M β-lactamases found that the introduction of admission and weekly surveillance combined with isolation of carriers resulted in a significant reduction in the percentage of patients infected or colonized with ESBL Enterobacteriaceae.[234] There was a significant reduction in ESBL-producing *K. pneumoniae* but not MDR *E. aerogenes*. The study has a number of important confounders, including an intervention to reduce the use of imipenem, encouraging prompt discharge and chlorhexidine bathing for isolated patients. A study in a French paediatric hospital evaluated a sequential change in isolation policy from placing patients in a single room to placing them in a cohort block, applying modelling to test the impact on transmission of ESBL producers.[235] No significant difference was identified between the two isolation protocols, but the model suggested that single-room or cohort isolation reduced transmission of ESBL producers. Finally, a 12-month cluster randomized study in 13 European ICUs evaluated chromogenic agar screening for ESBL Enterobacteriaceae on admission and isolation of carriers vs no admission screening.[236] A hand hygiene improvement programme for staff and chlorhexidine body washing for patients preceded the trial. Of the 2129 Enterobacteriaceae resistant to third- and fourth-generation cephalosporins found on screening, 29% were resistant to carbapenems. Screening and source isolation was not associated with any trend (during the intervention period) or step change (compared with a baseline period) in rate of ESBL transmission. However, individual species were not analysed separately.

Two studies have evaluated the impact of isolation on the transmission of *A. baumannii* and *P. aeruginosa*.[137,223] A seven-year before–after study in France evaluated the introduction of hospital-wide contact precautions, which included placing the patient in a single room or cohort with other patients, and the use of gloves and gowns or aprons, on the transmission of *A. baumannii*.[223] Isolation precautions were implemented for two years, then stopped for three years, then re-implemented for two years; the incidence of *A. baumannii* was significantly lower during the two periods when isolation precautions were in use. The implementation of isolation precautions was the only variable associated with lower incidence of *A. baumannii*

in multi-variate analysis. A follow-up study from the same group reported similar findings.[237] A study from an ICU in Brazil evaluated the introduction of contact isolation, including the use of gloves and gowns or aprons, cohorting of medical items (stethoscopes etc.), daily surface cleaning and disinfection on the rates of MDR *A. baumannii* and *P. aeruginosa*.[137] Bloodstream infections reduced significantly during the intervention, but species were not analysed separately. The study compared rates of infection at two points during the intervention, with no baseline period.

A number of other studies have implemented complex interventions that included increased isolation of patients.[116,221,222,224,238] One study in a US ICU implemented a multi-faceted intervention centred on improved isolation of patients, including admission screening for carbapenem-resistant *K. pneumoniae* but not for *A. baumannii* or *P. aeruginosa*.[221] There was a significant reduction in the rate of carbapenem-resistant *K. pneumoniae,* but no change in the rate of *A. baumannii* or *P. aeruginosa*. A study of various interventions in six US hospitals found that the only consistent predictor of successful control of *K. pneumoniae* with KPC carbapenemases was a shorter length of stay.[238] Three studies have evaluated the impact of a multi-faceted intervention to control the spread of *A. baumannii*. An intervention at US field hospitals in Iraq including improved hand hygiene, contact precautions, and cohorting patients and staff resulted in a significant reduction in *A. baumannii* ventilator-associated pneumonia.[222] A bundle of interventions including contact precautions, screening (on occasion) and regular staff briefings reduced the rate of MDR *A. baumannii* infections significantly overall, particularly bloodstream infections due to the organisms, in a Spanish hospital.[116] Finally, a study in a Thai ICU reported a significant reduction in MDR *A. baumannii* associated with the introduction of contact precautions, cohorting colonized patients, screening and environmental disinfection using bleach.[224] However, acinetobacter infections are more common in Thailand than in the UK.

Studies that have evaluated the impact of isolating patients in either single rooms or cohorts generally lack a concurrent control unit, making it difficult to be certain that changes in rate are attributable to isolation alone. The one study that included a concurrent control ward demonstrated no significant decrease in transmission of ESBL-producing Enterobacteriaceae.[236]

Evidence

Transmission of MDR Gram-negative bacteria is reduced by identifying patients who are infected or colonized, and placing them in single rooms or cohorts. 3

Recommendations

Identify and place infected and colonized patients in single rooms where available in this order of priority: carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *A. baumannii,* ESBL *Klebsiella* spp., carbapenemase-producing *P. aeruginosa*, ESBL *E. coli* and other Enterobacteriaceae, AmpC Enterobacteriaceae. Strong

If insufficient rooms are available, cohort isolate following local risk assessment. Conditional

Good Practice Recommendation

Establish a flagging system for patient notes. Conditional

9.4.4. Hand hygiene

Hand hygiene is an essential part of prevention of transmission of infection in health care, and it has been reviewed by Loveday *et al*.[176] Transient flora, including MDR Gram-negative bacteria, is acquired from touching the patient or environment, and is easily transferred to the next patient or surface. In turn, this causes colonization and later (potentially) infection. Use of alcohol hand rub (or liquid soap and water if hands are visibly soiled) has been shown to reduce the carriage of potential pathogens on the hands, and therefore is likely to reduce the number and likelihood of healthcare-associated infections.[174,176] Hand decontamination is considered to have a high impact on outcomes that are important to patients.[239] Training of all care workers, combined with audit and feedback of compliance rates, is central to current guidance on prevention of infection. The WHO Five Moments for Hand Hygiene has been widely used and supported by NICE, and describes the points at which hand hygiene is required.[239] Hand hygiene is often missed as the worker enters or leaves the patient environment, and on contact with potentially contaminated surfaces[240–242]

Despite many activities around the improvement of hand hygiene products, the introduction of alcohol-based hand rubs/gel, improving environmental factors, and extensive marketing and education campaigns, evidence of sustained compliance improvement is lacking. Globally, hand hygiene compliance is variable, with Creedon[243] and Larson *et al*.[244] suggesting a rate of approximately 50%. Therefore, improving compliance with hand hygiene is reliant upon an understanding of the interactions within health care between the individuals, the practices and procedures they perform, the environment that they work in, and the culture and safety awareness of the organization. These human factors should be applied to the infection prevention agenda to ensure that meaningful, sustainable interventions are adopted reliably to produce the greatest impact.[245]

Evidence

Hand hygiene is associated with reduction of carriage of potential pathogens on the hands. 2+

Recommendation

Hand hygiene is required before and after direct patient contact; after contact with body fluids, mucous membranes and non-intact skin; after contact with the immediate patient environment; and immediately after the removal of gloves. Strong

9.4.5. Environmental hygiene

9.4.5.1. When should the environment be sampled? The role of the environment in the transmission of healthcare-associated infection remains controversial and difficult to study. In recent years, much of the research around this has focused on high-profile Gram-positive pathogens such as MRSA, *Clostridium difficile* and glycopeptide-resistant enterococci, where a significant body of evidence for the importance of environmental sources has started to accumulate.[246,247] Reservoirs can be identified when infection control has failed to control an outbreak.[161] The general view has been that Gram-negative bacteria, particularly members of the Enterobacteriaceae, are not as successful at surviving in the environment for prolonged periods, and generally are relatively easily removed by

appropriate conventional cleaning and drying.[13] Nevertheless, Enterobacteriaceae, including carbapenem-resistant strains, are able to survive on dry surfaces for extended periods, sometimes measured in weeks and months.[248,249] Some strains of *E. coli* carrying ESBLs, for example, can survive for a median of 10 days in the environment.[250] In favourable conditions, *E. coli, Klebsiella* spp. and *Pseudomonas* spp. can survive for even longer.[161] *Acinetobacter* spp. have the capacity for long-term survival on dry surfaces.[14,251,252,253] *Pseudomonas* spp. are more traditionally associated with moist environments, and will be found around handwash basins and respiratory equipment.[35,254] There is considerable evidence that *P. aeruginosa* can contaminate waste water systems and spread from them, with these sometimes acting as reservoirs for carbapenemase-producing strains causing prolonged outbreaks.[255] If not adequately decontaminated (including the hand piece), endoscopes have been found to be responsible for outbreaks.[256]

MDR Gram-negative bacteria, including carbapenem-resistant organisms, Enterobacteriaceae and *A. baumannii*, can be cultured from sites surrounding infected and/or colonized patients.[106,257] Several studies have found that environmental contamination with resistant *K. pneumoniae* is more common than contamination with resistant *E. coli*.[258–260] No controlled studies have shown that an environmental intervention reduces the transmission of MDR Gram-negative rods. However, contamination with MDR Gram-negative bacteria can persist despite cleaning and disinfection.[106,261,262] Epidemiological data suggest that admission to an ICU room previously occupied by patients infected or colonized with *A. baumannii* or *P. aeruginosa* presents an increased risk for acquiring these organisms.[252] A significant correlation was noted between the number of environmental swabs in monthly screening and the number of patients with colonization/infection in the same month ($P = 0.004$).[103] However, this association was not seen for resistant Enterobacteriaceae, suggesting that the environment is less important in their transmission,[252,263] albeit with the caveat that these studies did not stratify by Enterobacteriaceae spp., which may be important given the increased capacity for *K. pneumoniae*, in particular, to contaminate and survive on hospital surfaces.

The evidence for the benefit of environmental screening is limited, and environmental sampling, in itself, will not limit transmission of MDR Gram-negative bacteria.[264] The purpose of screening may be to draw attention to failure of clearance of an outbreak strain by cleaning, or to point to a possible common source for a cluster or outbreak. Most of the evidence surrounding environmental screening is within reports of management of outbreaks, and there is often very little detail on sites sampled, sampling technique or method of culture.[265] When looking for small numbers of organisms or bacteria living in biofilms, the yield can be improved by using enriched culture, but this precludes quantitation. An alternative to specifically seeking the MDR outbreak strain(s) itself is to assess the degree of microbiological contamination by using surface contact plates and undertaking quantitative bacterial culture.[266] Surface contact plates using selective media for specific pathogens can suffer from low sensitivity, but use with non-selective media may be helpful. Choosing sites for sampling remains problematic as any sampling can only reflect a very small fraction of the relevant environment.[267] The general approach should be to choose sites that are likely to be relevant for cross-transmission, such as work surfaces close to the patient and equipment/surfaces that are likely to be touched frequently (e.g. computer keyboards, bed rails and door handles).[264,268]

Given the difficulties with microbiological sampling of the environment, there has been considerable interest in the availability of technologies that can indirectly assess microbial contamination. Adenosine triphosphate (ATP) bioluminescence has been used for many years in the food industry, where visual assessment is considered to be insufficient to assess the risk of infection from contaminated surfaces. It has a number of potential benefits, including simplicity and immediacy of feedback; however, it can give only indirect information on the likelihood of bacterial contamination, and bacteria themselves are not isolated for investigation and comparison with those causing outbreaks. Its use in hospitals can be problematic, as values can be variable and there is little consensus on an appropriate benchmark value to indicate inadequate cleanliness.[166,269,270] Results are prone to interference by different disinfectants.[271] Where ATP bioluminescence has been used most successfully has been on a continuous basis to improve compliance through feedback and improved cleaning.[272,273] Other methods of controlling the process of cleaning, such as the use of fluorescent gel markers, can be useful in auditing the cleaning process and feedback to cleaning staff.[274]

Evidence

Transmission of MDR Gram-negative bacteria, particularly non-fermenters, has been associated with contamination of the environment, water systems or equipment. 2–

Sampling of the environment can be useful in identifying sources of ongoing transmission or a single common source for an outbreak. 2–

Recommendation

Environmental screening should be considered where there is any unexplained transmission of MDR Gram-negative organisms or a possible common source for an outbreak. Strong

9.4.5.2. What is the evidence that respiratory equipment contributes to transmission? The respiratory tract of ventilated patients in critical care units is a frequent site of carriage of MDR Gram-negative bacteria. In one study in India, 87% of samples from ventilators, humidifiers, nebulizers and other respiratory equipment showed bacterial colonization, and 17/42 Gram-negative isolates were multi-drug resistant.[275] Endotracheal suctioning is a potential cause of cross-infection, as disconnection of the system may allow airborne spread to the patient's skin, staff hands and the immediate environment. In a crossover study in the Netherlands, there was no significant difference in acquisition of Gram-negative bacteria during periods of using closed suction vs open suction in ventilated patients.[276] However, antibiotic resistance rates were low and similar in each group. Comparing ventilated with non-ventilated patients in the ICU, non-fermenting and enteric Gram-negative bacilli were reported more frequently in ventilator-associated pneumonia, but this reflected the number of samples cultured per patient. The overall proportions of different pathogenic species were similar.[277]

Inappropriate washing of ventilator or endotracheal tubing in handwash sinks risks the spread of Gram-negative pathogens. Environmental sampling in two outbreaks of MDR *P. aeruginosa* showed it to have colonized the waste

water system, with blockages, splashback and spillage from showers being possible modes of spread.[35] Direction of water directly into the outlet caused spread of organisms from the sink drain trap. Sinks were fitted with a horizontal drain outlet at the back of the basin, resolving the problem. Sterile fluids (not tap water) must be used to clear suction equipment, as the latter may be contaminated with pseudomonads or Enterobacteriaceae, and these can be disseminated when the equipment is next used, including when catheters are changed.

Evidence

Gram-negative bacteria colonize respiratory equipment and may be washed into sink traps. 2+

Recommendation

Respiratory and other contaminated equipment should be decontaminated (or respiratory secretions discarded) away from the immediate bed area in designated cleaning sinks and not in handwash sinks. Strong

Good Practice Recommendation

Do not discard patient wash water, body fluids, secretions or exudates into handwash basins.

9.4.5.3. What is the evidence that sensor taps contribute to transmission? P. aeruginosa, including MDR strains, in water sources in ICUs has long been recognized as being associated with the development of bacteraemia and pneumonia in patients.[278] A recent systematic review demonstrated evidence of transmission of *P. aeruginosa* from water systems to patients and vice versa.[34] Point-of-use filters and increasing chlorine disinfection were effective interventions. Non-touch taps were identified as probable risk factors for biofilm formation and subsequent transmission to patients. Sinks and nurses' hands have been identified as possible vectors. In a neonatal unit, four neonates were infected and 44 were colonized by cross-infection with one clone.[124] In another neonatal unit in Germany, *P. aeruginosa* was isolated from the nasal prongs of 22 babies and from nine respiratory water reservoirs.[279] The hands of staff were the likely means of transmission. Other outbreaks have been due to contaminated detergent-disinfectant solution used in cleaning surfaces.[280] Studies have confirmed the effectiveness of point-of-use filters on water outlets in reducing infection in critical care units, but as they were descriptive cohort studies, they were subject to temporal variation.[281–283] Filters have to be replaced regularly, are expensive and can themselves be the source of contamination. *P. aeruginosa* is found in biofilm in flow straighteners, metal support collars and the adjacent parts of tap bodies. The level of contamination with *Pseudomonas* spp. is highest on complex flow straighteners, integrated mixers and solenoids.[278]

Sensor taps have been implicated in some outbreaks of pseudomonas bacteraemia and other infections in augmented care units as the flow is slow and controlled, and the internal mechanism is complex. Decontamination of sensor taps can be performed[284] and, in one major outbreak, all taps, mixer valves, flexible hoses and flow straighteners were replaced with simpler designs.[278]

Where there are vulnerable patients (i.e. augmented care), the Department of Health in England recommends a regular water-testing regimen; clinical surveillance is sufficient elsewhere. The frequency of testing depends on previous isolation of the organism and proximity to patients at risk (e.g. neonates). The purpose is to prevent colonization before the development of infection. A risk assessment to mitigate risks and a water safety plan are advised, including consideration of removal of thermostatic mixer valves and flow straighteners, and the design of the sink.[36] Infrequently-used taps should be flushed at full flow for 1 min daily or removed altogether. In the event of contaminated supplies, sterile water should be used for neonates, and single-use wipes should be used for other patient hygiene together with additional hand hygiene using alcohol gel after washing.

Evidence

The presence of infections with *P. aeruginosa* in patients is commonly associated with isolation of these bacteria from unit taps. 2–

The installation of point-of-use filters is associated with a reduction in pseudomonal infections. 3

Recommendation

For *P. aeruginosa*, including MDR strains, at a minimum in accordance with the organization's water safety plan, a risk assessment should be made when levels of patient colonization or infection rise, in order to determine if point-of-use filters should be installed or taps changed. Strong

9.4.5.4. Is any cleaning method more effective than others at removing multi-drug-resistant Gram-negative bacilli from the environment? The importance of persistence of transmission of MDR Gram-negative bacteria in the environment remains uncertain.[246,285] However, maintaining a clean environment and appropriately decontaminating all relevant equipment is an essential component of any infection prevention and control programme. The cleanliness of healthcare premises is an important component in the provision of clean safe care.[286] In England, the NHS Constitution pledges 'The NHS commits to ensure that services are provided in a clean and safe environment that is fit for purpose, based on national best practice'.[287] Whilst there have been significant improvements in the cleanliness of English healthcare premises, there is still room for improvement.[286]

The optimal methods for cleaning have been poorly studied with respect to MDR Gram-negative bacteria. Poor cleaning practice, such as inappropriate dilution of cleaning agent or inactivation of disinfectant by organic matter, is more likely to reduce cleaning efficacy than theoretical cross-resistance between disinfectant and antibiotic.[288] In extreme cases, agents used for cleaning can even become contaminated with Gram-negative bacteria, especially pseudomonads.[141,289–291]

Conventional decontamination is carried out by a human operator, and the reliance on the operator to select the correct product, dilution, distribution and surface contact time has the potential for decontamination failure. Debate around the use of chemical disinfectants vs detergents for routine cleaning is gathering momentum with concerns regarding chemical resistance, optimum disinfectant contact times, allergies amongst users and patients, and costs. In short, practice varies widely and monitoring is traditionally by visual inspection, which can be subjective. Efficacy is rarely measured.

Although persistence of Gram-negative organisms on reusable bedpans is a potential mode of spread, there are no

recent reviews of performance with respect to Gram-negative bacteria. In automated washer disinfectors, a combination of alkaline detergent and temperature over 85°C for 1 min is sufficient to eliminate *C. difficile* spores.[292] However, visible faecal soil can remain on 7–33% of bedpans, so appropriate education is important to ensure good practice.[293] Early studies showed that failure to attain 80°C was associated with persistence of *E. coli* and *P. aeruginosa*.[294] Transmission of pathogens via contaminated endoscopes is usually due to failure to comply with appropriate reprocessing practice guidelines.[295] Although some cases of transmission have been known, relatively few have been reported in peer-reviewed journals.

Assessment of the activity of disinfectants against MDR Gram-negative bacteria is hampered by the differences between testing against organisms in suspension and on a surface. *Acinetobacter* spp. and *Pseudomonas* spp. may survive in the ward environment even after bleach disinfection, and increase the risk of acquisition by patients.[106,252] In a prospective cohort study, cleaning the environment with sodium hypochlorite instead of detergent-disinfectant plus chlorhexidine bathing of patients reduced the risk of colonization with MDR *A. baumannii* becoming established beyond that achieved by improved contact precautions, cohorting, screening and antimicrobial stewardship.[296] Termination of acinetobacter outbreaks has been associated with closure and cleaning of units, and removal of a reservoir has been associated with termination of outbreaks for a variety of organisms.[161]

Most interventions to improve cleaning have been accompanied by a package of infection control precautions. Education campaigns and use of audit tools such as marking surfaces with fluorescent dye reduce contamination, but their effect may be transient.

Recently, there has been significant interest in the role of 'no-touch' automated disinfection systems as an additional measure for terminal cleaning of single rooms or hospital areas affected by clusters or outbreaks.[297] Various types of systems are currently marketed, including aerosolized or vapourized hydrogen peroxide, and ultraviolet radiation. Each has distinct microbiological and practical characteristics. A number of studies have shown improved efficacy of killing of various pathogens compared with cleaning alone, particularly in outbreaks, but there are limited data on whether this reduces acquisition rates of pathogens.[298–300] Hydrogen peroxide vapour from 30% H_2O_2 and, in one case, aerosolized hydrogen peroxide from 5% H_2O_2 have been used successfully as part of a bundle of interventions to prevent transmission of MDR *Acinetobacter* spp. in outbreaks,[105,107–109] and hydrogen peroxide vapour has been used to help control outbreaks of resistant Enterobacteriaceae, including those resistant to carbapenems.[262,301,302]

Patients admitted to rooms exposed to hydrogen peroxide vapour were significantly less likely to acquire any MDR organisms than patients admitted to rooms that had not been treated with hydrogen peroxide vapour in a cohort intervention study.[299] *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* in suspension are all susceptible to low concentrations of hydrogen peroxide, but efficacy is significantly reduced when the organisms are in biofilm; some *P. aeruginosa* in biofilm had prolonged survival following hydrogen peroxide exposure in the laboratory.[303] Hydrogen peroxide vapour (from 30% H_2O_2) is more effective in eradicating surface *A. baumannii* than aerosolized hydrogen peroxide (from 5% H_2O_2) (>6 log vs 1-4 log reduction).[304] It is essential to ensure that the area has been thoroughly cleaned first, because efficacy is affected by the presence of organic soiling.

Evidence

Cleaning is important in the control of outbreaks due to MDR *Acinetobacter* spp., and failure to clean specific areas or pieces of equipment has been associated with transmission of other MDR Gram-negative bacteria. However, evidence derived from before–after studies and outbreak reports is open to bias. 2–

Hydrogen peroxide vapour is effective in reducing environmental reservoirs of *Acinetobacter* spp. and other Gram-negative bacteria on surfaces (but not sink traps) if used in addition to standard cleaning. 2+

Recommendations

Terminal disinfection of vacated areas with hypochlorite should be used in the control of outbreaks of MDR Gram-negative infection. Conditional

Hydrogen peroxide vapour should be considered as an adjunctive measure to follow cleaning of vacated isolation rooms/areas. Conditional

Good Practice Recommendation

Increase cleaning frequency to at least twice daily, and every 4 h for high-contact surfaces in the presence of resistant Enterobacteriaece and *Acinetobacter* spp.

9.4.6. Selective decontamination: why is it not used? Is there a role?

SDD is an intervention that aims to reduce mortality and morbidity due to healthcare-associated infection in ICUs. It comprises application of non-absorbable antibiotics to the mouth and stomach together with a course of broad-spectrum intravenous antibiotic(s). A modification uses only the topical element of decontamination [selective oropharyngeal decontamination (SOD)]. The practice has been investigated extensively, largely in the Netherlands, and at least 12 meta-analyses of published papers have been produced.[305] Almost one-third of the trials suggest a significant reduction in the incidence of Gram-negative pneumonia, and one large randomized study demonstrated a small but significant reduction in mortality in a country with low levels of antibiotic resistance.[306] Despite issues with blinding, heterogeneity and compliance, both SDD and SOD appear to be associated with a reduction in pneumonia (OR 0.32, 95% CI 0.26–0.38) and mortality (OR 0.75, 95% CI 0.65–0.87).[307]

Nevertheless, only 5% of UK ICUs use SDD, largely because universal use of prophylactic antibiotics is counter to the tenets of antimicrobial stewardship.[308,309] In some studies with long-term surveillance, both SDD and SOD were associated with an increase in resistance to ceftazidime in Gram-negative flora of the respiratory tract, although systemic antibiotics were also given in many cases.[310–312] In the short term, however, a systematic review found a significant reduction in resistance of Gram-negative bacilli to third-generation cephalosporins during the use of selective decontamination.[208] The relevance of findings from one country to another is unclear when patterns and prevalence rates of resistant Gram-negative bacteria vary so widely. Preparation requires suitable manufacturing units, and administration can be labour intensive.

Recent high-quality studies have provided evidence that daily bathing using chlorhexidine gluconate in ICUs helps to reduce bacteraemia, but there is limited evidence of its specific effect on MDR Gram-negative bacterial infection.[313–316] This has formed part of successful bundles of interventions, but has not been tested as an isolated measure.[236,296,317–319] Some studies that have evaluated chlorhexidine as a single intervention including randomization have failed to demonstrate a reduction in Gram-negative bacteraemia.[314,316,320] There is no strong evidence that daily bathing with chlorhexidine reduces Gram-negative infection or colonization, and there is a risk of development of resistance.[114] The routine use of oropharyngeal chlorhexidine has been associated with an increase in mortality in one systematic review.[321]

Selective decontamination can temporarily suppress excretion of carbapenem-resistant organisms from the gastrointestinal tract and possibly supplement SICPs.[207] In a retrospective analysis of a German outbreak, SDD with colistin and gentamicin as oral solution and gel was used in 14 patients with proven carriage of carbapenemase-producing *K. pneumoniae* KPC-s-KP ST258.[311] Loss of carriage, as defined by three PCR screens 48 h apart, was found at a mean of 21 days in six treated patients (43%), but also in 30% of controls. Resistance to colistin and gentamicin in post-treatment isolates of *K. pneumoniae* rose 19% and 45%, respectively, compared with controls. In a randomized placebo-controlled trial of a regimen based on colistin and neomycin plus treatment of bacteriuria with nitrofurantoin, the detection of ESBL Enterobacteriaceae in rectal swabs was not affected significantly.[322] A randomized trial against placebo used oral gentamicin and topical oropharyngeal gentamicin and colistin for one week, and was directed against carbapenem-resistant *K. pneumoniae* carriage. It produced a significant reduction in carriage at two weeks but not at six weeks.[207] Mortality was not affected significantly, but throat carriage was reduced from 30% to zero in the intervention vs 35% to 30% with placebo ($P < 0.0001$). Isolates did not develop resistance. A lower-quality controlled study reported reductions in faecal and pharyngeal carriage of *Acinetobacter* spp. during colistin-/tobramycin-selective decontamination.[323] Against this, colistin/neomycin/nalidixic acid did not reduce infections or mortality due to MDR Enterobacteriaceae compared with no prophylaxis, but carriage was reduced ($N = 86$, RR 0.28, 95% CI 0.03–2.28).[324]

Evidence

The use of SDD or SOD is associated with a reduction in the incidence of pneumonia due to Gram-negative bacteria. 1+

The use of SDD or SOD is associated with a reduction in the mortality rate. 1+

SOD can be used to reduce excretion of MDR Gram-negative bacteria during an outbreak, but no effect on transmission of infection has been demonstrated, and application can be logistically difficult. 3

Recommendation

The routine use of SDD or SOD is not recommended for control of MDR Gram-negative bacteria. Conditional

9.5. What are the minimum standards to stop spread in public areas, primary care or care homes?

Care homes are recognized as potential reservoirs of MDR pathogens, which can spread among residents, generally as colonizers, and can be re-introduced into hospitals.[325] Publicity may alarm residents, their relatives and carers. Clear information on the standards of infection prevention and control should be available to promote confidence in the quality of care provided. Despite the relatively poor evidence base, guidelines are available for managers and carers.[239,326] In England, the Code of Practice[326] defines what is required to ensure compliance with Care Quality Commission registration requirements for cleanliness and infection control. Following general principles, owners are encouraged to contact the local health protection team in the event of outbreaks of infection, increase cleaning and hand hygiene compliance by patients and staff, conduct root cause analyses, and train staff in infection prevention and control. The prevalence of ESBL Enterobacteriaceae in the care home is usually unknown and sporadic. The need for a urinary catheter, if present, should be reviewed regularly and it should be removed as soon as it is not needed. The appropriate use of such devices to prevent healthcare-acquired infection has been reviewed elsewhere.[166] Residents with diarrhoea should be isolated in their room with a dedicated commode if no ensuite facilities are available. This isolation may require additional psychological support for the resident. Colonization with MDR organisms should not be construed as a reason to isolate or screen other residents. SICPs must continue to be applied.

Evidence

Institutionalized patients/residents are more likely to carry MDR organisms and present a risk on re-admission to hospital. 2+

Good Practice Recommendations

Care homes should adopt national recommendations for environmental and equipment cleanliness, and infection prevention and control.

Clear patient information on MDR Gram-negative infection must be provided in accessible formats to encourage good hand hygiene by patients and staff.

9.6. Are there organizational structures within a healthcare facility that play a role in the successful control of multi-drug-resistant Gram-negative bacilli?

In Israel, with prevalent ST258/KPC *K. pneumoniae*, a self-contained nursing unit with dedicated staff for patients carrying carbapenem-producing Enterobacteriaceae was effective as part of a package of measures including mandatory reporting and a centralized national monitoring system.[77] In the same way, a centralized national system was effective in the control of MRSA in the UK. Outbreaks of carbapenemase-producing *K. pneumoniae* appear to have been controlled by a package of measures, including nursing infected patients in separate units with different staff until discharge or cohorting.[197,262,327–329] The nursing units could be single

rooms, cohorts or wards, but the nurse did not cross between units during the shift. A sequential intervention study found that control was effective when patients were nursed in separate locations with dedicated nursing personnel. The shared medical staff had contact precautions vetted by the dedicated nurse before entering the area.[328] However, the intervention also included education, training, cleaning and screening.

Evidence

A separate unit with dedicated nursing staff rather than part of a ward with shared staff is effective in control packages. 3

Recommendation

Monitor hand hygiene of all staff when patient cohorting is being applied. Strong

10. Further research

- PCR-based tests for swift detection of MDR Gram-negative pathogens require further testing and development to allow cost-effective and rapid screening and detection of carriers.
- Consideration should be given to further investigation of screening patients of long-term care facilities as a means of tracking movement and defining reservoirs of organisms. Longitudinal assessment of patients carrying ESBL-producing organisms, particularly *E. coli* ST131, should be made on admission and discharge to hospitals and nursing homes to detect infection and spontaneous clearance of carriage.
- The risk factors for development of infection, such as urinary catheterization, should be separated from the risk factors for carriage, including hospitalization. Household contacts of cases of infection with *E. coli* ST131 or carbapenem-resistant *K. pneumoniae* should be examined for evidence of spread or subsequent infection.
- Given the recent changes in the prevalence of, for example, ESBL-producing *E. coli* ST131 and *K. pneumoniae* ST258 with KPC carbapenemases, the potential for screening patients for particular clones as means of controlling transmission in hospitals should be assessed. MALDI-ToF, serological and DNA-based detection methods should be developed, together with selective or indicator media for quinolone-resistant Enterobacteriaceae.
- Bacteraemia surveillance including sequence typing for strains resistant to cephalosporins, fluoroquinolones and carbapenems should be expanded and open to public and commissioning groups. This is essential for accurate risk assessment of patients transferred between healthcare facilities. In localities with outbreaks, typing and infection control should be extended to isolates from other anatomical sites.
- Randomized intervention studies of effectiveness measures, including single-room isolation, in the prevention of transmission of Gram-negative bacterial infection.
- The potential for endoscopes to disseminate MDR Gram-negative pathogens requires better understanding with reference to complex instruments such as those with wire-containing channels.

Conflict of interest statement

See full details in Section 4.4.

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Appendix 1. Glossary

Active surveillance: Admission regimen that involves testing of patients to detect the presence of multi-drug-resistant organisms.

AmpC β-lactamases: Clinically important cephalosporinases encoded by the chromosomes of many Enterobacteriaceae or (less often) by plasmids. High-level expression confers resistance to penicillins (except temocillin), cephalosporins (except cefepime), aztreonam and penicillin-β-lactamase inhibitor combinations.

Antimicrobial: A substance that kills or inhibits the growth of micro-organisms.

Augmented care area: Units where medical/nursing procedures render the patients susceptible to invasive disease from environmental and opportunistic pathogens (e.g. critical care, neonatal, burns and haematological wards).

Bacteraemia: The presence of micro-organisms in the blood stream.

safety, including exposure to infections (e.g. disposable gloves and disposable aprons).

Porins: Proteins that span the outer membrane of Gram-negative bacteria and mycobacteria forming pores that allow the entry of small water-soluble molecules, including antibiotics.

Screening of patients: Sampling potential colonization sites for multi-drug-resistant Gram-negative pathogens.

Selective decontamination: The prophylactic use of topical and systemic antibiotics to remove pathogenic organisms from the gastrointestinal tract to reduce the incidence of respiratory tract infections.

Standard infection control precautions (SICPs): Basic infection prevention and control measures necessary to reduce the risk of transmission of infectious agents from both recognized and unrecognized sources of infection. Sources of (potential) infection include blood and other body fluid secretions or excretions (excluding sweat), non-intact skin or mucous membranes, and any equipment or items in the care environment that could have become contaminated.

Appendix 2. Guideline development

The subject was identified by the Scientific Development Committee of HIS in February 2011 and approved by HIS in May 2011. The BSAC Council agreed a similar proposal at the same time. The BIA Council agreed to join in September 2011. The members were chosen to reflect the range of stakeholders, and were not limited to members of the three societies. The questions were decided at the first meeting of the Working Party Group in November 2011 from issues presented to the members and patient representatives by staff and patients in the preceding months. Each was debated by the Working Party Group before adoption. Enhance Reviews Ltd was paid for the search and data extraction. Working Party members were not paid, except for travel expenses.

Conflict of interest

Conflicts of interest were registered at the outset and renewed during the process. They are stated in the report. In the event of a potential conflict being identified, the Working Party agreed that the member should not contribute to the section affected.

Infection control: systematic review process

Patient Intervention Comparison Outcome process

Patients: All patient groups were included. The guideline is careful not to make recommendations that may prejudice clinical care based on sex, age, ethnicity or socio-economic status.

Interventions: Interventions were identified in the literature to generate intervention-specific recommendations.

Comparisons: Comparisons between intervention and standard management were used.

Outcomes: Objective referring to length of hospital stay, mortality, rate of acquisition or infection.

Systematic review questions

1. What is the definition of MDR Gram-negative bacilli?

β-lactamases: Enzymes produced by some bacteria that confer resistance to β-lactam antibiotics, such as penicillins and cephalosporins, by breaking down the central structure of the antibiotic.

Carbapenemases: β-lactamases that inactivate carbapenems such as meropenem; most also attack and confer resistance to penicillins and cephalosporins.

Care area: Any portion of a healthcare facility where patients are intended to be examined or treated.

Cleaning: Methods that physically remove soil, dust and dirt from surfaces or equipment.

Cohorting: Imposed grouping within an area of a hospital ward of patients or staff potentially exposed to designated diseases.

Colonization: Situation whereby micro-organisms establish themselves in a particular environment, such as a body surface, without producing disease.

Community-acquired: Infection or colonization that is acquired outside of hospitals.

Community-associated: Usually defined as infection or colonization detected in an outpatient or within 48 h of hospital admission.

Contact precautions: Hand hygiene is performed before touching the patient and prior to wearing gloves, and wearing gloves when touching the patient and the patient's environment. A single room is preferred; otherwise, discussion with infection control personnel to consider cohorting or not moving the patient. An apron/gown is worn for all patient interactions that may involve contact with the patient or potentially contaminated areas in the patient's environment. Donning personal protective equipment on room entry and discarding before exiting the patient room is required to contain pathogens, especially those that have been implicated in transmission through environmental contamination.[173]

Extended-spectrum β-lactamases (ESBLs): β-lactamases that attack cephalosporins with an oxyimino side chain (e.g. cefotaxime, ceftriaxone and ceftazidime), as well as the oxyimino-monobactam aztreonam. Unlike AmpC β-lactamases (q.v.), they are inhibited by clavulanic acid and tazobactam, and unlike carbapenemases (q.v.), they do not attack carbapenems

Healthcare-associated (acquired): Infection or colonization detected in an inpatient more than 48 h after hospital admission.

High-risk: Used to describe those patients or facilities where the risk of acquiring infection is in the upper 10% of the total patient population.

Infection: Invasion by and multiplication of pathogenic micro-organisms in the body, producing tissue injury and disease, requiring treatment.

Long-term care facility (care home/nursing home): Provides accommodation and meets the needs of patients with chronic illness or disability who cannot care for themselves.

Multi-drug-resistant (MDR) Gram-negative bacteria: Bacteria resistant to at least three different antibiotics.

Outbreak: At least two similar (i.e. not distinct) cases related in time and place.

Passive surveillance: Review of routine clinical samples of all patients by microbiologists on reporting results from the laboratory.

Personal protective equipment: The equipment a person wears to protect themselves from risks to their health or

2. What Gram-negative bacilli cause infection control problems?
3. What are the relative contributions of community and hospital acquisition?
4. What is the evidence for reservoir and spread of MDR Gram-negative bacteria in care homes and secondary care?
5. What is the role of agricultural use of sewage and antibiotic treatment in veterinary practice in spreading ESBLs?
6. What insights have national *E. coli* bacteraemia surveillance provided?
7. What is the role for screening in patients and staff?
8. What organisms should screening include?
9. Who, how and when to screen patients for MDR Gram-negative bacilli?
10. What can be done concerning patients unable to consent to a rectal swab?
11. How frequently does screening need to be performed?
12. Is there evidence for effective interventions on positive patients (i.e. can carriage be cleared)?
13. Selective decontamination: why is it not used? Is there a role?
14. When should the environment be sampled?
15. What is the evidence that respiratory equipment contributes to transmission?
16. What national surveillance is performed and how should it be developed?
17. What is the evidence that sensor taps contribute to transmission?
18. Is any cleaning method more effective than others at removing MDR Gram-negative bacilli from the environment?
19. What is the evidence that infection control precautions prevent transmission?
20. Are SICPs sufficient to stop transmission?
21. What are the minimum standards to stop spread in public areas, primary care or care homes?
22. Is there evidence for high-/low-risk areas within a healthcare facility?
23. Are there any organizational structures within a healthcare facility that play a role in the successful control of MDR Gram-negative bacilli?
24. How should we undertake local screening, why is it important and how should it be interpreted?
25. At what point should passive surveillance switch to active surveillance (i.e. screening)?
26. What is the role of isolation in care home/hospital settings?
27. Is there evidence of differences between organisms in respect of transmission, morbidity and mortality?

Antimicrobial chemotherapy: systematic review process

Systematic review questions

1. What is the clinical importance of carbapenemases vs Amp C and CTX-M strains?
2. What impact have returning travellers had on UK epidemiology?
3. What is the global epidemiology of MDR Gram-negative bacteria?
4. How do MDR Enterobacteriaceae differ from the non-fermenters in terms of their prevalence and associated resistance genes?
5. What is the efficacy of carbapenems, mecillinam, temocillin, fosfomycin and colistin against specific pathogens?
6. What are the recommended antibiotics for community/secondary/tertiary care?
7. What is the threshold level of resistance for changing choice of empirical treatment for urinary infection?

Databases and search terms used 23rd May 2014[358]

Databases

The Cochrane Library; MEDLINE; EMBASE; CINAHL

MeSH terms Appendix F, see online supplementary materiial

Search dates: Medline 1946–2014; Embase 1980–2012; CINAHL (1984–2012)

Search results (Figure 1)

Total number of articles located after duplicates removed = 2523

Sift 1 criteria

Abstract screening: systematic review, primary research, infection relates to MDR Gram-negative infection, informs one or more review questions

Articles retrieved

Total number of studies selected = 597

Sift 2 criteria

Full text confirms that the article is primary research (randomized controlled trial, non-randomized controlled trial, controlled before–after study, interrupted time series, case–control study, case series, prospective cohort, systematic review), informs one or more review questions

Articles selected for appraisal (10 full-text publications could not be retrieved)

Total number of studies selected = 49

Critical appraisal

Articles presenting primary research or a systematic review and meeting the sift criteria were critically appraised by two reviewers using SIGN and EPOC criteria. Consensus was achieved through discussion

Accepted and rejected evidence

No meta-analyses were available

Accepted after critical appraisal = 49

Rejected after critical appraisal = 0

Appendix 3. Consultation stakeholders

Antimicrobial Resistance and Hospital Acquired Infection Advisory Committee

British Medical Association

British Society of Antimicrobial Chemotherapy

British Infection Association

C. Diff Support

European Society of Clinical Microbiology and Infectious Diseases

Faculty of Intensive Care Medicine
Foundation Trust Network
Hand Hygiene Alliance
Healthcare Infection Society
Infection Prevention Society
Lee Spark Foundation
MRSA Action UK
NHS Confederation
NHS England
NHS Trust Development Authority
Patient's Association
Public Health England/Wales/Scotland/Northern Ireland
Royal College of Pathologists
Royal College of General Practitioners
Royal College of Nursing
Royal College of Physicians
Royal College of Surgeons
Service User Research Forum Healthcare Acquired Infections
UK Clinical Pharmacists Association
Unison

Appendix 4. Continuing Professional Development material

1. In deciding which patients to screen for carbapenem-resistant organisms, which two groups should take priority:

a) Admitted to intensive care
b) Admitted to surgical ward
c) Admitted from long-term care facility
d) Admitted to medical ward
e) Discharge to community

Answer: A C

2. For the purposes of national surveillance, which two antimicrobial susceptibilities for Gram-negative pathogens are most important to test?

a. Meropenem
b. Amoxicillin
c. Trimethoprim
d. Gentamicin

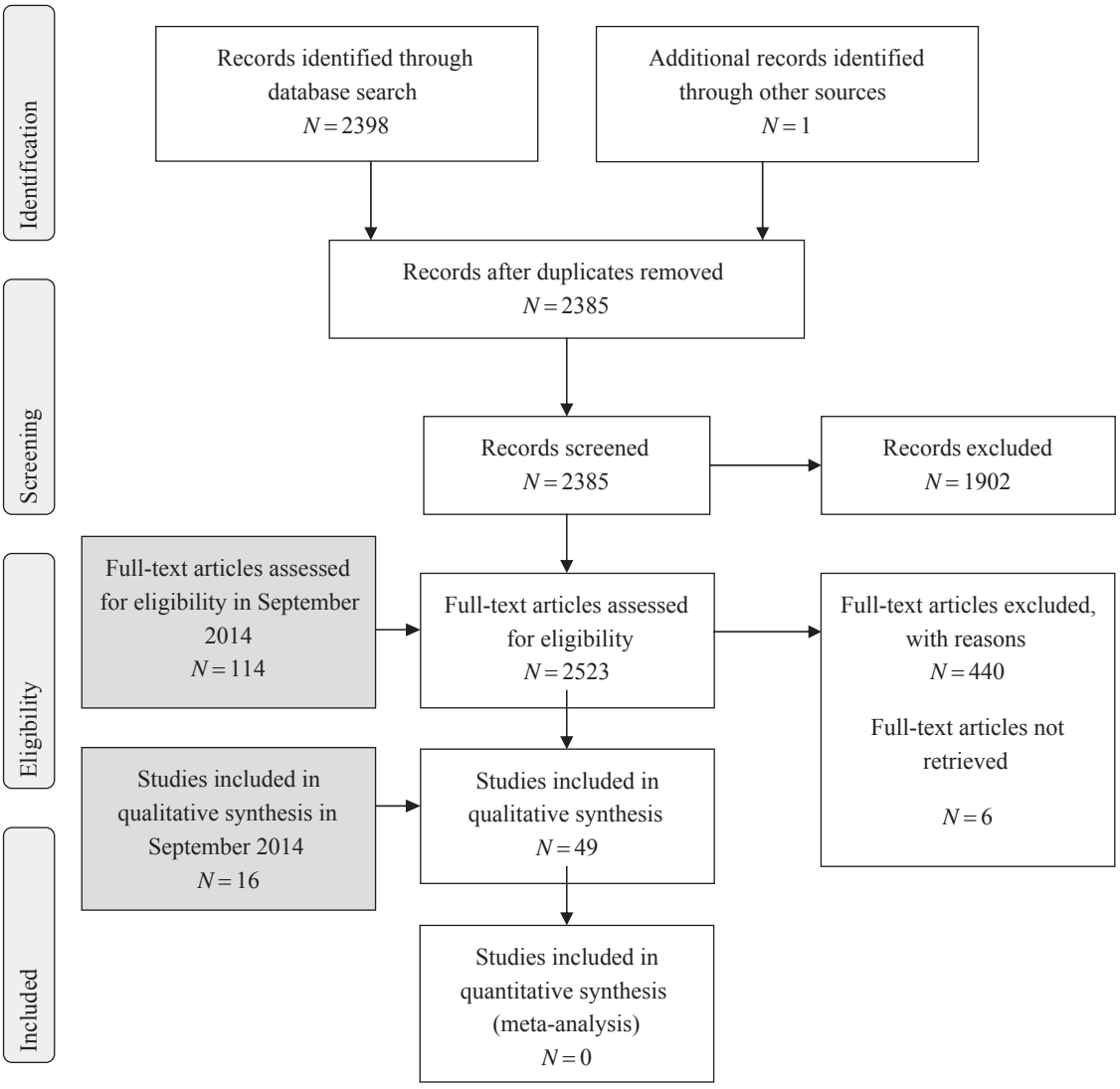

Figure 1. Flow chart of systematic review.

e. Cefpodoxime
Answer: A E

3. To prevent spread of multi-drug-resistant Gram-negative pathogens:
a. Assess all patients for risk of infection on arrival
b. Maintain standard infection control precautions in only those patients at high risk
c. Do not discard body fluids into hand basins
d. Minimize use of invasive medical devices
e. Audit and feedback staff compliance
Answer: A C D E

Appendices A—G. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jhin.2015.08.007.

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journal homepage: www.elsevier.com/locate/ipip

Outbreak Report

Role of the environment in transmission of Gram-negative bacteria in two consecutive outbreaks in a haematology-oncology department

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SUMMARY

In 2019—2020, two subsequent outbreaks caused by phenotypically identical ESBL-producing *Enterobacter cloacae* and multi-drug-resistant (MDR) *Pseudomonas putida* were detected in respectively 15 and 9 patients of the haematology-oncology department. Both bacterial species were resistant to piperacillin-tazobactam, used empirically in (neutropenic) sepsis in our hospital, and ciprofloxacin, used prophylactically in selective digestive decontamination for haematology patients. The *E. cloacae outbreak* was identified in clinical cultures of blood and urine. Despite intensified infection control measures, new cases were found in weekly point-prevalence screening cultures. Environmental samples of sinks and shower drains appeared positive in 18.1%. To diminish the environmental contamination burden, all siphons of sinks were replaced, and disinfection of sinks and shower drains was intensified using chlorine and soda on a daily basis. Replacement of shower drains was not possible. The outbreak of *P. putida* remained limited to rectal cultures only, and disappeared spontaneously without interventions. During both outbreaks, multiple strains of the incriminated bacterium were found simultaneously (demonstrated by Amplified-Fragment Length Polymorphism and/or Whole-Genome Multi-locus Sequencing Typing) in patients as well as the environment. It was experimentally shown that a biofilm on the toilet edge may act as a source for nosocomial transmission of Gram-negative bacteria. In conclusion, the drainage system of the hospital is an important reservoir of MDR bacteria, threatening the admitted patients. In existing hospitals, biofilms in the drainage systems cannot be removed. Therefore, it is important that in (re) building plans for hospitals a plan for prevention of nosocomial transmission from environment to patients is incorporated.

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Introduction

In recent years, many studies have demonstrated that sink drains, shower drains and toilets in hospitals can serve as a

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reservoir of Gram-negative bacterial infections in patients [1—3]. These bacteria reside in biofilms in and on water related devices, which are refractory to disinfection. Bacteria in the biofilm of drainage systems are under a prolonged antibiotic selective pressure, because antibiotics in faeces and urine of patients are absorbed by the biofilm [4]. Within these biofilms, containing several bacterial species, acquired mobile resistance elements are frequently transferred, resulting in highly resistant microorganisms, such as extended-spectrum β-lactamase and carbapenemase-producing Enterobacterales and Pseudomonas species [5,6].

Highly resistant, (as well as antibiotic susceptible), bacteria from biofilms can be transmitted to patients directly or indirectly via droplets, and go on to cause infections [7]. Furthermore, there have been a few reports of transmission via aerosols, but these remain a minority [8]. This transmission results in a serious threat to patients, especially critically-ill patients and immunocompromised hosts, leading to substantial morbidity and mortality, because treatment options are limited. Most Gram-negative healthcare-associated waterborne infections go undetected, unless they occur in outbreaks [2].

In Gram-negative nosocomial outbreaks, isolates found in patients and water-points can be identical, but the direction of transmission is usually unclear (water-point contamination originates from waste-water from the patient, or opposite direction). Therefore, the microbiomes of patients and water-points seem to be a continuum [9].

Usually, biofilms contain several bacterial species. The complete removal of biofilms from drains is often not possible, because they extend into the deeper parts of the water system, which cannot be reached. From this deeper compartment, biofilms grow back to the outlets, even from different patient rooms. Therefore, for infection control, mechanical cleaning and disinfection of the outlets of the system is often the maximum achievable goal.

We describe two subsequent outbreaks in 2019—2020 caused by ESBL-producing *Enterobacter cloacae* and multi drug-resistant *Pseudomonas putida* in the haematology-oncology ward of our hospital, which were related to contaminated water points. Both bacterial species were resistant to the antibiotics used for selective digestive decontamination (SDD), which is used to prevent systemic Gram-negative infection in neutropenic haematology patients, and also to the empirical antibiotic therapy for neutropenic fever.

The *E. cloacae* outbreak involved 15 patients (13 haematology and two oncology), whom were infected or colonized with a phenotypically identical strain (Figure 1), which proved to consist of several genotypes, found in both patients and the environment. Eventually, a bundle of several interventions, including replacement of siphons and intensive disinfection schemes resulted in the termination of this outbreak. The *P. putida* outbreak occurred one year later and involved nine haematology patients (asymptomatic rectal colonization only). This outbreak was also multi-clonal and involved patients and waterpoints, but this ended without interventions.

Methods and materials

Description of water points on the haematology-oncology department

All single and four-person rooms contain a separate sanitary room containing a sink, shower and toilet. The two-person rooms have a sanitary room with a sink and toilet. For these rooms; two extra shower rooms are created on the corridor of the ward (Figure 2). The sinks are situated directly below the outlet with a siphon. The shower drains are situated in such a way that patients do not stand on it. Before and during the outbreaks, all water points functioned properly.

Month	May					Jun				Jul				Aug					Sep				Okt				Nov			
Week	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Patient isolates		A					*A*					D																	
	A	*A*	A	NT	NT	A	C				A	A		E				C											
		Tested				50	50	18			11	55	57					40											40	
Environmental		Positive				9	9	6			7	12	4					8											5	
isolates		Genotyping- results				A	NT	NT			NT	C	A					A											F	
						A	NT	NT			NT	NT	E					A											G	
						NR	NT	NT			NT	NT	E					A											H	
						NT	NT	NT			NT	NT	NT					A											I	
						NT	NT	NT			NT	NT						A											J	
						NT	NT	NT			NT	NT						C												
						NT	NT				NT	NT						E												
						NT	NT					NT						NT												
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												NT																		
		% positive				18,0	18,0	33,3			63,6	21,8	7,0					20,0											12,5	
Interventions			1	2			3	4				5	6														7			

Figure 1. Epidemic curve ESBL-positive *E.cloacae* outbreak. Chronologic schematic overview of the epidemic. **Patient isolates**. Colour or rectangle: red; blood culture, yellow; urine, grey: rectal swab. Colour of character: black; haematology patient, red; oncology patient. **Environmental isolates:** 321 samples from various water points were tested; 58 (18%) were positive for ESBL-positive *E.cloacae* with the epidemic phenotype. **Genotyping results (patient and environmental isolates)**: Genotyping results were obtained by AFLP and/or wgMLST. A, C—E: genotype of ESBL-positive *Enterobacter cloacae* strain; italicized = genotyped by wgMLST. NT = not typed. NR = not related. **Interventions**: (1) contact-isolation in separate room for positive patients and all contact patients, (2) start of point prevalence screening of all patients on ward by rectal swab, (3) disinfection of sinks and showers with Na_2CO_3 on a weekly basis, (4) replacement of all syphons of sinks on the ward (replacement of shower drains not possible), (5) disinfection of shower drains with chlorine and Na_2CO_3 on a daily basis plus closure of culture positive shower drains in the corridor, (6) reopening of showers corridor, (7) termination of weekly point prevalence screening.

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Sampling of patients and environment

Environmental samples

During the two outbreak periods environmental sampling was performed on the ward, using Copan Transswab (108C USE)®. The interior of sink drains and shower drains were sampled by inserting the swab and rotating at a depth of about 6 cm. Toilets were sampled by swabbing under the edge. Furthermore, faucets and refrigerators inpatient rooms were swabbed.

Patient samples

Haematology patients were routinely screened twice a week for colonisation of throat and faeces with Gram-negative

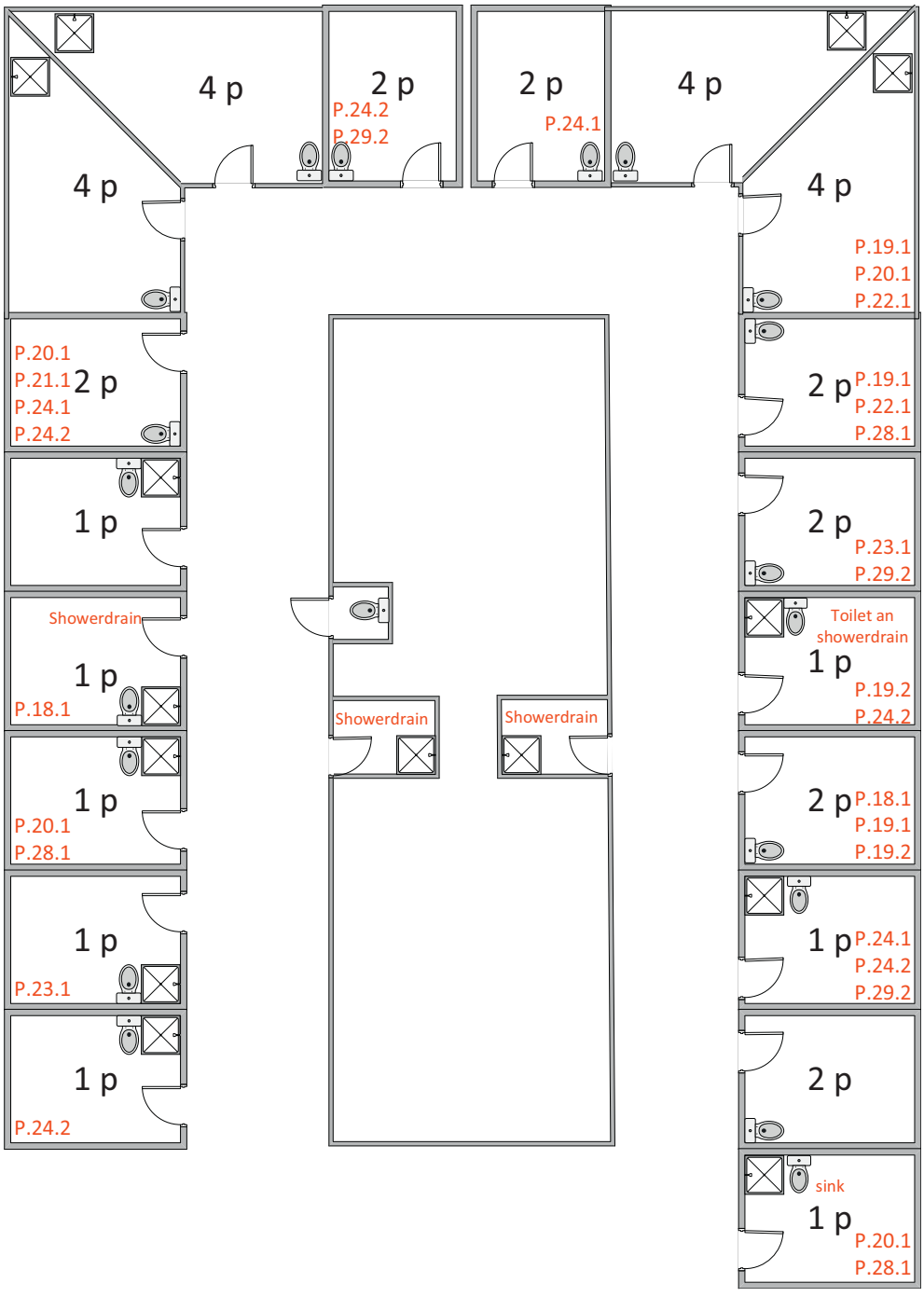

Figure 2. Overview environmental sampling E.cloacae. Situation in the haematology department in week 25. In this figure is indicated where patients and environmental samples tested positive for ESBL-positive *E.cloacae*. **Inner rectangles**: 1p/2p/4p = 1, 2, 4 persons patient room. Orange: environmental samples were positive for other Gram-negative bacteria. Red: environmental sample(s) positive for ESBL-positive *E. cloacae* and other Gram-negative bacteria. In the **outer rectangles** is indicated which patients had been admitted to the room before environmental sampling. **Two shower drains were both positive** at the corridor which were used by multiple patients were all positive for ESBL-positive *E. cloacae*.

bacteria, as a part of their intensive chemotherapy treatment. The oral combination of ciprofloxacin and fluconazole was used prophylactically for SDD, with the aim of preventing systemic Gram-negative or fungal infection during the neutropenic phase of their treatment. If bacteria resistant to ciprofloxacin were cultured from patients, oral colistin was added to bacterial SDD.

During the *E. cloacae* outbreak, present oncology patients were additionally screened weekly by rectal swab for *E. cloacae* carrier status. As no clinical infections occurred during the *P. putida* outbreak, oncology patients were not sampled.

Microbiological methods

Phenotypic investigation

Patient screening swabs were cultured overnight on blood agar and CLED agar at 35°C. As soon as outbreaks were identified, environmental samples and rectal swabs from oncology patients were eluted in Trypton Soya Broth (TSB) and cultured overnight at 35°C. The next day, the TSB was plated and cultured overnight on selective CHROMID® ESBL agar (bioMérieux, Marcy-l'Étoile, France) at 35°C. Bacterial strains were identified and investigated for antibiotic resistance using Vitek-2 (bioMérieux, Marcy-l'Etoile, France). ESBL-production from *E. cloacae* strains was confirmed by the double disk method with cefotaxime, ceftazidime and cefepime with and without clavulanic acid.

Genotyping of bacterial strains

Amplified Fragment Length Polymorphism (AFLP). Cultured isolates were typed by AFLP analysis as described elsewhere [10]. In brief, isolates were resuspended in 10 mM Tris-EDTA buffer pH 8.0. Restriction was performed with *MseI* and *EcoRI* restriction enzymes (New England Biolabs, Massachusetts, USA), adaptors were ligated and fragments were amplified by PCR with primers specific for the ligated adapters. Following restriction and amplification, DNA fragments were separated on an ABI Prism 3500XL Genetic Analyser (Applied Biosystems, Warrington, UK), after which data were analysed using the Pearson correlation coefficient and were clustered by unweighted pair-group matrix analysis (UPGMA) using BioNumerics software v. 7.6 (Applied Maths, St-Martens-Latem, Belgium). Isolates clustering above the threshold were assigned the same AFLP type number.

Whole Genome Multi Locus Sequence Typing (wgMLST). The first cluster of ESL E.cloacae isolates was also typed by whole genome sequencing (WGS). This was performed as described elsewhere [11]. Isolates clustering within an allelic difference of 20 or less were assigned to the same cluster. Sequencing data were deposited at European Nucleotide Archive (ENA) under BioProject PRJEB46126.

Environmental cleaning and disinfection on the ward

The sanitary facilities of the ward were cleaned daily with moist microfiber cloth and toilets with sanitary cleaner containing alkylalcoholethoxylate, aliphatic alcohol and 4-terr-butylcyclohexylacetate (Taski Sani 100, Diversey, Utrecht, The Netherlands). Floor drains of showers were disinfected with chlorine on a weekly basis for a duration of at least 5 minutes, and toilets were cleaned with fluid containing calcium carbonate (CIF, Unilever, Rotterdam, The Netherlands).

Experimental study

We investigated whether toilets might have been a driving force behind transmission on the ward. During flushing, droplets have the opportunity to spread to the environment, because the patient toilets in our hospital do not have a cover lid for practical reasons. The experiments were performed in a toilet room of an unused patient department (all toilets in the hospital are identical). *Experiment 1*: 90 mL Visirub® fluorescent concentrate (Hartmann, Nijmegen, The Netherlands) was mixed with the water present in the flushing cistern (estimated volume 9 L). Photographs were taken before and 5 minutes after flushing the toilet, with illumination of the toilet room using UV-light. *Experiment 2*: 24,25 mL Visirub® concentrate was mixed with 24,25 mL demineralized water and 1.5mL gel-former containing polyacrylamide, C13-14 isoparaffin and laureth-7 (Jojoli, Barendrecht, The Netherlands). The resulting fluorescent mixture was applied underneath the toilet edge. Again, photographs were taken in a similar manner.

Results

ESBL-positive E. cloacae

Description of the outbreak [Figure 1]

In May 2019, a cluster of three patients with multi-resistant ESBL-positive *E. cloacae* was detected on the haematology-oncology department of our hospital. The strains were resistant to the empiric antibiotic regimen on the ward (piperacillin-tazobactam) and had an identical antibiogram (ESBL+, ciprofloxacin R, trimethoprim-sulfamethoxazole R). One neutropenic patient had a positive blood culture with this strain, but recovered after switching the antibiotic regime to meropenem. Two of these patients were roommates and all three patients shared the same shower on the ward.

Six new cases occurred in the subsequent five weeks, despite ongoing enforcement of hand hygiene and contact isolation/cohorting of positive patients in a separate room.

By the end of June, disinfection of sinks and shower drains was intensified by replacing all daily and weekly cleaning products with chlorine at 250 ppm. Patients were instructed not to leave their personal belongings on the rim of the sink bowl, to prevent transmission from splashing water from the drain. In addition, all siphons of sinks on the ward were renewed. As environmental samples remained positive for *E. cloacae*, a weekly treatment with biofilm degrading liquid caustic soda (Senzora BV, Deventer, The Netherlands) for five minutes was added for every sink drain and toilet. After this intervention the incidence of newly colonized patients declined gradually over time.

In the peak of the outbreak, a change in the antibiotic regimen of SDD for neutropenic haematology patients, from ciprofloxacin to colistin, was considered an optional alternative intervention. The epidemic strain was resistant to ciprofloxacin, and more than half of the patients on the ward were treated with SDD, so this might have contributed to the rapid dissemination. Eventually, with the decline of new patient cases, this intervention was not effectuated.

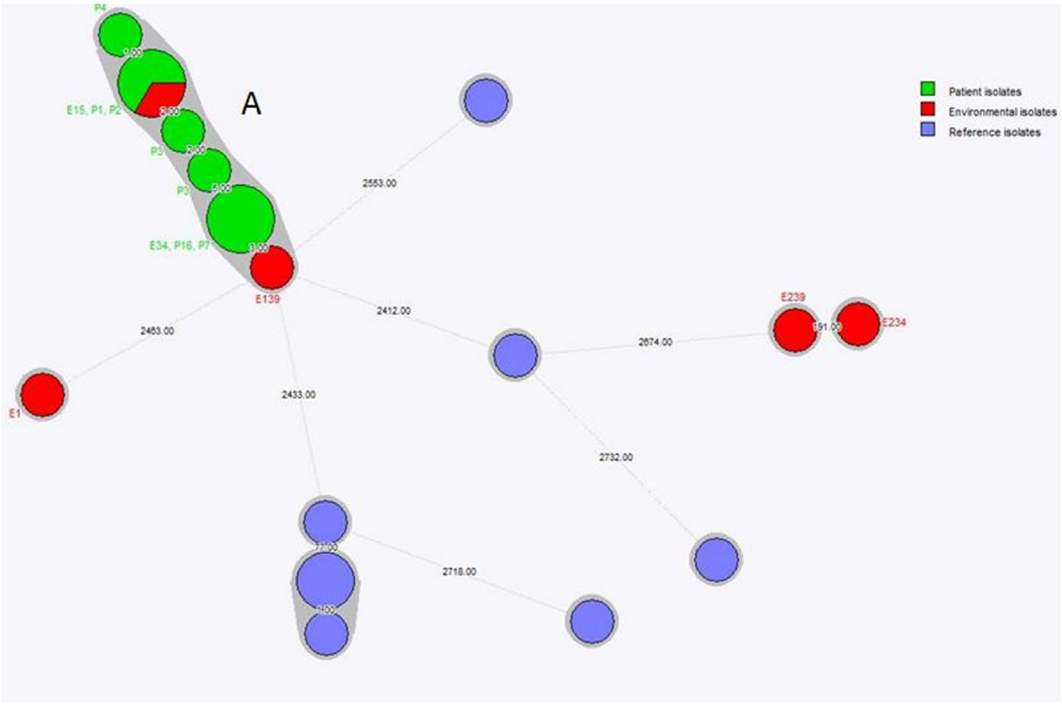

Figure 3. Whole Genome Sequencing results ESBL-*E.cloacae*. Minimum spanning tree of wgMLST of ESBL-positive *E. cloacae*. Each circle represents one or multiple isolates, depending on the circle size and lines between circles indicate the allelic distance between the indicated isolates. Isolates are coloured by origin: patient isolates (green), environmental isolates (red) and reference non-related isolates (blue). Isolates from patients and the environment that cluster together within the cut-off value are depicted with a grey line zone and indicated as cluster A.

A final round of environmental specimens (taken at the end of August) revealed eight positive environmental cultures from which five isolates belonged to genotype A. In retrospect, this could be explained by the suboptimal adherence to the intensified instructions for disinfection by the cleaning personnel. From week 35 on, genotype A *E. cloacae* was not identified in weekly screenings of patients, and environmental samples in November revealed only other genotypes.

Environmental samples and patient samples [Figure 2]

In June and July 2019, 47/241 (19.5%) of environmental specimens cultured *E. cloacae* with an identical antibiogram as the initial epidemic strain, among which three were typed as genotype A (three belonged to other genotypes and 41 were not typed). Positive patients were identified in the majority of patient rooms.

Genotyping [Figure 3]

All patient strains during the initial outbreak period in May (n= 4) appeared to be identical by AFLP and wgMLST (AFLP-type A). Additional isolates were therefore typed by AFLP only. In subsequent AFLP-analysis there appeared to be the simultaneous presence of multiple genotypes on the ward. In the period May—June, six of seven strains were identical (genotype A). In the period July—August three out of six strains were genotype A.

Typing of 18 environmental isolates demonstrated 3/6 genotype A in the period May—July, 7/8 genotype A in August and 0/4 genotype A in November.

Multi-drug-resistant Pseudomonas putida

Description of the outbreak [Figure 4]

In August 2020, a cluster of four patients with MDR *P. putida* was noticed in surveillance rectal cultures of haematology patients. The strains were resistant to the empiric antibiotic regimen on the ward (piperacillin-tazobactam) and had an identical antibiogram (ceftazidime I or R, meropenem R, aminoglycosides R, ciprofloxacin R, colistin S).

New cases (n=5) occurred in the subsequent two months, despite enforcement of hand hygiene and contact isolation/cohorting in a separate room of positive patients.

In contrast to the *E. cloacae* outbreak, no clinical cultures became positive with MDR *P. putida*, and the incriminated strains disappeared from rectal cultures of individual positive patients as soon as SDD was changed from ciprofloxacin to colistin. The incidence of colonization reduced spontaneously, so therefore interventions were not necessary. Suspected environmental sources, such as the shower and sink of a patient room that was shared by several positive patients, and the ice cube machine of the ward, were cultured but were negative.

In October, four new patients became positive with a phenotypically identical *P. putida*. Therefore, the 30 toilet edges in all rooms where positive patients had been admitted, were cultured for *P. putida*. Only one toilet edge was positive with a different genotype. It turned out that the four patient strains were different from each other and only one was colonized with the outbreak strain (genotype 3). Since then, no new patients carrying *P. putida* have been identified. Unexpectedly, in the last week of November four out of five genotyped

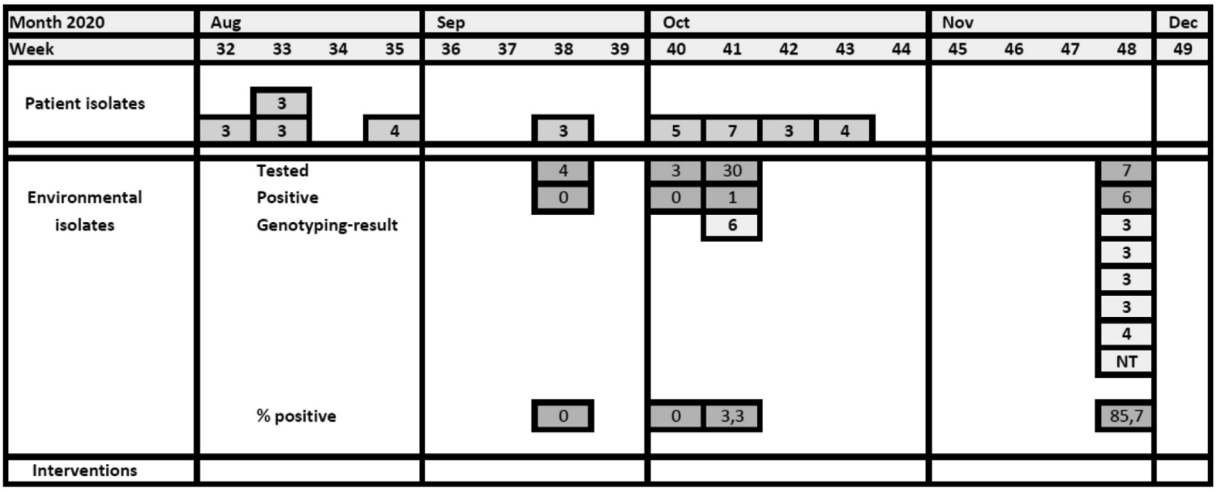

Figure 4. Epidemic curve multi-resistant *Pseudomonas putida* outbreak. Chronologic schematic overview of the epidemic. **Patient isolates**. Only positive cultures from rectal swabs for Selective Digestive Decontamination were involved. **Environmental cultures: *week 38***; shower and sink in patient room frequently occupied by positive patients, ***week 40***; ice cube machine; ***week 41***; various water tap points (water from toilets, siphons of basins, buckets used by cleaning personnel), ***week 48***; ridges of 7 toilets from patient rooms of positive patients (note: these cultures were negative for ESBL+ *E.cloacae*). **Genotyping results (patient and environmental isolates)**: 3–7: genotype of multi-resistant *Pseudomonas putida* strain. NT = not typed. **Interventions**: no interventions were carried out.

environmental strains from toilets were identified as the epidemic *P. putida (*genotype 3).

Environmental samples [Figure 4]

In September–November 2020, 7/44 (16%) of environmental cultures were positive for *P. putida* with an antibiogram identical to the strain found in the cluster in August. From these isolates four belonged to the epidemic genotype 3, two to other genotypes and one was not typed.

Genotyping

Three out of four strains of the initial cluster were determined to be identical by AFLP (genotype 3). During the outbreak different AFLP variants were found, from which some were shared between patients and some between patients and environment. Of the new cases in September–October, 2/5 typed isolates belonged to genotype 3 and of the seven positive environmental samples, four were genotype 3.

Experimental study [Figure 5]

Experiment 1: fluorescent remnants of flushing water were seen throughout the toilet bowl and some droplets outside were identified (Figure 5A). Experiment 2: flushing water was able to remove some fluorescent gel underneath the toilet edge, which could in theory be further transmitted to the environment by successive flushes (Figure 5B).

Discussion

In this study we describe the simultaneous colonization of patients and water related devices with ESBL-positive *E. cloacae* or MDR *P. putida* on the haematology-oncology ward of our hospital. A mix of genotypes were isolated in both cases, but a dominant genotype of either *E. cloacae* or *P. putida* was shared by both patients and environment, suggesting that environmental sources played a role in transmission. This hypothesis was supported by experimental results, which proved that by flushing toilets with a fluorescent dye, droplets were clearly disseminated to the environment in toilet rooms. For *E. cloacae*, various interventions resulted in the discontinuation of transmission among patients. For *P. putida*, transmission disappeared without other interventions beside changing SDD from ciprofloxacin to colistin in individual colonized haematology patients.

While the *E. cloacae* transmission was first discovered in clinical cultures of blood and urine, *P. putida* was only found in rectal surveillance cultures. *P. putida* rectal culture positivity was mainly noticed immediately after admission to the ward and disappeared as soon SDD was changed. For both outbreaks, environmental cultures of wet points remained positive for more than a month after the last positive patient was identified. The latter finding implicates that after disinfection of sink or shower drains, biofilm is still present in the deeper compartment of the hospital drainage system. Moreover, recontamination of the water from a biofilm underneath the toilet rim during flushing is a possibility (Figure 5). Bacteria in this biofilm remain an ongoing threat for colonization of patients during their stay in the hospital. As the drainage systems can often not be replaced, the most efficient solution is to "keep the monster inside" by removing the biofilm at the outlets at regular intervals combined with disinfection of these sites. The most efficient and achievable method is still under debate. Recent technical solutions, such as UV disinfection and ozonisation of water seem promising [12]. Innovative design of sinks can reduce the contamination of the near surroundings with bacteria originating from the biofilm [13]. The introduction of self-disinfecting siphons gave excellent results [14,15]. Furthermore, a critical appraisal of the necessity of all water points in the hospital is indispensable; unnecessary ones should be removed and the remaining points should be strictly divided into incoming and outgoing points to prevent cross-contamination [16]. For transmission prevention of highly resistant opportunistic plumbing premise pathogens, design

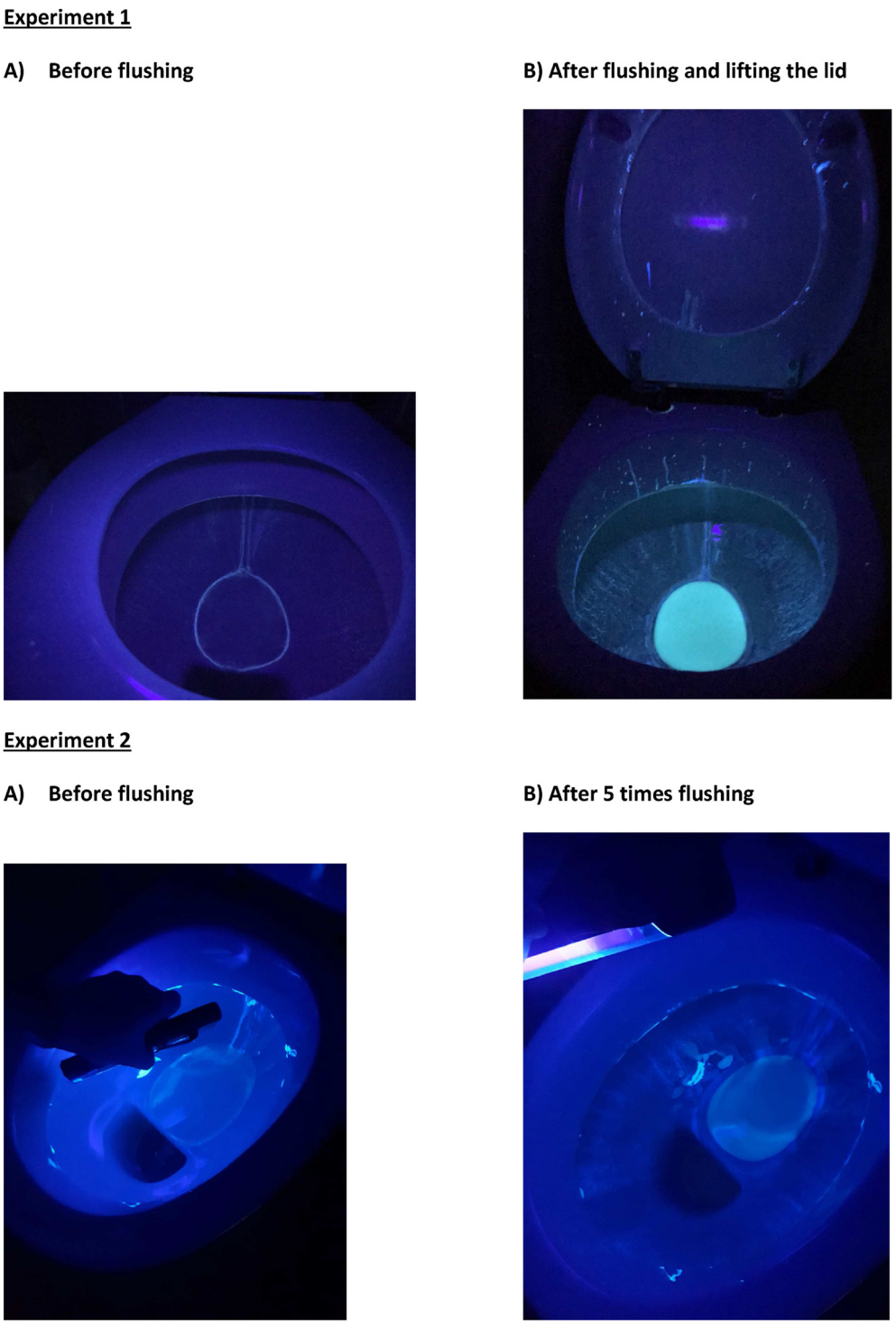

Figure 5. Experimental study flushing toilet. Experiments were performed in a toilet room of a unused patient department. *Experiment 1*: 90 mL Visirub® concentrate (Hartmann, Nijmegen, The Netherlands) was mixed with the water present in the flushing cistern (estimated volume 9 L). Photographs were taken before and 5 minutes after flushing the toilet, with illumination of the toilet room using UV-light. UV-positive remainders of droplets were identified inside as well as outside the toilet bowl. *Experiment 2*: 24,25 mL Visirub® concentrate was mixed with 24,25 demineralized water and 1,5 mL gel-former (Jojoli, Barendrecht, The Netherlands). The resulting fluorescent mixture was applied underneath the toilet edge. Again, photographs were taken in a similar manner. Flushing water was able to remove the gel from the toilet.

and construction of the water system of newly built hospitals is critical [17]. Also the implementation of waterless patient care is an expensive, but effective method to discontinue hospital transmission [18].

For situations wherein the former solutions are not feasible, the most effective intervention for disinfection and removal of biofilm is still under debate. Recently, Ledwoch *et al*. published a novel in vitro biofilm model to investigate this topic. In a study comparing four methods for disinfection, peracetic acid 4000 ppm was superior to alternatives, resulting in a >4 log reduction in viability in all drain sections [19]. Another weak point in manual disinfection is the correct implementation by the environmental services workers. In the *E. cloacae* outbreak, we discovered that the intensified disinfection strategy was not strictly followed by all members of the team, which contributed to the delayed termination of the outbreak. In this context, not only the type of disinfectant but also the contact time with the various parts of the drains is crucial. Education and feedback by regular audit are essential for high standard cleaning and disinfection in the hospital [20]. Furthermore, we concluded that biofilms do not only occur in the drainage system but also underneath the rim of the toilet seats. Technical solutions, such as rimless toilets, are helpful in prevention of biofilms that are difficult to remove.

High antibiotic use is a well-known risk factor for the selection of resistant bacteria. The majority of patients on the ward were haematology patients receiving SDD, as part of their treatment, for prevention of bacteraemia and mortality. In case of resistance in Gram-negative rods, ciprofloxacin is changed to a combination of trimethoprim-sulfamethoxazole and colistin. As a substantial part of the patient population was treated with an antibiotic for which the *E. cloacae* strains were resistant, this was considered as a possible driving force behind the ongoing transmission on the ward, because the colonization resistance in these patients was lowered. Switching to another antibiotic class could be an effective intervention. Frakking *et al*. used the discontinuation of ciprofloxacin prophylaxis in haematology patients in a bundle of interventions which ended a large and persistent outbreak caused by vancomycin-resistant *Enterococcus faecium*, which is less pathogenic than *Enterobacter* spp [21]. Verlinden *et al*. found no significant increase in serious infectious complications with the discontinuation of prophylaxis and a decrease in fluoroquinolone resistant and ESBL-producing Gram-negative bacteria [22]. Furthermore, the effectivity of ciprofloxacin prophylaxis in preventing mortality is questioned by several authors, and it might even cause selection for multi-drug resistant bacteraemia [23,24].

One limitation of our study is worth noting. We did not prove a causal relation as patients and water points were colonized at the same time period. The index-patient with the *E. cloacae* bacteraemia had been infected on the ward with the same strain one year earlier (data not shown), implying that the environment on the ward could have been contaminated for at least a year. This hypothesis is supported by the fact that at the end of our study in November, a part of the investigated environment was still positive for *E. cloacae*, despite the intensified cleaning and disinfection regime. Recently, Volling *et al*. described a tool for assessing the quality of evidence for transmission from water point to patient. In none of the 52 studies that were evaluated with this tool causality was conclusively demonstrated [25].

Conclusions

In conclusion, the patient environment is increasingly considered as a major source of transmission of nosocomial bacteria [26]. The drainage system of the hospital is an important reservoir of multi-resistant bacteria, threatening admitted patients. In existing hospitals, biofilms in the drainage systems can often not be removed. Therefore, it is important that in (re)building plans for hospitals a plan for prevention of nosocomial transmission from environment to patients is incorporated. For existing, maybe older, hospital buildings, a water safety plan for haematology-oncology departments should be instituted [27]. Furthermore, where technical solutions are not available, regular training of cleaning personnel is of utmost importance.

Credit statement of authorship

WC Van der Zwet: Conceptualization, methodology, formal analysis, writing original draft, writing review and editing. **IEJ Nijsen:** Methodology, formal analysis, visualization, data curation, writing review and editing. **C Jamin:** Investigation, visualization, writing review and editing. **LB Van Alphen:** Investigation, visualization, writing review and editing. **CJH Von Wintersdorff:** Investigation, visualization, writing review and editing. **AMP Demandt:** Writing review and editing. **PHM Savelkoul:** Writing review and editing, supervision.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Ethics and patient consent

Informed consent was not gained from patients involved in this outbreak. All patients were treated according to clinical judgement and infection control practices in order to treat them and control the outbreak according to local guidelines. Patients did not undergo randomisation or intervention for the purpose of this report. Data has been analysed and presented anonymously.

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Clinical Characteristics, Outcomes, and Risk Factors for Mortality in Patients with *Stenotrophomonas maltophilia* Bacteremia

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Abstract: This study aimed to establish the clinical features, outcomes, and factors associated with mortality in patients with *Stenotrophomonas maltophilia* (*S. maltophilia*) septicemia. The characteristics and outcome data used in this retrospective study were collected from medical records at Songklanagarind Hospital. Risk factors for survival were analyzed using χ^2-tests, Kaplan–Meier curves, and Cox regression. A total of 117 patients with *S. maltophilia* bacteremia were analyzed. The patients' median age was 45 years, 77 (70%) were male, 105 (90%) had comorbidities, 112 (96%) had previously undergone carbapenem therapy, and over half of the patients were on invasive medical devices. Trimethoprim-sulfamethoxazole (TMP-SMX) and fluoroquinolone showed high susceptibility rates to *S. maltophilia*, with 93% and 88% susceptibility, respectively. Patients who received appropriate empirical antibiotic treatment had significantly reduced 14-day, 30-day, and in-hospital mortality rates than those who did not ($p < 0.001$). The days of hospital stay and costs for those who received appropriate and inappropriate empirical antimicrobial treatment were 21 and 34 days ($p < 0.001$) and 142,463 and 185,663 baht, respectively ($p < 0.002$). Our results suggest that an appropriate empirical antibiotic(s) is significantly associated with lower 30-day mortality in hospitalized patients with *S. maltophilia* septicemia.

Keywords: *Stenotrophomonas maltophilia*; characteristics; outcomes; mortality; bacteremia

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1. Introduction

Stenotrophomonas maltophilia is a motile, glucose, non-fermenting, gram-negative bacterium that widely exists in hospital environments [1,2]. In the last few decades, it has been recognized as a significant pathogen responsible for hospital acquired infections in severely debilitated or immunocompromised individuals [3–8]. It usually causes pneumonia and bacteremia and, less frequently, it can cause infections of the urinary tract, intra-abdominal organs, and wounds [2,9].

Due to its inherent resistance to several antibiotic classes, treating *S. maltophilia* infections is difficult. *S. maltophilia* employs a wide spectrum of resistance mechanisms, including reduced permeability, expression of multidrug efflux pumps, and the synthesis of beta-lactamase, carbapenemase, and aminoglycoside-modifying enzymes [10,11]. Moreover, distinguishing between infection and colonization is challenging because of the bacterium's ability to colonize respiratory epithelial cells and invasive medical device surfaces. Hence, the administration of appropriate antibiotics is occasionally delayed, leading

to high mortality [10–12]. TMP-SMX is the preferred antibiotic for treating *S. maltophilia* infections [13,14]. However, fluoroquinolones and minocycline are also alternative management options [15–17]. According to a systematic review, infections with *S. maltophilia* are associated with high rates of mortality, ranging from 21 to 69% [18]. Prior reports have demonstrated the risk factors associated with mortality among *S. maltophilia* bacteremia patients, including septic shock, an Acute Physiology and Chronic Health Evaluation (APACHE) II score more than 15 points, indwelling invasive medical devices, mechanical ventilation, admission to intensive care units (ICU), prolonged hospitalization, and concurrent administration of immunosuppressive agents or broad-spectrum antibiotics [19,20].

To date, information regarding the clinical characteristics and outcomes of patients with *S. maltophilia* bacteremia in Thailand has rarely been reported. Thus, this study reviewed the data of patients with *S. maltophilia* septicemia at Songklanagarind Hospital to establish the clinical characteristics, outcomes, and risk factors associated with mortality. We find that treatment with appropriate empirical antimicrobial agent(s) is significantly associated with a lower 30-day mortality rate, and that a high APACHE II score is a significant independent predictor of mortality, suggesting that *S. maltophilia* bacteremia should always be considered in patients who exhibit potential risk factors.

2. Patients and Methods

2.1. Patients and Setting

This study was conducted retrospectively, using data collected at a tertiary care hospital in southern Thailand from 2010 to 2020. All hospitalized patients aged >15 years who had one or more episodes of infection with *S. maltophilia* isolated from blood culture were eligible for this study. Patients' clinical information was obtained from medical records, while microbiological data were extracted from a laboratory database. Only *S. maltophilia* septicemia as a first episode was included in the analysis to eliminate case duplication.

2.2. Bacterial Identification and Antimicrobial Susceptibility

Conventional biochemical tests, according to Bergey's Manual of Systematic Bacteriology, were performed to identify *S. maltophilia*. Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS) was further employed to confirm the identifications. The Kirby-Bauer disk diffusion method was used for antimicrobial evaluation of TMP-SMX and levofloxacin, while the Broth microdilution method was performed when testing ceftazidime and chloramphenicol. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) [21,22]. Regarding polymyxin susceptibility testing, there are no currently established polymyxin breakpoints for *S. maltophilia*, but Minimal Inhibitory Concentration (MIC) of $\geq$4 μg/mL is often considered resistance [23].

2.3. Study Design and Data Collection

Data Retrieval

Patients' electronic medical records were reviewed. Demographic and clinical variables including age, sex, body mass index, comorbidities, and immunocompromised status were obtained. Comorbidities included cerebrovascular disease, cardiovascular disease, chronic kidney disease, and diabetes mellitus. Immunocompromised status was defined as those who had an absolute neutrophil count $< 0.5 \times 10^9$/L for >two weeks or were on immunosuppressive therapy (chemotherapy within six weeks or corticosteroids at a dosage greater than 15 mg of prednisolone daily for >two weeks or disease-modifying antirheumatic drugs within four weeks after bacteremia). Clinical information included instances of pneumonia, initial ICU admission, the patient's APACHE II score, their history of previous exposure to antibiotics, and the use of invasive medical devices. Microbiological data included MICs. Treatment data included the appropriateness of empirical antimicrobial therapy and the duration of antibiotic treatment. An appropriate empirical

antibiotic was defined as the prescription of drug(s) that are effective against *S. maltophilia* isolates within 72 h of blood culture collection. TMP-SMX and fluoroquinolones are considered treatment options for infections with *S. maltophilia* [24]. The primary outcome was 30-day mortality, whereas secondary outcomes were in-hospital mortality, 14-day mortality, length of hospital stay, and hospital expenses. The length of in-hospital stay was defined as the duration of hospital stay after *S. maltophilia* septicemia was diagnosed. Hospital expenses were divided into two categories: antimicrobial pharmaceutical costs and hospitalization-associated costs.

2.4. Statistical Analysis

Data on demographics and clinical outcomes were analyzed. Continuous variables were reported as means ± SD (standard deviation), and categorical variables were reported as percentages (%) or frequency counts (n). Continuous variables were tested using the D'Agostino-Pearson test to check for normality, and if data were normally distributed, Student's *t*-tests were conducted and data were presented as means ± SD. Otherwise, the Mann–Whitney test was performed, and data were presented as medians (IQR). For categorical variables, χ^2-tests or Fisher's exact test were used. Univariate analysis was used to determine the crude odds ratio (OR) for mortality. A multivariate logistic regression model was used for 30-day mortality-related variables and variables with *p*-values ≤ 0.2. The significance level was set at $p \leq 0.05$. The adjusted OR and 95 percent confidence interval were used to express the relationship between variables and outcomes. A survival analysis using Cox proportional hazard regression was done to determine the factors associated with survival.

2.5. Ethical Statement

The institutional review board of the Faculty of Medicine, Prince of Songkla University, Thailand, approved this retrospective study (REC: 58368144). The authors were given permission to retrieve clinical and microbiological data from the hospital database with a consent waiver. Before being assessed and used, all data were anonymized. The researchers confirm that we conducted this research in line with the Declaration of Helsinki principles.

3. Results

From 2010 to 2020, 129 blood samples from 129 patients yielded positive cultures for *S. maltophilia*. Two or more organisms were isolated from one blood culture of 12 (9.3%) patients; therefore, they were excluded from the study. Figure 1 depicts the patient enrollment in this study. Of 117 patients with *S. maltophilia* septicemia, 54 (46.2%) received appropriate empirical antibiotic(s).

The records of 117 *S. maltophilia* bacteremia patients were included in the analysis. Table 1 reveals the baseline demographic characteristics and comparison of features between patients receiving and not receiving appropriate empirical antibiotics.

The patients' median age was 45 years (range, 40–51 years), and 66% (77/117) were men. *S. maltophilia* was predominantly found in hospitalized patients, with comorbidities in 90% of patients with underlying diseases. However, only six (5%) patients were immunocompromised. Most patients (70%) had a body mass index $\geq 30\,kg/m^2$, reflecting a high incidence of obesity among patients with *S. maltophilia* bacteremia. Also, 54 patients (46%) were critically ill, with an average APACHE II score of 19. In addition, over half (55%) of the patients had pneumonia and bacteremia. Most patients (96%) had a recent history of carbapenem exposure before *S. maltophilia* bacteremia, followed by cephalosporin, fluoroquinolone, beta-lactam/beta-lactamase inhibitor, and aminoglycoside exposure, with rates of 73%, 62%, 59%, and 34%, respectively.

We found significantly higher APACHE II scores, intensive care unit admission rates, rates of intravascular device and urinary catheter, and rates of empirical antimicrobial therapy with TMP-SMX, fluoroquinolone, and colistin for *S. maltophilia* bacteremia in patients who received appropriate antibiotic(s) than in those who did not. Other base-

line characteristics, including demographic data, did not significantly differ between the two groups.

Table 1. Characteristics of *S. maltophilia* bacteremia patients and comparison of features between patients receiving and not receiving appropriate empirical antibiotics.

Parameter	*S. maltophilia* Bacteremia Patients (n = 117)	*S. maltophilia* Bacteremia Patients Receiving Appropriate Empirical Antibiotics (n = 54)	*S. maltophilia* Bacteremia Patients Not Receiving Appropriate Empirical Antibiotics (n = 63)	*p*-Value [A]
Demographics				
Age, median (IQR)	45 (40, 51)	45 (36, 51)	45 (41, 51)	0.974
Male sex	77 (70)	40 (74)	37 (59)	0.121
Comorbidities	105 (90)	51 (94)	54 (86)	0.213
Immunocompromised status	6 (5)	3 (6)	3 (5)	0.997
Obesity	83 (70)	36 (67)	47 (75)	0.460
Previous exposure to antibiotics				
Carbapenem	112 (96)	50 (93)	62 (98)	0.158
Cephalosporin	85 (73)	41 (76)	44 (69)	0.462
Fluoroquinolone	72 (62)	32 (59)	40 (63)	0.639
β-lactam/β-lactamase inhibitor	69 (59)	36 (67)	33 (52)	0.119
Aminoglycoside	40 (34)	18 (33)	22 (35)	0.857
Clinical characteristics				
Initial ICU admission	54 (46)	32 (59)	22 (35)	0.014
APACHE II score, median (IQR)	19 (15, 23)	20 (13, 22)	16 (14, 18)	0.021
Pneumonia	55 (47)	26 (48)	29 (46)	0.966
Invasive medical devices				
Mechanical ventilator	95 (81)	48 (89)	47 (74)	0.054
Intra-vascular device	73 (62)	39 (72)	34 (54)	0.044
Urinary catheterization	101 (86)	52 (96)	49 (78)	0.008
Treatment				
Empirical treatment including				
Carbapenem(s)	110 (94)	50 (93)	60 (94)	0.551
Colistin	40 (34)	30 (56)	10 (15)	<0.001
TMP-SMX	30 (25)	30 (56)	0 (0)	<0.001
Fluoroquinolone(s)	31 (26)	28 (52)	3 (5)	<0.001
Duration of empirical treatment	3 (3, 4)	3 (3, 4)	3 (3, 4)	0.996

[A] Comparison between *S. maltophilia* bacteremia patients receiving and not receiving appropriate empirical antibiotics. Boldface entries indicate values that reached the significance level set at 0.05.

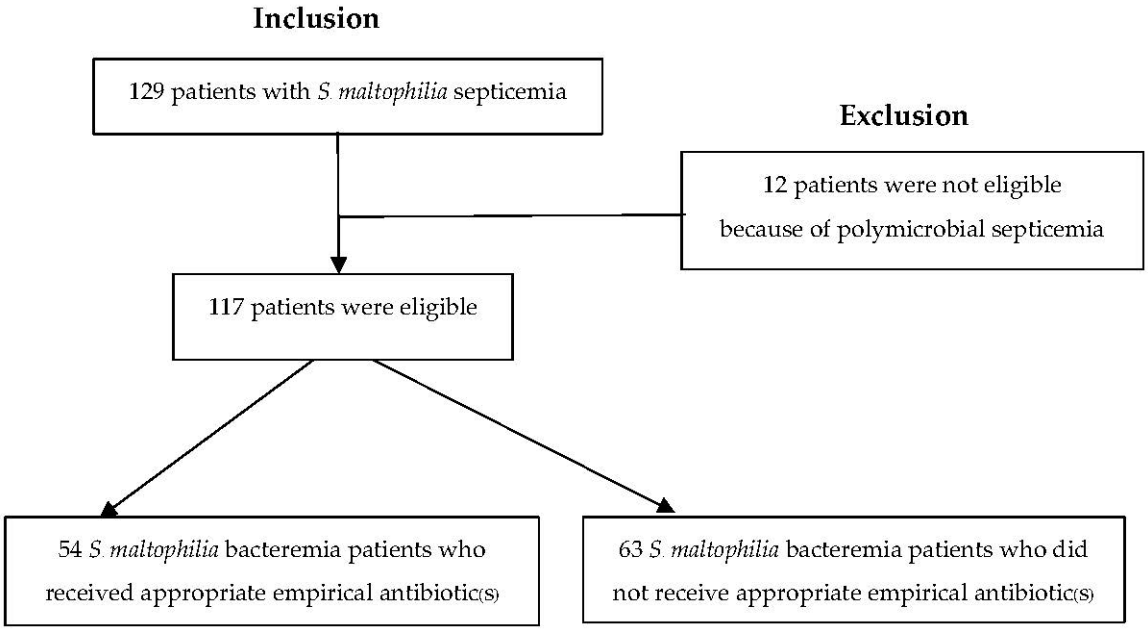

Figure 1. Study enrollment flow chart.

Only 54 (46.2%) patients received appropriate empirical antimicrobial agents, whereas 63 (53.8%) received inappropriate empirical antimicrobial agents. The majority of patients (94%) received empirical antibiotic therapy with carbapenem, which is ineffective against *S. maltophilia*, whereas one-third (34%) received empirical antibiotic treatment with colistin, and only one-fourth had received TMP-SMX or fluoroquinolone for empirical therapy. The duration of antibiotic treatment did not differ between the two groups.

Antimicrobial susceptibility testing of the *S. maltophilia* isolated from the 117 patients is shown in Table 2**.** The results indicated that TMP-SMX, levofloxacin, and chloramphenicol showed the highest susceptibility rate against *S. maltophilia*, with susceptibility values of 93%, 88%, and 82% respectively, compared with the 65% and 35% non-susceptible rate of ceftazidime and colistin. Thus, TMP-SMX was adopted as the most effective antibiotic against *S. maltophilia* bacteremia, followed by fluoroquinolone.

Table 2. Antibiotic-resistant profiles of 117 *S. maltophilia* isolates were obtained from the patients with bacteremia.

Antibiotics	No. of Resistant Isolates (%)
Chloramphenicol	21 (18)
Colistin	41 (35)
Levofloxacin	14 (12)
TMP-SMX	8 (7)
Ceftazidime	76 (65)

The clinical outcomes of the patients treated for *S. maltophilia* bacteremia are shown in Table 3**.** The 14-day, 30-day, and in-hospital mortality rates of those who received appropriate empirical antimicrobial treatment were significantly lower than the rates of those who received inappropriate treatment, with values of 7%, 7%, and 9% and 33%, 48%, and 51%, respectively ($p < 0.001$). Antimicrobial and non-antimicrobial expenses were the highest for patients who received appropriate antibiotic(s). The length of hospital stay from *S. maltophilia* bacteremia onset was significantly lower for patients who received appropriate empirical antibiotic (s) at 21 days, compared to 34 days for patients who did not ($p < 0.001$). Moreover, hospital costs were significantly lower for patients who received appropriate empirical antibiotic(s) (142,463 and 185,663 baht, respectively; $p = 0.002$).

Table 3. Comparison of outcomes between patients with *S. maltophilia* bacteremia who did or did not receive appropriate empirical antibiotic(s).

Outcomes		***S. maltophilia* Bacteremia Patients Who Received Appropriate Empirical Antibiotic(s) (n = 54)**	***S. maltophilia* Bacteremia Patients Who Did Not Receive Appropriate Empirical Antibiotic(s) (n = 63)**	***p*-Value [A]**
Clinical outcomes				
Mortality				
	14-day	4 (7)	21 (33)	0.001
	30-day	4 (7)	30 (48)	<0.001
	In-hospital	5 (9)	32 (51)	<0.001
Non-clinical outcomes				
Length of hospital stay (days) [median (IQR)]		21 (15, 31)	34 (29, 50)	<0.001
Cost (baht) [median(IQR)]				
	Total hospital	142,463 (125,008–212,389)	185,663 (159,223–200,685)	0.002
	Antimicrobial	30,854 (26,447–33,321)	31,854 (29,606–34,014)	0.031
	Non-antimicrobial	118,007 (97,245–175,004)	146,897 (131,488–190,884)	<0.001

[A] Comparison between patients with *S. maltophilia* bacteremia who did and did not receive appropriate empirical antibiotic(s). Boldface entries indicate values that reached the significance level set at 0.05.

The factors associated with 30-day mortality in patients with *S. maltophilia* bacteremia are shown in Table 4. A high APACHE II score was significantly associated with 30-day mortality (OR, 1.19; $p = 0.012$). The appropriate empirical antibiotic reduced 30-day mortality significantly (OR, 0.04; $p = 0.001$).

Table 4. Factors associated with 30-day mortality in 117 *S. maltophilia* bacteremia patients.

Variables	Values		Crude OR (95% CI)	Adjusted OR (95% CI)	*p*-Value [A]
	Survivors (n = 83)	Non-Survivors (n = 34)			
Age (years) [median (IQR)]	45 (42, 67)	47 (36, 73)	1.01 (0.99, 1.03)	1.01 (0.96, 1.02)	0.386
Male sex	57 (69)	20 (59)	0.65 (0.28, 1.49)	0.53 (0.25, 2.15)	0.567
Underlying disease(s)	76 (92)	29 (85)	0.53 (0.16, 1.81)	0.3 (0.10, 1.05)	0.615
Immunocompromised status	5 (6)	1 (3)	0.45 (0.05, 4.12)	0.41 (0.08, 3.71)	0.738
Obesity	58 (70)	25 (74)	1.19 (0.49, 2.04)	1.06 (0.35, 2.00)	0.905
APACHE II score [median (IQR)]	16 (13, 21)	18 (15, 21)	1.05 (1.01, 1.15)	1.19 (1.04, 1.35)	0.012
Pneumonia	40 (48)	15 (44)	0.84 (0.53, 2.63)	0.72 (0.50, 2.46)	0.546
Initial intensive care unit admission	38 (46)	16 (47)	1.05 (0.47, 2.32)	1.03 (0.25, 1.54)	0.131
Appropriate empirical antibiotic(s)	50 (60)	4 (12)	0.09 (0.03, 0.27)	0.04 (0.01, 0.16)	<0.001

[A] Comparison between survivors and non-survivors. Boldface entries indicate values that reached the significance level set at 0.05.

The 30-day Kaplan–Meier survival rates among patients with *S. maltophilia* who received appropriate empirical antibiotic(s) differed significantly from those of patients who did not ($p < 0.001$, log-rank test), as illustrated in Figure 2.

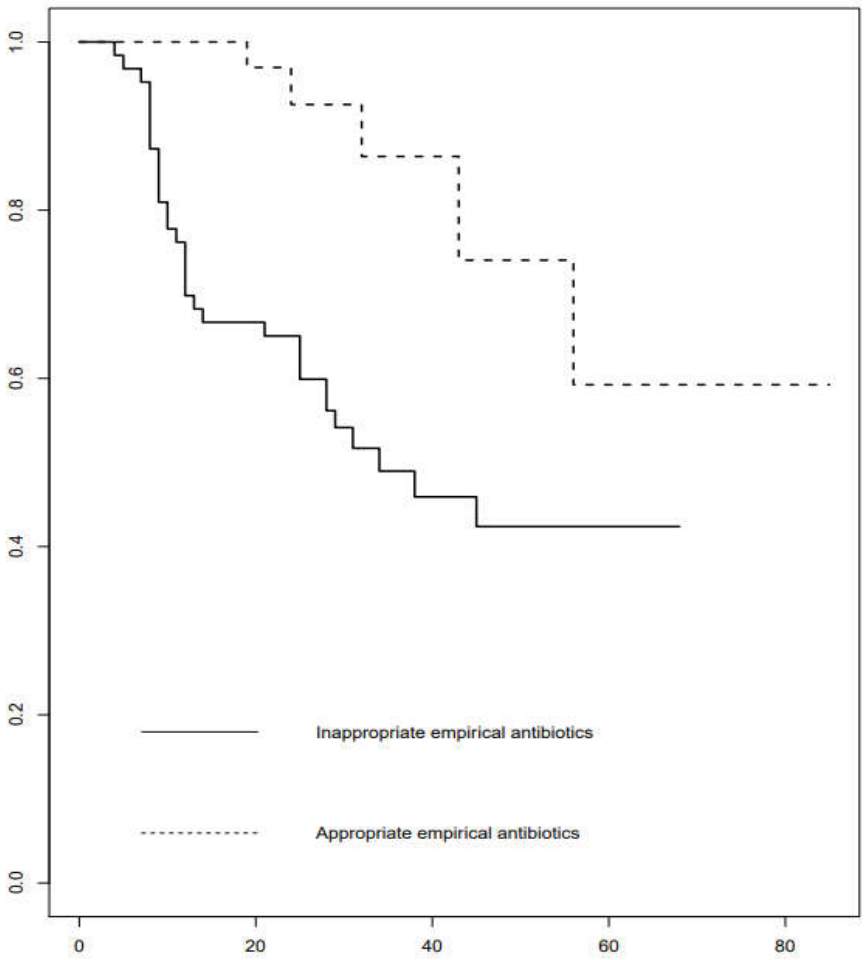

Figure 2. Kaplan–Meier survival curves of *S. maltophilia* bacteremia patients who received and did not receive appropriate empirical antibiotic(s).

4. Discussion

Since the extensive prescription of antimicrobial agents in the past decade, *S. maltophilia* has become the third most prevalent non-fermentative gram-negative bacterial isolate causing hospital acquired infections, preceded by *Pseudomonas aeruginosa* and *Acinetobacter* spp. *S. maltophilia* infections. These are associated with high mortality rates ranging from 21 to 69%, and the overall 30-day mortality rate of 29% observed in this study is comparable to previous reports.

Several characteristics of *S. maltophilia* bacteremia patients were similar to previous reports. Several reports showed that infection due to *S. maltophilia* commonly occurred among the critically ill patients who were admitted in ICUs, while less than half (46%) of the patients in this research were primarily admitted in ICUs [3–8,12]. This can be explained with the constrained ICU capacity in our setting. The patients in this study had relatively severe illness with a median APACHE score of 19, and most were subjected to a mechanical ventilator (82%), intravascular devices (62%) and urinary catheterization (86%), respectively. Thus, the acquisition of this organism frequently occurred among mechanically ventilated patients [12]. Most of the patients in this study (90%) had several medical morbidities. Similarly, risk factors of this infection including multiple debilitating illnesses were described in a previous report. Additionally, previous exposure to several broad-spectrum antibiotics were previously demonstrated to be a risk factor for infection due to *S. maltophilia*. This study shows that *S. maltophilia* mostly affects patients with prior use of antibiotics, especially carbapenem (96%). This suggests that the use of carbapenem in patients who are hospitalized may lead to the emergence of nosocomial infections

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caused by *S. maltophilia* as its intrinsic resistance to carbapenem and the selective growth of non-susceptible isolates. An antimicrobial susceptibility test in this study showed unfavorable resistance to commonly-used antimicrobial agents for nosocomial infection including ceftazidime and colistin. These findings are similar to previously published data [10,11]. The resistance seemed relatively low among levofloxacin (12%) and TMP-SMX (7%). The appropriate choices in our setting included TMP-SMX and fluoroquinolones. It is interesting that the resistance of colistin is relatively high at 35%. This finding is in line with the reports in several regions of the rising of colistin-resistant *S. maltophilia* [10,11,14]. Unlike the in vitro study of Enterobacteriaceae, the data on combination therapy of *S. maltophilia* remains unclear [25].

Previous reports have demonstrated that risk factors associated with mortality among *S. maltophilia* bacteremia patients include septic shock, APACHE II score ≥15 points, the indwelling of central venous catheters, mechanical ventilation, ICU admission, prolonged hospitalization, and receiving concurrent immunosuppressive agents or broad-spectrum antibiotics [19,20].

In our study, we identified that a high APACHE II score was also associated with higher rates of mortality, similar to previous literature [3,26–28]. Moreover, this study found that the appropriate empirical antibiotic(s) were a protective predictor to reduce mortality, based on the multivariate analysis results. However, some reports found that the inappropriate empirical antimicrobial treatment did not have a significant impact on mortality [12,18]. These discrepancies were probably due to the different antimicrobial susceptibility patterns. This study reported that TMP-SMX and levofloxacin remain highly active against isolates of *S. maltophilia* (93% and 88%, respectively), whereas the susceptibility rates of TMP-SMX and fluoroquinolones have varied in some previous reports (81–85%, and 21–82%, respectively) [18,29].

With regard to the emergence of infections of *S. maltophilia*, particularly in individuals who were admitted in ICUs, a previous report demonstrated the economic burdens among those patients with this infection including prolonged length of stay in hospital and hospital costs [30]. This current study did not only demonstrate only clinical benefits of appropriate antibiotics but also non-clinical benefits including length of hospital stay and hospital expenses. These findings suggest that the appropriate empirical antibiotic yielded an approximately 40% shorter length of hospital stay and an approximately 25% lower hospital cost. Even with the addition of appropriate empirical antimicrobial agents including fluoroquinolones and TMP-SMX into the commonly used regimens, the antimicrobial cost among those with appropriate empirical antimicrobial agents were still lower than those with inappropriate empirical antibiotics.

This study shows that *S. maltophilia* mostly affects patients with a history of the prior use of antibiotics, especially carbapenem (96%), reflecting the widespread but potentially problematic use of carbapenem in hospitalized patients, which may lead to the emergence of *S. maltophilia* infections due to its intrinsic resistance to carbapenem and the selective growth of non-susceptible isolates. Because *S. maltophilia* infections continue to increase worldwide and therapeutic options are limited, antimicrobial stewardship is essential to reduce the emergence of drug-resistant organisms and hospital costs.

Study Limitations

This study has some limitations. First, we enrolled patients from a single hospital, which could make it difficult to generalize our findings to other contexts. Second, the lack of data addressing the clinical judgment of physicians regarding antimicrobial therapy may have limited the scope of the analysis. Third, our study focused only on bloodstream infections and pneumonia, whereas other infection sites were not evaluated. Variations in the infection site might be associated with the outcome of *S. maltophilia* septicemia. Lastly, the design of this study was a retrospective cohort analysis, which may have contributed to information and selection biases. A well-designed prospective study should

be conducted to establish the impact of appropriate antimicrobial therapy on mortality outcomes more precisely.

5. Conclusions

Bacteremia due to *S. maltophilia* causes relatively high mortality and high resource utilization. This infection should of concern among patients with several medical comorbidities, comparatively severe clinical manifestations, with previous admission to intensive care units and previous exposures to broad-spectrum antimicrobial agents, particularly carbapenems. Appropriate empirical antimicrobial treatment yields a significant benefit on clinical outcomes including 14-day, 30-day and in-hospital mortality. Additionally, the patients who received appropriate empirical antimicrobial treatment had a substantially shorter length of hospital stay and lower hospital costs. According to antimicrobial susceptibility testing in this current study, the appropriate empirical antimicrobials agents include TMP-SMX and fluoroquinolone.

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Informed Consent Statement: Patient consent was waived because retrospective patient data was retrieved from the electronic hospital database.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the privacy and ethical reasons.

Conflicts of Interest: The authors declare no conflict of interest.

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Antimicrobial Resistance and Infection Control

RESEARCH Open Access

Evaluation of a *Stenotrophomonas maltophilia* bacteremia cluster in hematopoietic stem cell transplantation recipients using whole genome sequencing

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Abstract

Background: *Stenotrophomonas maltophilia* ubiquitously occurs in the hospital environment. This opportunistic pathogen can cause severe infections in immunocompromised hosts such as hematopoietic stem cell transplantation (HSCT) recipients. Between February and July 2016, a cluster of four patients on the HSCT unit suffered from *S. maltophilia* bloodstream infections (BSI).

Methods: For epidemiological investigation we retrospectively identified the colonization status of patients admitted to the ward during this time period and performed environmental monitoring of shower heads, shower outlets, washbasins and toilets in patient rooms. We tested antibiotic susceptibility of detected *S. maltophilia* isolates. Environmental and blood culture samples were subjected to whole genome sequence (WGS)-based typing.

Results: Of four patients with *S. maltophlilia* BSI, three were found to be colonized previously. In addition, retrospective investigations revealed two patients being colonized in anal swab samples but not infected. Environmental monitoring revealed one shower outlet contaminated with *S. maltophilia*. Antibiotic susceptibility testing of seven *S. maltophlia* strains resulted in two trimethoprim/sulfamethoxazole resistant and five susceptible isolates, however, not excluding an outbreak scenario. WGS-based typing did not result in any close genotypic relationship among the patients' isolates. In contrast, one environmental isolate from a shower outlet was closely related to a single patient's isolate.

Conclusion: WGS-based typing successfully refuted an outbreak of *S. maltophilia* on a HSCT ward but uncoverd that sanitary installations can be an actual source of *S. maltophilia* transmissions.

Keywords: *Stenotrophomonas maltophilia*, Outbreak investigation, Whole genome sequence-based typing, Immunocompromised hosts

Background

Stenotrophomonas maltophilia, an intrinsically multidrug resistant gram negative pathogen, is widely distributed in aqueous habitats such as, sink drains, endoscopes and hemodialysis water within clinical settings [1, 2]. Usually, this pathogen is not highly virulent in immunocompetent individuals but can cause severe infections including bacteremia, peritonitis and meningitis in immunocompromised hosts resulting in complications, e.g. septic shock, respiratory failure, tissue necrosis and septic thrombophlebitis [3]. Recipients of hematopoietic stem cell transplantations (HSCT) are especially at risk and suffer from pulmonary hemorrhage resulting in higher mortality rates compared to non-HSCT patients [4].

In recent years, an increasing incidence of *S. maltophilia* is reported on oncologic wards [5]. Moreover, several outbreaks and pseudo-outbreaks caused by this pathogen have been reported [6, 7]. In addition to epidemiological

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investigations, different typing methods, e.g. pulse field gel electrophoresis (PFGE) and multi locus sequence typing (MLST) are used to identify the genetical relationship among different *S. maltophilia* isolates [8–11]. These techniques are useful in excluding outbreak scenarios, if MLST sequence types (ST) or PFGE patterns of isolated pathogens differ. In case of identical STs or highly similar PFGE patterns both methods reach their discriminatory limits. In these cases an outbreak is assumed, although more precise methods uncover and accidental cluster. Additionally, inter-laboratory comparability is limited using PFGE. Therefore, whole genome sequence-based typing (WGS) approaches are increasingly seen as gold standard method for highly discriminatory typing [12] and the superiority of WGS in comparison to other typing methods was already demonstrated for several bacterial pathogens [13–17]. Moreover, we could recently demonstrate the high reproducibility of WGS, which is another prerequisite for the applicability in clinical routine [18].

Here we evaluated a cluster of *S. maltophilia* bacteremia in recipients of allogeneic stem cell grafts using WGS-based typing.

Methods

Cluster detection, epidemiological investigations and infection control measures

The 1500-bed University Hospital Münster includes two HSCT-units each comprising 10 patient rooms, which are all HEPA-filtered and equipped with separated bathrooms for each patient (Fig. 1). In total, 193 patients were admitted to the HSCT-units during 2016. Patients usually receive an allogenic HSCT.

S. maltophilia bloodstream infections (BSI) were defined as one or more blood cultures positive for *S. maltophilia* obtained from patients with clinical signs of infection (fever >38 °C, chills, hypotension) according to the critieria given by the European Centre for Disease Prevention and Control [19]. Four *S. maltophilia* BSI were detected in patients (P1, P3, P4, P6) in the HSCT ward between February and July 2016. As this number exceeds the average baseline of 0.5 *S. maltophilia* BSI per year detected during 2011–2015, an epidemiological investigation was initiated. Routine environmental screening of aqueous habitats (shower heads, shower outlets, washbasins and toilets) within patients' rooms in the HSCT unit, normally focusing on multidrug resistant

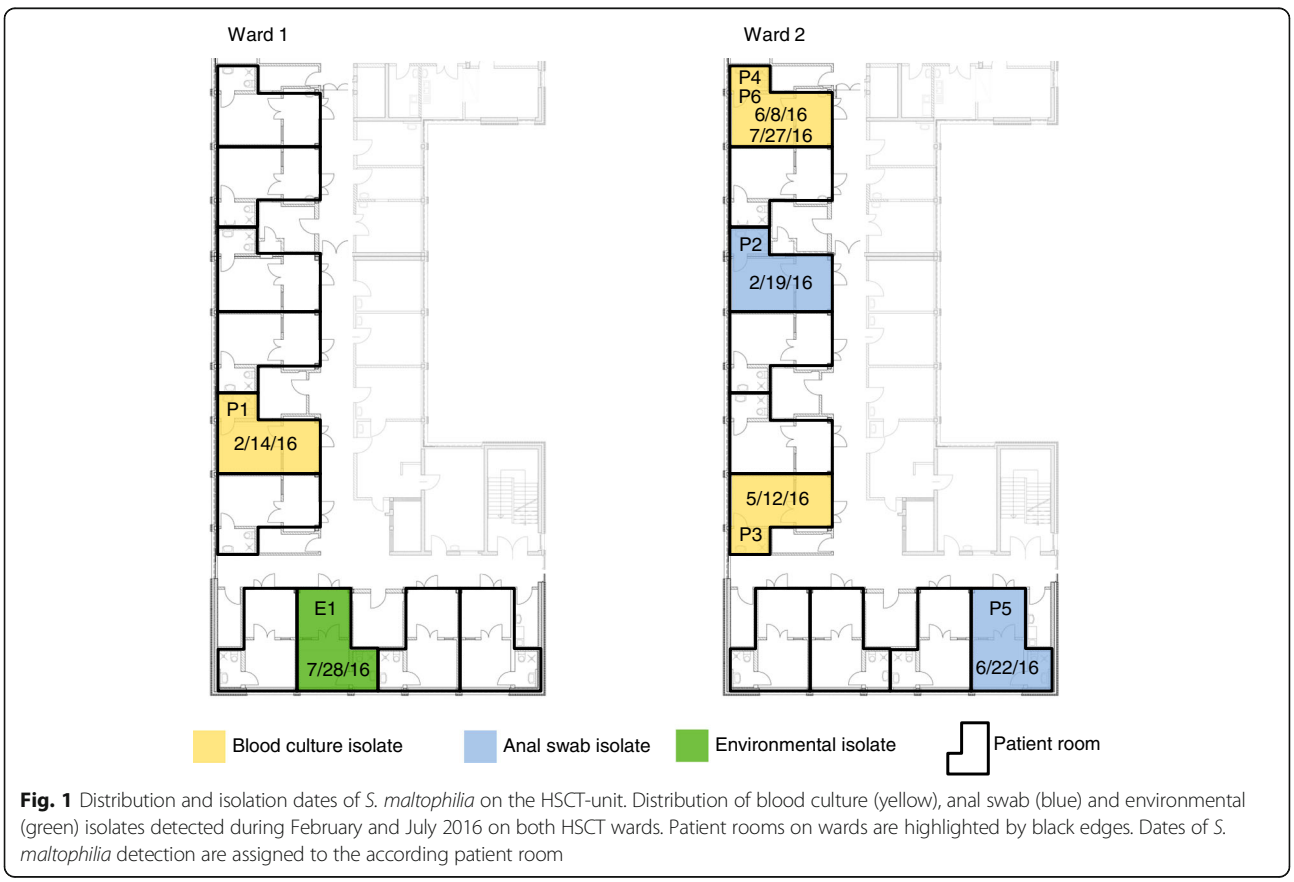

Fig. 1 Distribution and isolation dates of *S. maltophilia* on the HSCT-unit. Distribution of blood culture (yellow), anal swab (blue) and environmental (green) isolates detected during February and July 2016 on both HSCT wards. Patient rooms on wards are highlighted by black edges. Dates of *S. maltophilia* detection are assigned to the according patient room

Pseudomonas aeruginosa, was expanded to *S. maltophilia.* Patients colonized with *S. maltophilia*, coincidentally detected in anal/rectal swabs or stool samples (as screening of HSCT-patients concentrates on multidrug resistant *Pseudomonas aeruginosa*), were retrospectively identified. Moreover, hand hygiene and surface disinfection measures were intensified.

Identification and susceptibility testing

Positive blood culture samples were detected using an automated blood culture system (BACTEC™ 9240, Becton Dickinson GmbH, Heidelberg, Germany). Environmental samples were plated on Columbia sheep blood agar (Oxoid, Wesel, Germany) and MacConkey agar (Becton Dickinson, Heidelberg, Germany) after enrichment in Tryptic Soy Broth (Becton Dickinson) for 24 h at 36 °C. Subsequent species identification was performed by Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF MS; Bruker, Bremen, Germany). Susceptibility testing for trimethoprim-sulfamethoxazole (TMP-SMX) was performed using disk diffusion method in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards and interpreted using EUCAST clinical breakpoints (version 6).

Whole genome sequencing (WGS) based typing

To determine the clonal relationship of *S. maltophilia* strains isolated from blood cultures, the isolates were subjected to WGS using the Illumina MiSeq platform (Illumina Inc., San Diego, USA) as described previously [13]. Retrospectively identified colonization isolates were not subjected to WGS as they were no longer available in contrast to BSI isolates, that are stored for a longer time period. Using SeqSphere$^+$ software version 2.0 beta (Ridom GmbH, Münster, Germany), all coding regions were extracted and compared in a gene-by-gene approach (core genome multilocus sequence typing, cgMLST) using *S. maltophilia* K279a strain (GenBank accession number AM743169.1) as a reference sequence. Instead of a published cgMLST scheme, which is not yet available, this ad hoc scheme was used to differentiate the cluster. SeqSphere$^+$ software was used to display the clonal relationship in a minimum-spanning tree (MST). For backwards compatibility with classical molecular typing, i. e. MLST, the MLST sequence types (ST) were extracted from the WGS data in silico.

Results

Epidemiological investigation and susceptibility testing

Chronological order and spacial distribution of isolated *S. maltophilia* is displayed in Fig. 1. Isolates were obtained from blood culture samples of patients on both wards. Two of the four isolates (isolated from P4 and P6) were detected within short intervals in two patients both admitted to room 1 on ward 2. Of the four patients suffering from *S. maltophilia* bacteremia, three (P1, P4 and P6) were previously tested positive for *S. maltophilia* in stool samples or anal swabs. Additionally, two colonized patients (anal swabs; P2, P5) admitted to ward 2 were uncovered by the retrospective analysis (Table 1). One environmental isolate (E1) could be detected in a shower outlet of a patient room that was a patient with *S. maltophilia* infection. Susceptibility testing revealed all patient isolates to be susceptible to TMP-SMX, except isolate P3. The environmental isolate was resistant to TMP-SMX (Table 1).

Clinical characteristics and outcome of patients with *S. maltophilia* bacteremia

All patients with *S. maltophilia* BSI suffered from acute myeloid leukemia as the underlying disease. P2 and P5, who were only colonized by *S. maltophilia,* suffered from chronic lymphatic leukemia and myeloproliferative neoplasm. Except P1, all patients had already received allogenic SCT at the time of *S. maltophilia* detection. P1 and P6 developed an acute respiratory distress syndrome. P1, P3, and P6 died due to *S. maltophilia* bacteremia.

WGS-based typing

We analyzed four bloodstream isolates and the environmental isolate by WGS. Comparison via cgMLST, based on 1876 genes present in all isolates, revealed no genetical relationship among isolates originating from patients (Fig. 2). In contrast, *S. maltophilia* strains isolated from P3 and E1 showed a similar genotype with only four alleles difference, suggesting a relationship between these two isolates. P3 and E1 harbored the MLST ST 94. All other isolates harbored new MLST STs.

Discussion

S. maltophilia has emerged as an important nosocomial pathogen associated with increased mortality rates in patients suffering from hematological malignancies and receiving hematopoietic stem cell transplantations [20, 21]. In this study, WGS was used to evaluate a cluster of four *S. maltophilia* BSI in patients partially suffering from pulmonary dysfunction associated with the infection due to this

Table 1 Patients with *S. maltophilia* colonizations and blood stream infections during February and July 2016

Patient no.	Anal colonization	Blood stream infection	Strain susceptibility to TMP-SMX	WGS performed
P1	+	+	S	+
P2	+	–	S	–
P3	–	+	R	+
P4	+	+	S	+
P5	+	–	S	–
P6	+	+	S	+

TMP-SMX – trimethoprim-sulfamethoxazole, WGS – whole genome sequencing

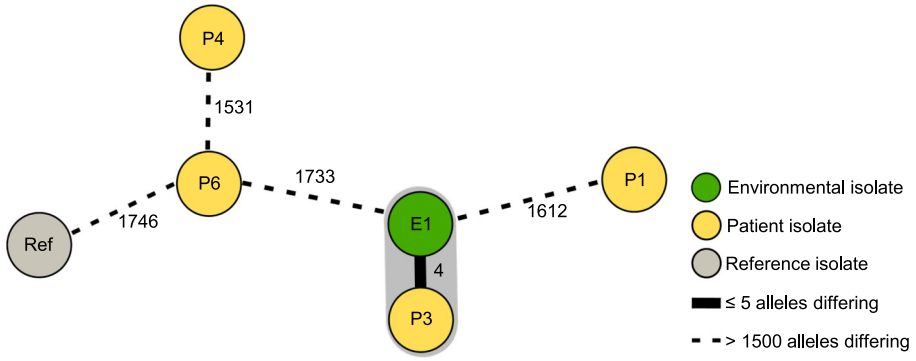

Fig. 2 Minimum spanning tree of *S. maltophilia* isolates. Minimum spanning tree of four blood culture isolates (P, *yellow*), one environmental isolate (E, *green*) from the HSCT unit and one outgroup isolate (Ref, non-HSCT unit, isolated in May 2016, *grey*) based on up to 1876 target genes, pairwise ignoring missing values. Genotypes are consecutively numbered, starting with P1 (isolated in February 2016). Each dot represents one genotype and is colored according to its origin. Different connecting lines and numbers on these lines show the number of alleles differing between two genotypes

pathogen [4]. Susceptibility testing, normally used as a first indication of nosocomial transmission of bacterial strains, could not exclude a spread of one single clone. WGS and subsequent cgMLST analysis, methods that have shown to provide detailed information in evaluating the epidemiology in nosocomial clusters of other pathogens [22], excluded a genetical relation and therefore an outbreak scenario.

A number of different sources are possibly responsible for the increasing number of *S. maltophilia* BSI. Due to its multidrug resistant nature, selection of this pathogen can easily occur in patients after receiving broad-spectrum antibiotics [23]. Moreover, *S. maltophilia* infections occur frequently in combination with the distinct immunocompromised status of HSCT recipients [24]. On the other hand, several environmental sites such as sink drains could be detected as sources of *S. maltophilia* within hospital settings [25].

To what extent these habitats are origins for hospital-acquired colonizations or infections of *S. maltophilia* could not yet be shown in detail. In this study we documented a genetical relation between an environmental and a patient blood stream infection isolate by analyzing WGS data. Although there was no local proximity, *S. maltophilia* could be transmitted from the environment to a patient or vice versa, at least giving the possibility of further spread via aqueous habitats in the HSCT unit.

Conclusion

WGS- analysis can be used to precisely refute an outbreak of *S. maltophilia*. Additionally, WGS- based typing documented sanitary installations in HSCT units to be an actual source or result of transmission between environmental and human habitats. Hence, in addition to classical infection control strategies, monitoring of aqueous habitats has to be established within the rooms of HSCT recipients in order to prevent transmission of these multidrug resistant organisms, as recently also shown for *P. aeruginosa* [26].

Abbreviations

BSI: Blood stream infection; cgMLST: Core genome multilocus sequence typing; HSCT: Hematopoietic stem cell transplantation; MST: Minimum spanning tree; PFGE: Pulsed field gel electrophoresis; ST: Sequence type; WGS: Whole genome sequencing

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Availability of data and materials

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Authors' contributions

SK, AP, EAI were responsible for all data collection. SK, MHP, AK and AM were involved in data analysis and interpretation. All authors contributed to and approved the final draft of manuscript.

Ethics approval and consent to participate

All strategies and investigations were performed in accordance with the recommendations of the legally assigned institute for infection control and prevention (Robert Koch Institute). For the present retrospective analysis formal consent is not required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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SYSTEMATIC REVIEW
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Risk Factors for Acquired *Stenotrophomonas maltophilia* Pneumonia in Intensive Care Unit: A Systematic Review and Meta-Analysis

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Background and Aims: *Stenotrophomonas maltophilia* is increasingly found in critically ill patients, but it is considered a pathogen of limited pathogenicity and therefore it is not often targeted. We systematically evaluated risk factors for *S. maltophilia* pneumonia in ICU patients for better clinical management.

Methods: Prospective and retrospective studies of *S. maltophilia* infection in the ICU from database establishment to August 8, 2021, were searched through PubMed, web of science, Cochrane Library Embase and CNKI. The literature was independently screened and extracted by two authors according to inclusion and exclusion criteria, evaluated for quality by the NOS scale, and meta-analyzed by stata 14.0 software.

Results: A total of eight studies with a sample size of 2,320 cases were included. Meta-analysis showed that APACHE-II score > 20 (OR = 10.98, 95% CI: 5.67 ~ 21.26), COPD (OR = 3.97, 95% CI: 2.39 ~ 6.61), malignant tumor (OR = 2.15, 95% CI: 1.03 ~ 4.50), mechanical ventilation (OR = 8.75, 95% CI: 2.59 ~ 29.58), tracheotomy (OR = 6.12, 95% CI: 2.06 ~ 18.18), endotracheal intubation (OR = 4.25, 95% CI: 2.30 ~ 7.84), β- Lactamase inhibitors (OR = 9.98, 95% CI: 1.51 ~ 65.96), aminoglycosides (OR = 4.01, 95% CI: 2.06 ~ 7.80), carbapenems (OR = 2.82, 95% CI: 1.49 ~ 5.31), and quinolones (OR = 2.17, 95% CI: 1.21 ~ 3.89) were risk factors for ICU-acquired *S. maltophilia* pneumonia.

Conclusion: Many risk factors are associated with *S. maltophilia* pneumonia in ICU patients. Clinical workers should pay more attention to assessing the risk of infection in ICU patients and enhance the prevention and management of high-risk groups, which will help reduce their risk of *S. maltophilia* infection.

Keywords: *Stenotrophomonas maltophilia*, ICU-acquired pneumonia, risk factor, meta-analysis, infection

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INTRODUCTION

S. maltophilia is a non-fermentable Gram-negative bacterium that is an opportunistic agent. It is naturally resistant to many commonly used antibiotics, such as carbapenems and aminoglycosides (1, 2). It is due to such characteristics, in the context of drug resistance, that *S. maltophilia* is becoming an important pathogen of hospital infections in the ICU, which can lead to infections in the lungs, bloodstream and many other important parts of the body, even life-threatening (3, 4).

According to the CHINET bacterial resistance surveillance data in 2020, *S. maltophilia* accounted for 2.98% of all strains and ranked 9th, 7th among Gram-negative bacteria, and 3rd among non-fermentative bacteria, after *Bacillus immobilis* and *Pseudomonas aeruginosa*. *S. maltophilia* has the highest resistance to ceftazidime (38.5%), followed by levofloxacin (10.8%), compound sulfamethoxazole (6.7%) and tigecycline (2.7%), and the lowest resistance to minocycline (2.3%) (5). Clinically, it often causes mixed infections with other bacteria, mainly *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, so *S. maltophilia* infections are difficult to be treated and the mortality rate is high. Muder et al. (6) reported a mortality rate of 21% in patients with *S. maltophilia* bacteremia. Paez et al. (7) reported a direct mortality rate of 26.7% due to *S. maltophilia* infection and a mortality rate of 21–69% associated with *S. maltophilia* infection.

The study of risk factors for ICU-acquired *S. maltophilia* pneumonia is of great practical significance for the in-depth study of the hazards of this bacterium and the adoption of appropriate preventive and control measures. Currently, Several studies at home and abroad have investigated the risk factors for the occurrence of *S. maltophilia* infection in ICU patients, but there are drawbacks such as small sample size and incomplete risk factor indicators, and the significance of clinical guidance is limited. This study aims to systematically evaluate the risk factors of hospital-acquired *S. maltophilia* pneumonia by Meta-analysis, and provide a theoretical basis for clinical formulation of prevention and control strategies to reduce the morbidity and mortality of *S. maltophilia* infection.

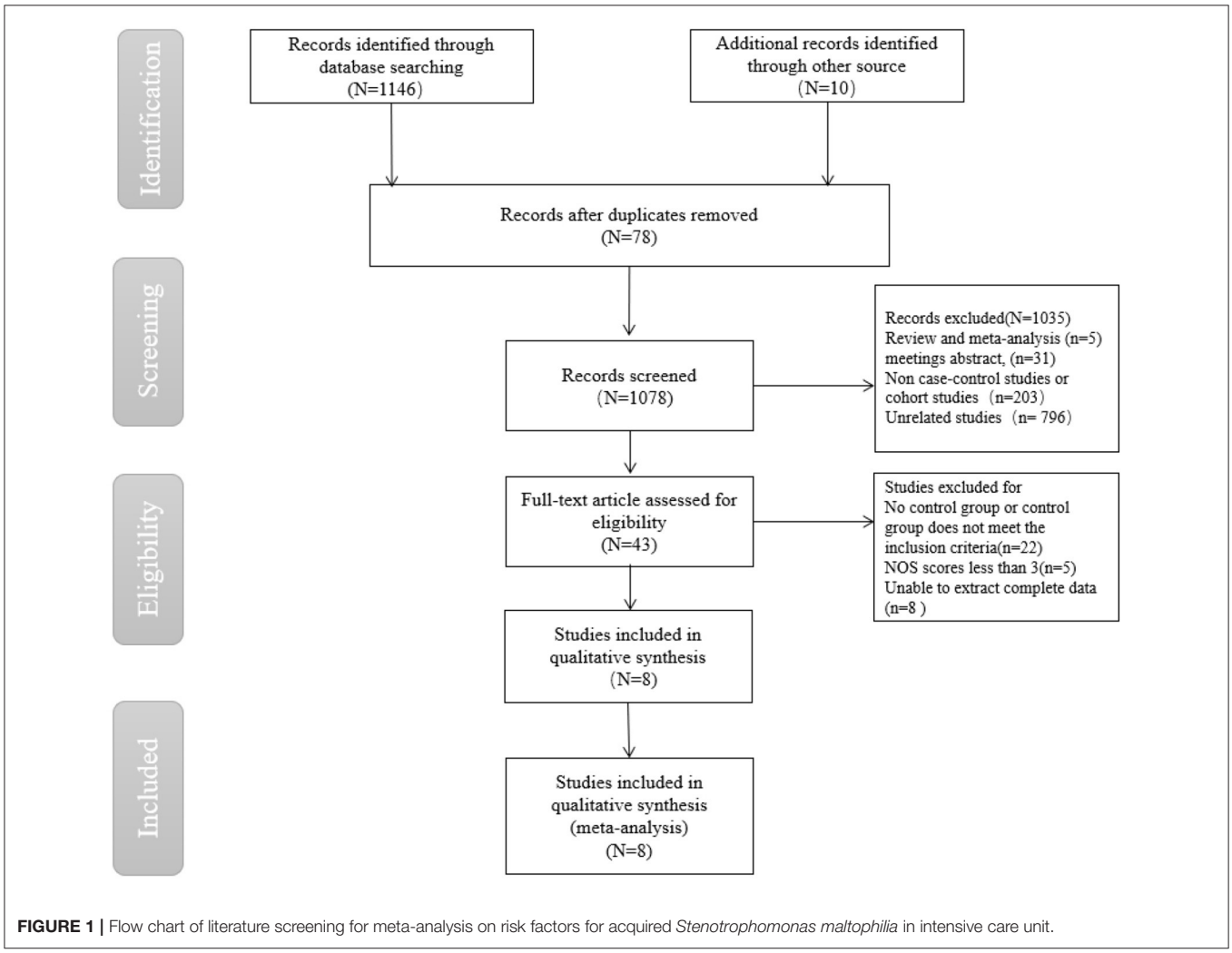

FIGURE 1 | Flow chart of literature screening for meta-analysis on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

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TABLE 1 | Characteristics of the included studies.

Study	Year	Design study	Area	Infection group	Non-infection group	Risk factors
Stang et al. (8)	2002	Case-control	USA	26	137	01.02.03.07.20.21.22.24
Hanes et al. (9)	2006	Cohort	France	30	60	01.02.07.08.09.11.14.15.16.17.18.21.22.23.24.25
Nseir et al. (10)	2011	Case-control	China	35	140	01.02.04.11.14.15.16.19.21.23
Xu et al. (11)	2012	Case-control	Germany	36	28	01.02.03.08.09.10.12.13.14.15.17.18
Guo et al. (12)	2014	Case-control	China	42	84	01.02.04.06.08.09.11.14.15.16.19
Saugel et al. (13)	2016	Case-control	Netherlands	6	15	01.02.03.08.09.10.11.13.14.16
Ibn Saied et al. (14)	2019	Case-control	China	29	58	01.02.03.09.10.11.13.14.17.18.21.22.23.24.25
Lei et al. (15)	2020	Case-control	USA	102	1,492	01.02.05.06.07.11.12.13.20

01, Age, years; 02, Gender; 03, APACHE-II score; 04, APACHE-II score >20; 05, Glasgow score; 06, Glucocorticoid; 07, Length of ICU stay, days; 08, COPD; 09, Diabetes; 10, Malignancy; 11, Cardiovascular disease; 12, kidney dysfunction; 13, Immunosuppression; 14, Mechanical ventilation; 15, Tracheal intubation; 16, Tracheotomy; 17, Central venous catheterization; 18, Urinary catheter; 19, Nasogastric tube; 20, Operation; 21, Carbapenems; 22, β-lactamase inhibitor; 23, Aminoglycosides; 24, Quinolones; 25, Nitroimidazoles.

TABLE 2 | Risk of bias assessment of the included studies according to the Newcastle-Ottawa Scale (NOS).

NOS items/Study ID	Hanes et al.	Xu et al.	Saugel et al.	Guo et al.	Scholte et al.	Shi et al.	Saied et al.
Is the case definition adequate?	*		*	*	*	*	*
Representativeness of the cases	*	*	*	*	*		*
Selection of controls	*	*	*		*	*	*
Definition of controls	*	*	*	*	*	*	*
Compatibility	*	*	*	*	*	*	*
Ascertainment of exposure	*	*	*	*	*	*	*
Same method of ascertainment for cases and control	*	*	*	*			*
Non-response rate	*	*			*	*	*
Total score	8	7	7	6	7	6	8

**Representative studies meet this criteria.*

MATERIALS AND METHODS

Literature Search Strategy

PubMed, Web of Science, Cochrane Library, Embase and CNKI were searched from the time of database establishment to August 8, 2021. A combination of subject terms, free words, and Boolean logical operators was used for the search terms: *Stenotrophomonas maltophilia*, hospital-acquired pneumonia, ventilator-associated pneumonia, intensive care, risk factors, etc. The English databases were searched for the following terms: (*Stenotrophomonas maltophilia* OR *S. maltophilia* OR SMA) AND (nosocomial infection OR hospital infection OR hospital acquired infection OR cross infection OR VAP OR ventilator associated pneumonia OR ventilator-associated pneumonia) AND (ICU OR Intensive Care OR NICU OR PICU OR CCU) AND (risk factor OR factor). A manual search of relevant content reviews and references of included literature was conducted to identify potential studies that met the inclusion criteria.

Literature Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) The type of literature was a cohort study or case-control study published nationally and internationally; (2) The study population was divided into two groups based on whether they were infected with *S. maltophilia*, and the diagnosis criteria for *S. maltophilia* pneumonia in this study were described below; (3) Risk factors for *S. maltophilia* pneumonia in ICU patients were present in the literature, such as comorbid underlying diseases, invasive procedures undergone, and use of broad-spectrum antibiotics; (4) Outcome indicators for risk factors for *S. maltophilia* pneumonia in ICU patients could be expressed as odds ratios (OR), and their 95% confidence intervals (CI) were calculated. Studies were excluded when meeting one of the following criteria: (1) duplicate reports, conference reports, and reviews; (2) abnormal or missing data; (3) low quality of literature [Newcastle-Ottawa Scale (NOS) score≤3].

In this study, infection and colonization were considered ICU-acquired if they were diagnosed more than 48 h after ICU admission. Pneumonia was defined as follows: (1) new or progressive pulmonary infiltrates. (2) Temperature > 38°C or <36.5°C, leukocyte count >12,000 μl^{-1} or <4000 μl^{-1}, purulent endotracheal aspirate or sputum. (3) Positive respiratory sample. (4) Decreased oxygenation.

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TABLE 3 | Meta-analysis results of risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

Exposure factors	Included studies	Heterogeneity	*p*	Fixed-effect model (FEM)	*p*	Random-effect model (REM)	*p*
General condition							
Age, years	8	0	0.93	−0.76 (−2.62∼1.10)	0.42	−0.76 (−2.62∼1.10)	0.42
Gender	8	0	0.83	0.77 (0.59∼1.02)	0.07	0.77 (0.58∼1.01)	0.06
APACHE-II score	3	0	0.83	2.80 (−0.31∼5.82)	0.08	2.80 (−0.31∼5.82)	0.08
APACHE-II score >20	2	0	0.39	10.98 (5.67∼21.26)	<0.001	11.49 (6.02∼21.92)	<0.001
Glasgow score	2	0	0.66	−0.50 (−1.91∼0.90)	0.49	−0.50 (−1.91∼0.90)	0.49
Glucocorticoid	3	42	0.58	0.91 (0.51∼1.61)	0.74	0.98 (0.42∼2.29)	0.97
Length of ICU stay, days	4	0	0.50	1.65 (0.70∼2.60)	0.001	1.65 (0.70∼2.60)	0.001
Pre-existing medical conditions							
COPD	4	77.8	0.004	3.97 (2.39∼6.61)	<0.001	3.99 (1.19∼13.32)	0.03
Diabetes	5	8.1	0.36	1.50 (0.89∼2.63)	0.13	1.47 (0.82∼2.61)	0.20
Malignancy	3	0	0.99	2.15 (1.03∼4.50)	0.04	2.15 (1.03∼4.50)	0.04
Cardiovascular disease	7	48.2	0.07	0.92 (0.66∼1.29)	0.63	1.0 (0.61∼1.75)	0.92
kidney dysfunction	3	0	0.87	1.20 (0.69∼2.07)	0.52	1.21 (0.70∼2.07)	0.50
Immunosuppression	3	49.6	0.14	1.38 (0.87∼2.21)	0.17	1.70 (0.38∼7.69)	0.49
Invasive procedures							
Mechanical ventilation	5	71.4	0.007	8.22 (4.82∼14.03)	<0.001	8.75 (2.59∼29.58)	<0.001
Tracheal intubation	3	0	0.52	4.25 (2.30∼7.84)	<0.001	4.08 (2.22∼7.51)	<0.001
Tracheotomy	4	67.2	0.03	6.10 (3.54∼10.52)	<0.001	6.12 (2.06∼18.18)	0.001
Central venous catheterization	3	82.7	0.003	3.22 (1.62∼6.42)	0.001	2.30 (0.37∼14.41)	0.37
Urinary catheter	3	0	0.89	2.14 (0.79∼5.84)	0.14	2.10 (0.77∼5.76)	0.15
Nasogastric tube	3	78.3	0.03	3.28 (1.85∼5.83)	<0.001	3.36 (0.95∼11.87)	0.06
Operation	3	0	0.75	0.78 (0.36∼1.70)	0.53	0.80 (0.36∼1.76)	0.57
Antimicrobial agents							
Carbapenems	4	23	0.27	2.82 (1.49∼5.31)	0.001	2.82 (1.30∼6.09)	0.008
β-lactamase inhibitor	3	85.9	0.001	7.88 (4.41∼14.09)	<0.001	9.98 (1.51∼65.96)	0.02
Aminoglycosides	3	27.4	0.25	4.01 (2.06∼7.81)	<0.001	4.12 (1.75∼9.70)	0.001
Quinolones	3	39.1	0.19	2.17 (1.21∼3.89)	0.009	2.25 (1.03∼4.93)	0.04
Nitroimidazoles	2	60.6	0.11	1.63 (0.43∼6.24)	0.48	1.75 (0.14∼22.69)	0.67

Data Extraction and Quality Evaluation

The retrieved literature was screened by two authors independently according to the inclusion and exclusion criteria, and the following data information was extracted: name of the first author, time of publication, source of the literature, basic characteristics of the included cases, and possible risk factors for *S. maltophilia* pneumonia in ICU patients. If the opinions of two reviewing authors do not agree, they discuss. If there was still disagreement after discussion, a third party opinion was sought. The quality of the included literature was also evaluated according to NOS score (8), and the evaluation items include three aspects of population selection, comparability and exposure evaluation, with a score out of 9. A score of 7 and above was considered as high-quality literature, 4–6 as moderate quality literature, and 1–3 as low-quality literature.

Data Analysis

Statistical analysis was performed by stata14.0 software. I^2 was used to determine the heterogeneity of the included literature, and the fixed-effects model was used when $p > 0.1$ and $I^2 < 50\%$; otherwise, the random-effects model was applied. OR and its 95% CI were calculated for count data, while weighted mean difference (WMD) and its 95% CI were calculated for measurement data, and differences were considered statistically significant at $p \leq 0.05$. Sensitivity analysis was performed by calculating the OR and 95% CI for both fixed-effects and random-effects models and comparing the results of the two groups. Sensitivity analysis was performed by changing the data analysis model. If there was no substantial change after the model change (no opposite conclusion was reached after changing the model), the consolidated result was considered to be stable. Begg's test was used to test for publication bias when the number of included papers for individual risk factor analysis was ≥3.

RESULTS

Literature Search

Firstly, 1,156 papers were initially searched in the database through the search strategy, and then 43 papers were selected through title, abstract and keywords, etc. Finally, eight papers

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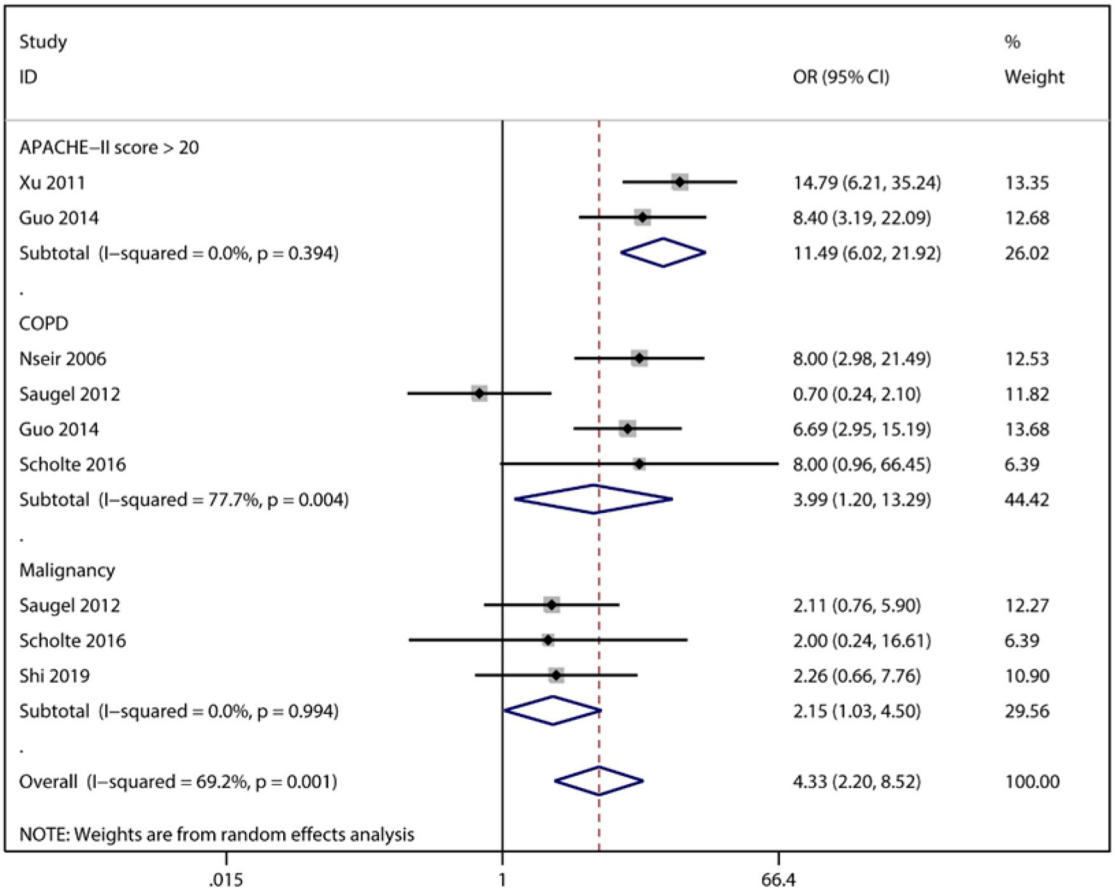

FIGURE 2 | Meta-analysis results of impact of general condition and combined underlying diseases on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

(9–16) were further screened by reading the full text, including five papers in English and three papers in Chinese (**Figure 1**).

Baseline Characteristics of the Studies

The eight included papers included in the study were published between 2002 and 2020, seven of which were case-control studies and one was a cohort study involving 2320 patients, 306 in the *S. maltophilia*-infected group and 2014 in the non-infected group, and 25 exposure factors for *S. maltophilia* infection were extracted. The quality of the eight papers was evaluated using the NOS scale, including five high-quality papers and tree moderate-quality papers. The basic characteristics of the included literature are shown in **Tables 1**, **2**.

Meta-Analysis of Exposure Factors for *S. maltophilia* Pneumonia

Heterogeneity was tested for exposure factors such as age, gender, APACHE-II score, length of ICU stay, Glasgow score, glucocorticoid use, diabetes, malignancy, cardiovascular disease, renal insufficiency, immunodeficiency disorders, tracheal intubation, indwelling catheters, surgery, and use of carbapenems, quinolones, and aminoglycosides. Heterogeneity was acceptable ($p > 0.10$, $I^2 < 50\%$), and effect sizes were combined using a fixed-effects model. heterogeneity was present for APACHE-II scores >20, COPD, tracheotomy, mechanical ventilation, indwelling nasogastric tube, central venous line, use of β-lactamase inhibitors and nitroimidazole antibiotics ($p < 0.10$, $I^2 > 50\%$), random-effects model combinations of effect sizes were performed.

The meta-analysis showed that risk factors for *S. maltophilia* pneumonia in the ICU included APACHE-II score > 20 (OR = 10.98, 95% CI: 5.67 ~ 21.26), COPD (OR = 3.97, 95% CI: 2.39 ~ 6.61), malignant tumor (OR = 2.15, 95% CI: 1.03 ~ 4.50), mechanical ventilation (OR = 8.75, 95% CI: 2.59 ~ 29.58), tracheotomy (OR = 6.12, 95% CI: 2.06 ~ 18.18), endotracheal intubation (OR = 4.25, 95% CI: 2.30 ~ 7.84), β- Lactamase inhibitors (OR = 9.98, 95% CI: 1.51 ~ 65.96), aminoglycosides (OR = 4.01, 95% CI: 2.06 ~ 7.80), carbapenems (OR = 2.82, 95% CI: 1.49 ~ 5.31), and quinolones (OR = 2.17, 95% CI: 1.21 ~ 3.89). There was no obvious correlation between risk factors and *S. maltophilia* pneumonia, such as age, gender, APACHE-II score, length of stay in ICU, combined diabetes, combined cardiovascular disease, combined renal insufficiency, combined immunodeficiency disease, indwelling

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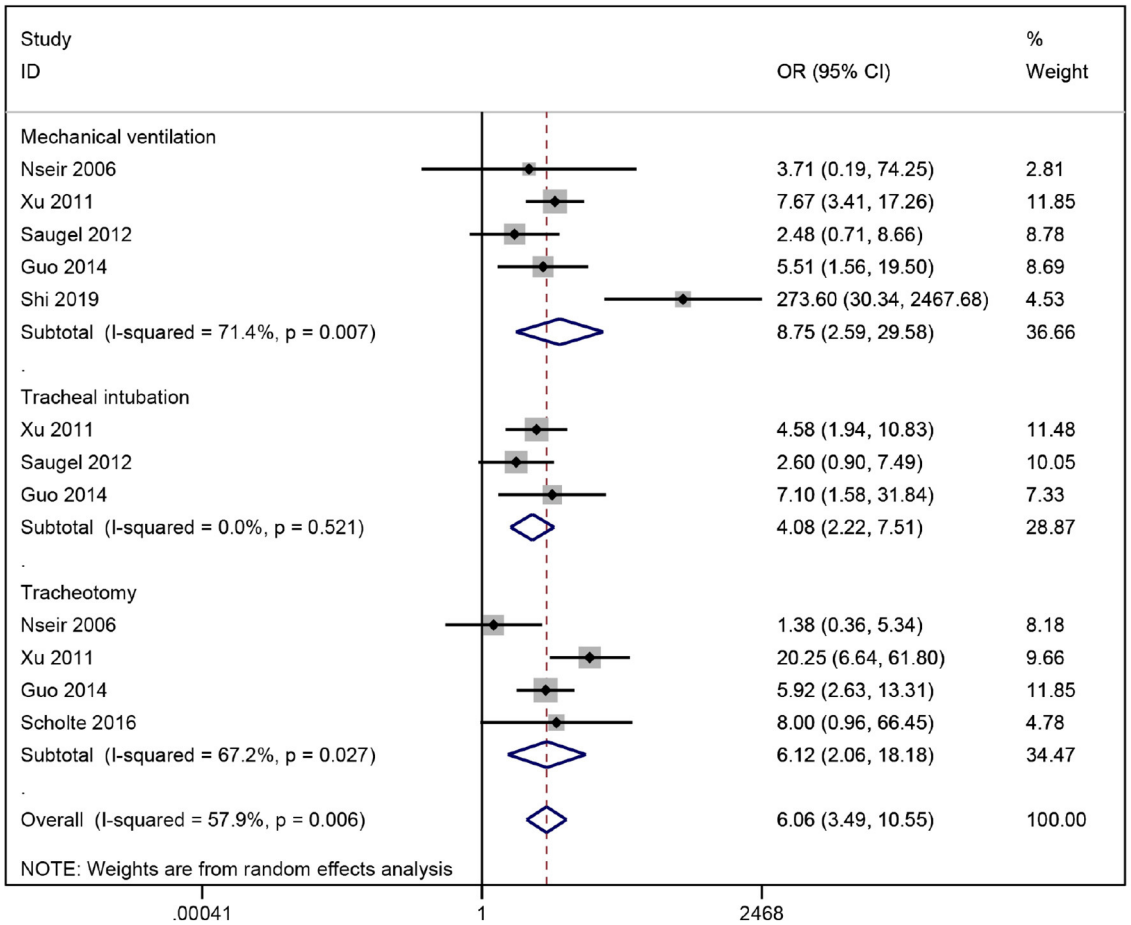

FIGURE 3 | Meta-analysis results of impact of invasive operations on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

catheter, operation, central venous line, indwelling nasogastric tube, corticosteroids, and nitroimidazole antibiotics.

Sensitivity Analysis and Publication Bias

Sensitivity analysis suggested that the results of the meta-analysis were stable for all outcome indicators except for two exposure factors, central venous placement and indwelling nasal cannula (**Table 3**). Begg's test was used to test for publication bias when the number of included papers for individual risk factor analysis was $\geq$3. The results showed $p > 0.05$, indicating that the publication bias of included papers was not significant.

DISCUSSION

Stenotrophomonas maltophilia is widely distributed in natural environments, such as soil, water, and hospital environments, and can also parasitize the human skin, respiratory and digestive tracts. It is a very common conditional pathogen. The results of studies over the past few years have shown that the detection rate of *S. maltophilia pneumonia* is increasing year by year and has become an important pathogen of ICU infections. ICU-acquired infections associated with *S. maltophilia* are an independent risk factor for mortality in the ICU, and therefore knowledge of the risk factors for *S. maltophilia* pneumonia in the ICU and early targeted empirical treatment are key to reducing mortality from *S. maltophilia* pneumonia. In this study, we conducted a meta-analysis to screen the risk factors for *S. maltophilia* pneumonia in the ICU regarding general condition, co-morbid underlying diseases, invasive procedures, and use of antibiotics.

Correlation of the Patient's General Condition, Co-morbid Underlying Diseases and ICU-Acquired *S. maltophilia* Pneumonia

S. maltophilia pneumonia was associated with the patients' underlying disease status, and among the various underlying diseases, patients with COPD had the highest risk of infection (OR = 3.99), followed by malignant tumor (OR = 2.15). In contrast, underlying diseases such as immunodeficiency disorders, diabetes mellitus, and renal insufficiency were not associated with *S. maltophilia* pneumonia in ICU patients

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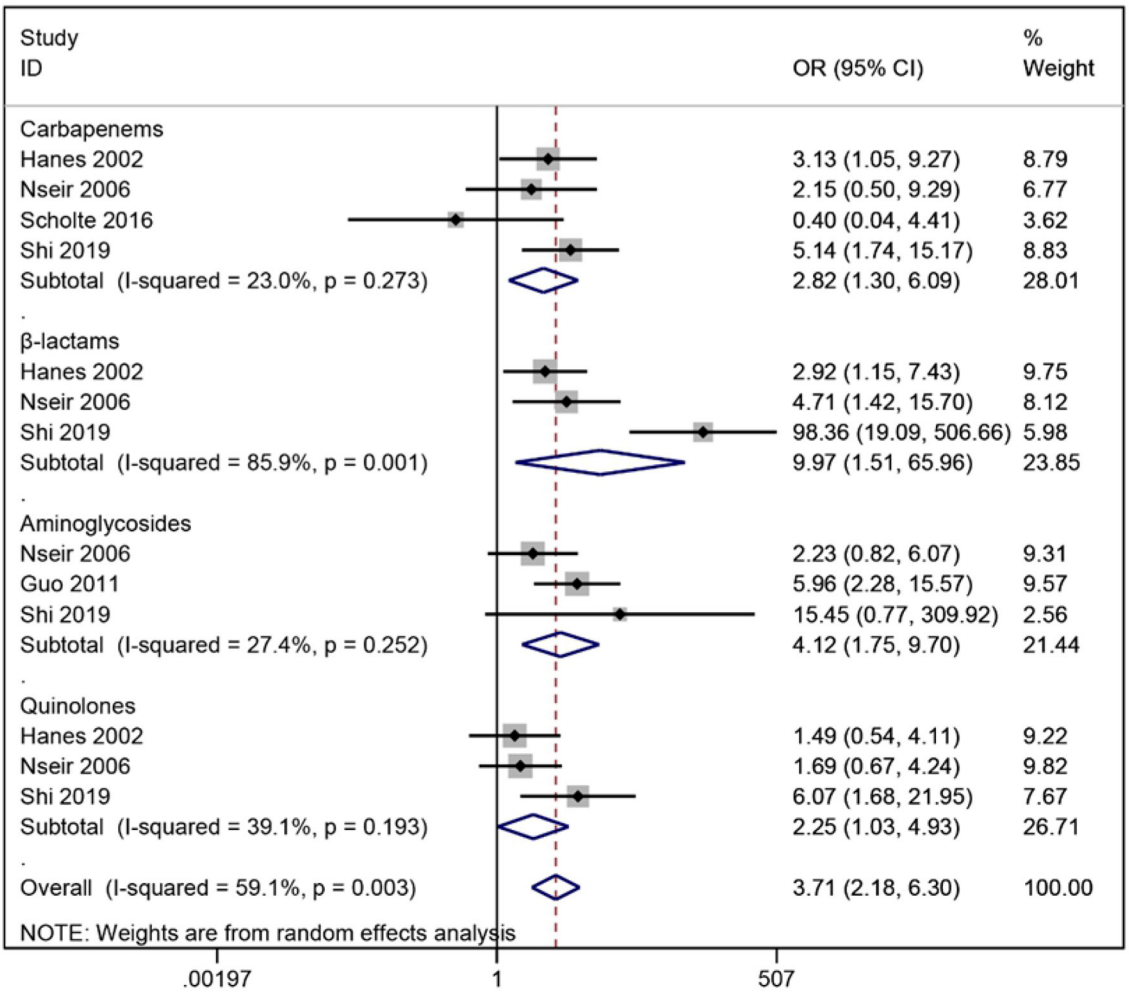

FIGURE 4 | Meta-analysis results of antimicrobial drug impact of on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

(**Figure 2**). Severity of illness is also an important factor in *S. maltophilia* pneumonia, and APACHE-II score is one of the most widely used tools for critical illness assessment (17). Furthermore, by combining the APACHE-II score of the experimental and control groups, we did not find a statistical difference between the two groups, unlike that reported by a few individual studies (9, 15). However, further analysis informed that there was a hierarchical effect between APACHE-II scores and *S. maltophilia* pneumonia, suggesting that *S. maltophilia* pneumonia is closely related to the severity of the disease. In recent years, the trend of glucocorticoid abuse in clinical practice has become more serious, and related studies have reported that long-term high-dose glucocorticoid use is a high-risk factor for multi-drug-resistant bacteria and fungal infections (18–20). However, we did not find that glucocorticoids increased the incidence of *S. maltophilia* pneumonia in the ICU, there may be bias due to different doses of glucocorticoids, course of treatment and patients' treatment response.

Invasive Procedures and ICU-Acquired *S. maltophilia* Pneumonia

Our meta-analysis indicated that among the risk factors involved in invasive maneuvers, mechanical ventilation was strongly associated with ICU-acquired *S. maltophilia* pneumonia (OR = 8.75), followed by tracheotomy (OR = 6.10) and tracheal intubation (OR = 4.25), demonstrating that invasive procedures such as mechanical ventilation, tracheal intubation and tracheotomy are high risk factors for *S. maltophilia* pneumonia in the ICU (**Figure 3**). Invasive procedures can breach the body's basic defense barriers. *S. maltophilia* colonized in the oral pharynx tends to form bacterial biofilms in the lining of indwelling catheters and tends to enrich at oxygen storage sites, increasing the risk of pulmonary *S. maltophilia* infection (21). In addition, the longer the duration of invasive procedures such as mechanical ventilation, the greater the risk of *S. maltophilia* infection. Guo et al. (12) have reported that the duration of invasive ventilator ventilation (>14 d) is an independent risk factor for ICU-acquired *S. maltophilia*

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infection. Therefore, clinical practitioners need to strictly follow the indications for invasive procedures and reduce unnecessary invasive operations, while tracheal intubation should be removed as early as possible when conditions allow to help reduce the risk of *S. maltophilia* infection. In our study, the combination of two exposure factors, central venous cannulation and indwelling nasal cannula, was more heterogeneous and less robust than other invasive procedures, and the combined results should be viewed with caution.

The Association Between Antimicrobial Drug Use and ICU-Acquired Pneumonia With *S. maltophilia*

At present, for the treatment of antibiotic-resistant *S. maltophilia* infection, Chinese experts recommended three combined treatment modes, which are based on compound sulfamethoxazole, combined with ticarcillin clavulanate potassium, cefoperazone sulbactam, fluoroquinolone, minocycline, ceftazidime or polymyxin. Or ceftazidime-based fluoroquinolones, ticarcillin clavulanate potassium or cefoperazone sulbactam regimen; Alternatively, a polymyxin-based regimen combined with ticarcillin clavulanate potassium can be adopted (22). However, the use of antibiotics is a double-edged sword. *S. maltophilia* is naturally resistant to carbapenems, while its AAC(6')-lz acetyltransferase and pumping system make it highly resistant to aminoglycosides, and these characteristics could explain the use of both drugs to passively screen the bacterium for hospital-acquired infections due to the proliferation of dominant bacteria (23). Nseir et al. (10) found that broad-spectrum antibiotics such as fluoroquinolones and cephalosporins can also increase the rate of *S. maltophilia* pneumonia and concluded that broad-spectrum antibiotic use is more significant than carbapenems alone. This study showed that the use of β-lactamase inhibitors had the largest combined OR associated with *S. maltophilia* pneumonia (OR = 7.88), followed by aminoglycosides (OR = 4.01) and carbapenems (OR = 2.82), suggesting that the possibility of *S. maltophilia* pneumonia should be considered when clinical treatment with these three drugs is not effective (**Figure 4**). The long-term heavy use of broad-spectrum antibiotics is an important medical factor for hospital-acquired infections of *S. maltophilia.* Broad-spectrum antibiotics increase the risk of infection by killing other pathogens while screening out dominant species, including *S. maltophilia*. A study by Xu et al. (11) concluded that the use of ≥3 antibiotics for more than 1 week was an independent risk factor for *S. maltophilia* pneumonia in ICU patients, and therefore care should be taken to monitor the risk of infection in ICU patients using multiple antimicrobials simultaneously.

Limitation of this Study

There are a few limitations in this study: (1) Due to the limited number of domestic and international studies on risk factors for *S. maltophilia* pneumonia in ICU and the uneven quality of the literature. Eight papers were screened strictly according to the inclusion and exclusion criteria, including five in English and three in Chinese, which may have a certain degree of publication bias. (2) Due to limited literature, some risk factor indicators in this study were not combined effectively, which may affect the study results. (3) At present, the research on related risk factors in China is not deep enough, and there is a lack of relevant prospective cohort studies. Therefore, more rigorous design, large samples, and multicenter studies are needed to clarify the risk factors for *S. maltophilia* pneumonia in the ICU.

CONCLUSION

In recent years, the disease burden of hospital-acquired *S. maltophilia* pneumonia in ICU patients has been high, and resistance to the organism is increasing. *S. maltophilia* pneumonia occurs in patients with severe disease, comorbid COPD, malignancy, high APACHE-II scores, undergoing invasive procedures, and in ICU patients on broad-spectrum antibiotics due to a combination of host and medical factors. From the host side, these patients are characterized by impaired immune function, severe disease, and the need for prolonged hospitalization, which objectively contributes to the infection of conditional pathogens such as *S. maltophilia* (24). Therefore, strengthening the monitoring, prevention, and control of patients with risk factors of *S. maltophilia* infection is beneficial to reduce the risk of infection and death in ICU patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

NW and CT: screening, data extracting, and writing. NW, CT, and LW: analysis. NW and LW: manuscript revision. LW: manuscript finalization. All authors contributed to the article and approved the submitted version.

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Review

Stenotrophomonas maltophilia: The Landscape in Critically Ill Patients and Optimising Management Approaches

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Abstract: The aim of this review is to synthesise the key aspects of the epidemiology, current microbiological diagnostic challenges, antibiotic resistance rates, optimal antimicrobial management, and most effective prevention strategies for *Stenotrophomonas maltophilia* (SM) in the intensive care unit (ICU) population. In recent years, resistance surveillance data indicate that SM accounts for less than 3% of all healthcare-associated infection strains, a percentage that doubles in the case of ventilator-associated pneumonia (VAP). Interestingly, SM ranks as the third most isolated non-glucose fermenter Gram-negative bacilli (NFGNB). Although this NFGNB genus has usually been considered a bystander and colonising strain, recently published data warn about its potential role as a causative pathogen of severe infections, particularly pneumonia and bloodstream infections (BSI), not only for the classical immunocompromised susceptible host patients but also for critically ill ones even without overt immunosuppression. Indeed, it has been associated with crude 28-day mortality as high as 54.8%, despite initial response following targeted therapy. Additionally, alongside its intrinsic resistance to a wide range of common antimicrobials, various worldwide and local surveillance studies raise concerns about an increase in ICU settings regarding resistance to first-line drugs such as cotrimoxazole or tigecycline. This scenario alerts ICU physicians to the need to reconsider the best stewardship approach when SM is isolated in obtained samples from critically ill patients. Despite the coverage of this multidrug-resistant bacterium (MDRB) provided by some traditional and a non-negligible number of current pipeline antimicrobials, an ecological and cost-effective strategy is needed in the present era.

Keywords: ICU; critically ill; sepsis; antimicrobial; multidrug-resistant bacteria; sepsis; antimicrobial stewardship; *Stenotrophomonas maltophilia*

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1. Introduction

The genus *Stenotrophomonas* comprises at least eight species, of which *Stenotrophomonas maltophilia* (SM) is the most predominant and the only one known to cause human disease [1]. SM is an environmental non-glucose fermenter Gram-negative bacillus (NFGNB) isolated from aqueous-associated sources both inside and outside the hospital setting [2]. It is ubiquitous and often encountered as a coloniser of plants and medical devices owing to its site adhesion and biofilm formation abilities [3]. Not typically found in the common gut flora, it can colonise the oral cavity and the respiratory tract epithelial cells of cystic fibrosis (CF) and hospitalised patients, causing chronic infection of the airways in this population that contributes to inflammation, lung damage, and premature mortality.

Interestingly, although understudied, SM ranks as the third most isolated unusual NFGNB (after *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) in various reports [4,5].

Most SM isolates are hospital-acquired, with a median delay of 8–14 days of hospital stay, but community-acquired isolates have also been described [4,6].

To date, distinguishing between SM colonisation and true infection represents a significant challenge, especially when more than one pathogen is isolated. This yet unresolved issue needs to be elucidated because although other host and severity clinical factors enable physicians to tailor management decision-making, finding a balance between antimicrobial overuse and the risk of worse prognosis due to absent or delayed treatment remains complicated [7]. SM infection rates have been confirmed to range from 13 to 60% according to definitions for surveillance of healthcare-associated infection established by the National Healthcare Safety Network managed by the Centers for Disease Control and Prevention (CDC/NHSN) [8,9]. Moreover, in a prospective multicentre cohort (33% ICU patients), Fihman V et al. observed that SM caused infection more often than colonisation [RR 1.74 (95% CI 1.15–2.63), p = 0.01] [4]. In a previous review of 13 cohort studies, a considerable mortality rate (up to 37.5%) was attributed to SM infection. These authors warned clinicians against underestimating its clinical significance, even redefining the term "SM colonisation" [10].

As an unusual NFGNB, SM has often been considered less pathogenic than many other nosocomial organisms. However, this is without considering immunocompromised patients or those with chronic respiratory diseases such as CF, who represent a debilitated population at high risk of suffering from severe and mainly nosocomial infections from this opportunistic pathogen [4,11].

Furthermore, SM infections are notable in the ICU population. All patients admitted to ICU are at high risk of infection by MDRB, even without a known immunosuppressed status, and when infected, need timely appropriate treatment due to an increased risk for mortality. In an earlier, classic study in 1995, Laing FPY et al. found that ICU patients were more frequently infected than those in non-ICU settings [12]. Although ICU-acquired colonisation and/or infection related to SM was considered uncommon among immunocompetent ICU patients (two patients per 1000 ICU days) [6], it was nonetheless recognised as a potentially worrisome emerging ICU pathogen; indeed, an ICU-acquired infection related to SM was independently associated with ICU mortality in this study. A recently published study confirms this ominous SM infection prognosis. It has been associated with crude 28-day mortality as high as 54.8% despite initial response after targeted therapy, especially if respiratory-focused [13].

Several studies have investigated the risk factors for SM infection in ICU patients, some of which are related to illness severity and underlying comorbidities such as APACHE-II score > 20, older age, and chronic obstructive pulmonary disease (COPD). Other risk factors that are concerned with invasive procedures at ICU admission include indwelling intravascular catheter devices, mechanical ventilation, tracheotomy, and tracheal intubation. Finally, SM isolation within 30 days, hospitalisation within the last three months, and prolonged antibiotic treatment (specially carbapenems [CP], but also β-lactamase inhibitors, antipseudomonal cephalosporins and aminoglycosides) have been associated with SM infections; COPD and CP use have been demonstrated as independently predictive factors [4,6,7,11,14–16].

Defining the clinical risk factors for SM infection is, therefore, a challenge, but one that would be highly beneficial for adequate antimicrobial treatment in the current MDRB era. Initial CP regimens covering extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) bacteria causing community and healthcare infections could lead to inappropriate SM treatment due to its intrinsic CP-resistant profile. Indeed, an association between inappropriate initial antimicrobial treatment and mortality has previously been reported in SM-infected, either critically ill or non-critical cases [4,6,10].

This current situation is a challenge for doctors, who need to raise the index of suspicion. Moreover, knowledge about local epidemiology is essential to become familiar with antibiotic resistance rates and accordingly plan an optimal antimicrobial stewardship strategy. Finally, updated infection control measures such as those included in the Spanish

"Zero Resistance" program [17] should accompany these bundles to improve the prognosis of SM infections in ICU patients.

This review aims to highlight key issues over the last twenty years to help provide clinical guidance for when SM is isolated in critically ill patients.

2. Relevant Sections

2.1. Commensal vs. Pathogenic Status of SM: Relevant Aspects

2.1.1. Why Should We Consider Highlighting the Importance of Colonisation by SM?

While microbial culture isolation of a putative pathogen in the laboratory is often associated with infectious syndromes such as BSIs and other sterile body site infections, the correlation is not well defined for NFGNB when the cultured specimen is from body sites with a high colonisation/contamination potential, especially when more than one pathogen is isolated [7].

In contrast, colonisation by an MDRB has been associated with a significantly higher frequency of subsequent infection with the same MDRB and increased mortality in the ICU population. A meta-analysis including a total of 13 studies (with 15,045 ICU patients) found the risk for subsequent ESBL-PE infection to be almost 50-fold higher in colonised than non-colonised patients. These authors concluded that ESBL-PE colonisation of the digestive tract could be a useful marker to predict ESBL-PE infection [18]. In a single-centre retrospective observational study including 3463 surveillance cultures from 250 haematological patients (11 from ICU), Torres I and colleagues documented MDRB colonisation in 33.7% of admissions, most frequently by NFGNB (55.7%). Colonisation by any MDRB was independently associated with an increased risk of MDRB-BSI (HR, 3.70; 95% CI, 1.38–9.90; $p = 0.009$) [19].

SM is rarely present in the oropharynx microbiome of healthy patients but can often be recovered from the oropharynx of hospitalised and CF patients. In the specific case of SM, infection was predicted (96% sensitivity and 93% specificity) by a relative abundance of 36% of oral *Stenotrophomonas*, as assessed via 16SrRNA gene quantification in a cohort of 90 acute myeloid leukaemia (AML) patients receiving remission induction chemotherapy [14]. An SM infection incidence of 9% was reported in this immunosuppressed non-ICU population. In a previous similar study, which analysed SM colonisation during allogeneic hematopoietic stem cell transplantation, the authors observed that SM colonised patients had a worse prognosis, with a poorer overall survival from 6 to 60 months due to higher non-relapsed mortality after allo-transplant (6 months: 15% vs. 4.8% and 60 months: 40.1% vs. 16.2%, $p = 0.003$) [20]. SM colonised in the oral pharynx tends to form bacterial biofilms in the lining of indwelling catheters, increasing the risk of pulmonary SM infection. The results obtained from immunosuppressed non-ICU patients are quite different from those described with *Enterobacterales*, non-SM NFGNB, and *Enterococcus* spp., which usually involve the importance of negative predictive value for suspected infection, frequently BSI caused by gastrointestinal translocation due to rectal colonisation [18,19].

Several classic prospective case-control studies in the ICU population contain significant findings and suggestions focused on the patient risk profile and outcomes of SM infection. Regarding the former, four studies stand out. First, research performed by the Grupo Andaluz para el Estudio de Enfermedades Infecciosas (GAEI) in six tertiary hospitals (with one-third of patients in ICU) revealed a pooled incidence of SM colonisation or infection of 5.7 cases per 10,000 admissions. About half were considered infected, with pneumonia as the most frequent type of infection in ICU patients (two-thirds VAP). The results of qualitative and quantitative analysis of variables independently associated with an increased risk of acquiring SM pointed to SM as a potential cause of nosocomial infections in patients who had previously received a prolonged course of a carbapenem, ceftazidime, or quinolone, particularly if they were under mechanical ventilation [9]. Second, a more recently published meta-analysis including 2320 ICU patients, of which 306 (13%) were SM-infected, showed a particular risk profile with highly suspected SM origin in ICU-acquired pneumonia, specifically including the above-mentioned criteria. These authors

drew attention to a subset of critically ill patients with underlying comorbidities (COPD, malignancy, and high APACHE-II score) who had undergone invasive procedures related to artificial airways and were on broad-spectrum antibiotics due to a combination of host and medical factors [15]. Third, during the pandemic period, a systematic Japanese review of COVID-19-associated SM infection (most patients requiring ICU admission) reported these patients as having similar risk factors as in the above-mentioned studies of patients without COVID-19 [21]. Finally, revisiting the risk factors for the second most common SM infection, bacteraemia, a retrospective study comparing 54 SM with 167 *P. aeruginosa* and 69 *Acinetobacter* species bacteraemic patients showed SM isolation within 30 days prior to infection onset as an independent risk factor for SM bacteraemia, with earlier SM isolation in 66.7%. Nearly half of these patients were considered to have secondary bacteraemia. The thirty-day all-cause mortality of 33.3% among the SM group was significantly higher than that of the other NFGNB groups, and catheter-related infections were associated with survival [16].

Likewise, the outcomes of SM infections acquired during ICU stay have prompted clinical physicians to consider antimicrobial treatment. In a prospective observational case-control study among immunocompetent cases with ICU-acquired infection related to SM (2% of overall incidence), 80% of patients had prior colonisation related to this microorganism [6]. The study reported a significantly higher mortality rate in cases with ICU-acquired SM infection than those with ICU-acquired SM colonisation (70% vs. 25%, OR = 2.5 [1.2 to 4.9], $p = 0.029$). Furthermore, ICU-acquired SM infection was independently associated with ICU mortality. Despite evidence suggesting that clinicians should consider the importance of the predictive value of proven colonisation by SM in critically ill patients, there is no evidence supporting the benefit of antimicrobial use; instead, it likely incurs an ecological cost in this scenario. Therefore, close monitoring of these colonised patients should be considered a daily best practice.

2.1.2. Why and When Should We Consider SM as a Real-Life Pathogen in the ICU? The Importance of VAP Cases in the Critically Ill

SM can cause serious infections, including bacteremia, tracheobronchitis, pneumonia, meningitis, endocarditis, endophthalmitis, skin and soft tissue, urinary tract, and oral cavity infections. Although this bacterium is normally nosocomial, community-onset infections have also been reported [3]. Worldwide, the most common clinically observed specimen is blood, with a frequency of 36.84%, followed by the eye and respiratory tract (19.29% and 15.78%, respectively) [11]. In the classic multicentre case-control study from the GAEI group, the authors underline a difference between ICU and cancer patients, with the most frequent infections being respiratory (especially VAP) in the former and primary or catheter-related bacteriemia in the latter [9].

From a global perspective, according to CHINET bacterial resistance surveillance data, in 2020, SM accounted for 2.98% of all strains and ranked third among NFGNB [5]. In the same way, in the recently published European surveillance, SM accounted for 0.8% of all healthcare-associated infections, rising to 1.9% in cases of pneumonia/lower respiratory infections [22]. Likewise, it is noteworthy that SM is usually linked to pulmonary infections in critically ill patients. In fact, in a 2021 CDC/NHSN updated national summary of pathogens distributions, SM appeared among the most frequently possible VAP pathogens not usually reported from other device-associated infections, accounting for 4% in hospital ICUs and ranking ninth for this type of healthcare infection. Similarly, in the Spanish national surveillance programme of ICU-acquired infection (ENVIN-HELICS registry) in 2023, SM represented 2.68% of isolated microorganisms among the main ICU-acquired infections. SM was especially important in the case of VAP (5.88%), ranking fourth after *P. aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [23].

Besides the above-mentioned risk factors for SM infections in critically ill patients, some evidence points to the length of ICU stay as associated with acquiring SM during hospitalisation, suggesting that empiric treatment should be considered for this microorganism

in this setting. The GAEI group described a median duration of 17 days (range 0–180) before SM isolation [9]. A subsequent study reported a mean time of 14 ± 11 days between ICU admission and the first ICU-acquired SM [6]. The duration of invasive procedures (such as mechanical ventilation >14 days) also seems important, having been identified as an independent risk factor for ICU-acquired SM infection in a neurological ICU [24].

In a recently published retrospective bi-centre study including 103 ICU patients with SM infection, pneumonia (hospital-acquired pneumonia [HAP] and VAP) was the predominant clinical syndrome (72.8%), while 22% of cases were in haemato-oncology patients. In multivariable analysis, only increasing age and haemato-oncologic disease were shown to be independent risk factors for 28-day mortality SM infection, which was as high as 54.8% despite initial clinical and laboratory response after targeted therapy [13].

The possibility of developing fatal haemorrhagic pneumonia caused by SM has also been observed in the population with hematologic malignancies with SM bacteraemia. It is a rare presentation, with only 91 reported cases worldwide from 1990 to 2023, but with a very high associated 30-day mortality of 90.1%, even after prompt antibiotic treatment [25–27]. Due to its severity and rapid progression, after analysing the few survival cases, some authors emphasise the importance of early suspicion and suggest combination therapy, including TMP-SMX, for this clinical picture in this specific population, although no clinical studies are available [25,26]. The exact mechanism by which SM causes haemorrhage is unknown. Windhorst et al. propose pathogenic factors such as StmPr1, a protease with broad specificity secreted by SM, leading to tissue invasion, destruction, and haemorrhage [28]. Moreover, neutropenia (<500/mm^3) and thrombocytopenia (<50,000/mm^3) have been identified as risk factors for haemorrhagic pneumonia, which could partly explain the bleeding progression in patients with haematologic malignancies with SM bacteraemia [25].

2.2. Antibiotic Resistance Traits and Microbiological Diagnostic Challenges: Clinical Impact on the ICU Population

2.2.1. Basis of SM Antibiotic Resistance

Numerous intrinsic and acquired resistance traits make SM-caused infections notoriously difficult to treat. This bacterium is intrinsically resistant to a wide range of antibiotics, including most β-lactams, fluoroquinolones, tetracycline derivatives (except for doxycycline, minocycline, or tigecycline), chloramphenicol, all aminoglycosides (including kanamycin, tobramycin, amikacin, and neomycin) and trimethoprim [29]. SM employs a wide spectrum of antibiotic resistance mechanisms, which include overexpression/mutation of multidrug resistance efflux pumps, reduced membrane permeability, the chromosomally encoded Sm*qnr* gene (that protects both gyrase and topoisomerase IV from quinolones), and the production of beta-lactamase, carbapenemase, and aminoglycoside-modifying enzymes [11,29]. It is noteworthy that the high carbapenem resistance of SM is primarily caused by the expression of two chromosomally encoded extracellular ß-lactamases, bla_{L1} (a class B metallo-β-lactamase which is not inactivated by aztreonam) and bla_{L2} (a class A clavulanic acid susceptible cephalosporinase, which is susceptible to inhibition by serine-β-lactamase inhibitors such as clavulanic acid and avibactam), which would explain the clinical benefit of combined ceftazidime–avibactam if administered in combination with aztreonam [30].

Among its acquired resistance mechanisms, SM can develop cefiderocol resistance through different genetic pathways, such as mutations in the *tonB* gene or in the *smeT* promoter [29]. An increasing presence has also been noted of *sul*1, *sul*2, and *dfrA* genes that have been found both chromosomally and in plasmids, which contribute to SM resistance to trimethoprim-sulfamethoxazole (TMP/SMX). These genes are often associated with Class 1 integrons, which favour their mobility and even carry multiple resistance genes, which are conducive to multi-resistance.

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2.2.2. Considerations for Empirical Antimicrobial Decision-Making

This nosocomial pathogen shows low susceptibility to many antibiotics commonly used to empirically treat hospital-acquired infections. In contrast, antimicrobials with a priori in vitro susceptibility, such as TMP/SMX, quinolones, and tetracyclines derivatives (minocycline and tigecycline), are not covered in usual clinical practice in the ICU setting. Gram-negative bacteria are the organisms most commonly responsible for serious infections in the ICU; however, dual empirical coverage usually includes *P. aeruginosa* and *Enterobacteriaceae* as the organisms most frequently involved, whereas SM is responsible for only a minority of cases. SM is, therefore, not typically covered and is likely to be subject to initial inappropriate treatment (ITT). Quinolone is sometimes suggested as an option in empiric combination therapy, such as in the PANNUCI algorithm for nosocomial pneumonia management in the ICU, in which cases SM may be covered [31]. However, quinolones are not always recommended for severe nosocomial infections with sepsis and septic shock, especially given that the resistance rate to ciprofloxacin and levofloxacin in *P. aeruginosa* currently exceeds 30% in most Spanish hospitals [32]. In a large retrospective analysis of ICU patients with bacteraemia and severe sepsis/septic shock caused by a Gram-negative organism, IIT was a key determinant of short-term mortality with a three-fold increase, and MDR was strongly associated with IIT [33]. Notably, SM was specifically identified as a predictor of IIT in this study population.

SM has been associated with infections after various prolonged antimicrobial treatments, especially with carbapenems, which probably would be selected for SM because of its intrinsic resistance to most of them [9,11]. As well as its prior use perse, cumulative exposure to meropenem has been associated with increased infection risk (hazard ratio, 1.17; 95% CI, 1.01–1.35; $p = 0.03$) in non-critically ill patients, resulting in a 17% increased risk of SM infection with each additional day of meropenem use [14]. Furthermore, a study by Xu et al. concluded that the use of three antibiotics for more than one week was an independent risk factor for SM pneumonia in ICU patients [34]. It seems that not only the type but also the time of exposure and the number of antimicrobials can impact SM infection development.

Interestingly, SM has been identified as the most frequent carbapenem-resistant (CR) Gram-negative species in hospitalised patients with HAP and VAP [35]. In this retrospective cohort study, which included 8969 patients, a total of 11.8% of isolates were CR, with nearly 60% of this requiring ICU admission. *P. aeruginosa* was the most common Gram-negative pathogen overall (21.1%) but was second to SM (16.8 vs. 38.7%) among CR species. In terms of clinical impact, it is noteworthy that CR was associated with an excess of 3.0 days (95% CI, 1.4–4.6) in overall length of stay and an excess of $8921 (95% CI, 3864–13,977) in hospital costs [35].

SM production of inducible carbapenemase, which hydrolyses and detoxifies antibiotics, is also involved in polymicrobial infections, especially among SM and *P. aeruginosa*, although the pathogenic role of SM is difficult to ascertain in this scenario. There is limited understanding of the way antimicrobial resistance evolves in the presence of non-targeted microbial species and how ecological interactions likely alter the effectiveness of antibiotic treatment. CF represents the paradigm of this co-infection, which affects up to a quarter of patients and is often associated with increased mortality [36]. A 24-day-long in vitro experimental study with two-species model CF communities highlighted some aspects related to bacterial eco-evolutionary dynamics in the absence of and under clinically relevant β-lactam imipenem concentrations. Firstly, they found that a clinical SM strain protected sensitive *P. aeruginosa* from imipenem. Secondly, the presence of SM led to an equal or increased rate of imipenem resistance development via parallel loss of function mutations in the OprD porin gene. Finally, and unexpectedly, they observed that resistant *P. aeruginosa* drove SM into extinction due to increased production of pyocyanin in the presence of imipenem, which was cytotoxic to SM [37]. Interestingly, there is some clinical evidence illustrating the way interactions between members can affect the prognosis of this specific polymicrobial infection. SM and *P. aeruginosa* can produce biofilm in the lungs,

creating a thriving environment for both. In a Chinese retrospective analysis including severe pneumonia patients, a higher mortality rate and longer hospital stay were reported for those co-infected with *P. aeruginosa* and SM compared with those infected with only one or not infected [38].

2.2.3. Related Microbiological Diagnostic Challenges

Caution should be applied when considering in vitro results of SM susceptibility based on antimicrobial susceptibility testing (AST) guidelines (the Clinical and Laboratory Standards Institute [CLSI], the European Committee on Antimicrobial Susceptibility Testing [EUCAST], and Food and Drug Administration [FDA]), which are not standardised. CLSI has established minimum inhibitory concentration (MIC) interpretation criteria for seven antibiotics, including TMP-SMX, ticarcillin–clavulanate, ceftazidime, cefiderocol, levofloxacin, minocycline, and chloramphenicol. In contrast, the EUCAST has defined MIC and disc breakpoints only for TMP-SMX (an antimicrobial with nearly identical breakpoints for EUCAST (>4 mg/L) and CLSI ($\geq$4 mg/L)). The FDA only recognises ceftazidime breakpoints for SM. In real life, only five agents with interpretable antibiotic MIC data are available to clinicians for clinically relevant application in AST (TMP-SMX, ceftazidime, cefiderocol, levofloxacin and minocycline) [29,39]. As a result, differences among SM resistance rates to these antimicrobials become apparent among prevalence studies, and most importantly, the lack of adequate information available may have a considerable impact on patient treatment.

Accordingly, a recent report including 223 studies in which CLSI recommendations were used evaluated the prevalence of antibiotic resistance in clinical isolates of SM worldwide. The authors observed that the most frequent antibiotic resistance worldwide was against levofloxacin (14.4%) among prevalence studies, with an increased resistance especially in China, and against TMP/SMX (36.84%) among case reports/case series studies, with the highest resistance rate reported in Asia [11]. A more recent worldwide meta-analysis showed a low global prevalence of resistance to TMP-SMX (14.7%), suggesting that this antimicrobial remained effective as a first-line treatment for SM. Other findings were a relatively low resistance rate to levofloxacin (16%), a significant increase in resistance to tigecycline, and the lowest resistance rates documented for minocycline (3%) and cefiderocol (4%) [39]. Regarding the ICU population, the above-mentioned ENVIN-HELICS registry showed a trend towards increased SM resistance to TMP/SMX during the last two years (2022–2023) compared with the pre-pandemic period (2018) (6.2% and 13.5% vs. 0%, respectively), similar to tigecycline (60% and 80% vs. 0%), in contrast to levofloxacin that has remained the same or even decreased (13.65 and 13.3% vs. 15.38%, respectively). Moreover, this report showed an SM resistance of 53.8% to ceftazidime/avibactam and 50% to cefiderocol among 13 and 4 out of 113 tested strains, respectively, within 2022 and 2023 in the participating Spanish ICUs [23].

Despite the MDR profile of SM, clinicians should be aware of the epidemiologic and previous colonisation situation in their local institutions to ensure appropriate treatment. Familiarity with microbiological tests, from the simple Gram stain microscopy to new methods for rapid SM identification (such as Matrix-assisted laser desorption ionisation–time of flight [MALDI-TOF] mass spectrometry), as well as a deeper understanding of the value of syndromic panels to detect causative pathogens by molecular methods, are also essential to avoid ITT in the ICU setting (see Table 1). Regarding molecular detection, there are currently few methods that allow the detection of SM directly from biological samples. For the diagnosis of bacterial infections in the lower respiratory tract, the only platform that allows this detection from a direct sample is Unyvero. Furthermore, a recent randomised control trial involving patients with pneumonia in the ICU observed that the growth of 'off-panel' pathogens was unusual and most commonly due to *Citrobacter* species and SM. The lack of detection of these organisms by the pneumonia panel did not adversely affect patients' antimicrobial treatment in this trial [40]. Accordingly, a stewardship strategy for guiding an adjustment of empirical antimicrobial therapy based on

the results of rapid microbiological testing like Biofire® Filmarray® Pneumonia Panel plus (BioFire Diagnostics, LLC, Salt Lake City, UT, USA) seems imperative in the management of critically ill patients [41]. Likewise, next-generation metagenomic sequencing (mNGS) has emerged as a promising tool in the field of medicine. Although it shows promising diagnostic performance for severe pneumonia in critically ill patients, facilitating precise diagnoses and treatments, the clinical value of this technology remains controversial due to challenges such as the lack of common standards and guidelines. Continued research and the development of standardized protocols are necessary to maximise its potential and ensure its clinical applicability [42].

Table 1. New microbiological diagnostic methods for rapid SM identification.

Diagnostic Test	Pathogen/ Resistance	Sample Type	Time Required to Detection (Hours)	Detection Limit	Sensitivity (%)	Specificity (%)	Robustness	Reference
Unyvero A50 System HPN (Curetis, Holzgerlingen, Germany)	21 Gram-negative and Gram-positive bacteria/17 antibiotic-resistant markers	Sputum, bronchoalveolar lavage, tracheal and bronchial secretions	<5 h	NR	84	98	NR	Sun et al., 2021 [43]
Unyvero A50 System BCU (Curetis)	34 Pathogens, 16 antibiotic resistance markers	Positive blood culture	<5 h	NR	96.8	99.8	NR	Burrack-Lange et al., 2018 [44]
Biofire®Filmarray® BCID2 (bioMeriéux, Craponne, France)	33 Gram-negative, Gram-positive bacteria, Candida spp., and 10 antibiotic-resistant markers	Positive blood culture	1 h	NR	>80 for SM	99.8	NR	Peri et al., 2022 [45]
Sepsis Flow Chip (VITRO Master Diagnóstica, Granada, Spain)	17 Gram-negative and Gram-positive bacteria, 1 yeast, and 19 antibiotic-resistant markers	Positive blood culture	3 h	NR	100 for SM	100	NR	Galiana et al., 2017 [46]
hemoFISH (Miacom Diagnostics, Düsseldorf, Germany)	19 Gram-negative and Gram-positive bacteria	Positive blood culture	>5 h	NR	<80–90 for SM	>95	NR	Reitz et al., 2018 [47]
Magicplex Sepsis Real-Time (Seegene, Seoul, Republic of Korea)	73 Gram-positive, 12 Gram-negative, 6 fungal, and 3 antibiotic-resistant markers	Whole blood	3–6 h	NR	65	92	NR	Carrara et al., 2013 [48]
MALDI Biotyper System (Bruker, Billerica, MA, USA)	Bacterial and fungal species or species groups	A single colony from isolate positive culture or positive whole blood cultures	minutes	-	73 for SM	99	NR	Gautam et al., 2017 [49]
LiDia-SEQ NGS (DNA Electronics Ltd., Carlsbad, CA, USA)	Bacterial and fungal pathogens	Whole blood	>4 h	1 CFU/mL	NR	NR	NR	Not found

Note: SM: *Stenothophomonas maltophilia*; NR: not reported; CFU: colony forming unit.

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2.3. Best Evidence-Based Approach to SM Severe Infections. Antimicrobial Stewardship in the Critically Ill

As mentioned above, SM infection management can be hampered either by high-level intrinsic resistance to many antibiotic classes and the increasing occurrence of acquired resistance to the first-line drug cotrimoxazole (TMP/SMX) [3] or by a lack of coverage by the most used empirical antimicrobial strategies for ICU infections, which in turn can cast a shadow over its prognosis.

Different treatment guidelines for infections caused by multidrug-resistant Gram-negative microorganisms have recently been published, including recommendations on SM treatment in guidelines by the Infectious Diseases Society of America (IDSA) and the Society of Infectious Diseases and Clinical Microbiology (SEIMC) [30,50,51]. Published data on the optimal treatment for SM infections are limited. Despite the lack of randomised controlled trials comparing TMP/SMX with any other available treatment for SM infections, it is still currently considered the drug of choice based on high in vitro susceptibility rates [52].

For severe infection, the Spanish Society recommends considering combination therapy in immunocompromised patients (strength of recommendation B and quality of evidence II), preferably including TMX/SMX [50]. Likewise, IDSA recommendations from 2022 were recently updated in the 2023 guidelines on the treatment of antimicrobial-resistant Gram-negative infections [53]. The authors directly suggested two approaches: (1) combined use of two of the following agents: TMP-SMX, minocycline/tigecycline, cefiderocol, or levofloxacin, at least until clinical improvement is observed, primarily because of limited and conflicting in vitro and clinical data (limited to observational studies) supporting any individual agent and the need to increase the likelihood of appropriate treatment. (2) The combination of ceftazidime–avibactam plus aztreonam in situations of significant clinical instability following clinical failure with or intolerance to other agents.

It is noteworthy that the authors recommend a second approach, including ceftazidime–avibactam and aztreonam, in situations of significant clinical instability. This has been considered a reasonable treatment option for severe infections despite limited available clinical data and few case reports, based on the above-mentioned explanation that this combination can overcome the activity of both β-lactamases intrinsic to SM. The IDSA panel expressly advised avoiding ceftazidime as a treatment option because of the presence of β-lactamase genes intrinsic to SM that are expected to render this antimicrobial inactive. The panel also changed the aztreonam regimen in terms of suggested antibiotics dosing from the previous publication [30,53]. Regarding the ICU population, in an Italian case series of 26 COVID-19 ICU patients with VAP due to difficult-to-treat NFGNB, with SM isolated in six of these patients (mostly as part of polymicrobial infection), ceftazidime–avibactam was employed in different regimen combinations. The authors suggest that SM appears as a true pathogen in this patient population, promoting the development of haemorrhagic pneumonia or bacteraemia. A surprising finding was a 30-day overall mortality of 60.8% vs. 16% in an SM-specific subgroup [54].

The 2023 American guidelines proposed TMX-SMX and minocycline as the preferred components for combination. Minocycline has slightly more favourable in vitro data, availability of CLSI breakpoints, oral formulation, and likely improved tolerability compared with tigecycline. Moreover, minocycline has been associated with lower adverse effects and mortality than TMP/SMX in a retrospective study dealing with severe patients based on the APACHE-II score [55]. However, tigecycline is also a treatment option for SM infections [30], with extensive penetration into lung tissue at high doses, as demonstrated in critically ill patients [56]. In several series, moreover, the reported MICs for eravacycline are two- to four-fold lower than for tigecycline and frequently mirror minocycline for SM.

Fluoroquinolones are commonly used as an alternative for cases with TMP/SMX-resistant SM or with intolerance due to adverse side effects, especially in HIV-infected patients. Several retrospective observational studies comparing monotherapy with fluoroquinolones and TMP/SMX (with a quarter of patients in the ICU at the time of culture) have suggested that levofloxacin had similar efficacy [57] or even lower OR of death and length

of hospital stay than TMP/SMX in patients with lower respiratory tract infections [50,58]. However, because of suboptimal results with fluoroquinolone monotherapy in in vitro studies, known mechanisms of resistance of SM to fluoroquinolones, the emergence of resistance during therapy, and inherent biases in the observational data, the IDSA panel suggests levofloxacin be used only as a component of combination therapy when prescribed for treatment of SM infections [53]. It is noteworthy that treatments (e.g., quinolones) that select mutants leading to multidrug efflux pump overexpression, such as SmeDEF, can reduce SM susceptibility to several antimicrobials simultaneously. This would explain co-resistance to quinolone and cotrimoxazole. However, it does not appear to affect susceptibility to either minocycline or tigecycline, which could thus be candidate antimicrobials in this context [29,52,59]. This could also be the rationale behind the use of antimicrobial combinations in severe infections.

Regarding second-line agents against SM infections, SEIMC experts have recommended their use for their in vitro activity against the isolate (B-II) [50]. In particular, the IDSA proposes cefiderocol because, despite the limited availability of clinical data, in vitro data and animal models are encouraging for its use in treating SM infections, initially in combination with a second active agent [51,53,60]. Clinically, a Spanish retrospective observational study [61] and a French case series [62] assessing the effect of cefiderocol treatment for severe infections due to difficult-to-treat resistant Gram-negative bacilli in critically ill patients reported a 30-day overall mortality rate of nearly 30%. Among the few patients infected with SM (5 and 4, respectively), mortality increased to 80%, although most were immunosuppressed, and related mortality was not specified. Lastly, the ongoing international retrospective medical chart review PROVE study, with a higher sample size regarding SM infections and with more than 70% of patients admitted to ICU, will soon shed further light on the real-life situation.

2.4. Preventive Measures: Theoretical Basis for Clinical Formulation of Prevention and Control Strategies to Reduce the Morbidity and Mortality of SM Infection in the ICU

SM is widely distributed in natural environments, such as soil, water, and hospital environments, and can also parasite the human skin, respiratory and digestive tracts. Since environmental and clinical strains are genetically closely related, soil might be a likely source for community-acquired infections [2]. Moreover, sink drains, faucets, water, and sponges have also been identified as environmental sources of SM in hospitals, reflecting the ability of this pathogen to survive on any humid surfaces and colonise them over the long term by forming biofilm and resisting commonly used biocides [2,29,36,63]. SM possesses multiple virulence factors that allow it to colonise and produce infection, and among the most important of these is the production of pili, flagella, fimbria structures, and adhesins, which contribute to adherence, auto-aggregation, and colonisation of biotic and abiotic surfaces. This scenario allows SM to become a potentially important pathogen in the ICU setting, where healthcare-associated infections are a global challenge.

Guided by whole genome sequencing analysis, there is provided evidence that sink drains serve as antibiotic-resistant organism persistent reservoirs (in particular, SM and *P. aeruginosa* but not *Enterobacterales* species) in ICUs, and moreover that this was associated with human clinical infections, specifically bacteriemia by some *P. aeruginosa* clones [64]. In another previously published study, patients with SM pneumonia were associated with clones originating from sinks and medical devices in a Swedish ICU outbreak [65]. Therefore, disinfection of all possible sources is imperative to avoid healthcare-associated infections with this pathogen [29]. In addition, SM can be transferred via healthcare providers by hand contact. This reinforces the fact that healthcare personnel need to improve infection control practices and keep a close surveillance system [17,66].

Finally, a hospital-acquired BSI has been observed that is seasonal with summer spikes. A national Belgian cohort study on this infection showed associations between ambient climate variables and hospital-acquired BSI incidence rates per microorganism, with Gram-negative but not Gram-positive incidence increasing by 13% for every 5 °C increase in

temperature. Stenotrophomonas, Acinetobacter, and Klebsiella spp. demonstrated the highest correlation with increased temperature, and Stenotrophomonas and Bacteroides spp. had higher relative humidity. These factors also warrant further examination to improve patient safety in our units [67].

3. Discussion and Future Directions

SM has been identified as a novel nosocomial pathogen associated with life-threatening invasive infections and a high mortality rate in some patient populations, especially those who are severely ill or immunocompromised [10,25]. Clinicians should be made aware of the implications of isolating this organism and should interpret their findings considering clinical settings.

The ICU population is especially vulnerable to this pathogen because of patients' conditions and an environment conducive to SM colonisation and subsequent patient transmission. As mentioned above, SM can often be recovered from the oropharynx of hospitalised and CF patients. We could hypothesise that critically ill mechanically ventilated patients can mimic CF status, as in both cases, SM causes chronic infection of the airways, which contributes to inflammation and lung damage. Moreover, prolonged ICU stays are accompanied by numerous antimicrobial rounds: these induce ecological effects on microbiota that condition the emergence and frequently the coexistence of MDRB. CF represents the paradigm of bacterial–bacterial community interactions, including Pseudomonas spp. and SM, whose prevalence can be correlated with or sometimes be independent of the use of antimicrobials such as carbapenems [7]. Moreover, poor clinical outcomes in co-infected cases warrant further research to find better antibiotic treatment strategies and case management to reduce co-infection mortality [38].

SM diagnosis also raises many challenges. It is imperative to establish a reproducible, accurate, sensitive, and predictive standard method to detect clinically important susceptibility profiles in contemporary isolates. Moreover, molecular rapid methods should be included in guidelines, but these should preferentially be universal and available for all centres.

As previously mentioned, the use of carbapenem in daily practice in the ICU represents one of the most important risk factors for SM infections from a different perspective. Classically, Del Toro MD et al. hypothesised that antimicrobials would select for SM because of its intrinsic resistance to most antibiotics, thus making colonisation easier to detect with clinical cultures and favouring the possibility of infection in patients debilitated by invasive procedures [9]. Therefore, implementing an antimicrobial stewardship program (ASP) in the ICU attending to a combination of measures related to antimicrobial selection, PK/PD optimisation, and short time duration, is one of the main keys to optimising and dealing with the healthcare problem of increasing antibiotic resistance [68]. Furthermore, ASPs are widely accepted in the ICU, and it has been demonstrated that antimicrobials can be administered without compromising patient safety [69].

It is noteworthy that although TMP-SMX is the treatment of choice for SM infections, it is not routinely included in empirical treatment regimens due both to its adverse event profile and the relative rarity of SM infections in comparison to other Gram-negative bacilli. Consequently, a risk stratification strategy seems important. The use of clinical scoring tools while awaiting final culture results may help identify patients at increased risk for a particular infection, with the intent of reducing time to appropriate antimicrobial therapy. Accordingly, a risk score has been developed that predicts hematologic malignancy patients at increased risk for SM BSI to guide early TMP-SMX therapy [70]. Twenty percent of the 337 patients included were admitted to the ICU. The StenoSCORE, incorporating five variables (AML, absolute neutrophil counts, mucositis, central venous catheter, and carbapenem receipt for $\geq$3 days), performed moderately well in predicting this infection. Afterward, it was externally validated with a single-centre cohort study with hematologic malignancy, including 36 patients with SM BSI [71]. The authors also evaluated alternative variables that better predicted SM BSI and ICU admission within 12 h of index culture

included in the so-called StenoSCORE2. ROC curve analysis of the StenoSCORE2 performance produced an AUC of 0.84 (95% CI 0.76–0.92), which was higher than the previously published StenoSCORE. A StenoSCORE2 $\geq$ 4 had a sensitivity of 86%, specificity of 76%, and accurately identified 77% of SM BSIs.

Overall, combination therapy with effective antibiotics has been suggested when SM infections are suspected [53]. Unlike the current recommendation of combination therapy for empiric treatment of suspected or proven septic shock in ICU patients and monotherapy for definitive treatment when culture results and susceptibility tests are available, even for NFGNB, combination therapy could be discussed in patients with proven difficult-to-treat pathogens such as SM or carbapenem-resistant *Enterobacteriaceae* during ASP in the ICU, following clinical practice guidelines [68,72].

Opening further lines of research may help control the spread of SM in the ICU setting, with the optimisation of infection control policies deserving special attention [17].

Table 2 shows the most relevant findings and evidence-based key messages regarding severe SM infections in the critically ill population.

Table 2. Most relevant findings and key messages regarding severe SM infections in the critically ill population.

Scenario	Clinical Evidence
Epidemiology	Recent surveillance data indicate that SM accounts for less than 3% of all strains of healthcare-associated infections, a percentage that doubles in the case of VAP [5,22,23]. Crude 28-day mortality is 54.8% [13], increasing to 90% if hemorrhagic pneumonia is present [25], and it is further increased with co-infection by *Pseudomonas aeruginosa* [38]
Risk factors	Importance of stratification strategy: pre-existing medical conditions (COPD, malignancy); length of ICU stay; invasive procedures and antimicrobial agents, especially CP [15]. StenoSCORE2 for SM bacteriemia prediction [71]
Colonisation	Oral SM relative abundance of 36% predicts infection [14]. Previous SM colonisation was found in 80% of patients with pneumonia and 67% of patients with bacteremia caused by SM [6,16]
Antibiotic resistance	SM is intrinsically resistant to a wide range of commonly used antibiotics [29]. The resistance surveillance data reflect an increase, especially for cotrimoxazole and tigecycline [11,23,39]
Diagnostic challenges	There is no consensus among antimicrobial susceptibility testing guidelines [29,39]. An ICU-ASP for guiding an adjustment of empirical antimicrobial therapy based on rapid microbiological testing seems imperative [41,68]
Empirical therapy	SM predicts initially inappropriate antibiotic therapy [33]
Directed therapy	Combination therapy is recommended until clinical improvement [51,53]
Prevention measures	SM establishes ambiental ICU reservoirs and is related to patient infection [64,65]. Optimisation of infection control policies deserves special attention [17]

Note: SM: *Stenothophomonas maltophilia*; VAP: ventilator-associated pneumonia; COPD: chronic obstructive pulmonary disease; ICU: intensive care unit; CP: carbapenems; ASP: antimicrobial stewardship program.

This paper has several limitations inherent to narrative review methodology, such as potential incomplete coverage, lack of quantitative analysis, and positive results bias. Heterogeneity in study designs and the lack of explicit criteria for study selection may

have also introduced some bias. Nevertheless, to our knowledge, no previous study has comprehensively evaluated evidence on Stenotrophomonas maltophilia infections in critical patients. However, data are limited, and relevant clinical questions remain outstanding, such as the microbiological diagnostic challenges, proper antimicrobial stewardship in the critically ill, and concrete preventive measures in these settings.

4. Conclusions

SM infection remains a challenge for critical care physicians. A high index of suspicion and deep knowledge of both the local epidemiological situation and the new rapid diagnostic tools linked to the implementation of an ICU-ASP in daily practice, as well as control measures, would improve the ominous prognosis of this bacterium's presence in the ICU setting.

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Clinical presentation and outcomes of bloodstream infection with intrinsically carbapenem-resistant non-fermenting gram-negative organisms: *Stenotrophomonas maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp. in Singapore, from 2012 to 2024

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Abstract

Background Bloodstream infections with the non-fermenting Gram-negative organisms *Stenotrophomonas maltophilia*, *Elizabethkingia* spp. or *Chryseobacterium* spp. are observed in nosocomial settings. Comparative description of their clinical presentation, microbiological characteristics, treatment options and outcomes remain to be investigated.

Methods We performed a retrospective single-centre analysis of bloodstream infections with the abovementioned three organisms from 1 Jan 2012 to 30 Jun 2024.

Results A total of 349 distinct encounters (from 322 unique patients) were identified with bacteraemia. *Stenotrophomonas maltophilia* was the commonest (197/349, 56.4%), followed by *Elizabethkingia* spp. (127/349, 36.4%) and *Chryseobacterium* spp. (25/349, 7.2%). Prior carbapenem exposure was observed in 59.9% of cases. The majority were related to central lines (58.2%). Most cases were nosocomial in onset (82.5%), and a third were from the intensive care unit (32.1%). A significant proportion of our *Stenotrophomonas maltophilia* (32.8%) and *Chryseobacterium* spp. (22.7%) isolates were resistant to levofloxacin, while a majority of the organisms retained

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susceptibility to trimethoprim-sulfamethoxazole (TMP-SMX) and minocycline. Dual antibiotics were used in a minority of encounters (23/349, 6.6%). Mortality was high across infections with the three organisms, but highest amongst those with *Stenotrophomonas maltophilia* bacteraemia (41.6%), followed by *Elizabethkingia* spp. (29.9%) and *Chryseobacterium* spp. (20.0%).

Conclusions *Stenotrophomonas*, *Elizabethkingia or Chryseobacterium spp* bacteraemia was associated with significant mortality. Most cases were nosocomial in acquisition, with prior carbapenem exposure or indwelling central catheters. Fluoroquinolone resistance was common for *Stenotrophomonas maltophilia* and *Chryseobacterium* spp., but less prevalent in *Elizabethkingia* spp., while TMP-SMX and minocycline retained susceptibility. Monitoring these trends would be critical in guiding empiric therapy for these organisms.

Keywords Stenotrophomonas maltophilia, Elizabethkingia spp., Chryseobacterium spp., Clinical outcomes, Bacteraemia

Introduction

Three non-fermenting carbapenem resistant Gram-negative organisms – *Stenotrophomonas maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp. may cause invasive bloodstream infections in certain clinical contexts. These three organisms share common risk factors for the development of infection including prior carbapenem exposure, indwelling central catheters, immunocompromised states or critical illness in the intensive care [1–3]. Historically, there are also specific infections that are associated with each of these organisms. For example, *S. maltophilia* typically causes infections of the respiratory tract and bloodstream infections, while *Elizabethkingia* spp. is an important cause of meningitis in the paediatric population, and *Chryseobacterium* spp. is known to cause nosocomial bloodstream infections, often in the context of indwelling devices [4–6]. These organisms are frequently difficult to treat because they are intrinsically resistant to carbapenems and most beta-lactam antibiotics [1, 7]. They thus require therapy with other antimicrobial agents such as fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX) or tetracyclines (minocycline in particular). In fact, due to difficulty in predicting susceptibility patterns in patients with severe *S. maltophilia* infections, guidance from the Infectious Diseases Society of America (IDSA) recommends dual antimicrobial therapy [8]. However, clinical data is scarce. Similarly, invasive *Elizabethkingia* spp. infections may be fulminant, and some experts also recommend combination therapy initially [9]. Invasive infections with *Chryseobacterium* spp. are less frequent, but can also confer significant mortality and morbidity [4].

From a global perspective, infections with these three organisms are on the rise, with evolving trends in antimicrobial susceptibility. Unfortunately, optimal treatment remains ill-defined and clinical outcomes remain poor. In part, clinical outcomes are poor for patients with bacteraemia with these organisms as they present as invasive infections in immunocompromised hosts [10–12]. In patients with haematologic malignancies, *S. maltophilia* often causes a severe haemorrhagic pneumonia [13]. In other hosts, these organisms can cause invasive infection in those critically ill and also present as part of a co-infection with additional virulent pathogens [3, 4, 8, 14]. Therefore, we aimed to describe and examine the comparative characteristics of the background, clinical presentation of bloodstream infections with *S. maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp., in addition to describing trends in antibiotic treatment regimens and clinical outcomes. This would be informative to guide optimal treatment of infections with these organisms and also form the basis for future prospective study.

Methods

We performed a retrospective single-centre cohort study to describe the clinical characteristics, antimicrobial susceptibility patterns and treatment outcomes for bloodstream infections with *S. maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp. This study included distinct patient encounters with positive blood cultures identifying any of the three organisms from 1st January 2012 to 30th June 2024. All patients were adults (age $\geq$ 21 years old) hospitalised in our tertiary specialist institution (National University Hospital, Singapore). The positive blood cultures were identified through the laboratory information system, for which species level identification was performed using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker MALDI Biotyper, Bruker, Massachusetts, US). We excluded positive cultures from other sites (e.g. respiratory samples) and only examined positive blood cultures. We excluded duplicate blood culture isolates from the same patient hospitalisation encounter within the study period. Relapse of bacteraemia or recurrent bloodstream infection was tabulated separately, if it occurred in a different hospitalisation encounter. Susceptibility testing was performed by Etest. The tested antimicrobials varied during the included time period with the most frequently tested antimicrobials being trimethoprim-sulfamethoxazole (TMP-SMX), levofloxacin,

and minocycline. Ceftazidime testing for *S. maltophilia*, and piperacillin-tazobactam testing for *Elizabethkingia spp.* and *Chryseobacterium* spp. was also performed. The laboratory uses the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. However, EUCAST breakpoints were only available for *S. maltophilia* testing for TMP-SMX [15]. Non-species PK/PD breakpoints were used for levofloxacin, ceftazidime, and piperacillin-tazobactam interpretation. As there are no EUCAST non-species breakpoints for minocycline and TMP-SMX (other than for *S. maltophilia)*, Clinical and Laboratory Standards Institute (CLSI) breakpoints were used.

Baseline clinical data was tabulated, including the clinical presentation, demographics, past medical history and outcome data. Nosocomial onset of bacteraemia was defined as bacteraemia occurring ≥ 48 hours into the hospitalisation. We defined prior carbapenem exposure as having received a carbapenem within one month prior to the episode of bacteraemia. Microbiological findings included the speciation of each organism, as well as the antibiotic resistance patterns. Laboratory markers were tabulated if obtained from the same blood draw, or the closest results that were obtained within 48 h of the positive blood culture, including the full blood count, kidney function, liver function testing, and C-reactive protein levels. For each episode of bloodstream infection, antibiotic choice and duration was recorded. The initial empiric antibiotic choice was defined as the antibiotic that was chosen in response to the identification of one of these three organisms in blood culture, prior to antibiotic susceptibility testing results. The empiric antibiotic choice was labelled as an "appropriate" choice, if the clinical isolate eventually demonstrated susceptibility to one or more of the chosen agents. Clinical outcomes such as the length of hospital stay, need for intensive care, mechanical ventilation, vasopressor support, recurrence of bacteraemia within a year from the index episode and in-hospital all-cause mortality were also evaluated. We recorded all episodes of bloodstream infection (bacteraemia). If the same individual had two separate hospital encounters with bacteraemia, they were recorded as two distinct episodes. Co-infection with other pathogens were also recorded if they were concomitantly isolated from blood cultures in the same setting as the positive blood culture with *S. maltophilia*, *Elizabethkingia* spp. or *Chryseobacterium* spp. Adequate source control of infection was recorded if patients had undergone a procedure such as central line removal, debridement of infected tissues, or abscess drainage, where appropriate.

We then stratified the patients into three groups (*S. maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp.) and compared them across clinical, demographic, laboratory parameters as well as clinical outcomes. Continuous parameters were examined by one-way analysis of variance and presented as means ± one standard deviation. Categorical variables were presented in frequencies and percentages, and compared by Chi-squared tests. Where statistical significance across the three groups was observed, post-hoc pairwise comparisons between the groups were conducted, with Bonferroni correction. A p-value of < 0.05 was considered significant. All data analyses for this study were carried out on SPSS version 20.0 (SPSS, Inc., Chicago, Illinois). This study had been approved by the National Healthcare Group Domain Specific Review Board (DSRB 2024–3132) prior to its conduct. The study was done in accordance with the principles laid out by the Declaration of Helsinki. All data was anonymised and a waiver of written informed consent had been obtained from the institutional review board prior to the conduct of the study, as this was a retrospective study and did not involve the human participants directly.

Results

A total of 349 distinct patient encounters involving 322 patients with bloodstream infections caused by *S. maltophilia*, *Elizabethkingia* spp., and *Chryseobacterium* spp. were analyzed. The majority of these infections were attributed to *S. maltophilia* (56.4%), followed by *Elizabethkingia* spp. (36.4%), then *Chryseobacterium* spp. (7.2%) (Fig. 1).

Demographics and clinical presentation

Patients with *Elizabethkingia* spp. bacteremia were significantly older. Immunocompromised status was most common in patients with *S. maltophilia* (62.4%) compared with the other two organisms (*Elizabethkingia* spp. (37.8%) or *Chryseobacterium* spp. (48.0%). Additionally, prior carbapenem exposure within the preceding month was reported in 59.9% of cases, with no significant differences observed among the three pathogens. Central lines were present in most patients, particularly those with *S. maltophilia* (89.8%). Total parenteral nutrition was rare (2.9%) and predominantly associated with *S. maltophilia* as well, although this was not statistically significant. Most infections were nosocomial in onset (82.5%), and 32.1% of cases occurred in the intensive care unit (Table 1).

Microbiological characteristics

Co-infections were observed in 25.8% of cases, most commonly with *Chryseobacterium* spp. (44.0%). Enterococci ($n = 14$) and Coagulase negative Staphylococci were the most common co-pathogens ($n = 13$), followed by *Pseudomonas* spp. ($n = 11$), *Candida* spp. ($n = 7$), and *Acinetobacter* spp. ($n = 7$). Catheter-related infections accounted for a majority (58.2%) of cases. Amongst these

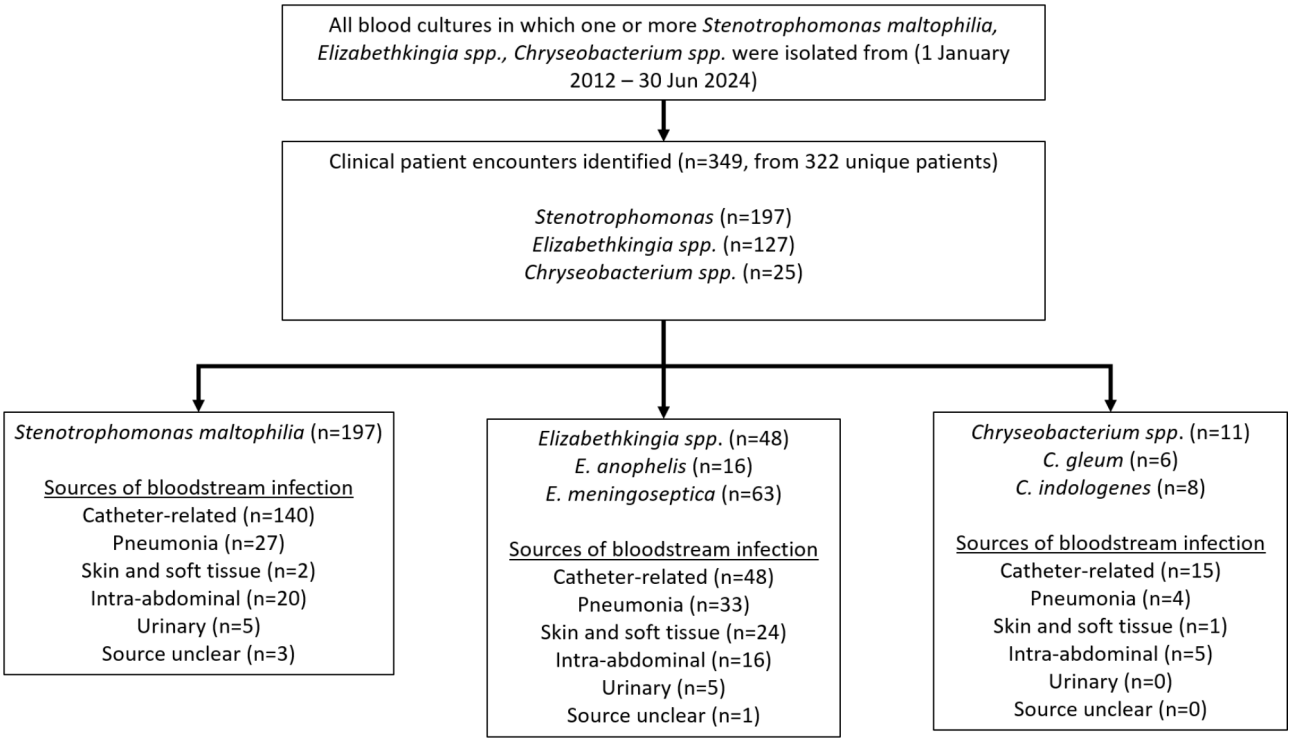

Fig. 1 Flowchart describing patient population by organism and sources of bloodstream infection

three organisms, the organism to present most frequently with skin and soft tissue infection as its source of infection was *Elizabethkingia* spp. A majority of these skin and soft tissue infections were a result of recent peripheral intravenous cannulation and subsequent thrombophlebitis. Intra-abdominal infection had the highest frequency with *Chryseobacterium* spp. (Table 1). Intraabdominal infections tended to occur after surgery or an endoscopic procedure, such as endoscopic retrograde cholangiopancreaticography for biliary obstruction. All episodes of urinary infection were associated with urological procedures or stenting.

Across the three organisms, 23% of isolates were resistant to levofloxacin, and 4.2% were resistant to TMP-SMX. Eight of the 349 isolates (2.3%) were resistant to both TMP-SMX and levofloxacin. Of these, 7 were *S. maltophilia* isolates and the remaining one was a *Chryseobacterium* isolate. Amongst the *S. maltophilia* isolates, resistance to levofloxacin in our institution was high (32.8%), although a majority retained susceptibility to TMP-SMX (93.1%) and minocycline (98.8%). Similarly, amongst the *Chryseobacterium* spp. isolates, 22.7% were resistant to levofloxacin, while a majority remained susceptibile to TMP-SMX (95.7%) and minocycline (100%). For *Elizabethkingia* spp., levofloxacin resistance was observed in 7.9%, while no isolates were resistant to TMP-SMX or minocycline. (Table 2).

Antimicrobial treatment and clinical outcomes

Initial antibiotic regimens commonly included TMP-SMX (37.0%) or levofloxacin (39.0%), with dual therapy used in only 6.6% of cases. Empiric therapy was appropriate in 84.2% of encounters, though 17.5% required regimen changes due to antibiotic intolerance or inappropriate initial choices. Patients received an average of 12 (±7) days of appropriate antibiotics, with no significant differences among the three pathogens (Table 2).

The overall mean length of hospital stay was 48 ± 107 days. Most patient encounters required a procedure for source control (219 out of 349, 62.8%). Of these procedures, 208 patients underwent central line removal, and the remaining 11 patients either had debridement of infected issues, or drainage of intra-abdominal abscesses. In-hospital mortality was high in this cohort at 35.8% (125 out of 349). Mortality was highest amongst patients with *S. maltophilia* bacteraemia (41.6%) versus *Elizabethkingia* spp. (29.9%). and *Chryseobacterium* spp. (20.0%, $p = 0.023$). Recurrence of bacteraemia within 1 year of the index episode was observed in 12 out of 349 patient encounters (3.4%), and a majority of these were in patients with *S. maltophilia* (Table 2).

Discussion

Overall, we described important trends in hospitalised patients presenting with bloodstream infection with *S. maltophilia*, *Elizabethkingia* spp. or *Chryseobacterium*

Table 1 Demographics and clinical characteristics by organism

Parameter	Overall cohort (*n* = 349)	*Stenotrophomonas maltophilia* (*n* = 197)	*Elizabethkingia* spp. (n = 127)	*Chryseobacterium* spp. (n = 25)	*p*-value
Age (years)	63.7 (± 5.0)	60.7 (± 14.9)	68.7 (± 14.2)[#]	61.9 (± 13.1)	< 0.01
Age < 60 years, n	131 (37.%)	87 (44.2%)	34 (27.0%)[#]	10 (40.0%)	0.008
Male sex, n	227 (65%)	125 (63.5%)	84 (66.1%)	18 (72.0%)	0.664
Smoking, n	27 (7.8%)	11 (5.6%)	13 (10.2%)	3 (12.0%)	0.225
Hypertension, n	184 (52.7%)	92 (46.7%)	80 (63.0%)	12 (48.0%)	0.015
Hyperlipidaemia, n	144 (41.3%)	71 (36.0%)	66 (52.0%)[#]	7 (28.0%)	0.007
Diabetes mellitus, n	120 (34.4%)	63 (32.0%)	47 (37.0%)	10 (40.0%)	0.537
End-stage kidney disease, n	63 (18.1%)	35 (17.8%)	21 (16.7%)	7 (28.0%)	0.398
Chronic liver disease, n	31 (8.9%)	17 (8.6%)	11 (8.7%)	3 (12.0%)	0.851
Ischaemic heart disease, n	97 (27.8%)	40 (20.3%)	49 (38.6%)[#]	8 (32.0%)	0.001
Immunocompromised host, n	183 (52.4%)	123 (62.4%)	48 (37.8%)[#]	12 (48.0%)	< 0.001
Type of immunocompromised host					< 0.001
Solid organ transplant	7 (2.0%)	5 (2.5%)	2 (1.6%)	0 (0.0%)	
Bone marrow transplant	17 (4.9%)	11 (5.6%)	2 (1.6%)	4 (16.0%)	
Haematological disorder	76 (21.8%)	59 (29.9%)	15 (11.8%)	2 (8.0%)	
Solid tumours	76 (21.8%)	44 (22.3%)	27 (21.3%)	5 (20.0%)	
Rheumatological disorders	8 (2.3%)	6 (3.0%)	2 (1.6%)	0 (0.0%)	
Others	3 (0.9%)	2 (1.0%)	0 (0.0%)	1 (4.0%)	
Prior carbapenem exposure within one month of bacteraemia, n	209 (59.9%)	128 (65.0%)	68 (53.5%)	13 (52.0%)	0.086
Duration of prior carbapenem exposure (days)	9 (± 7)	10 (± 7)	8 (± 5)	9 (± 7)	0.089
Presence of central line, n	263 (75.4%)	177 (89.8%)	68 (53.5%)[#]	18 (72.0%)	< 0.001
Total parenteral nutrition, n	10 (2.9%)	9 (4.6%)	1 (0.8%)	0 (0.0%)	0.093
Nosocomial onset of bacteraemia, n	288 (82.5%)	155 (78.7%)	114 (89.8%)	19 (76.0%)	0.025
Onset of bacteraemia in Intensive Care Unit, n	112 (32.1%)	78 (39.6%)	30 (23.6%)[#]	4 (16.0%)	0.002
Co-infection (bacteraemia) with other organisms	89 (25.8%)	60 (30.8%)	18 (14.4%)[#]	11 (44.0%)[@]	< 0.001
Source of bacteraemia					< 0.001
Catheter-related	203 (58.2%)	140 (71.1%)	48 (37.8%)	15 (60.0%)	
Pneumonia	64 (18.3%)	27 (13.7%)	33 (26.0%)	4 (16.0%)	
Skin and soft tissue	27 (7.7%)	2 (1.0%)	24 (18.9%)	1 (4.0%)	
Intraabdominal	41 (11.7%)	20 (10.2%)	16 (12.6%)	5 (20.0%)	
Urinary	10 (2.9%)	5 (2.5%)	5 (3.9%)	0 (0.0%)	
Unclear source	4 (1.1%)	3 (1.5%)	1 (0.8%)	0 (0.0%)	
Temperature (℃)	38.3 (± 0.7)	38.2 (± 0.8)	38.3 (± 0.7)	38.4 (± 0.5)	0.502
Duration of fever (days)	4.6 (± 3.7)	5.3 (± 4.3)	3.7 (± 2.6) [#]	3.2 (± 1.9) [#]	< 0.001
Respiratory rate (per minute)	22 (± 6)	22 (± 7)	22 (± 3)	21 (± 3)	0.766
Pulse rate (per minute)	94 (± 20)	95 (± 20)	93 (21)	95 (± 16)	0.500
Systolic blood pressure (mmHg)	121 (± 25)	119 (± 26)	122 (± 23)	131 (± 29)	0.054
Diastolic blood pressure (mmHg)	69 (± 16)	66 (± 14)	71 (± 17) [#]	77 (± 19) [#]	0.001
Oxygen saturations (%)	98 (± 3)	98 (3)	98 (3)	97 (± 3)	0.643
Haemoglobin (g/dL)	9.6 (± 2.1)	9.1 ± 1.9)	10.2 (± 2.2) [#]	10.3 (± 2.0) [#]	< 0.001
Total white cell count (x10^9/L)	9.8 (± 8.1)	8.1 (± 7.2)	12.3 (± 8.8) [#]	11.1 (± 7.9)	< 0.001
Absolute neutrophil count (x10^9/L)	8.2 (± 7.3)	6.7 (± 6.6)	10.3 (± 7.9) [#]	8.2 (± 7.3)	< 0.001
Absolute lymphocyte count (x10^9/L)	0.81 (± 0.69)	0.69 (± 0.65)	0.97 (± 0.71) [#]	0.95 (± 0.71)	0.001
Platelet count (x10^9/L)	171 (± 136)	143 (± 124)	205 (± 139) [#]	223 (± 165) [#]	< 0.001
Serum creatinine (μmol/L)	173 (± 186)	155 (± 175)	196 (± 197)	196 (± 206)	0.129
Aspartate aminotransferase (AST, U/L)	151 (± 758)	89 (± 241)	259 (± 1214)	99 (± 309)	0.145
Alanine aminotransferase (ALT)	118 (± 780)	61 (± 109)	215 (± 1287)	88 (± 206)	0.229
Lactate dehydrogenase (U/L)	960 (± 2221)	802 (± 1213)	1272 (± 3341)	615 (± 280)	0.197
C-reactive protein (mg/L)	106 (± 91)	116 (± 100)	95 (± 79)	85 (± 74)	0.089

[#]post-hoc pairwise comparison against *Stenotrophomonas maltophilia, where p < 0.05*

[@]post-hoc pairwise comparison against *Elizabethkingia* spp., where *p* < 0.05

Table 2 Microbiology and treatment outcomes of patients by organism

Parameter	Overall cohort (*n* = 349)	*Stenotrophomonas maltophilia* (*n* = 197)	*Elizabethkingia* spp. (*n* = 127)	*Chryseobacterium* spp. (*n* = 25)	*p*-value
Microbiology					
Resistant to levofloxacin	79/344 (23.0%)	64/195 (32.8%)	10/127 (7.9%)[#]	5/22 (22.7%)	< 0.001
Resistant to TMP-SMX*	14/336 (4.2%)	13/189 (6.9%)	0/124 (0.0%)[#]	1/23 (4.3%)	0.012
Resistant to minocycline	1/194 (0.5%)	1/92 (1.1%)	0/85 (0.0%)	0/17 (0.0%)	0.573
Resistant to piperacillin-tazobactam	15/90 (16.7%)	-	12/75 (16.0%)	3/15 (20.0%)	0.704
Resistant to ceftazidime	40/78 (51.3%)	25/62 (40.3%)	12/12 (100.0%)	3/4 (75.0%)	< 0.001
Therapeutic strategies					
Initial empiric antibiotic choice in response to bacteraemia					< 0.001
TMP-SMX* containing	129 (37.0%)	93 (47.2%)	32 (25.2%)	4 (16.0%)	
Levofloxacin containing	136 (39.0%)	77 (39.1%)	52 (40.9%)	7 (28.0%)	
Minocycline containing	11 (32.0%)	5 (2.5%)	3 (2.4%)	3 (12.0%)	
Piperacillin-tazobactam containing	39 (1.1%)	0 (0.0%)	34 (26.8%)	5 (20.0%)	
Others	34 (9.7%)	22 (11.2%)	6 (4.7%)	6 (24.0%)	
Initial dual antibiotics regimen	23 (6.6%)	13 (6.6%)	8 (6.3%)	2 (8.0%)	0.952
Initial antibiotic choice appropriate	294 (84.5%)	163 (83.2%)	112 (88.2%)	19 (76.0%)	0.227
Required switch in antibiotics from empirical regimen	61 (17.5%)	33 (16.8%)	24 (18.9%)	4 (16.0%)	0.866
Final antibiotic regimen choice					< 0.001
TMP-SMX* containing	144 (41.3%)	95 (48.2%)	43 (33.9%)	6 (24.0%)	
Levofloxacin containing	137 (39.3%)	66 (33.5%)	64 (50.4%)	7 (28.0%)	
Minocycline containing	19 (5.4%)	13 (6.6%)	3 (2.4%)	3 (12.0%)	
Piperacillin-tazobactam containing	15 (4.3%)	0 (0.0%)	11 (8.7%)	4 (16.0%)	
Others	34 (9.7%)	23 (11.7%)	6 (4.7%)	5 (20.0%)	
Final dual antibiotics regimen	20 (5.7%)	12 (6.1%)	6 (4.7%)	2 (8.0%)	0.767
Final antibiotic choice effective	305 (87.6%)	169 (86.2%)	116 (91.3%)	20 (80.0%)	0.191
Duration of appropriate antibiotics (days)	12 (± 7)	12 (± 7)	13 (± 7)	11 (± 4)	0.216
Appropriate antibiotic duration two weeks or longer, n	194 (56.1%)	99 (50.8%)	83 (65.9%)[#]	12 (48.0%)	0.020
Required procedure for source control (e.g. central line removal, debridement of infected tissues, drainage of abscesses)	219 (62.8%)	141 (71.6%)	58 (45.7%)[#]	20 (80.0%)[@]	< 0.001
Clinical outcomes					
Length of hospital day (days)	48 (± 107)	48 (± 76)	53 (± 148)	27 (± 30)	0.534
Length of intensive care unit stay	24 (± 27)	28 (± 29)	16 (± 23)	11 (± 6)	0.087
Required vasopressor support	107 (30.7%)	73 (37.1%)	29 (22.8%)[#]	5 (20.0%)	0.012
Required mechanical ventilation	104 (29.8%)	68 (34.5%)	31 (24.4%)	5 (20.0%)	0.082
Required intensive care	115 (33.0%)	80 (40.6%)	30 (23.6%)[#]	5 (20.0%)	0.002
In-hospital mortality	125 (35.8%)	82 (41.6%)	38 (29.9%)	5 (20.0%)	0.023
Recurrence of bacteraemia within 1 year	12 (3.4%)	11 (5.6%)	0 (0.0%)[#]	1 (4.0%)	0.026

*TMP-SMX: trimethoprim-sulfamethoxazole

[#]post-hoc pairwise comparison against *Stenotrophomonas maltophilia, where p < 0.05*

[@]post-hoc pairwise comparison against *Elizabethkingia* spp., where $p < 0.05$

spp. over the 13 years from 2012 to 2024. Broadly, the risk factors for acquisition of these organisms were similar. A majority (> 80%) were nosocomial in acquisition, and a third were acquired in critically ill patients in the intensive care unit. Furthermore, close to 60% had prior carbapenem exposure before developing bacteraemia with these organisms. Prolonged carbapenem exposure has been associated with invasive infections with these organisms as they are intrinsically resistant to carbapenems. Selection pressure from prolonged carbapenem exposure likely eradicates normal flora and allows for proliferation of these opportunistic non-fermenting intrinsically carbapenem-resistant Gram negative organisms [2–4, 16].

Nevertheless, there were sizeable number of catheter-related bloodstream infections amongst patients with end-stage kidney disease on haemodialysis that presented from the community. Given that they were community acquired infections, a majority of these patients did not have carbapenem exposure prior to infection. Compared with *Staphylococcus aureus* and coagulase negative *Staphylococci*, *S. maltophilia*, *Elizabethkingia* spp. and *Chryseobacterium* spp. are generally thought to be uncommon causes of catheter-related bloodstream

infections patients on dialysis. However, outbreaks in dialysis centres have been reported, which may be related to contaminated water sources [17, 18]. In our cohort these episodes appeared to be sporadic and did not appear to cluster to any specific timeframe. We also did not observe recurrence of bacteraemia in the same patients who would return to visit the same dialysis centre after hospital admission, which may more clearly suggest a contaminated water source.

Solid organ tumours also formed an important risk factor for bloodstream infections with these organisms, accounting for approximately 20% of encounters. These patients often have central lines for delivery of chemotherapy, or biliary obstruction and endoscopic procedures which have been stented. Translocation of bacteraemia from the biliary tree into the bloodstream has been reported after biliary procedures, particularly when the procedure was performed in the context of biliary obstruction [19]. Carbapenem or broad-spectrum antibiotic use peri-procedurally then may have subsequently pre-disposed to bacteraemia with one of these three organisms.

Elizabethkingia spp. bloodstream infections were also observed amongst patients who were immunocompetent and not critically ill. The source of these infections was often pulmonary, in the form of hospital acquired pneumonias, or skin and soft tissue infection, in the context of thrombophlebitis after recent intravenous cannulation [20]. In our adult population, we did not identify meningitis or central nervous system infection, which had been commonly observed in the paediatric setting [5].

Overall, we found these three organisms to be important nosocomial pathogens. The in-hospital mortality in our cohort was high, consistent with data from other observational cohorts [3, 4, 14]. Amongst the three, mortality was highest in *S. maltophilia* bloodstream infections. Optimal treatment approaches for these infections remain unclear. In our centre, for infections with *S. maltophilia*, the first-line empiric antibiotic choice was TMP-SMX, due to the high rates of fluoroquinolone resistance.

S. maltophilia harbour beta lactamases that hydrolyse penicillins, cephalosporins, carbapenems and aztreonam [21–23]. In addition, they also demonstrate intrinsic resistance to aminoglycosides by chromosomal aminoglycoside acetyl transferase enzymes [24]. In addition to the intrinsic antimicrobial resistance, *S. maltophilia* is also known to develop resistance while on therapy, by upregulating efflux pumps [25–27]. Similarly, both *Elizabethkingia* spp. and *Chryseobacterium* spp. in addition to beta-lactamases, can also express efflux pumps which confer resistance to other antibiotics [28, 29]. As such, with the intention to avoid the development of antimicrobial resistance, some experts recommend the use of combination antibiotic therapy [8, 30]. The clinical evidence for this practice remains scarce and controversial. One reasonable approach may have been to stratify patients by disease severity, where it appeared that patients with more severe infection were more likely to demonstrate benefit from the use of combination antimicrobial therapy [31].

In contrast to *S. maltophilia*, clinical outcomes and characteristics are less well studied in *Elizabethkingia* spp. and *Chryseobacterium* spp., probably because they are less frequent causes of invasive infection. Consequently, there are no treatment guidelines for infections with *Elizabethkingia* spp. or *Chryseobacterium* spp. Dual antibiotic therapy is sometimes used for these infections, particularly, when the infection is severe and while susceptibility testing results are pending, to increase the likelihood of at least one appropriate empiric agent. In our retrospective review, dual therapy was rarely used for infections with these three organisms. Therefore, the numbers were too small to conclude or evaluate if outcomes associated with empiric dual therapy were better or worse than with monotherapy.

In addition to antibiotic choice, the optimal duration of antibiotic therapy for bloodstream infections with these organisms also remains to be elucidated. Duration of therapy is likely guided by the patient's clinical syndrome (e.g. pneumonia, skin and soft tissue infection, catheter-related infection, etc.), and clinical progress on antimicrobial therapy. The IDSA guidelines recommend line removal, and 7 to 14 days of antibiotics for catheter-related bloodstream infections with Gram negative organisms [32]. In most of the patients in our cohort, central lines were removed in a timely fashion, which likely provided an adequate degree of source control for the infection. The patients in our cohort received an average of 12 ± 7 days of appropriate antibiotics. With this, we observed that relapse of bacteraemia within a year was low in our cohort (3.4%). Future study is important to define appropriate antibiotic duration for this infection. To examine this, patients may have to be stratified by source of infection. For example, in clinical contexts in which the infection complicated and source control is less adequate (e.g. an infected biliary stent that cannot be removed), a longer duration of antibiotic therapy may be required.

Limitations

Our study reflects a single-centre experience of patients being managed for bloodstream infections with *S. maltophilia*, *Elizabethkingia* spp. or *Chryseobacterium* spp. MALDI-TOF MS could reliably identify *Elizabethkingia* spp. to a genus level, so this group was analyzed as a whole. Species-level identification to further comparing the differences in clinical characteristics and outcomes

among distinct species would require further diagnostic methods (for example, 16s rRNA sequencing) [33]. Source of infections were determined by the primary managing team, as documented in the clinical charts on retrospective review. The clinical and demographic data obtained therefore relied on the quality of documentation in the patients' electronic records. Importantly, this is a cross-sectional study, and we did not prospectively follow-up each of these patients. Furthermore, as this was a retrospective and observational cohort, treatment decisions, such as antibiotic choices and durations were left to the discretion of the managing physicians. As such, we could not systematically study the impact or difference of various treatment options on clinical outcomes as well. Most empiric antibiotics were administered intravenously, but we did not capture the timing of oral switch in therapy. We also only reported the antimicrobial susceptibility testing for the index episode of bacteraemia. There were some subjects with multiple blood cultures that returned positive over time. We did not capture if resistance to antibiotics had developed while on therapy.

Our findings were exploratory and descriptive. As such, we did not build a multivariable model to adjust for confounding variables that may have explained some of the observed trends. For example, although the prevalence of prior carbapenem exposure was common in bacteraemia with these three organisms, we did not have a control group of bacteraemia with other Gram-negative organisms to compare against. Nevertheless, we report real-world observational findings in terms of the clinical presentation and outcomes from these infections.

Furthermore, we also used all-cause in-hospital mortality as an outcome measure. It was not always possible to elucidate if the bacteraemia episode was the main contributor to an individual's mortality. Therefore, an individual's underlying co-morbidities such as underlying metastatic cancer may also have contributed to the adverse outcomes. We did not specifically study the trends in mortality over time. However, our study examined a long period of time (close to 13 years). The changes in critical care practices and other unmeasured factors may also have contributed to differences in clinical outcomes. Furthermore, in some immunocompromised hosts, fluoroquinolone or trimethoprim-sulfamethoxazole prophylaxis may have been used. Understanding the specific antibiotic therapy that the patient had been on prior to the bacteraemia episode may importantly influence the clinical outcomes, as well as the antimicrobial susceptibility profile of infections with these three organisms.

Our study focused only on infections with these three organisms, and we were not able to compare the prevalence of these infections with bloodstream infections with other Gram-negative organisms., although our findings are exploratory, they may form the important basis for randomised controlled trials to evaluate optimal antimicrobial treatment regimens and duration in these infections. Additionally, to understand the evolution and development of antibiotic resistance, future prospective study on the mechanisms of resistance in these organisms are important.

Conclusions

Mortality and morbidity were high in our cohort, particularly in patients with *Stenotrophomonas maltophilia* bloodstream infection. Dual therapy was rarely used as part of the initial treatment regimen, and it remains unclear if this would have an impact on clinical outcomes. Fluoroquinolone resistance was common in *S. maltophilia* and *Chryseobacterium* spp., but was less frequently identified in *Elizabethkingia* spp. isolates. Understanding local trends in resistance patterns may thus help to guide empirical antibiotic choice for infections with these organisms. Monitoring local susceptibility patterns for these three organisms over time would be helpful to guide treatment protocols, and prospective studies evaluating optimal treatment regimens and duration, and evolving resistance mechanisms are warranted.

Abbreviations

CLSI	Clinical and Laboratory Standards Institute
DSRB	Domain Specific Review b\Board
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IDSA	Infectious Diseases Society of America
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry
TMP-SMX	Trimethoprim-sulfamethoxazole

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Author contributions

MCYK, JNN contributed to the conception, data collection, data analysis, writing of the original draft and critical review of the manuscript. NS, KLC, LHWL and DMA contributed to the conception, data analysis, and critical review of the manuscript.

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Data availability

Data will be made available on reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

This study was approved by the hospital's institutional review board (National Healthcare Group (NHG) Domain Specific Review Board (DSRB) 2024–3132). A waiver of written informed consent was obtained from the hospital's institutional review board (National Healthcare Group (NHG) Domain Specific Review Board (DSRB) 2024–3132) prior to conduct. The study was carried out in accordance with the principles laid out by the Declaration of Helsinki.

Consent for publication

Not Applicable.

Competing interests
The authors declare no competing interests.

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REVIEW

Burkholderia cepacia Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products

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SUMMARY *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) was once thought to be a single bacterial species but has expanded to the *Burkholderia cepacia* complex (Bcc), comprising 24 closely related opportunistic pathogenic species. These bacteria have a widespread environmental distribution, an extraordinary metabolic versatility, a complex genome with three chromosomes, and a high capacity for rapid mutation and adaptation. Additionally, they present an inherent resistance to antibiotics and antiseptics, as well as the abilities to survive under nutrient-limited conditions and to metabolize the organic matter present in oligotrophic aquatic environments, even using certain antimicrobials as carbon sources. These traits constitute the reason that Bcc bacteria are considered feared contaminants of aqueous pharmaceutical and personal care products and the frequent reason behind nonsterile product recalls. Contamination with Bcc has caused numerous nosocomial outbreaks in health care facilities, presenting a health threat, particularly for patients with cystic fibrosis and chronic granulomatous disease and for immunocompromised individuals. This review addresses the role of Bcc bacteria as a potential public health problem, the mechanisms behind their success as contaminants of pharma-

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ceutical products, particularly in the presence of biocides, the difficulties encountered in their detection, and the preventive measures applied during manufacturing processes to control contamination with these objectionable microorganisms. A summary of Bcc-related outbreaks in different clinical settings, due to contamination of diverse types of pharmaceutical products, is provided.

KEYWORDS *Burkholderia cepacia* complex, pharmaceutical contamination

INTRODUCTION

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative nonfermenting betaproteobacteria (1) that are broadly distributed in the environment, colonizing diverse niches, whether natural or human-made (2–6). This feature is attributed to their genotypic and phenotypic plasticity and consequent capacity for rapid mutation and adaptation to challenging environments (1, 7, 8). This group of bacteria has emerged as a worrying opportunistic pathogen, with high potential to cause serious respiratory infections in patients with underlying illnesses, namely, cystic fibrosis (CF) (9–11) and chronic granulomatous disease (CGD) (12, 13). Bcc infection also has been reported in immunocompromised patients (debilitated elderly people, HIV-positive individuals, cancer patients undergoing chemotherapy, etc.) (14–16). Bcc bacteria can adapt to the stressful conditions that characterize the CF lung environment, which makes them virtually impossible to eradicate, leading to unpredictable and variable outcomes, ranging from asymptomatic carriage to a quick and sometimes unexpected deterioration of the patient's condition, culminating in a fatal necrotizing pneumonia (termed "cepacia syndrome") (3, 17).

Remarkably, Bcc bacteria also possess the capacity to survive and proliferate in water-based environments, such as water bodies, lakes, rivers, drinking water, and liquids containing small amounts of nutrients (18–21). In addition to this capacity, these bacteria are described as major contaminants of sterile (e.g., intravenous drugs and solutions) and nonsterile pharmaceuticals (e.g., nasal sprays, water-based products, mouthwash, preoperative skin solutions, and hand sanitizers), being the cause of numerous nosocomial outbreaks registered in the last 2 decades (22–26). Many of these contamination episodes have been associated with the ability of Bcc bacteria to thrive in the presence of antimicrobials and disinfectants, particularly the biocides used in pharmaceutical products' formulations (24, 27–31). Benzalkonium chloride (BZK) solutions have been described as one of the most frequent sources of Bcc contamination (32). Bcc bacteria have developed an array of strategies to cope with the presence of biocides, including their active extrusion from the bacterial cell through the action of efflux pumps or the inactivation by catabolic enzymes and subsequent use as carbon sources for bacterial growth (33–36).

Since Bcc bacteria can survive under nutrient-deprived conditions, water constitutes the most common environment in which they can cause contamination, raising a particular concern when the water supplies of pharmaceutical companies are affected (37, 38). The clinical relevance of Bcc bacteria and their status as one of the most prevalent contaminants in pharmaceutical industries has led authorities, namely, the United States Food and Drug Administration (FDA), to propose the inclusion of these bacteria in the "Objectionable Microorganisms" category (39, 40). In addition to the fact that Bcc bacteria are inherently difficult to detect and identify, due to their specific metabolic requirements and low growth rate, the majority of phenotypic and genotypic methods also demonstrate significant limitations in their capacity to detect Bcc organisms. Therefore, it is important to understand and control the presence of Bcc in pharmaceutical settings, including raw materials and finished products, to avoid their propagation into health care settings, which greatly compromises the treatment options and general quality of life of susceptible patients. Figure 1 provides an overview of the main topics that will be addressed in this review, with special focus on pharmaceutical products' contamination and the associated implications in terms of public health.

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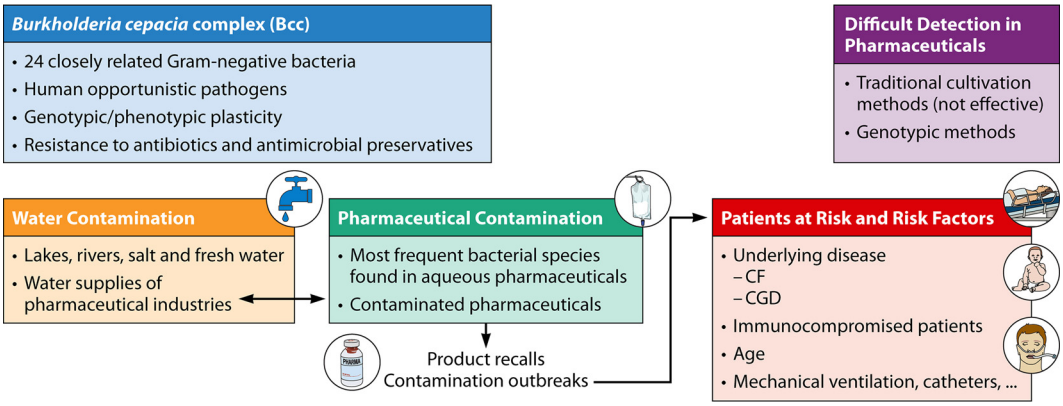

FIG 1 Overview of the main topics addressed in this review, from the description and characterization of *Burkholderia cepacia* complex (Bcc) bacteria and their survival and proliferation in diverse environments, including water supplies and pharmaceutical products, to the related nosocomial outbreaks, especially involving susceptible patients, as well as the difficulty and complexity associated with Bcc detection and identification based on phenotypic and genotypic methods.

Bcc—DIVERSITY AND ECOLOGY

The *Burkholderia cepacia* complex (Bcc) is a group of 24 phylogenetically related Gram-negative betaproteobacteria that are widely distributed in the environment (41–45). They carry complex genomes (with sizes ranging from 6 to 9 Mb and typically 3 chromosomes) and have the ability to rapidly adapt through mutation, which translates into a remarkable genotypic and phenotypic plasticity (1, 3, 7). Consequently, Bcc bacteria are known for their metabolic diversity and capacity to endure environmental stresses (33, 46).

Bcc bacteria are frequently found in natural environments, occupying diverse niches, such as soil, water (including seawater), plant rhizosphere, and agricultural products (2–6). However, the distribution of Bcc species is not homogeneous among these habitats. For instance, *B. cepacia*, *B. cenocepacia*, *B. vietnamiensis*, and *B. ambifaria* are the most representative Bcc species inhabiting the rhizosphere of plants (3–6, 8), while *B. cepacia*, *B. cenocepacia*, *B. vietnamiensis*, *B. anthina*, and *B. seminalis* are commonly found in water-based environments worldwide (6, 19–21). Nevertheless, the precise environmental origin of some Bcc species, namely, *B. multivorans*, is currently unclear, and the reports concerning its presence in different environmental samples are rather contradictory; some describe water as the most common source of this bacterium (19, 21), while others indicate that soil is the most common environmental niche (47). These discrepancies might be due to the limitations associated with the isolation methods (48).

Due to their metabolic diversity and ability to establish mutualistic and symbiotic interactions with plants, the use of *Burkholderia* species for biological control, plant growth promotion, and bioremediation has been attempted, but their pathogenic potential raises concerns (5). Since Bcc species are phylogenetically related, the exchange of genetic material between different strains/species might occur, creating the possibility for apparently innocuous strains to acquire pathogenic characteristics (6). This hypothesis has not yet been described in natural settings but has been demonstrated *in vitro* (6). Field tests have shown that Bcc bacteria can proliferate within the rhizosphere of various economically relevant crops, including rice (where *B. vietnamiensis* has been found in high numbers), pea roots (colonized by *B. ambifaria*), and wheat (with *B. cepacia* and *B. cenocepacia* inhabiting its rhizosphere) (4). Some of these species are also capable of N_2 fixation, thereby contributing to plant growth (8). Another important capacity is the production of biopesticides that protect crops against other bacteria, protozoa, nematodes, and fungal diseases, such as root rot or seed-damaging infections (2, 5). Due to their ability to use complex and diverse carbon sources, some *Burkholderia* isolates potentially can degrade different xenobiotic com-

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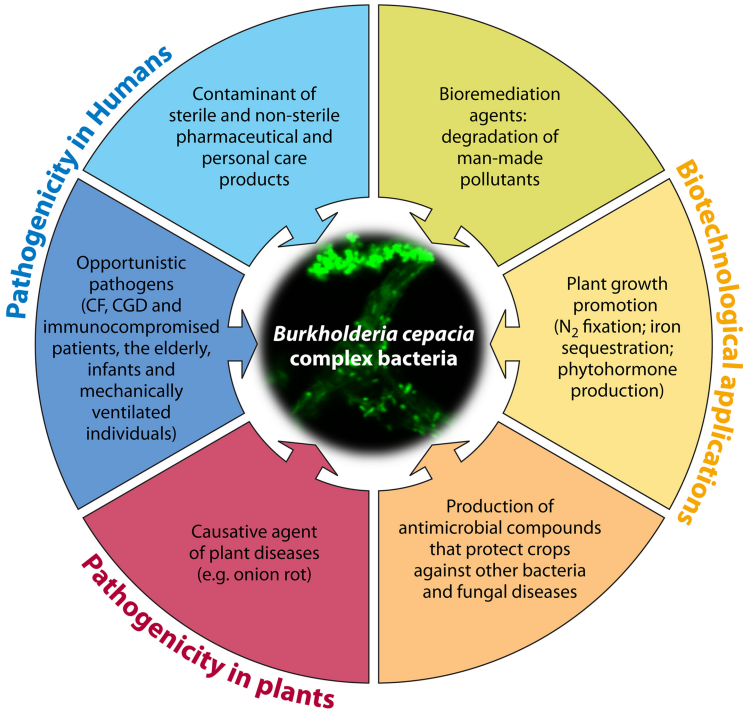

FIG 2 Strains of Bcc bacteria have potentially beneficial ecological and biotechnological applications, including their role in biocontrol, bioremediation, and plant growth promotion. However, their application in environmental settings raises concern, since members of the Bcc are human opportunistic pathogens and have been associated with infection outbreaks due to contaminated pharmaceutical products. These bacteria also can cause disease in plants.

pounds and organic pollutants, including constituents of crude oils, pesticides, phthalates, and solvents, like trichloroethylene (TCE) (3, 8).

Unlike the majority of opportunistic pathogens, members of the Bcc are not prone to commensal carriage, and infections typically are acquired in hospital settings or directly from the environment. In the case of chronically infected CF patients, person-to-person transmission might also occur outside the context of health care facilities (3). In 2002, a soil isolate was proven to be genetically indistinguishable from an epidemic *B. cenocepacia* clone (at the time referred to as *B. cepacia* genomovar III) responsible for widespread infection in CF patients, proving that strains that are pathogenic to humans do not necessarily differ from those found in the environment (49). A number of studies have confirmed the presence of Bcc bacteria in outdoor environments with which people may have regular contact. For example, PCR analysis of DNA extracts from soil and rhizosphere samples, collected from 91 sites located in 3 large U.S. cities (playgrounds, athletic fields, parks, hiking trails, residential yards, and gardens), revealed that around 90% of the samples examined were positive for Bcc (50). Moreover, *B. cenocepacia*, *B. anthina*, *B. multivorans*, and *B. pyrrocinia* strains were isolated from soil and water samples collected in the house and gardens of four CF patients, suggesting that these bacteria are also common in the home environment, possibly acting as a reservoir for infection (51). Therefore, further understanding of the role played by the natural environment in Bcc infection acquisition is of the utmost importance. The positive and negative impacts of the presence of Bcc bacteria in natural and human-made environments are summarized in Fig. 2.

Bcc BACTERIA AS HUMAN OPPORTUNISTIC PATHOGENS

Despite their potential application in bioremediation, the large-scale use of Bcc bacteria deserves attention from the scientific community, especially concerning their release in the environment. This is important, since the multiple bacterial species that

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constitute the Bcc may lead to serious respiratory infections among CF (9–11) and CGD patients (12, 13). Immunocompromised individuals, infants, and the elderly also are susceptible to Bcc infection (14–16). Bcc strains rarely cause respiratory infection in healthy (immunocompetent) individuals, since they are cleared by normal airway mucociliary activity (8). In the case of hospitalized non-CF patients, the most commonly reported risk factors comprise the use of venous and urinary catheters, endotracheal tubes (in mechanically ventilated patients), hemodialysis, and long hospitalization periods in intensive care units (17). Patient-to-patient transmission is thought to occur through spreading of aerosol particles, direct physical interaction with infected people, or upon contact with contaminated surfaces (3).

The accumulation of mucous secretions is recurrent in CF patients' airways due to a mutation in the CF transmembrane conductance regulator (CFTR) gene (52). The resulting lung environment is prone to colonization by Bcc bacteria and other pathogens (52), often presenting selective pressures that induce phenotypic and genotypic variation, conferring evolutionary and adaptive advantage (53). Such adaptation mechanisms might include increased antimicrobial resistance, the development of alternative metabolic pathways, and marked genomic expression reprogramming (53–55).

Although a smaller proportion of CF patients are infected with Bcc than with *Pseudomonas aeruginosa*, they represent a major concern, since the clinical outcome is very unpredictable, even if patients are infected with clonal strains (3, 17). Although the Bcc comprises 24 bacterial species, the two most prevalent among the CF community are *B. cenocepacia* and *B. multivorans* (4, 10, 17). However, the less represented species, in particular, *B. dolosa*, *B. stabilis*, *B. contaminans*, and *B. cepacia*, also may result in poor clinical outcomes (11, 56, 57).

B. gladioli, although not a member of the Bcc, is a very closely related bacterial species and accounts for a significant percentage of opportunistic respiratory CF infections (56). This pathogen also has been identified in CGD (58) and other non-CF patients (59, 60). However, to our knowledge, there are no reports on *B. gladioli*-related nosocomial outbreaks, and contamination of pharmaceutical products with this species has not been registered. Contamination episodes involving the presence of *B. gladioli* have been registered only in the water supply system of a laboratory mouse housing facility (61) and within endotracheal tubes used for mechanical ventilation (59). Unlike Bcc species, *B. gladioli* is highly susceptible to certain antibiotics, such as piperacillin-tazobactam, aminoglycosides, quinolones, carbapenem, imipenem, and ticarcillin-clavulanic acid (59, 62, 63), which might, to some extent, explain the lack of contamination outbreaks related to this species.

Bcc BACTERIAL SURVIVAL IN WATER AND UNDER NUTRIENT-DEPLETED CONDITIONS

Besides being widely distributed in the soil, Bcc bacteria also can survive and proliferate in water-based environments, such as water bodies, lakes, rivers, drinking water, and liquids containing small amounts of nutrients. This characteristic is mainly attributed to the genetic and nutritional diversity of these species, which might be responsible for the ability of certain Bcc bacteria to metabolize the organic matter present in oligotrophic aquatic environments (18).

Examples of Bcc Survival in Water Environments

In a study carried out in the province of Bologna (Italy), *B. cepacia* was found to be present in 3.5% of the eighty-five samples of drinking water that were collected from public and private buildings, growing at a temperature of approximately 24°C (20). Additionally, it was demonstrated that the organic matter from the biofilm that is normally formed around the inner walls of water pipes was probably metabolized by the bacterial cells (20). When twenty-eight water samples, collected from the European rivers Schelde and Leie, were analyzed, ten of them were positive for the presence of Bcc bacteria, namely, *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. vietnamiensis*, and *B. anthina* (19). More recently, the presence of Bcc bacteria in water bodies of the West

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Lake in China was confirmed, where 40% of the 670 bacterial isolates analyzed belonged to five representative Bcc species: *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, and *B. seminalis* (21).

Besides being able to survive and proliferate in water-based environments, Bcc bacteria also have been reported to tolerate substantial temperature variations. Early studies performed on three different strains of *P. cepacia* (now *B. cepacia* or another Bcc species), incubated in distilled water, revealed optimum growth rates at 37°C (64). Nonetheless, high population yields were also observed during long-term incubation at temperatures ranging from 18°C to 42°C (64). Moreover, two other *B. cepacia* strains were able to grow in distilled water at temperatures as low as 10°C and as high as 50°C, even surviving under those conditions for 21 days and 48 h, respectively (64). Another study involving six *B. cenocepacia* strains revealed that all of them were able to proliferate or survive in distilled water for at least 40 days at 18°C and 23°C (18).

Mechanisms of Adaptation to Water and Nutrient Scarcity

Several factors can influence bacterial survival in water, namely, their physiological state, intrinsic tolerance to nutrient scarcity, interaction with other bacteria, and temperature variations (18). However, in the case of Bcc bacteria, the reasons behind their remarkable ability to persist in water environments for long periods of time still have not been thoroughly assessed, and to our knowledge, there are no published studies pertaining to the molecular mechanisms behind Bcc bacterial survival in water. Relevant clues about this topic can be extrapolated only from other bacterial species within the *Burkholderia* genus, namely, *B. pseudomallei*, a pathogen endemic to tropical and subtropical regions and the causative agent of melioidosis disease in humans and animals (65). Despite not being a member of the Bcc, it is the best-studied *Burkholderia* species in terms of survival in water environments. Results suggest that the ability to maintain an intact outer membrane architecture is relevant for *B. pseudomallei*'s prolonged survival in water environments (66). A switch of morphology from typical Gram-negative rods to cocci/coccobacilli and the transition into a viable but nonculturable state were also considered to contribute to *B. pseudomallei*'s survival in distilled water at 25°C for 16 years (67). A recent study also suggested that *P. aeruginosa* is capable of long-term survival in a nutrient-deprived environment by existing in a dormant state (68). During long-term incubation in water, *P. aeruginosa*'s cells were found to exhibit a decreased metabolic activity to convert from a rod to a coccoid shape and to change the membrane lipid composition, leading to decreased outer membrane permeability and consequent increased tolerance to polymyxin B (68). A global expression analysis indicated that the majority of genes were repressed, including those required for DNA replication (68). However, a number of genes had increased expression, in particular, amino acid, fatty acid, and phospholipid metabolism genes, suggesting that these compounds can be used as alternative carbon and energy sources under nutrient starvation (68). Water constitutes a hostile environment, presenting several challenges to bacterial growth, including a lack of nutrients and low osmolarity (66). The fact that Bcc bacteria can persist under such harsh conditions is indicative of their remarkable metabolic capacity and deserves special attention, as they can pose serious threats to public safety and health.

Bcc BACTERIA AS CONTAMINANTS OF PHARMACEUTICAL PRODUCTS

Product Recalls Associated with the Presence of Bcc Bacteria

One of the most commonly reported contaminants of nonsterile pharmaceutical products is *Burkholderia cepacia* (69), whether it was definitively identified as *B. cepacia* (the type species) or was just a member of the *Burkholderia cepacia* complex, since most of the literature available on the topic dates back to the pre-Bcc (or even the pre-*Burkholderia*) era. This is a particular concern for recently described members of the Bcc, which may have been involved in contamination episodes in the past, but their role cannot be recognized due to the deficient taxonomic knowledge at the time. Those contaminants can be transmitted through raw materials, water, and machine surfaces

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used in pharmaceutical manufacturing. According to United States Food and Drug Administration (FDA) recall data, from 1998 to 2006, *B. cepacia* was identified as the cause of 22% of nonsterile product recalls (22). This tendency has grown over recent years, with this species being involved in 34% of the nonsterile product recalls between 2004 and 2011 (22). *B. cepacia* was considered the most common microbial contaminant found in nonsterile products between January and July 2012, corresponding to 39% of the bacterial species isolated from contaminated samples, while other bacterial genera, including *Pseudomonas*, *Staphylococcus*, and *Enterobacter*, were less prevalent within the collected samples (69). Bcc bacteria have been isolated from diverse types of pharmaceutical and personal care products, including nasal sprays (70, 71), multiple lotions and oils (72), water-based products (73), mouthwash (74, 75), cleansing washcloths and baby wipes, preoperative skin solutions, hand sanitizers (76), and gas relief liquid drops (22, 69).

In an epidemiological survey performed by our group between 1995 and 2002, which included patients receiving treatment at the major Portuguese CF center within a central Lisbon hospital, an unusually high prevalence of *B. cepacia* and *B. contaminans* in sputum cultures was detected (57, 71). Later, a market surveillance conducted by INFARMED, the National Authority of Medicines and Health Products, in 2003 and in 2006, detected Bcc bacteria contamination in several batches of nonsterile saline solutions for nasal application, which are often administered to CF patients for inhalant therapy. Further analysis confirmed that the clinical clones and the strains isolated from contaminated saline solutions were indiscernible based on molecular typing methods (57, 77).

Looking at the FDA's list of recalls, market withdrawals, and safety alerts (78) in recent years, several warnings have been launched concerning the presence of Bcc bacteria in a wide variety of products. In October 2016, the FDA, in collaboration with the Centers for Disease Control and Prevention (CDC), detected the presence of *B. cepacia* in the water system used for oral liquid docusate sodium manufacture. This led to a national alert, and the product was voluntarily recalled from the market by its producing pharmaceutical company (79). In 2017, the FDA detected a potential *B. cepacia*-related contamination of decongestant relief syrups for the treatment of cough, cold, and allergies and advised the voluntary recall of the products from the market (80). More recently, in 2018, the FDA detected the presence of several microbial contaminants in homeopathic drug products, including *B. multivorans*, and the recall of all water-based products from the company in question was recommended (81).

Ability of Bcc Bacteria To Survive and Proliferate in Pharmaceutical Products

Sterile pharmaceutical products typically are intended for intravenous administration (e.g., injection or infusion) or to be applied directly in the eyes. Due to the high infection risk associated with administration through these routes, such products must be manufactured in an environment that is free from bacteria, viruses, and other potentially infectious microorganisms (82). Nonsterile products (e.g., docusate, mouthwash, and body wash) are usually administered orally or topically and, theoretically, have a lower potential to cause infection. For that reason, those compounds do not require sterilization before use (83). Nevertheless, microbiological control of nonsterile products is particularly important, since they are more prone to microbial contamination, which can reduce product quality and, above all, cause drug-related infections (84).

Given their ability to use numerous organic compounds as carbon and energy sources, several of them xenobiotics that are very difficult to catabolize (4, 75), Bcc bacteria can grow and proliferate in a wide variety of medicinal drugs (24). One example of such metabolic diversity is their ability to grow on nitroaromatic and aromatic compounds through the oxidation of aromatic structures and breakdown of halogenated compounds by monooxygenases and dioxygenases (76, 77). Since nitroaromatic compounds make up a vast array of pharmaceutical drugs, Bcc bacteria can lead to the degradation of active ingredients and excipients, compromising drug

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stability and purity, as well as their potency and effectiveness (24, 78). Apart from altering the chemical, physical, and organoleptic properties, drug degradation might also lead to the formation of toxic substances (84). This constitutes a serious risk for the consumers of such products, who typically suffer from other pathological conditions. Bcc-contaminated products constitute a vehicle for transmission of these opportunistic pathogens to susceptible individuals, favoring the development of life-threatening chronic infections.

Reasons Underlying Bcc Contamination in Pharmaceutical Settings and Preventive Measures

The persistence of Bcc bacteria in pharmaceutical products is, in part, attributed to the lack of good manufacturing practices. Pharmaceutical companies are responsible for ensuring quality control of their products as well as for monitoring the processing steps and components that are used (26). However, the lack of adequate cleaning procedures; the use of an unsuitable grade of water, associated with poor water control and design systems; the use of the same disinfectants for long periods of time; insufficient microbiological controls; and inadequate testing, specification, and validation guidelines constitute some of the causes of bacterial contamination in pharmaceutical settings (69).

According to the Parenteral Drug Association (PDA) technical report no. 67, *Exclusion of Objectionable Microorganisms from Nonsterile Pharmaceutical and Over-the-Counter (OTC) Drug Products*, *Medical Devices and Cosmetics*, an objectionable organism is defined as a microorganism that can proliferate in a certain pharmaceutical product, causing adverse effects on its chemical, physical, functional, and therapeutic properties (39). This definition also comprises microorganisms with a pathogenic character that, when present in a certain pharmaceutical product in large numbers, can cause infection after administration (22, 39, 40). The high prevalence of product recalls due to contamination with Bcc bacteria and the numerous nosocomial outbreaks verified throughout the years constitute the main reasons why members of the Bcc are considered objectionable organisms (22–24, 62). Moreover, their role as human opportunistic pathogens (affecting both CF and non-CF patients) (3, 17), as well as their resistance to antibiotics and antimicrobial preservatives, also contribute to their inclusion in that category (30, 33, 38, 77).

The U.S. Pharmacopeia (USP) provides several guidelines and methodologies to detect the presence of specific indicator organisms in nonsterile products, namely, the USP guidelines 61, *Microbial Enumeration Tests* (85), and 62, *Tests for Specified Microorganisms* (86). However, despite the fact that Bcc bacteria are widely recognized as objectionable organisms, the USP does not provide any specific test to determine their presence in nonsterile drug products (38, 69). Due to the lack of information and adequate detection methods, Bcc contamination is often disregarded, since these microorganisms are not considered a priority for pharmaceutical manufacturers, who are often unaware of their impact and the consequences that might result from releasing Bcc-contaminated products into the market (38). The concern raised by the undetected presence of Bcc in pharmaceutical products is not a new topic and has been the object of discussion by the FDA since 1981, when the regulatory agency detected irregularities in the validation and control processes of a water deionization system, which resulted in the contamination of a drug product with *B. cepacia* (38, 69). Another example dates back to 1993 and concerns the recall of a metaproterenol sulfate inhalation solution contaminated with *B. cepacia*, which, according to the USP, required no microbial testing before being released onto the market (38, 69). More recently, a chemically preserved oral fexofenadine antihistaminic suspension was found to be contaminated with *B. cepacia* 6 months after the manufacturing process (87). The objectionable organism had not been detected earlier because it was not included in the routine testing described by USP guideline 62 and was not considered a threat, even when its presence had been detected once in the manufacturing company's water system (87). The microbiological counts obtained during product stability testing were

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acceptable for 6 months, after which a considerable increase of CFU numbers was registered. *B. cepacia* was subsequently identified through Gram staining and biochemical assays (87). These case reports highlight the urgent need for pharmaceutical companies and regulatory agencies to work together in order to reformulate the current protocols and legislation regarding the presence of Bcc bacteria in pharmaceutical products.

Presently, the FDA continues to warn pharmaceutical companies about the contamination hazard posed by Bcc bacteria when present in sterile and nonsterile pharmaceutical products, reinforcing the fact that the number of product recalls due to Bcc contamination remains high (88). On May 2017, the regulatory agency provided new regulatory guidance for manufacturers of nonsterile water-based drug products, stating the importance of establishing guidelines to prevent contamination with such objectionable organisms, including correct process design and quality assurance of incoming materials, as well as the monitoring of storage conditions and cleaning procedures (88).

In the specific case of Bcc bacteria, the number of product recalls reported in the literature confirms that antimicrobial preservatives are not effective as a way of preventing contamination, since several strains can proliferate in preserved solutions (38). Therefore, manufacturers cannot rely on the addition of antimicrobial preservatives for contamination control and must instead apply stringent in-process control and guarantee sterile conditions during every step of the manufacturing process instead of testing only the finished products (26, 37, 38). Water is the most commonly used raw material in the pharmaceutical industry and is considered a frequent source of Bcc contamination. In fact, several Bcc outbreaks that occurred throughout the years have identified pharmaceutical-grade water as the root cause of product contamination, whereas contamination of raw materials and inadequate quality control procedures were less implicated in product recalls (37, 38). Therefore, in industrial settings, every source of water should be considered a potential reservoir (38), and every piece of equipment used for processing (tanks, pumps, and filling lines) should be properly cleaned, disinfected, and dried, especially the product contact surfaces (37).

Both the FDA and the USP have provided technical guidelines concerning the water used for pharmaceutical manufacturing. The FDA advises manufacturers inspect the source of water used for wet granulations or aqueous liquid preparations, which should be chemically and microbiologically purified USP water (89). Moreover, potable water might only be used for bulk drug manufacturing and not to prepare USP-compliant dosage-form products or laboratory reagents. Nonpotable water systems, such as cooling water for air conditioning or fire sprinklers, should be correctly identified, and cross-connections with potable water must be avoided (89). Regular collection of water samples from piping systems should be a common practice, since these constitute a major source of water contamination. Maintaining the water circulation systems at constantly high temperatures (typically 80°C) is also advised to prevent significant microbial growth (89). Additionally, during the formulation of aqueous oral and topical dosage-form products, reduced water activity (a_w) is important to prevent microbial contamination and guarantee the product's self-preservation capacity (90). Pharmaceutical products with an a_w below 0.75 are significantly less prone to contamination, while nasal inhalants, hair shampoos, and antacids, which have higher water activities (a_w of 0.99), are very susceptible to contamination by Gram-negative bacteria, namely, Bcc (90).

UNIQUE ABILITY OF Bcc TO OVERCOME BIOCIDE ACTION

Bacterial Resistance to Biocides and Outbreaks Associated with Contaminated Solutions

The term biocide is generally employed to describe a chemical agent that destroys or inhibits the growth or activity of living organisms. While antiseptics are substances with antimicrobial activity that are applied to the skin/living tissue in order to reduce the microbial flora, disinfectants are applied to nonliving objects to destroy harmful

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microorganisms. Preservatives are added to a wide variety of products, some of them not required to be sterile (e.g., medicines, food, and cosmetics), to prevent microbial growth (27, 32). These chemical agents are also employed at different concentrations, depending on whether the purpose is disinfection or preservation. For example, the biocide biguanide chlorhexidine is used for surface disinfection at concentrations in the range of 0.5% to 4% (vol/vol), for antisepsis at 0.02% to 4% (vol/vol), and for preservation at 0.0025% to 0.01% (vol/vol) (91).

Antiseptics and disinfectants (biocides) are widely used in hospital settings as a way of controlling infections. In general, biocides have a broader spectrum of activity and tend to act upon multiple targets, whereas antibiotics usually have specific intracellular targets (27). The most commonly used biocides include alcohol, iodine, iodophores, triclosan, chloroxylenol, chlorohexidine gluconate (CHX), and quaternary ammonium compounds, such as benzalkonium chloride (BZK) (32). One of the most worrying features of Bcc populations is that they can remain viable and/or proliferate within the currently commercialized biocide formulations, displaying low susceptibility to a variety of compounds, including CHX, cetylpyridinium chloride, triclosan, BZK, and povidone (30). Several studies also have demonstrated that the biocide concentration in many commercial products is insufficient to kill Bcc bacteria, especially when they form biofilms. This is the case for CHX, hydrogen peroxide, and BZK (92–94). For example, the typical BZK and CHX concentrations applied in commercial products range from 0.02% (200 μg/ml) to 5% (50,000 μg/ml), which might not be sufficient to eradicate resistant strains (85). Based on susceptibility assays, proper elimination of Bcc bacteria might require the use of biocide concentrations 25 times higher than those currently applied (24).

Most outbreaks have been associated with improper product utilization, namely, the use of contaminated water during manufacturing processes, overdilution of antiseptic solutions, use of outdated products, prolonged storage periods after opening, and improper storage conditions (32, 93). The use of biocides beyond the expiration date is risky, since changes in the chemical concentration or composition are likely to occur, leading to a decreased bactericidal/bacteriostatic potency (93). Contaminated solutions may appear clear and, therefore, are difficult to distinguish from truly sterile solutions (88). The ability of Bcc bacteria to survive and thrive for long periods of time in biocide-containing solutions raises an alarming concern in terms of public health, since they can be transmitted to patients through the use of contaminated products and cause subsequent problems.

The mechanisms of biocide resistance can be intrinsic or acquired through mutation events or horizontal gene transfer (27, 28). Gram-negative bacteria generally have a higher inherent resistance to biocides than Gram-positive bacteria due to the structure of their outer membrane, which acts as a barrier against the entry of several antimicrobial agents. The presence of an intact and highly charged lipopolysaccharide layer is also an intrinsic Bcc characteristic that helps prevent the diffusion of hydrophobic antimicrobials (27, 28). The presence and activation of efflux pump systems is among the major mechanisms of intrinsic biocide resistance. The most relevant family of efflux pumps comprises the resistance-nodulation-cell division (RND) transporters (95). These are usually chromosomally encoded and composed of a periplasmic membrane fusion protein and an outer membrane factor, allowing efflux of solutes across the inner and outer membranes, thereby reducing accumulation in the periplasm (95). The role of these efflux systems has been described for some Bcc species, conferring resistance to BZK and CHX in *B. cenocepacia* (33, 96) and to methylisothiazolinone-chloromethylisothiazolinone (M-CMIT) and fluoroquinolones in *B. lata* (97). Genomic expression analysis of two sequential *B. cenocepacia* isolates recovered from a CF patient undergoing antibiotic therapy revealed an increased transcription of several genes encoding efflux pumps in the highly resistant clonal isolate, namely, those encoding RND transporters, drug efflux pumps of the major facilitator superfamily (MFS), and ABC transporters (54). The same highly antibiotic-resistant isolate displayed

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reduced outer membrane permeability, which was associated with the downregulation of genes encoding porins that may function as channels for the entry of antibiotics (54).

Phenotypic adaptations, such as biofilm growth, also can be considered intrinsic resistance mechanisms. Biofilm formation in Bcc bacteria has been linked to an increased ability to cause persistent infections, especially in CF patients (98, 99). The composition of the biofilm matrix, rich in polymeric substances, might contribute to the development of resistant bacteria by restricting the diffusion of antimicrobial agents (98). However, some compounds, including antibiotics, still can penetrate through biofilms, depending on the nature of both the compound and the biofilm (98, 100). The antimicrobial peptide nisin was able to penetrate through the biofilm structure, causing bacterial death, even though the viability loss was more pronounced in the case of planktonic cells than in biofilm-grown bacterial populations (101). Within the biofilm, the development of a microenvironment characterized by lower nutrient/oxygen availability and reduced bacterial growth is likely to occur together with the production of enzymes that can be involved in the degradation/neutralization of chemicals (27). Overall, biocides like CHX and hydrogen peroxide fail to significantly reduce the viability of sessile cells compared to the effects on planktonic cells (92). The exposure of *B. cenocepacia* biofilms to high levels of oxidizing agents, namely, hydrogen peroxide and sodium hypochlorite, led to the upregulation of several genes, some of which are involved in oxidative stress and general stress responses, as well as others that encode proteins required for the repair of reactive oxygen species (ROS)-induced cellular damage (102). The treatment of *B. cenocepacia* biofilms with CHX also led to the transcriptional upregulation of genes encoding membrane-related and regulatory proteins as well as drug resistance determinants, including sessile-specific RND efflux pumps (96). Bacteria within the biofilms might also resist the action of antimicrobial agents by existing in a slow-growing state, typically characterized by lower antimicrobial susceptibility (98).

Acquired resistance can occur through mutations in genes involved in the biosynthesis of the cell wall, membrane lipids, porins, or outer membrane proteins (OMPs) (28). Horizontal gene transfer may contribute to the acquisition of mobile genetic elements, such as plasmids or genes encoding proteins responsible for modification/degradation of the biocide (e.g., *o*-phosphotransferases, *o*-adenyltransferases, and *n*-acetyltransferases) (28). Another mechanism by which microorganisms can gain biocide resistance is through molecular target alteration. For instance, in *Escherichia coli*, a missense mutation in the FabI protein is sufficient for altering the target site for triclosan, conferring resistance to that compound (28).

Biocides will continue to play an important role in preventing the spread of infection in the health care environment, but it is critical to reevaluate the type of products and the concentrations at which they should be added. For successful biocidal formulations and regulation policies, knowledge about the mechanisms of action and possible development of bacterial resistance is of the utmost importance.

Mechanisms of Benzalkonium and Chlorhexidine Resistance in Bcc Bacteria

BZK and CHX are two of the most common biocides used in health care settings and personal care products, being classified as low-level antiseptics with a broad spectrum of activity against Bcc bacteria (28, 32). BZK is commonly used as an antimicrobial preservative for pharmaceutical products, being the biocide of choice for the majority of multidose aqueous nasal, ophthalmic, and optic products (103). It is a quaternary ammonium compound, composed of a positively charged nitrogen covalently bonded to three alkyl group substituents and a benzyl substituent (34, 104). Bcc-contaminated BZK solutions have been pointed out as the major cause of outbreaks, unlike any other biocide available in the market (32). BZK's effects occur mainly at the level of membrane permeability, starting with its adsorption onto and penetration of the bacterial cell surface, followed by membrane destabilization/disorganization and consequent leakage of cellular constituents, culminating in cell death (27, 104, 105).

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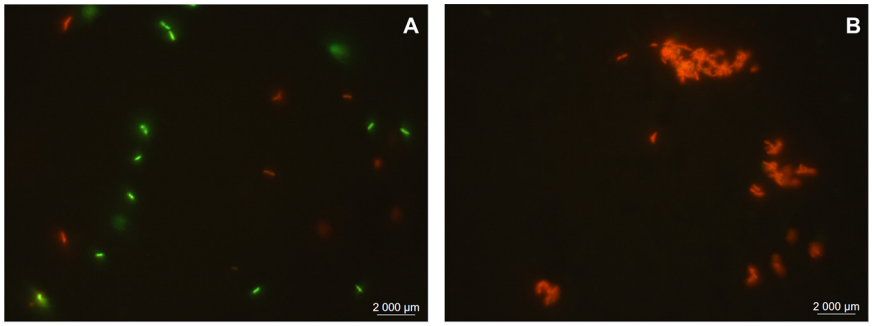

FIG 3 Microscopic observation of cells costained with SYTO9 (green) and propidium iodide (red), showing the effect that the presence of the biocide benzalkonium chloride (BZK) has on *B. cepacia* bacterial viability and aggregation into large clumps that settle on the bottom of the incubation flasks when incubated for several months under conditions mimicking pharmaceutical storage in control saline solution (0.9% [wt/vol] NaCl) (A) or saline solution with 0.05% (wt/vol) BZK (B).

CHX is typically used for hand hygiene and as a component of mouthwash solutions (27, 32, 93). It is a biguanide disinfectant that exerts its effects at the cell membrane level, compromising its structure through the breakdown of associated divalent cations and disruption of lipopolysaccharides, which results in the loss of cytoplasmic components and periplasmic enzymes (93, 96). Several Bcc-related nosocomial outbreaks also have been attributed to the use of contaminated CHX solutions, especially in recent years (106–109).

Early studies identified a *B. cepacia* strain that remained viable for 14 years under nutrient limitation in a saline solution supplemented with 0.05% (wt/vol) BZK (110). More recently, the BZK and CHX susceptibility of 36 different Bcc strains that had been incubated in distilled water for 40 days was assessed through MIC assays and compared to the values at initial inoculation (day zero) (94). Although the 40-day incubation period in distilled water, prior to the addition of the biocides, resulted in a higher susceptibility to both BZK and CHX, six *B. cenocepacia* strains still retained a high level of resistance (94). The same study also reported that clinical strains of *B. contaminans*, *B. multivorans*, *B. vietnamiensis*, and *B. ambifaria* were better recovered after 14 days in the presence of BZK rather than during the first 24 h (94). These observations suggest that BZK was inactivated by the bacteria and was even used as carbon and energy sources for bacterial growth and metabolism (94). Long-term incubation (18 months) with BZK (at 53 μg/ml and 500 μg/ml) also induced structural and organizational alterations in *B. cepacia* cell suspensions incubated in aqueous saline solutions (0.9% [wt/vol] NaCl), including the formation of cellular aggregates, which could be visualized both macro- and microscopically, as evidenced in Fig. 3 (our unpublished results). When the antimicrobial effects of sublethal concentrations of BZK (10 to 50 μg/ml) and CHX (2 to 10 μg/ml) were compared between six *B. cenocepacia* strains, all of them remained viable, exhibiting low susceptibility toward the antiseptics upon incubation at 23°C for 28 days (93). In another study, short-term exposure of a *B. lata* strain to 50 μg/ml of both CHX and BZK resulted in reduced susceptibilities to ceftazidime, ciprofloxacin, imipenem, and, to a lesser extent, meropenem (35). The upregulation of transporter and efflux pump genes, namely, of an outer membrane protein and an ABC transporter, was registered, suggesting that drug efflux plays an important role in reducing the intracellular concentration of these particular antimicrobial peptides (35). Repeated exposure of a *B. cepacia* strain to sublethal concentrations of CHX during 10 passages in the presence of the biocide was reported to induce a 7.3-fold decrease in bacterial susceptibility (111).

An extensive study on the resistance of Bcc bacteria to BZK examined the potential involvement of efflux pumps, the presence of a putative BZK degradation pathway, and the proteome changes induced in response to that biocide. All 20 of the Bcc strains tested were able to partially degrade (4.7 to 42.6% degradation) BZK in a period of

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7 days (33). Quantitative proteomic analysis revealed that treatment with BZK resulted in a rapid alteration in the expression patterns of several proteins at a genome-wide level. Proteins belonging to the major facilitator superfamily and to the RND family, presumably involved in multidrug efflux, were upregulated (33). This suggests an intrinsic ability of *B. cenocepacia* to resist BZK's action, which may involve extrusion of the biocide, catalyzed by efflux pumps, and synthesis of metabolic enzymes involved in its degradation. These two mechanisms appear to work synergistically, accelerating the development of resistance (33). The cleavage of the C-N bond in the early steps of the degradation pathway also resulted in reduced BZK toxicity. Finally, the ability of Bcc bacteria to generate acetyl-coenzyme A from BZK, which can be used in central carbon metabolism, allows its utilization as energy and carbon sources, besides being an effective way of degrading BZK without the accumulation of intermediates or toxic metabolites (33).

DETECTION AND IDENTIFICATION OF Bcc BACTERIA IN PHARMACEUTICAL PRODUCTS OR COMPONENTS

Complexity of the Problem

The correct detection of Bcc bacteria is of extreme importance in pharmaceutical industries, allowing the identification of contamination sources and subsequent implementation of corrective measures. The ideal detection method should be rapid and easy to perform due to the clinical relevance of Bcc organisms and the need to ensure the quality and safety of pharmaceutical products. Since Bcc bacteria are adapted to survive and proliferate in water-based environments, as well as under nutrient limitation, they may not grow as well (or at all) if transferred to nutrient-rich culture media (18, 64). In pharmaceutical industries, microorganisms are subjected to multiple stresses, associated not only with the underlying manufacturing processes but also with the lack of nutrients that characterize the raw materials, air, and water, as well as the temperatures applied for manufacturing and storage procedures, which might be below or above room temperature (26). Bcc bacteria have developed several strategies to cope with environmental stresses, among which is the induction of a viable but nonculturable (VBNC) state, a reversible process in which metabolic activity drops to very low levels and no replication occurs (112, 113). Under these conditions, cells might be viable and maintain their integrity and pathogenic potential but cannot be cultured (112, 113). In addition, bacteria adapted to stressful environments, such as nutrient limitation or the presence of antimicrobial agents, often undergo metabolic and morphologic alterations that might interfere with the processes used to eradicate/control their presence. One example is the development of bacterial subpopulations, designated small-colony variants (SCVs), which are characterized for being approximately one-tenth the size of colonies originating from wild-type bacteria and for growing at a rate nine times lower than that of their progenitor cells (114–116). These SCVs have been identified in the respiratory tract of cystic fibrosis patients after lung transplantation, including representative strains of *B. multivorans*, *B. cepacia*, *B. stabilis*, and *B. vietnamiensis* (116). When grown in petri dishes, the reduced size of the colonies may render their visualization with the naked eye difficult, and sometimes even impossible, if a standardized incubation time is used (Fig. 4) (our unpublished results). Moreover, many Gram-negative pathogens alter their characteristic rod-shaped forms to smaller coccoid-like forms after incubation for days or weeks in nutrient-poor environments (117), as observed during long-term colonization of the CF lungs with *B. cenocepacia* (118). Therefore, during membrane filtration processes, those smaller bacterial forms might be able to pass through the 0.2-μm-pore-size filters, which theoretically retain all bacterial species (26). As a result of the referenced limitations, the presence of Bcc bacteria is frequently overlooked during pharmaceutical manufacturing and in the final products, frequently leading to false-negative results (38). Difficulties in detecting the presence of Bcc bacteria within CF patients' respiratory secretions also arise due to their growth rate being lower than that of other coinfecting microorganisms, which might result in the overgrowth of other bacterial populations,

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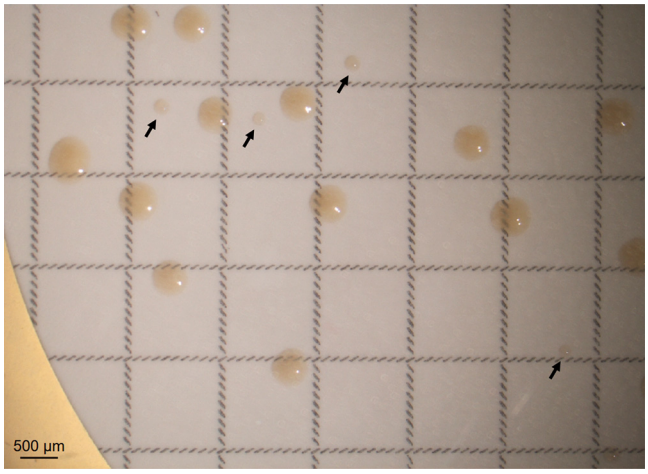

FIG 4 Section of a filter disk corresponding to a *B. cepacia* cell population incubated for several months in a saline solution (0.9% [wt/vol] NaCl) containing benzalkonium chloride (BZK) at 0.05% (wt/vol) under conditions mimicking pharmaceutical storage. The presence of small-colony variants (SCVs), indicated by black arrows, that were absent from the cell population at time zero can be observed on the filter disk following incubation in agar plates (our unpublished results).

masking the presence of Bcc (119). However, the correct detection and identification of Bcc bacteria is of paramount importance for the application of adequate infection control policies as well as to evaluate the most suitable treatment options (120, 121).

Detection and Identification by Phenotypic Methods

Reference laboratories responsible for the quality control of pharmaceutical products typically perform standard phenotypic methods for bacterial isolation and identification (122). In general, these methods rely on enrichment, cultivation, and isolation of microorganisms, requiring long incubation times and extensive manipulation (24). Additionally, traditional cultivation methods and phenotypic assays do not provide enough sensitivity and often underestimate microbial communities, given that some bacteria are not able to grow in certain substrates or do it at such low rates that the incubation periods are not sufficient to allow proper detection (24, 26, 122). Conventional biochemical methods based on catalase, gluconate, malate, phenylacetate, and leucine arylamidase activity are not useful for identification, since Bcc and non-Bcc bacteria cannot be distinguished (123). Many identification errors arise from the phenotypic similarities between *Burkholderia* and other bacterial genera, such as *Cupriavidus*, *Ralstonia*, *Achromobacter*, *Brevundimonas*, *Comamonas*, *Pandoraea*, and *Delfia*, which are frequently misidentified as Bcc species (123, 124). Automated identification systems have also been developed, such as the Vitek MS and the Bruker Biotyper, based on matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (123). This technique consists of the spectral analysis of bacterial ribosomal proteins, which are ionized by laser irradiation of bacterial cells (123). It has a high initial investment cost but provides results in a matter of minutes, as opposed to days, when traditional methods are used (37). Despite the advances registered in this field, further developments still are required for the successful identification of Bcc members, including the development of a database of mass-charge fingerprints, which requires continuous updates to comprise more microorganisms, including Bcc species, which are currently underrepresented (37).

Apart from the correct identification of Bcc bacteria, it is also relevant to know if these contaminants are viable or not. Flow cytometry is a widely used technique that can be useful to characterize bacterial viability by assessing cell membrane integrity (125). Usually, the fluorescent probes SYBR green I (for total cell staining) and propidium iodide (for staining dead cells) are used for nucleic acid costaining. It is a cultivation-independent approach that is particularly useful to detect Bcc bacteria

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residing in a viable but nonculturable state (125). Fluorescence species-specific identification could be achieved by labeling with antibodies that bind specifically to Bcc bacterial cell surface molecules. The combination of flow cytometry with fluorescence species-specific detection, although time-consuming, potentially is useful in the pharmaceutical industry (113).

Detection and Identification by Genotypic Methods

Due to the challenges associated with Bcc bacterial identification using traditional phenotypic methods, the application of molecular methods, based on DNA or RNA analysis, is of great importance for quality control in pharmaceutical industries, providing higher resolution, easier analysis, and faster results (123, 126). PCR-based methods are the most widely used for DNA analysis, and their implementation in quality control laboratories has grown over the years. The first reports of the use of PCR-based assays for bacterial detection in artificially contaminated pharmaceutical products date back to 1998, when Jimenez et al. developed the BAX system, which allowed the detection of *Salmonella enterica* serovar Typhimurium within a 30-h period, instead of the 5- to 6-day period associated with traditional cultivation methods (127). Two years later, the same researchers reported a simplified method with PCR beads, which allowed the detection of bacteria in artificially contaminated pharmaceutical products in 27 h (128). The majority of the genotypic methods are based on single genes, such as *recA* (129), *fur* (130), and *hisA* (131), with *recA* being the most widely used. Bcc bacteria exhibit 94 to 95% similarity in terms of *recA* gene sequences among different species and 98 to 99% between sequences of the same species, making it a good candidate for species identification (129). Despite being commonly used for bacterial identification, the 16S rRNA gene sequence has a high similarity level (98 to 100%) among Bcc species, providing only limited resolution for differentiation at the species level (129). A method for the rapid identification of Bcc species based on differences in the *recA* gene sequence, detected by rapid-cycle PCR and through the use of specific fluorescence resonance energy transfer (FRET) probes, was proposed in 2006 that provided results within an hour (132). An expanded multilocus sequence typing (MLST) for the identification of Bcc species, with PCR primers targeting different housekeeping genes (*atpD*, *gltB*, *gyrB*, *lepA*, *phaC*, *recA*, and *trpB*), was proposed later (133). MLST analysis has proven very useful for epidemiological studies, being able to correctly identify Bcc at the genus level and presenting very good results in terms of species identification (134).

To improve the sensitivity of PCR-based methods for Bcc identification, other approaches have been developed, including seminested PCR (SN-PCR) and real-time PCR (RT-PCR). SN-PCR consists of two PCR rounds with two sets of primers, in which the second round uses one of the primers applied in the first round (135). One hundred randomly collected commercial syrup preparations were analyzed by both SN-PCR and conventional methods. The PCR assay detected the presence of *B. cepacia* in two of the samples that had not been detected by standard approaches, highlighting the advantages of using PCR-based methods (136). RT-PCR also has been used for the identification of *B. cepacia* in pharmaceutical products (137). This technical approach allowed bacterial identification in all of the artificially contaminated samples within 30 h (137). The main advantages of RT-PCR include high sensitivity and accuracy, reproducible data, low contamination risk, time efficiency, low labor intensity requirements, and a lack of the need for post-PCR analysis (138).

In recent years, metagenomic approaches, based on next-generation sequencing, have proven very useful for pathogen detection in clinical laboratories by allowing the identification of virtually all pathogens present in a clinical sample without requiring prior cultivation (139). There are at least two strategies for performing metagenomic studies, including deep amplicon sequencing, which involves PCR-based amplification of a highly conserved taxonomic marker, typically the 16S rRNA gene in the case of bacteria, or shotgun metagenomics, where total metagenomic DNA first is extracted and sequenced by a next-generation platform, followed by its assembly and annotation

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(139, 140). This approach does not require prior knowledge of microbial identity (139). The main advantages of metagenomics in these settings include quick pathogen identification and the ability to detect less abundant pathogens (139). The generation of complete genome assemblies also allows thorough microorganism characterization, contributing to the development of novel diagnostic methods and the prevention of future contamination outbreaks (139). To our knowledge, there are no current reports on the use of metagenomics for the detection of Bcc bacteria in pharmaceutical or clinical settings, but this approach has successfully detected the presence of bacteria from the *Burkholderia* genus in stream water samples of a polluted urbanized area in São Pedro, Brazil (141).

CONTAMINATION OUTBREAKS IN HEALTH CARE SETTINGS AND CONTROL MEASURES

Throughout the years, a growing body of evidence has confirmed the impact of Bcc bacterial outbreaks, with multiple case studies being reported in order to identify the sources of contamination and their implications in clinical settings. The majority of the cases in which Bcc bacteria have been detected in clinical samples have an environmental origin, typically from contaminated pharmaceutical/personal care products. Contamination of medical products can be classified as extrinsic, when it is introduced during product use, or intrinsic, meaning that the product was already contaminated before use. The easy cross-transmission of Bcc bacteria is particularly worrying, since it facilitates the rapid spreading of infections between different wards within the same facility or even between hospitals situated in different regions of a country (142). The types of products identified as sources of Bcc contamination are diverse, including anesthetic eye drops (143), mouthwash solution (75), fentanyl infusion (144), ultrasound gel (145), antiemetic drugs (15), chlorhexidine solutions (106, 108, 109), liquid soap (76), caffeine citrate (146), distilled water (73), dextrose solutions (147), catheters (16), liquid docusate (148, 149), filters and water oxygen humidifiers (150), washing gloves (151), ventilators (152), and saline flush syringes (153) (Table 1).

Outbreaks and sporadic failures associated with biocides also may be due to user error rather than microbial contamination itself. Common errors include the use of overdiluted solutions and outdated products, the use of tap water to prepare biocide solutions, refilling of small-volume dispensers from large-volume stock containers, and improper product selection (38). Nonsterile products, especially multidose containers, should be handled with extra care, since most of them lose physical integrity after being opened for the first time, becoming more prone to microbial contamination (87). Training of end-users and hospital personnel health care settings should be indispensable (38). Concerning infection control and prevention, there are certain measures that should be followed to minimize the risk of cross-contamination, especially among CF patients. Although associated with anxiety and psychosocial consequences, the segregation of Bcc-positive patients has contributed to a reduction of *B. cepacia* complex-related infections. Additionally, regular microbiological surveillance in clinical settings should include examination for Bcc organisms. Bcc-infected patients should attend different clinics according to the strains they are infected with to avoid superinfections (154). Good hygiene practices are mandatory, including handwashing, disinfection with alcohol rubs, and the use of disposable gloves. Extra care should be taken when handling patient samples, such as sputum specimens and throat swabs. Patients should have well-ventilated, single rooms, and any medical procedure involving respiratory function tests, nebulizations, and airway clearance should be carried out in a separate room (154).

CONCLUSIONS AND PERSPECTIVES

Pharmaceutical products contaminated with Bcc bacteria constitute a serious risk for susceptible patients, particularly those suffering from CF and CGD. Bcc infection also has been reported in immunocompromised individuals (e.g., cancer patients submitted to chemotherapy, HIV/AIDS patients, mechanically ventilated patients, and infants/the

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TABLE 1 *Burkholderia cepacia* complex-related outbreaks[a]

Species	Product	Location	Time period	Patient information	No. of patients affected	Reference
Bcc bacteria	Anesthetic eye drops	Madurai, India (Aravind Eye Hospital)	December 2011–February 2012	Patients who underwent cataract surgery and developed acute postoperative endophthalmitis	13	143
B. cepacia	Intrinsically contaminated alcohol-free mouthwash solution	General medical intensive care unit, Vozandes Hospital, Quito, Ecuador	March 2011–May 2012	Ventilated; 3 developed ventilator associated-pneumonia, non-CF patients	13	75
B. contaminans	Continuous fentanyl infusion prepared by institutional compounding pharmacy	Duke University Hospital, Durham, NC	31 August–6 September 2012	1 Child, 6 adults, anemia, cirrhosis, hepatic failure, trauma, HIV, infectious endocarditis, peritonitis	7	144
B. cepacia	Ultrasonic couplant	Hospital in Zhongshan, China	May 2013	Post-C-section patients	4	155
Bcc bacteria	Antiemetic drug granisetron	Mumbai, India (daycare unit of private hospital)	End of 2009	Cancer patients with tunneled catheters	13	15
Bcc bacteria	0.5% Chlorhexidine solution	Seoul, Republic of Korea (Boramae Medical Centre)	10 October–16 December 2013	NR	40	106
B. stabilis, *B. contaminans*, and *B. ambifaria*	Intrinsically contaminated ultrasound gel	Neonatal and adult intensive care units, Argentina	April–July 2013	7 Preterm neonates with respiratory distress, 3 ICU patients who had undergone recent cardiovascular surgery, 1 general ward patient	11	145
B. stabilis	NR[b]	500-Bed tertiary-care hospital in Beijing, China, ophthalmology and otolaryngology	19 March–20 September 2013	Chronic bacterial or fungal sinusitis	53	156
B. cepacia	NR	Hemodialysis center, La Linea de la Concepción, Cadiz, Spain	November 2013–February 2014	NR	7	157
B. cepacia and *B. contaminans*	Nonsterile saline solutions for nasal application	CF care unit of a Lisbon central hospital	2003–2005	CF patients		57, 77
Bcc bacteria	Liquid soap	Near East University Hospital, Nicosia, Cyprus	November 2013	Sickle cell anemia	1	76
B. cepacia	Caffeine citrate	Neonatal intensive care unit, India	September–October 2015	Preterm infants	7	146
B. cepacia	Intravenous solutions of 5% dextrose, normal saline (opened bottles), and continuous-positive-airway-pressure humidifier water	Neonatal intensive care units of 2 hospitals, India	January–March 2014	Neonates, 10 preterm, 11 showed signs of sepsis	12	147
Bcc bacteria	Distilled water	Pediatric intensive care unit, Father Muller Medical College, India	NR	Pediatric patients, 1 with subaortic ventricular septal defect, 1 with rhabdomyosarcoma, on chemotherapy, 1 with pneumonia and pneumothorax	3	73
Bcc bacteria	Central venous catheter, pneumonia	U.S. Veterans Health Administration medical centers	1 January 1999–31 December 2015	Non-CF, advanced age, chronically ill, severe disease	248	16
Bcc bacteria	Liquid docusate	629-Bed, tertiary-care, pediatric hospital in Houston, TX	February 8–July 4, 2016	Pediatric, non-CF	24	148
Bcc bacteria	NR	Urology clinics, Jordan	NR	3 Uncomplicated infections + 1 recurrent infection	4	158
B. cepacia	Flow filter, hemodialysis water, water-oxygen humidifier	Hemodialysis unit, Regional Hospital Coronel Oviedo, Paraguay	November 2013–February 2014	NR	NR	150

(Continued on next page)

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TABLE 1 (Continued)

Species	Product	Location	Time period	Patient information	No. of patients affected	Reference
B. cenocepacia	Gel packaged in sachets for use within the sterile ultrasound probe cover	4 Hospitals across Australia	26 March 2017–7 April 2017	Patients with significant preexisting morbidities	11	159
B. stabilis	Commercially available washing gloves	9 Institutions in Switzerland	May 2015–August 2016	Bloodstream and non-bloodstream infections	46	151
B. cepacia	Ventilators	General 1,600-bed hospital, Beijing, China	1–14 June 2015	Ventilator-associated pneumonia	4	152
Bcc bacteria	Rubber stopper of sealed multidose amikacin injection vials	Pediatric ICU and pediatric ward of a tertiary-care hospital, India	June 2012–January 2013	Children with peripheral interavenous catheters	76	160
B. cepacia	Ultrasound probe gel	Referral hospital in Riyadh, Saudi Arabia	8 January–15 June 2016	Non-CF patients	15	161
Bcc bacteria	Oral liquid docusate sodium	Pediatric intensive care unit, The Johns Hopkins Hospital, Baltimore, MD	19 May 2017–30 July 2017	Infants with multiple chronic medical conditions	8	149
Bcc bacteria	Octenidine mouthwash solution	Cardiothoracic intensive care unit in Germany	August–September 2018	Critically ill, postcardiac surgery patients	3	74
Bcc bacteria	Saline flush syringes	59 Nursing facilities in 5 U.S. states	September 2016–January 2017	NR	162	153
B. lata	Chlorhexidine mouthwash	2 Tertiary-care hospitals in Australia	May–June 2016	Intensive care patients	8	107
Bcc bacteria	Prefilled syringe containing liquid docusate	16 Facilities in USA	February 2016	Infants, children, adults and older adults 80 mechanically ventilated and 41 with feeding tubes	108 (63 confirmed and 45 suspect cases)	162
B. cepacia	Intrinsically contaminated commercial 0.5% chlorhexidine solution	600-Bed university-affiliated teaching hospital in South Korea	November 2014–January 2015	Infants, mostly preterm, with respiratory distress syndrome	21	108
B. cepacia	4% Chlorhexidine aqueous body wash	Peritoneal Dialysis Unit, Middlemore Hospital, Auckland, New Zealand	NR	Dialysis patients with peritoneal catheters	9	109
B. cepacia	NR	Tertiary-care hospital, Turkey	2013–2018	NR	46	163
B. cepacia	NR	Neurotrauma critical care unit of a level 1 trauma care center in India	August–November 2014	Mostly patients with head and spinal trauma	48 (15 of which with central line-associated bloodstream infections)	164

[a]*Burkholderia cepacia* complex-related outbreaks that have occurred in different settings and the types of products identified as sources of Bcc contamination, as described in the literature published during the past 5 years. The majority of the contaminations are attributed, in the corresponding publications, to Bcc in general, with no information at the species level, or to the type species *Burkholderia cepacia*. However, since a significant number of reports predate the year in which each Bcc species has been described, the impact of the more recently described species in the contamination outbreaks is currently unknown. The 24 species that currently comprise the *Burkholderia cepacia* complex (and the year of each species description in the literature [41–45, 153, 154, 165–171]) are the following: *B. alpina* (2017), *B. ambifaria* (2001), *B. anthina* (2002), *B. arboris* (2008), *B. catarinensis* (2017), *B. cenocepacia* (2003), *B. cepacia* (1950), *B. contaminans* (2009), *B. diffusa* (2008), *B. dolosa* (2004), *B. lata* (2009), *B. latens* (2008), *B. metallica* (2008), *B. multivorans* (1997), *B. pseudomultivorans* (2013), *B. paludis* (2016), *B. puraquae* (2018), *B. pyrrocinia* (2002), *B. seminalis* (2008), *B. stabilis* (2000), *B. stagnalis* (2015), *B. territorii* (2015), *B. ubonensis* (2000), and *B. vietnamiensis* (1995).
[b]NR, no result.

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elderly). Over recent years, both sterile and nonsterile pharmaceutical products have been recalled from the market due to Bcc contamination and subsequent nosocomial outbreaks. The difficulties in terms of detection and correct identification of Bcc bacteria by traditional cultivation techniques or even by genotypic methods, their capacity to grow under conditions of low nutrient availability, and an inherent resistance to chemical preservatives (that can even be used as carbon and energy sources) reinforces their potential to cause disease, hampering the application of adequate infection control policies and therapeutic approaches. Since water is the most common source of this contaminant, aqueous pharmaceutical products represent a particular risk for spreading infection. The negative impact of Bcc bacterial contamination in clinical settings and their prevalence as the number one contaminant in pharmaceutical industries has led authorities, namely, the United States Food and Drug Administration (FDA), to propose the inclusion of these bacteria in the "Objectionable Microorganisms" category. Therefore, it is essential and urgent to prevent Bcc contamination in pharmaceutical manufacturing settings, including the raw materials and finished products, and to develop efficient methods to detect and correctly identify members of this complex when their presence is suspected.

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Handwashing sinks as reservoirs of carbapenem-resistant *Acinetobacter baumannii* in the intensive care unit: a prospective multicenter study

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Introduction: The extent to which sinks are contaminated by carbapenem-resistant *Acinetobacter baumannii* (CRAB) in intensive care units (ICUs) and the association between these contaminated sinks and hospital-acquired CRAB infections during the non-cluster period remains largely unknown. Here, we performed a prospective multicenter study in 16 ICUs at 11 tertiary hospitals in Chengdu, China.

Methods: We sampled sinks, collected CRAB clinical isolates, and conducted whole-genome sequencing and analysis.

Results: A total of 789 swabs were collected from 158 sinks, and 16 CRAB isolates were recovered from 16 sinks, resulting in a contamination rate of 10.16%. Twenty-seven clinical isolates were collected during the study period. The majority (97.67%, 42/43) of the CRAB isolates belonged to ST2, and 36 (83.72%) of them had both bla_{OXA-23} and bla_{OXA-66}. The 43 strains belonged to 12 clones. One certain clone caused multiple contaminations of seven sinks in one GICU. Two clones of ST2 bla_{OXA-23} and bla_{OXA-66}-carrying sink strains were likely the sources of the two clusters in the two GICUs, respectively. Five ST2 bla_{OXA-23}-carrying isolates were found to be common clones but were recovered from two hospitals.

Conclusion: The contamination rate of CRAB in handwashing sinks is high in some local ICUs, and the contaminated sinks can serve as environmental reservoirs for CRAB clusters.

KEYWORDS

carbapenem-resistant *Acinetobacter baumannii*, intensive care units, handwashing sinks, environmental reservoir, outbreak source

1 Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a common healthcare-associated infection (HAI) pathogen and has been classified as a critical priority by the World Health Organization due to the urgent need for new antibiotics to combat it (1). A systematic review conducted by Antimicrobial Resistance Collaborators showed that the CRAB contributed to 57,700 deaths globally in 2019 (2). In the intensive care unit (ICU), CRAB is particularly common and is mainly responsible for HAIs and hospital outbreaks (3–5), causing a wide range of infections, including ventilator-associated pneumonia and bloodstream infections (6–8).

The most common mechanism by which CRAB strains exert carbapenem antibiotic resistance is the production of oxacillinase (OXA)-type carbapenem-hydrolyzing class D β-lactamases, which are encoded by intrinsic $bla_{\text{OXA-51-like}}$ genes (e.g., $bla_{\text{OXA-51}}$, $bla_{\text{OXA-64}}$, $bla_{\text{OXA-65}}$, $bla_{\text{OXA-66}}$, and $bla_{\text{OXA-98}}$,) and/or acquired genes, such as $bla_{\text{OXA-23-like}}$ (e.g., $bla_{\text{OXA-23}}$, $bla_{\text{OXA-27}}$, $bla_{\text{OXA-49}}$, and $bla_{\text{OXA-225}}$), $bla_{\text{OXA-24/40-like}}$ (e.g., $bla_{\text{OXA-24}}$, $bla_{\text{OXA-25}}$, $bla_{\text{OXA-26}}$, $bla_{\text{OXA-40}}$, and $bla_{\text{OXA-72}}$), $bla_{\text{OXA-58-like}}$ (e.g., $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-164}}$), $bla_{\text{OXA-235-like}}$ (e.g., $bla_{\text{OXA-235}}$ and $bla_{\text{OXA-237}}$), and $bla_{\text{OXA-143-like}}$ genes (9–12). The most prevalent acquired carbapenemase gene reported is $bla_{\text{OXA-23}}$, followed by $bla_{\text{OXA-24/40}}$ (12–16). In addition, class B metal β-lactamase genes (e.g., bla_{NDM}, bla_{VIM}, and bla_{IMP}) have also been reported to exist in a very small number of CRAB strains (9, 10).

Handwashing sinks play an important role in infection prevention and control systems and are common in all hospitals, including in the ICU. In China, a multicenter investigation conducted in 14 provinces showed that the total installation rate of handwashing sinks increased from 69.30% in 2010 to 77.20% in 2016 in 200 hospitals (17). However, handwashing sinks can be contaminated by multi-drug-resistant organisms and cause outbreaks in hospitals (18). Previous studies have identified that certain parts of handwashing sinks, such as sink traps and sink splashing (19, 20), sink bowls and drains (21, 22), faucet aerators (23), and water taps (24), can be contaminated by CRAB during the outbreak period.

However, few studies have focused on the level of handwashing sink contamination by CRAB in different ICUs during the non-outbreak period, and the degree to which contaminated handwashing sinks are associated with hospital-acquired infections in CRAB remains largely unknown. In this study, we conducted a prospective multicenter investigation across 11 hospitals in Chengdu, with the following three objectives: (1) to evaluate the prevalence of CRAB contamination in the sinks of local ICUs during a non-outbreak period; (2) to characterize the carbapenem-resistant genes and molecular epidemiological features of CRAB strains isolated from infected patients and from handwashing sinks in these ICUs; and (3) to assess the potential role of CRAB-contaminated sinks in contributing to patient infections during the non-outbreak investigation period.

2 Materials and methods

2.1 Study setting and design

This multicenter prospective investigation was performed in 16 ICUs at 11 hospitals in Chengdu, Sichuan Province, China. The 16 ICUs included 9 general ICUs (GICUs, with a total of 279 beds and 103 handwashing sinks) and 7 neonatal ICUs (NICUs, with a total of 282 beds and 55 handwashing sinks) (Table 1). From March 2019 to August 2019, all handwashing sinks in the included ICUs were sampled once to screen for CRAB. The clinical CRAB isolates recovered from patients were collected between 2 weeks before and 3 months after the date of sampling in ICUs. If one or more sinks were positive for CRAB, the clinical isolates from this ICU or from other participating ICUs at the same hospital were recovered and transferred to West China Hospital for identification again using the Vitek II automated system (bioMérieux, Marcy l'Etoile, France). All the isolates were subjected to whole-genome sequencing (WGS) to detect homology.

The study was approved by the Ethical Committee of the West China Hospital, and the need to obtain informed consent was waived.

2.2 Sample sites and sampling methods

The sample points included the surfaces, faucets, bowls, drains, overflows, and drain holes of the handwashing sinks. A total of 100 cm^2 of the sink surface and the bowl were sampled using sterile rayon swabs (Copan, Brescia, Italy) moistened with tryptic soy broth (TSB, Hopebio, Qingdao, China). A 5 × 5 cm caliper was used to confirm the sampling area. The sample points for the faucet included a surface area of 5 cm from the outer wall of the water outlet and 3–5 cm from the inner wall. For the bubbled faucets, the bubbler was removed, and the samples were sampled using sterile rayon swabs. The drain holes were sampled 3–5 cm below the drain by the swabs. All the swabs were immediately placed into 15 mL sterile tubes containing 6 mL of TSB.

2.3 Microbiological methods

All the tubes were incubated at 37°C overnight and centrifuged as described in our previous study (25). The supernatant was subsequently discarded, and the precipitants were resuspended in 1 mL of TSB. A 50 µL suspension was inoculated onto *Acinetobacter* selected agar plates (CHROMagar™, Paris, France) containing 4 µg/mL meropenem to screen for CRAB. Isolates that were pink, round, smooth, raised, and moist on the selected agar plates were suspected and identified using a matrix-assisted laser desorption/ionization–time of flight mass spectrometry (Bruker, Billerica, Massachusetts). The minimum inhibitory concentrations (MICs) of meropenem were determined using the broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI) (26).

WGS was performed for all identified isolates using the Illumina HiSeq X10 platform (Illumina, San Diego, California). Subsequent reads were assembled into contigs using SPAdes version 3.14.0 (27). Precise species identification was established based on average nucleotide identity between the strains and type strains of *Acinetobacter* species using JSpeciesWS (28). The sequence of the STs was determined using the genome sequence to query the multilocus sequence typing database.[1] Antimicrobial-resistant genes were identified using ResFinder.[2] The draft genomes of the strains have been deposited in GenBank under BioProject number PRJNA977829. Single-nucleotide polymorphisms (SNPs)

1 https://pubmlst.org/bigsdb?db=pubmlst_abaumannii_seqdef
2 http://genomicepidemiology.org/

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TABLE 1 Characteristics of the participating ICUs and CRAB isolates from sinks and clinical samples.

Hospital	ICU no[a]	ICU beds	Sink no.	Positive sinks (%)	CRAB clinical isolate no.
Chengdu First People's Hospital	1G	60	13	2(15.4)	16
	1N	36	8	0(0)	0
Chengdu Second People's Hospital	2G	22	11	7(63.6)	4
	2N	20	4	1(25.0)	0
The First Affiliated Hospital of Chengdu Medical College	3G	28	11	4(36.4)	4
Sichuan Provincial People's Hospital	4G	32	9	2(22.2)	3
	4N	45	15	0(0)	0
Affiliated Hospital of Chengdu University	5G	22	10	0(0)	…
	5N	16	4	0(0)	…
Chengdu Public Health Center	6G	16	16	0(0)	…
Chengdu Women and Children's Hospital	7N	80	6	0(0)	…
Sichuan Women and Children's Hospital	8N	80	16	0(0)	…
Sichuan Integrative Medicine Hospital	9G	25	15	0(0)	…
	9N	5	2	0(0)	…
Chengdu Third People's Hospital	10G	24	4	0(0)	…
West China Hospital, Sichuan University	11G	50	14	0(0)	…
Total	16	561	158	16(10.1)	27

CRAB, carbapenem-resistant *Acinetobacter baumannii*; ICU, intensive care unit.
[a]In this column, G indicates general ICU; N indicates neonatal ICU; no sink was positive for CRAB at the participating ICUs and not to collect CRAB clinical isolates.

among the same ST strains were analyzed using Snippy version 4.6.0.[3] Recombination regions were predicted using Gubbins version 2.4.1 (29) under the GTRGAMMA model with a maximum of 50 iterations. A cutoff of equal to or less than 11 high-quality SNPs was used to define clones within the same ST strains, as described in a previous study (30).

3 Results

3.1 The contamination of sinks by CRAB

In total, 789 swabs were collected from 158 handwashing sinks in 16 ICUs at 11 hospitals, and 16 CRAB isolates were recovered from 16 handwashing sinks in 5 ICUs at 5 hospitals (Table 1). The total contamination rate of CRAB in the handwashing sink was 10.16% (16/158, 95% CI 5.40–14.83%). The contamination rate in different ICUs ranged from 0 to 63.6%, and the sink contamination rate in the GICU was 15.29% (95% CI 7.75–21.38%), while it was 3.57% (95% CI 0.0–5.35%) in the NICU. Of the 16 sink CRAB isolates, 15 were recovered from the GICUs and belonged to ST2 (12 isolates carrying both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$ carbapenemase genes, 1 isolate harboring both $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-98,}}$ and 2 isolates carrying only $bla_{\text{OXA-23}}$). The remaining CRAB isolate was recovered from a NICU and belonged to ST203, and this isolate harbored both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$ (Table 2). The 16 sink isolates could be assigned to 6 clones.

3.2 Characteristics of clinical isolates

A total of 27 CRAB clinical isolates were recovered from 4 of the 5 ICUs with CRAB-contaminated sinks. The vast majority of the 27 CRAB clinical isolates were recovered from sputum ($n = 21$, 77.8%), whereas the remaining were from bronchoalveolar lavage fluid (BALF, $n = 5$, 18.5%) and cerebrospinal fluid ($n = 1$, 3.7%). All of the clinical isolates belonged to ST2. Twenty-four of them harbored both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$ carbapenemase genes, while the other three carried only $bla_{\text{OXA-23}}$ (Table 2). The 27 clinical isolates could be assigned to 12 clones.

3.3 Diverse clonal backgrounds of CRAB isolates and sink as a source of transmission in a GICU

A total of 18 ST2 CRAB isolates were recovered from ICU 1G, including 2 isolates from two sinks, 15 isolates from sputum, and 1 isolate from BALF. The 18 isolates could be assigned to 6 clones. Seven clinical isolates recovered from sputum, along with one sink isolate (090698), harbored both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-6}}$. These strains shared 1–7 SNPs, and therefore belonged to a common clone (clone 2b) (Figure 1), indicating an outbreak. Four (090713, 090714, 090715, and 090736) of these seven clinical isolates were recovered before the sink sampling date, and the other three clinical isolates (090739, 090740, and 090745) were recovered after the sink sampling date. These three isolates shared smaller SNPs (2, 4, and 2, respectively) with the clinical isolate (090713) than with the sink

3 https://github.Com/tseemann/snippy

TABLE 2 CRAB recovered from sinks and clinical samples.

Isolate	ICU[a]	ST	Clone[b]	Carbapenemase	Meropenem MIC, mg/L	Source
090737	1G	2	2a	OXA-23	64	Sputum
090738	1G	2	2a	OXA-23	64	Sputum
090698	1G	2	2b	OXA-23, OXA-66	32	**Sink**
090713	1G	2	2b	OXA-23, OXA-66	32	Sputum
090714	1G	2	2b	OXA-23, OXA-66	32	Sputum
090715	1G	2	2b	OXA-23, OXA-66	64	Sputum
090736	1G	2	2b	OXA-23, OXA-66	32	Sputum
090739	1G	2	2b	OXA-23, OXA-66	32	Sputum
090740	1G	2	2b	OXA-23, OXA-66	32	Sputum
090745	1G	2	2b	OXA-23, OXA-66	64	Sputum
090697	1G	2	2c	OXA-23, OXA-66	64	**Sink**
090742	1G	2	2c	OXA-23, OXA-66	64	Sputum
090747	1G	2	2c	OXA-23, OXA-66	64	Sputum
090748	1G	2	2c	OXA-23, OXA-66	64	BALF
090741	1G	2	2d	OXA-23, OXA-66	32	Sputum
090743	1G	2	2d	OXA-23, OXA-66	32	Sputum
090746	1G	2	2e	OXA-23, OXA-66	64	Sputum
090744	1G	2	2f	OXA-23, OXA-66	32	Sputum
090685	2G	2	2g	NDM-1, OXA-98	64	**Sink**
090686	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090688	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090690	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090693	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090695	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090696	2G	2	2g	OXA-23, OXA-66	64	**Sink**
090718	2G	2	2g	OXA-23, OXA-66	64	Sputum
090767	2G	2	2g	OXA-23, OXA-66	64	CSF
090768	2G	2	2g	OXA-23, OXA-66	64	Sputum

(Continued)

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TABLE 2 (Continued)

Isolate	ICU[a]	ST	Clone[b]	Carbapenemase	Meropenem MIC, mg/L	Source
090766	2G	2	2h	OXA-23, OXA-66	32	Sputum
090694	2 N	203	…	OXA-23, OXA-66	64	**Sink**
090700	3G	2	2i	OXA-23, OXA-66	32	**Sink**
090701	3G	2	2i	OXA-23, OXA-66	64	**Sink**
090717	3G	2	2i	OXA-23, OXA-66	64	Sputum
090749	3G	2	2i	OXA-23, OXA-66	64	BALF
090750	3G	2	2i	OXA-23, OXA-66	32	BALF
090699	3G	2	2j	OXA-23, OXA-66	32	**Sink**
090703	3G	2	2j	OXA-23, OXA-66	32	**Sink**
090716	3G	2	2k	OXA-23, OXA-66	64	BALF
090704	4G	2	2a	OXA-23	64	**Sink**
090705	4G	2	2a	OXA-23	64	**Sink**
090769	4G	2	2a	OXA-23	32	Sputum
090770	4G	2	2L	OXA-23, OXA-66	64	Sputum
090771	4G	2	2m	OXA-23, OXA-66	64	BALF

BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; ICU, intensive care unit; MIC, minimum inhibitory concentration; ST, sequence type; CRAB, carbapenem-resistant *Acinetobacter baumannii*. Strains from sinks are highlighted in bold.

[a]ICU designation is available in Table 1.

[b]ST number and clone designation (e.g., 2a representing clone a of ST2).

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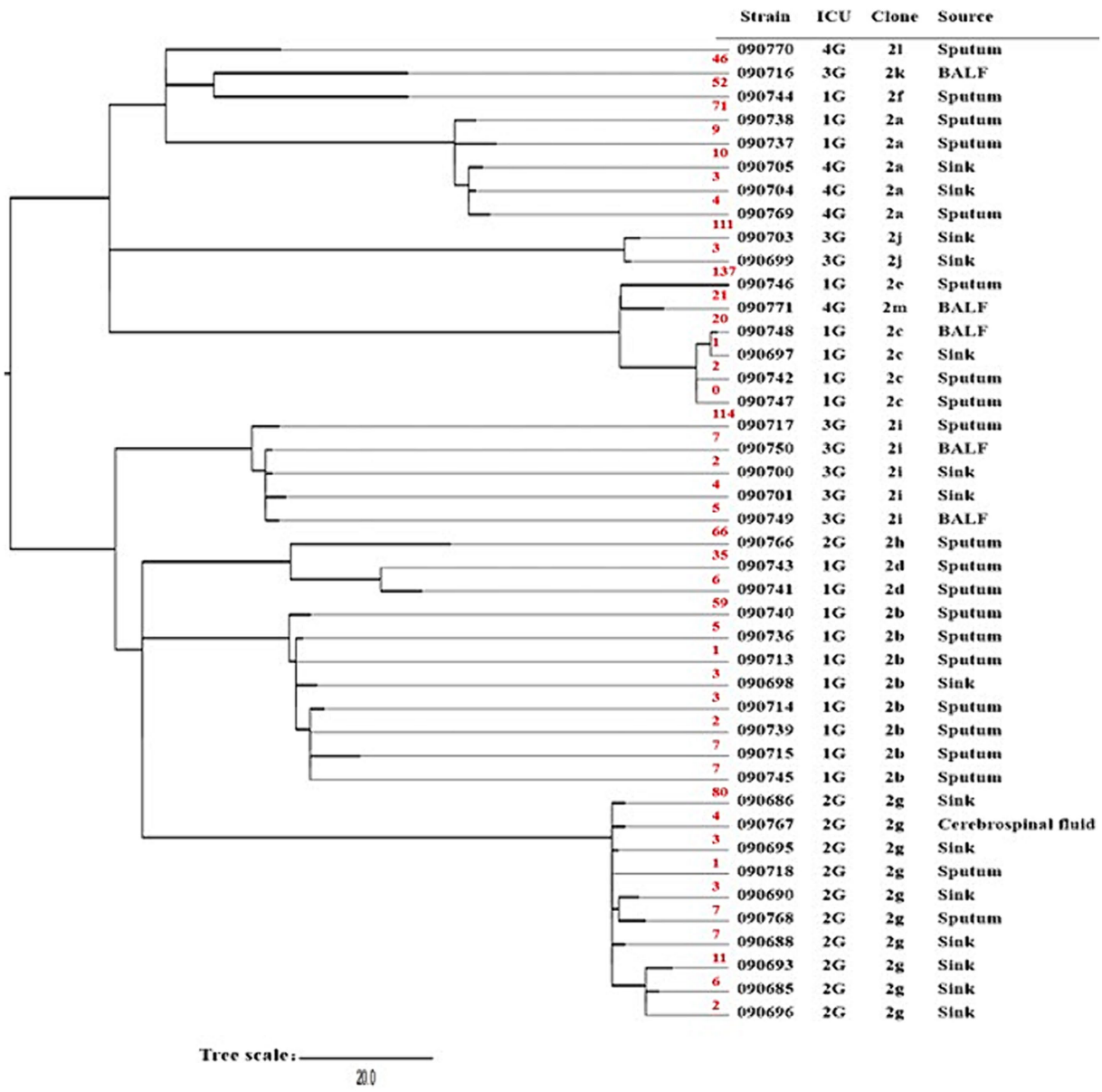

FIGURE 1
Phylogenetic trees of sequence type (ST) 2 carbapenem-resistant *Acinetobacter baumannii* strains. Shown are the strain name, the ICU where the strain was recovered (see Table 1), and the clone to which the strain belonged (see Table 2). The number of single-nucleotide polymorphisms between the adjacent two strains is indicated in red. Abbreviations: BALF, bronchoalveolar lavage fluid; ICU, intensive care unit.

isolate (5, 7, and 5, respectively); therefore, the sink isolate (090698) was likely to be contaminated but not the exact route of transmission.

Another isolate (090697) from another sink shared 0–2 SNPs with three clinical isolates (two isolates were recovered from sputum, and one was recovered from BALF) and therefore belonged to a common clone (clone 2c) (Figure 1), also indicating an outbreak in the ICU 1G. All three clinical isolates were recovered at a later date than the sink sampling date; therefore, the sink was likely to be the route of infection transmission. However, the two sink isolates in this ICU shared 117 SNPs, indicating that they were assigned to two strains. The remaining six clinical isolates belonged to four clones but were not the same as the two sink isolates from ICU 1G.

3.4 A clone of ST2 bla_{OXA-23} carrying CRAB transmission in two GICUs

There were five isolates (two clinical isolates from ICU 1G, one clinical isolate, and two sink isolates from ICU 4G) that only carried the bla_{OXA-23} gene and shared 3–10 SNPs (clone 2a) (Figure 1), suggesting interhospital transmission. These two sink isolates (090704 and 090705) were recovered approximately 3 months prior to the clinical isolate (090769); therefore, the sinks were likely to constitute the route of patient transmission. Although the two clinical isolates (090704 and 090705) from ICU 1G were recovered approximately 1 month prior to the two sink isolates from ICU 4G, the exact route of interhospital transmission remains unclear.

3.5 A high contamination rate of CRAB in the NICU and GICU at a hospital

There were 8 (7 from ICU 2G and 1 from ICU 2N) of the 15 sinks recovered CRAB isolates with a CRAB contamination rate as high as 53.3% in hospital 2. One sink isolate (090694) recovered from ICU 2N belonged to ST203, but no clinical isolate was obtained during the period of the study in this ICU. Therefore, ST203 CRAB was not a common cause of infection among patients. Seven sink isolates (six harboring both bla_{OXA-23} and bla_{OXA-66} and one isolate harboring both bla_{NDM-1} and bla_{OXA-98}) and three clinical isolates (two recovered from sputum and one recovered from cerebrospinal fluid) recovered from ICU 2G belonged to ST2, sharing 1–11 SNPs and belonging to a common clone (clone 2g) (Figure 1). Only one of the three clinical isolates (090718) was recovered earlier date than the sink sampling date and shared 2–5 SNPs with the other two clinical isolates (090767 and 090768). Compared to the seven sink isolates, the two isolates (090767 and 090768) shared 3–7 SNPs and 6–14 SNPs, respectively. Therefore, the sink was likely to be contaminated but not the exact route of the transmission. The remaining clinical isolate (090766) from ICU 2G belonged to a distinct clone (2h) and shared 35–142 SNPs with other isolates.

3.6 A cluster of ST2 bla_{OXA-23} and bla_{OXA-66}-carrying CRAB in a GICU

In ICU 3G, four sinks were contaminated by CRAB, which belonged to the ST2 type and carried both bla_{OXA-23} and bla_{OXA-66} genes. Two of the four sink isolates (090700 and 090701) shared 2–7 SNPs with three clinical isolates (090717, 090749, and 090750), indicating the presence of a common clone (clone 2i). Although one (090717) of the three clinical isolates was recovered at an earlier date than the sink sampling date, the other two (090749 and 090750) clinical isolates shared fewer SNPs (3–5 and 2–4) with the two sink isolates than with the clinical isolate (8, 7). Therefore, these two contaminated sinks were likely to be the route of the transmission. The other two sink isolates (090699 and 090703) shared three SNPs and belonged to another common clone (2j) (Figure 1). The remaining clinical isolate (090716) in this ICU belonged to clone (2k) alone, sharing 46–122 SNPs with other isolates.

4 Discussion

To our knowledge, this study was the first multicenter investigation on handwashing sink contamination by the CRAB in China. Unlike most studies in which the handwashing sink was sampled during an outbreak period, this study was conducted during a non-outbreak period in the included ICUs. The results showed that the contamination rate of handwashing sinks ranged from 0 to 63.6% in different ICUs, while other investigations did not find CRAB contamination in handwashing sinks during non-outbreak scenarios (31, 32). It has been well documented in the literature that the contamination level of handwashing sinks is related to the improper use, the size and depth of the sink, and the frequency of environmental cleaning (33). This study further revealed that the contamination rate of CRAB in handwashing sinks was significantly higher than that of carbapenem-resistant *klebsiella pneumoniae* (CRKP) or carbapenem-resistant *klebsiella oxytoca* (CRKO), with a rate of 10.1, 3.8, and 3.8%, respectively, during the same period, as we previously reported (25). This disparity may be attributed to the biofilm-forming capability of *Acinetobacter baumannii*, which enables it to survive on various surfaces more effectively than CRKP (34). In addition, the higher prevalence of CRAB compared to CRKP in local settings likely contributed to this difference (15).

All 27 clinical strains of CRAB obtained in our study were sourced from GICU, and this may be attributed to several factors: Patients in the GICU suffer from more severe illnesses, have longer hospital stays, experience higher rates of CRAB colonization, and are subjected to increased antibiotic usage (35, 36). Furthermore, a lack of awareness regarding the prevention and control of HAIs and insufficient cleaning and disinfection practices in GICU environments, including handwashing sinks, may contribute to CRAB infections among GICU patients (22, 37). In China, hospitals have already possessed relatively good resources for neonatal care and treatment (38), which likely results in fewer infections occurring in the NICU. It has been reported that CRAB strains carrying class B metallo-β-lactamase genes (e.g., bla_{NDM}, bla_{VIM}, and bla_{IMP}) are prevalent in pediatric populations (16, 39). However, our findings indicate that the dominant strains identified in the GICU sinks carried class D β-lactamase genes, specifically bla_{OXA-23}, with only one strain carrying bla_{NDM-1} isolated from a GICU sink.

In this study, we found that sink contamination by CRAB was not the major source of CRAB clinical infections in most ICUs in this study. However, at least three clones (clone 2c in ICU 1G, clone 2a in ICU 4G, and clone 2i in ICU 3G) of ST2 CRAB sink isolates were demonstrated to be the sources of patient infections or outbreaks. The results indicated that these three ICUs should take some proven measures to reduce the microbial contamination rate of sinks, including disinfection, use of drain covers, or even rebuilding the handwashing sink (21, 40, 41). In addition, we identified a transmission of clone 2a between two hospitals, along with two sink strains that were detected prior to a clinical strain in one of the hospitals. This finding underscores the need to pay close attention to hospital sewage contaminated with antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) (42). Hospital sewage may serve as a potential reservoir for CRAB, highlighting the importance of strengthening disinfection, management, and monitoring protocols for hospital sewage in local settings (43).

ST2 is the most dominant type of CRAB in the world as well as in China (42). The main mechanism of carbapenem resistance in *Acinetobacter baumannii* is the acquisition of carbapenem-hydrolyzing oxacillinase-encoding genes, and bla_{OXA23} is by far the most widespread in most countries (44). In this study, all of the clinical isolates and all but one of the sink isolates belonged to ST2. The bla_{OXA-23} gene was also the most common (42/43, 97.7%) oxacillinase-encoding gene. In addition, we found that most CRAB isolates (37/43, 86.0%) carried both bla_{OXA-23} and bla_{OXA-66}, similar to the findings of other studies (45–48). One ST2 CRAB strain isolated from a sink, carrying both bla_{NDM-1} and $bla_{OXA-51-like}$ gene bla_{OXA-98}, was not reported previously, which was alarming.

A strain of ST203 CRAB was recovered from a sink in a NICU. Although ST203 *Acinetobacter baumannii* has been found in cats in France and Japan (49, 50), this is the first report of ST203 CRAB recovered from a hospital environment and harboring both bla_{OXA-23} and bla_{OXA-66}. Although there were no patients infected with this strain in our study, rigorous monitoring is needed to prevent the spread of ST203 CRAB from the sink to humans, especially neonates.

This study also has several limitations. First, the P traps of handwashing sinks were not sampled in our study, which means potential colonization by CRAB in these areas could have been missed. However, we sampled the drainage pipe 3–5 cm below the drainage tube,

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and the results can reflect the contamination degree of the drainage pipe to a certain extent. Second, the clinical strains were collected only within 2 weeks before and 3 months after sink sampling. There might have been additional cases of CRAB associated with sinks, which could have been missed. Third, sinks were only sampled once, and other environmental surfaces were not sampled; additional isolates from the sinks may have been missed. Despite these limitations, this study revealed the level of handwashing sink contamination by CRAB in different ICUs and the association of contaminated CRAB with hospital-acquired infections.

5 Conclusion

This study revealed that the contamination rate of CRAB in handwashing sinks was high in some local ICUs, and the contaminated sinks could serve as environmental reservoirs for CRAB clusters.

Data availability statement

The data presented in the study are deposited in the GenBank under BioProject repository, accession number PRJNA977829, available at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA977829.

Ethics statement

The studies involving humans were approved by the Ethical Committee of the West China Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because The study only used clinical strains and did not require sample collection for patients.

Author contributions

LW: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. YF: Writing – review & editing, Software, Formal analysis. JL: Writing – review & editing, Resources, Investigation. XK: Writing – review & editing, Resources, Investigation. HZ: Writing – review & editing, Resources, Investigation. HW: Writing – review & editing, Resources. SR: Writing – review & editing, Investigation. LZ: Writing – review & editing, Investigation. YZ: Writing – review & editing, Investigation. QX: Writing – review & editing, Investigation. YL: Writing – review & editing, Investigation. XW: Writing – review & editing, Investigation. XD: Writing – review & editing, Investigation. WZ: Writing – review & editing, Investigation. QL: Writing – review & editing, Investigation. HG: Writing – review & editing, Investigation. CT: Writing – review & editing, Methodology, Conceptualization. FQ: Writing – review & editing, Writing – original draft, Methodology, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Brief report

Outbreak of drug-resistant *Acinetobacter baumannii* ST219 caused by oral care using tap water from contaminated hand hygiene sinks as a reservoir

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Key Words:
Drug-resistant *Acinetobacter baumannii* ST219
Hand hygiene sink
Intensive care unit
Oral care
Outbreak
Tap water

An outbreak of amikacin- and ciprofloxacin-resistant *Acinetobacter baumannii* ST219 in Tokai University hospital's emergency intensive care unit was caused by its colonization in water systems and subsequent spread through oral care using tap water. The outbreak was successfully controlled after replacement of the water system and implementation as of daily cleaning of water taps and oral care with a dry method. It is important to strictly manage the water system in critical care areas.

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Acinetobacter baumannii has the capability of long-term survival in both dry and wet hospital environments.[1] This pathogen produces a biofilm in water and has been isolated from water systems in medical facilities.[2] We experienced an outbreak of amikacin- and ciprofloxacin-resistant (hereinafter designated drug-resistant [DR]) *A baumannii* in the emergency intensive care unit (EICU) at Tokai University Hospital. The drinking tap water had been contaminated as a reservoir, and thus oral care with this tap water was a route of transmission. Here we describe the study of this outbreak of DR *A baumannii* in the EICU and measures implemented to control infection.

MATERIALS AND METHODS

The advanced emergency and critical care center at Tokai University Hospital accepts approximately 7,000 ambulances and 2,600 admissions per year. The center has a total of 58 beds, including a 19-beds EICU, a 36-beds high care unit in the emergency ward, and a 3-beds burn unit.

In the bacteriological investigation of the transmission route, samples from environmental surfaces of critical and noncritical areas were collected by wiping with a sterile swab moistened with sterile normal saline and then inoculated on a 5% sheep's blood agar plate (Kyokuto Pharmaceutical Industrial Co, Ltd, Tokyo, Japan). Bacterial identification and antimicrobial sensitivity tests were performed with the MicroScan WalkAway 96 Plus System (Siemens, Erlangen, Germany). To confirm the identification of *A baumannii*, OXA-51−like genes were examined by polymerase chain reaction (PCR).

The DR *A baumannii* isolates were screened for gene homology according to repetitive-sequence-based (rep) PCR as described previously.[3] The genealogical tree was determined using DendroUPGMA (http://genomes.urv.cat/UPGMA/). Multilocus sequence typing (MLST) was performed as described previously.[4] The amplification products were purified with a Qiagen DNA Purification Kit (Qiagen, Venlo, The Netherlands). DNA sequencing was performed using an ABI PRISM 3730 DNA analyzer (Life Technologies, Carlsbad, CA). The MLST sequences were uploaded to the *A baumannii* MLST sequence type database (http://pubmlst.org/abaumannii/) to determine the alleles and sequence types. Clonal

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Conflicts of interest: None to report.

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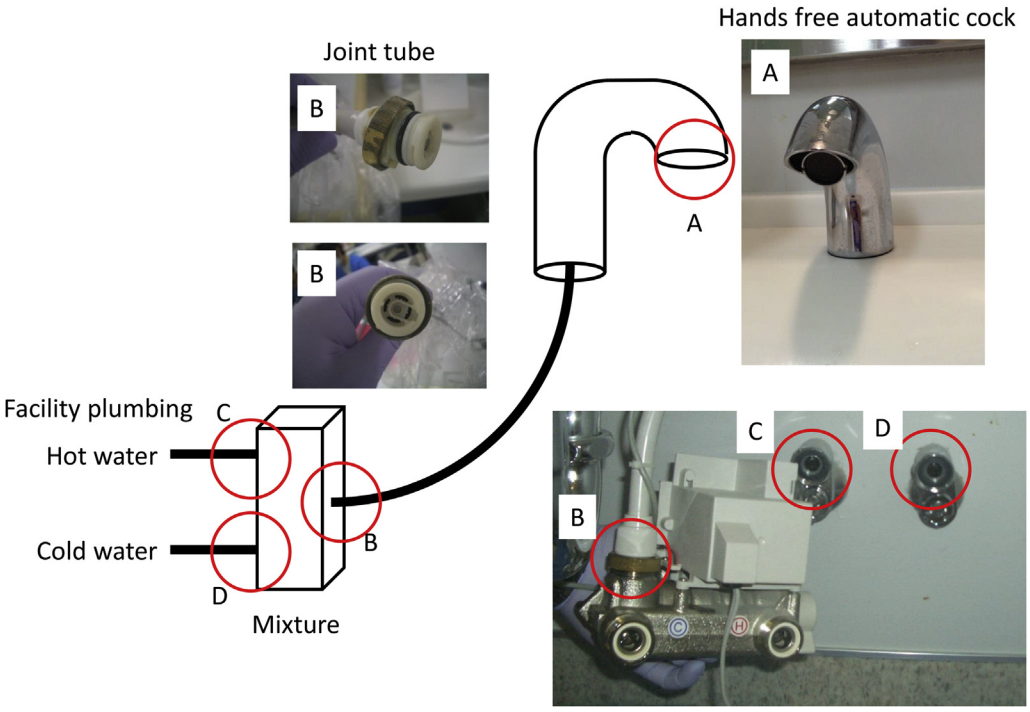

Fig 1. Bacterial investigations of parts of a hand hygiene sink in the EICU. DR *A baumannii* ST219 was isolated from a hands-free automatic tap (A) and water mixture side of the joint tube (B), but not the plumbing for either hot water (C) or cold water (D).

complexes were defined as single-locus variants or double-locus variants.

RESULTS

An outbreak of DR *A baumannii* was initially recognized in September 2013 from the aspirated sputum of tracheal intubation in a series of 3 patients under mechanical ventilation. An epidemiologic study revealed nothing shared by the 3 patients, including the patient flow, bed, respirator (closed airway suctioning system), medical staff, or care areas. Moreover, bacterial investigation of 29 medical staff hands was negative for the detection of DR *A baumannii* ST219. On environmental screening, DR *A baumannii* was detected on hands-free automatic taps of hand hygiene water sinks in critical areas of the EICU. Each component of the water sinks was subjected to a bacteriological investigation (Fig 1). DR *A baumannii* was isolated from the hands-free automatic tap (Fig 1A) and the joint tube on the mixture side (Fig 1B), but not from the plumbing for either hot water or cold water (Fig 1C and D). In addition to hand hygiene sinks, DR *A baumannii* was detected from other critical areas in the EICU environment, including the bronchoscope cabinet, the expectoration supporting device (CoughAssist), and plate sampling test.

On day 23 of the outbreak, use of all 10 hand hygiene water sinks was prohibited. The sinks, automatic taps, tubes, and hot and cold water mixture unit were replaced. Cleaning of the water tap was added to the daily sink cleaning routine. On day 26, the method of oral care was changed to a waterless technique, performed by wiping the teeth and gingiva with a swab after moistening the tissue with sterile water (dry oral care) under the guidance of a dental hygienist. Up to that time, conventional oral care had been performed by nurses using a toothbrush, toothpaste, and tap water while suctioning (wet oral care).

Finally, DR *A baumannii* was detected in sputum from 15 patients under tracheal intubation without pneumonia, all of whom had been receiving wet oral care. After the outbreak was controlled, DR *A baumannii* was not detected in the EICU in routine active surveillance cultures of any patients over the next 6 months.

DR *A baumannii* was detected in OXA-51–like genes by rep-PCR. The rep-PCR analysis revealed a high level of similarity in the patterns of all patient isolates and the hand hygiene sink samples (hands-free automatic tap and joint tube) (Fig 2A and B). The MLST analysis of all isolates of DR *A baumannii* obtained from the patients and the water system revealed the ST219 strain (Fig 2B). The clinical isolates of drug-sensitive *A baumannii* demonstrated the ST679 strain.

DISCUSSION

In this outbreak of DR *A baumannii*, oral care using contaminated tap water was identified as a transmission route. A waterless mouth care method proved useful in preventing further transmission. Oral hygiene is recommended as means of reducing the incidence of ventilator-associated pneumonia.[5] We had been adopting routine oral care using tap water, which was considered safe in Japan even without daily cleaning of taps. On the other hand, water sources in our hospital had recognized reservoirs for water-transmissible pathogens such as *A baumannii*.[6] Design and daily cleaning of sink and tap are important for prevention of latent colonization of *Acinetobacter* in the hand hygiene sink. To prevent such infections, wide and deep hand hygiene sinks with hands-free automatic water taps should be installed.[7]

MLST typing of DR *A baumannii* identified the outbreak of the ST219 strain. A few cases of infection with *A baumannii* ST219 have been reported as well.[8] *A baumannii* ST208 was implicated in an outbreak in the EICU and burn unit of Tokai University Hospital in 2010, where the air fluidity bed was identified as a reservoir.[9] It is likely that the EICU environment is readily contaminated with *A baumannii*, leading to a risk of contamination in the tap water in the hand hygiene sink via the hands of health care workers. To prevent future outbreaks, it is important to minimize the risk in the wet environmental reservoir, including sanitary strict management of water systems, particularly in critical care areas.

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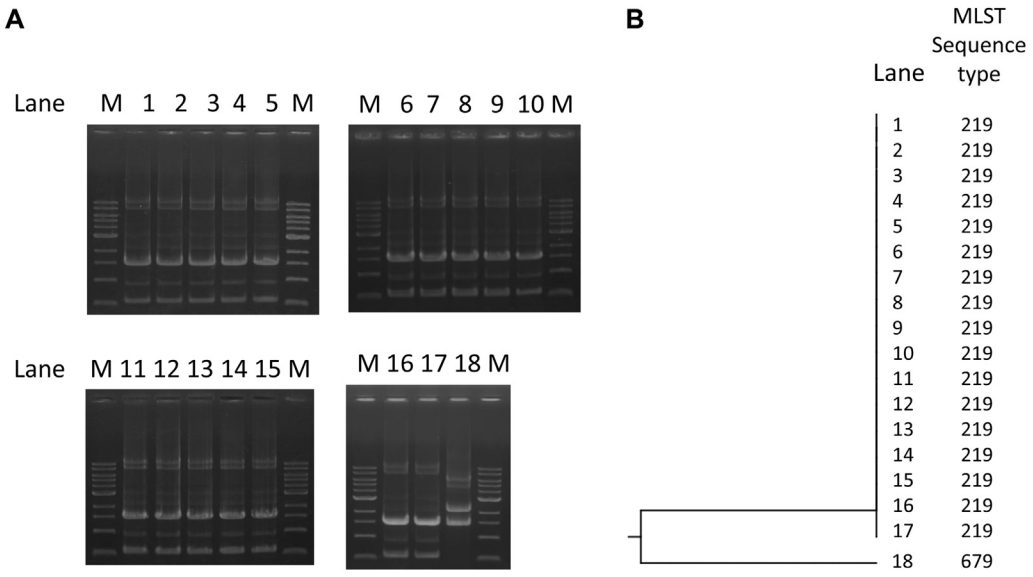

Fig 2. Molecular typing of the clinical isolates of DR *A baumannii* using rep-PCR and MLST. All of the isolates of DR *A baumannii* were subjected to molecular typing using rep-PCR, as shown in the electrophoretic analysis (A), as well as a genealogical tree and molecular typing using MLST (B). Lanes 1-15 indicate DR *A baumannii* isolated from the patients. Lanes 16 and 17 indicate DR *A baumannii* isolated from hands-free automatic tap and water mixture side of the joint tube, respectively. Lane 18 indicates a clinical isolate of drug-sensitive *A baumannii*. Lane M is a Marker.

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Journal of Antimicrobial Chemotherapy

OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 to 2014

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Objectives: OXA-48-like carbapenemases have spread worldwide since 2001. We analysed patient and microbiological data for UK isolates with these enzymes as confirmed by the national reference laboratory from November 2007 to December 2014.

Methods: MICs were determined using BSAC agar dilution. Isolates with reduced susceptibility or resistance to at least one carbapenem and high-level resistance to both piperacillin/tazobactam (MICs ≥64 mg/L) and temocillin (MICs ≥128 mg/L) were screened by PCR for $bla_{\text{OXA-48-like}}$ genes. The genomes of about half of the isolates were sequenced, with MLST types, resistance genes and plasmid replicon types inferred. Patient data provided by sending laboratories were reviewed.

Results: Isolates ($n = 741$) with OXA-48-like carbapenemases were submitted from 111 UK laboratories, representing 536 patients. Almost all (99%; 736 of 741) were Enterobacteriaceae, predominantly *Klebsiella pneumoniae* (55%; 408), and most (80%; 595) were from inpatients. WGS of 351 non-duplicate isolates identified $bla_{\text{OXA-48}}$ as the most common variant, found in two-thirds (235 of 351) of isolates, followed by $bla_{\text{OXA-181}}$ (68), $bla_{\text{OXA-232}}$ (32), $bla_{\text{OXA-244}}$ (10), $bla_{\text{OXA-484}}$ (5) and $bla_{\text{OXA-245}}$ (1). Among *K. pneumoniae* (163 of 351), *Escherichia coli* (114 of 351) and *Enterobacter cloacae* (42 of 351), 119 STs were identified. Mapping analyses revealed that 63% (222 of 351) of isolates harboured plasmids that shared >99% identity to one of four known plasmids [pOXA-48a (44%; 154 of 351), pOXA-232 (10%; 34 of 351), pOXA181 (9%; 30 of 351) and pKP3-A (1%; 4 of 351)]; the remaining 37% of isolates harboured $bla_{\text{OXA-48-like}}$ in unknown environments.

Conclusions: OXA-48-like carbapenemases are an increasing problem in the UK. This study highlights both the role of successful plasmids and the polyclonal nature of their dissemination.

Introduction

OXA-48-type carbapenemases were first identified in 2001 in a carbapenem-resistant *Klebsiella pneumoniae* isolate from the urine of a patient hospitalized in Istanbul, Turkey.[1] Since then, reports of infections caused by OXA-48-producing Enterobacteriaceae have escalated—particularly in Europe, Asia and Africa, and less so in the Americas.[2–5] There are six carbapenem-hydrolysing class D β-lactamase (CHDL) subgroups that are clinically significant: OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58 and OXA-143.[6] All except the OXA-48 group are predominantly found in *Acinetobacter* spp. isolates,[6] whereas OXA-48 enzymes are usually found in Enterobacteriaceae.[5] To date, 14 OXA-48-like variants have been described (OXA-48, -162, -163, -181, -199, -204, -232, -244, -245, -247, -370, -405, -436 and -484) of which 11 are CHDLs. These vary in sequence by one to five amino acids from the 'classical' OXA-48 variant and hydrolyse penicillins and carbapenems, but not extended-spectrum cephalosporins (e.g. cefepime and ceftazidime).[1] In contrast to their CHDL counterparts, OXA-163, -247 and -405 also differ from OXA-48 by one or two amino acids, but additionally have a four amino acid deletion in the active site region; as a result they lack significant carbapenemase activity, but do exhibit increased activity toward extended-spectrum cephalosporins.[7–9]

$bla_{\text{OXA-48}}$ has only been found in Enterobacteriaceae and has been associated with outbreaks in Turkey, the Middle East and North Africa.[5,10] Other variants have different geographical associations: in particular $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-232}}$ have been epidemiologically linked to the Indian subcontinent and are often co-harboured with NDM enzymes.[11,12]

The proliferation of OXA-48-like enzymes has been attributed both to successful clones and to plasmid spread. In 2011, OXA-48-positive *K. pneumoniae* ST395 was identified in patients in Morocco,

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the Netherlands and France, suggesting the country-to-country transfer of this clone,[13] with patient-linked transfer of OXA-48-producing *Enterobacter cloacae* from Morocco to France also documented.[14] On the other hand, pOXA-48a, a 61.9 kb self-conjugative IncL/M plasmid, was shown to be the primary vehicle for dissemination of bla_{OXA-48} in several outbreaks.[5,15] This broad host range plasmid harbours bla_{OXA-48} within Tn*1999* and occurs across several enterobacterial species.[5,15]

This study describes the epidemiology of OXA-48-like carbapenemase producers submitted to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit between 2007 and 2014.

Materials and methods

Bacterial isolates, identification and susceptibility testing

Isolates were submitted to PHE's AMRHAI Reference Unit from clinical laboratories across the UK between November 2007 and December 2014 for investigation of unusual resistance, including to carbapenems.

Bacterial identification was carried out using chromogenic agars [CHROMagar™ Orientation (CHROMagar, Paris, France) and Brilliance UTI (Oxoid, Basingstoke, UK)] together with API-20E tests (bioMérieux SA, Marcy-l'Étoile, France) or, since August 2012, by MALDI-TOF MS (Bruker Microflex LT; Bruker Daltonik GmbH, Bremen, Germany).

Antibiotic susceptibilities (MICs) were determined by BSAC agar dilution[16] using AMRHAI's standard Gram-negative antibiotic panel, which includes ertapenem, meropenem and imipenem (the latter with/without 320 mg/L EDTA to detect metallo-carbapenemases), and interpreted using EUCAST breakpoints.[17]

Screening for carbapenemase genes

Isolates displaying high-level resistance to piperacillin/tazobactam (MICs ≥64 mg/L) and temocillin (MICs ≥128 mg/L), as well as reduced susceptibility or resistance to any carbapenem, were tested for $bla_{OXA-48-like}$ genes by in-house PCR[1] and/or with a commercial microarray (Check-MDR CT102; Check-Points, Wageningen, The Netherlands).[18] In instances where imipenem potentiation by EDTA was observed, isolates were also tested by PCR for the presence of metallo-carbapenemases (bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{SIM} and bla_{SPM}) by in-house PCRs.[19]

WGS and analyses

Three hundred and seventy isolates, temporally and geographically distributed throughout the study, were selected for WGS. Genomes were sequenced using the Nextera sample preparation method with the standard 2× 100 base sequencing protocols on a HiSeq instrument (Illumina, San Diego, CA, USA). Data were analysed using an in-house bioinformatics pipeline as previously described.[20] STs were inferred from WGS data where MLST schemes exist.

For plasmid analysis, sequencing reads were mapped against known OXA-48 plasmids, namely pOXA-48a (NC_019154), pOXA-232 (JX423831), pKP3-A (NC_019160) and pOXA181 (KP400525).

Analysis of patient demographic information

Patient data were obtained from the request forms sent with submissions from referring laboratories. A patient was categorized as 'new' if they were found to have OXA-48-like-positive isolates detected by AMRHAI for the first time and 'known' if any OXA-48-like-positive isolate, irrespective of species, had previously been identified from the patient by AMRHAI.

Results

Demographics of affected patients and distribution

During the study period, AMRHAI confirmed 741 OXA-48-like positive isolates from 111 laboratories throughout the UK and obtained from 536 patients. Figure 1 illustrates the temporal distribution of these isolates among 'new' and 'known' patients, and among submitting laboratories. The first OXA-48-like positive isolate was a *K. pneumoniae*, submitted to AMRHAI in November 2007 and obtained from the urine of a patient previously hospitalized in Turkey.[10]

Isolates with OXA-48-like carbapenemases were submitted from laboratories across all UK regions. The national distribution of affected patients was as follows: England ($n = 514$), Scotland ($n = 13$), Northern Ireland ($n = 6$) and Wales ($n = 3$). The greatest number of affected patients was in the London region ($n = 203$), followed by the North West ($n = 143$).

Most source patients were hospitalized (79%; 421 of 536), but a few were outpatients (7%; 38 of 536), in primary care (8%; 41 of 536) or in unknown settings (7%; 36 of 536). The mean patient age was 59.5 years and 54% (289 of 536) were male.

A travel history was reported for 130 of 536 (24%) patients. Of these, 55 patients had documented foreign travel to the following destinations: India (12), Turkey (9), Pakistan (5), Egypt (4), Libya (4), Spain (4), Kuwait (3), Malta (3), Sri Lanka (2), Tunisia (2) and single patients had travelled to Cyprus, Kenya, Morocco, Russia, Saudi Arabia, Singapore and Syria. Twenty patients were known to have been hospitalized while abroad in Egypt (4), Libya (4), Turkey (4), India (3), Pakistan (2), Cyprus (1), Spain (1) and Sri Lanka (1).

Single OXA-48-like-positive isolates were referred from 408 of 536 (76%) patients and multiple isolates from the remaining 128 (24%). Among the patients with multiple OXA-48-like-positive isolates, 43 of 128 (34%) yielded isolates of different species or genera and 63 of 128 (49%) had isolates referred from different anatomical sites. The OXA-48-like-positive isolates were referred over a period of <14 days in 65 of 128 (51%) instances, for 17 of 128 over a period of 14–28 days, 38 of 128 over a period of 1–6 months and over a period >6 months from 8 patients. Seventeen percent (127 of 741) of isolates were submitted from a single laboratory (laboratory 1), which serves three hospitals (to be subsequently known as hospitals A, B and C), over a 2 year period. Further details of the isolates from this laboratory are given below.

Microbiology

Most (99%; 736 of 741) submitted isolates were Enterobacteriaceae, comprising: *K. pneumoniae* (55%; 408 of 741), *Escherichia coli* (29%; 218 of 741), *Enterobacter* spp. (9%; 68 of 741), *Klebsiella oxytoca* (3%; 24 of 741), *Citrobacter* spp. (2%; 14 of 741), *Serratia marcescens* (>1%; 3 of 741) and one *Raoutella ornithinolytica*. There were also five non-Enterobacteriaceae isolates comprising *Pseudomonas aeruginosa* ($n = 3$; all from one patient)[21] and *Shewanella* spp. ($n = 2$; OXA-48-like enzymes are intrinsic in this genus).

If samples rather than patients were considered as the denominator, most were taken in hospitals (89%; 656 of 741), but some were general practice urines (6%; 45 of 741) and a few from unknown settings (5%; 40 of 741). The most frequently reported specimen type was urine (29%; 215 of 741), followed by faeces or

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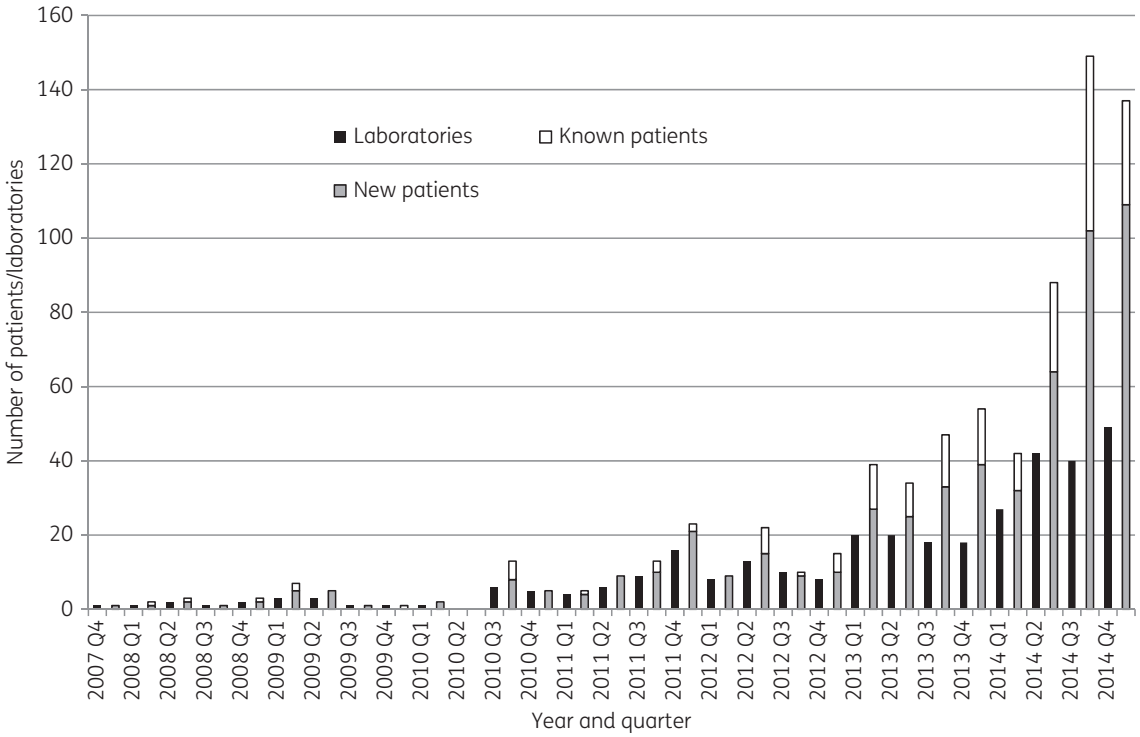

Figure 1. Numbers of new and known affected patients and laboratories sending OXA-48-positive isolates per quarter during the study period.

rectal swabs (27%; 202 of 741). Fifteen percent (110 of 741) of isolates were obtained from tissue and fluid samples, 11% (83 of 741) from blood cultures and line tips, 9% (68 of 741) from screening swabs, 5% (40 of 741) from respiratory samples and 3% (23 of 741) were unknown specimen types (Table 1).

Carbapenemase alleles and typing of the isolates

WGS was undertaken for 351 non-duplicate isolates from single patients and their STs (where MLST schemes exist) and carbapenemase alleles were defined. These comprised: *K. pneumoniae* ($n = 163$), *E. coli* ($n = 114$), *E. cloacae* ($n = 42$), *K. oxytoca* ($n = 13$), *Citrobacter* spp. ($n = 11$), other *Enterobacter* spp. ($n = 5$), *S. marcescens* ($n = 2$) and *P. aeruginosa* ($n = 1$). Their carbapenemase genes comprised: $bla_{\text{OXA-48}}$ (66%; 230 of 351), $bla_{\text{OXA-181}}$ (18%; 62 of 351), $bla_{\text{OXA-232}}$ (7%; 24 of 351), $bla_{\text{OXA-244}}$ (3%; 10 of 351), $bla_{\text{OXA-245}}$ (<1%; 1 of 351), $bla_{\text{OXA-484}}$ (1%; 5 of 351), $bla_{\text{OXA-48}} + bla_{\text{NDM-1}}$ (1%; 5 of 351), $bla_{\text{OXA-181}} + bla_{\text{NDM-1}}$ (2%; 6 of 351) and $bla_{\text{OXA-232}} + bla_{\text{NDM-1}}$ (2%; 8 of 351). The carbapenemase variants and allele combinations OXA-48, OXA-181, OXA-48 + NDM-1 and OXA-181 + NDM-1 were found in multiple species. OXA-232, OXA-245, OXA-484 and OXA-232 + NDM-1 were found only in *K. pneumoniae* isolates, while OXA-244 was found only in *E. coli* isolates. Numerous other genes encoding resistance to several other classes of antibiotics were also detected throughout all species (Table 2). Overall, 19 of 351 (5%) isolates had OXA-48-like enzymes together with another carbapenemase, 266 of 351 (76%) also harboured a predicted ESBL or plasmid-encoded AmpC and 85 of 351 (24%) had OXA-48-like enzymes without any of these additional β-lactamase types.

K. pneumoniae

One hundred and sixty-three non-duplicate *K. pneumoniae* isolates, from 56 laboratories across 10 UK regions, were sequenced. Individual laboratories submitted between 1 and 15 isolates. Forty-nine STs were identified, the most frequent being ST14 (27 isolates from 17 centres over 16 months), followed by ST231 (18 from 14 centres over 15 months), ST147 (17 from 13 centres over 7 years), ST101 (13 from 10 centres over 4 years), ST11 (10 from 7 centres over 16 months) and ST16 (6 from 5 centres over 3 months). The remaining 43 STs were each represented by five isolates or fewer. Five OXA-48-like variants were identified [$bla_{\text{OXA-48}}$ ($n = 86$), $bla_{\text{OXA-181}}$ ($n = 31$), $bla_{\text{OXA-232}}$ ($n = 24$), $bla_{\text{OXA-484}}$ ($n = 5$) and $bla_{\text{OXA-245}}$ ($n = 1$)] and 16 isolates produced more than one carbapenemase [$bla_{\text{OXA-48}} + bla_{\text{NDM-1}}$ ($n = 3$), $bla_{\text{OXA-181}} + bla_{\text{NDM-1}}$ ($n = 5$) and $bla_{\text{OXA-232}} + bla_{\text{NDM-1}}$ ($n = 8$)].

Multiple plasmid replicon types were identified including IncL/M, IncA/C, several IncF variants, IncHI2, IncX3 and ColE-like replicons. Plasmid mapping revealed that 70 of 86 (81%) isolates with OXA-48 enzymes and 1 with OXA-245 had plasmids exhibiting >99% sequence identity to pOXA-48a, whereas among the 31 with OXA-181 enzymes 8 (26%) and 5 (16%) had plasmids with >99% sequence identity to pOXA181 and pKP3-A, respectively. All 32 isolates with OXA-232 enzymes had plasmids exhibiting >99% sequence identity to pOXA-232. pOXA-48a sequences were found in 37 STs, most frequently ST11 ($n = 8$) and ST101 ($n = 9$), whereas pOXA181 sequences were found in five STs (ST11, ST61, ST25, ST307 and ST709), each with one to three representatives; pKP3-A sequences were found in two STs, with one representative (ST395) and four representatives (ST147), respectively. pOXA-232 sequences were found in seven STs (ST14, ST15, ST16,

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Table 1. Sources of OXA-48-like-positive isolates

	Hospital setting								
Species	urines	screening swabs	blood cultures and line tips	respiratory	tissue and fluid	faeces/ rectal swabs	not known	General practice urines	Total
K. pneumoniae	91	27	48	28	58	110	8	15	385
E. coli	47	22	18	7	28	58	2	25	207
Enterobacter spp.	6	15	9	1	9	16	10	1	67
K. oxytoca	2	2	7	0	2	6	0	4	23
Citrobacter spp.	5	2	1	0	0	4	0	0	12
S. marcescens	0	0	0	0	1	0	0	0	1
R. ornithinolytica	0	0	0	0	0	1	0	0	1
Other spp.[a]	0	0	0	2	2	1	0	0	5
Total	151	68	83	38	100	196	20	45	701

	Unknown setting							
Species	urines	screening swabs	blood cultures and line tips	respiratory	tissue and fluid	faeces/ rectal swabs	not known	Total
K. pneumoniae	9	0	0	1	7	4	2	23
E. coli	8	0	0	0	2	1	0	11
Enterobacter spp.	1	0	0	0	0	0	0	1
K. oxytoca	0	0	0	1	0	0	0	1
Citrobacter spp.	1	0	0	0	0	1	0	2
S. marcescens	0	0	0	0	1	0	1	2
Total	19	0	0	2	10	6	3	40

[a]Other species comprise *P. aeruginosa* ($n = 3$) and *Shewanella* spp. ($n = 2$).

ST147, ST231, ST307 and ST395), most often ST14 (15 isolates from 13 centres), ST231 (11 from 8 centres) and ST147 (3 from 2 centres).

The earliest UK isolate with an OXA-48-like enzyme dated from 2007 and was from a patient who had previously been hospitalized in Turkey.[10] It was shown here to belong to ST147 and to harbour a pOXA-48a-like plasmid.

The most frequent submitter, laboratory 1, sent 15 isolates that were subjected to WGS over 22 months—13 of which were obtained from hospital A. These 15 represented 11 STs and all harboured plasmids with >99% sequence identity to pOXA-48a.

E. coli

One hundred and fourteen non-duplicate *E. coli* isolates, from 49 laboratories across seven UK regions, were sequenced. Laboratories submitted between 1 and 16 isolates and 37 STs were identified. The most frequent of these were ST38 (53 isolates from 34 laboratories over 42 months) and ST410 (12 from 9 laboratories over 20 months). The remaining 35 STs were each represented by four isolates or fewer. Three OXA-48-like variants were identified [$bla_{\text{OXA-48}}$ ($n = 74$), $bla_{\text{OXA-181}}$ ($n = 30$) and $bla_{\text{OXA-244}}$ ($n = 10$)].

Multiple plasmid replicon types were identified including IncL/M, several IncF replicons, IncB/O, IncHI2, IncK and IncX3. Plasmid analyses revealed that 22 of 74 (30%) *E. coli* with OXA-48 enzymes had plasmids exhibiting >99% sequence identity to pOXA-48a, and 22 of 30 (73%) of those with OXA-181 had plasmids that exhibited >99% sequence identity to pOXA181. pOXA-48a sequences were found in 17 STs, each with one to four representatives. pOXA181 sequences were found in 10 STs, predominantly ST410 ($n = 11$), which were submitted from eight laboratories across five regions. No plasmid could be identified in 70 isolates. Of these, 50 belonged to ST38, 41 of them harbouring $bla_{\text{OXA-48}}$ and 9 harbouring $bla_{\text{OXA-244}}$. The most frequent submitter, laboratory 1, sent 16 isolates that were subject to WGS over a 21 month period (14 of which were obtained from hospital A); these represented 14 STs, all had OXA-48 and 12 of 16 (75%) harboured pOXA-48a sequences.

Enterobacter spp.

Forty-seven non-duplicate *Enterobacter* spp. isolates, sent from 15 laboratories across seven UK regions, were sequenced. These comprised: *E. cloacae* ($n = 42$), *Enterobacter aerogenes* ($n = 4$) and *Enterobacter hormaechei* ($n = 1$). Individual laboratories submitted between 1 and 27 isolates. Forty-five isolates harboured $bla_{\text{OXA-48}}$ and the remaining two isolates harboured $bla_{\text{OXA-48}} + bla_{\text{NDM-1}}$. Thirteen STs were identified among the *E. cloacae* isolates, each with between 1 and 17 representatives. The most frequently

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Table 2. Characteristics of 351 non-duplicate isolates that were subjected to WGS

		Carbapenemases				Other resistance genes		
Species	No. of isolates	OXA-48-like alleles (no.)	OXA + NDM alleles (no.)	STs (no. if >1)	Replicons	β-lactamases (variants)	aminoglycoside resistance genes	others
K. pneumoniae	163	OXA-48 (86), OXA-181 (31), OXA-232 (24), OXA-245 (1), OXA-484 (5)	OXA-48 + NDM-1 (3), OXA-181 + NDM-1 (5), OXA-232 + NDM-1 (8)	11 (10), 14 (27), 15 (4), 16 (6), 17, 25 (2), 35, 36, 39, 43, 45 (4), 48 (2), 101 (13), 104, 105, 111, 133 (2), 147 (17), 152, 187 (2), 231 (18), 253, 294, 299, 307 (7), 323, 327, 336 (3), 359, 392 (3), 395 (2), 405 (2), 461 (2), 659, 685, 709, 831 (2), 922, 985, 1141, 1164, 1473 (5), 1680 (2), 1819, 1821, 1825, 1827, 1834, 2205	A/C, ColKP3, FIA, FIB, FII, HI2, L/M, X3	bla_{TEM-1}, bla_{SHV} (1,11,28,33,39,75, 76,100,103,159), bla_{CTX-M} (14,15,16), bla_{OXA} (1,9), bla_{DHA-1}, bla_{CMY-4}	*strA*, *strB*, *aadA1*, *aadA2*, *aadA3*, *aadA5*, *armA*, *aac(3)-lla*, *aac(3)-lld*, *aac(6')lb-cr*, *rmtF*	*oqxA*, *oqxB*, *qnrB1*, *qnrS1*, *qnrB66*, *arr-2*, *arr-3*, *sul1*, *sul2*, *fosA*, *mphA*, *msrE*, *ereA*, *ereB*, *ereC*, *ermB*, *mdfA*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA14*, *catA1*, *catB3*, *cmlA1*, *sat2*, *tetA*, *tetD*
E. coli	114	OXA-48 (74), OXA-181 (30), OXA-244 (10)		10 (4), 28, 38 (53), 58, 59, 69, 73, 83, 95, 127, 131, 167, 205 (2), 224, 227 (2), 354 (2), 361, 399 (3), 401, 405 (4), 410 (12), 428, 648, 681, 940 (3), 1170, 1284 (2), 1431, 1722, 2139, 2164, 2179, 3221, 3541, 6328, 6329, 6330	B/O, FIA, FIB, FII, HI2, K, L/M, X3	bla_{TEM} (1,33,169,190), bla_{CTX-M} (14,15,24,27,82), bla_{OXA} (1,10), bla_{CMY} (2,42,44,54,59,61)	*strA*, *strB*, *aadA1*, *aadA2*, *aadA3*, *aadA5*, *aadA23*, *aac(3)-lld*, *aac(3)-lla*, *aph(6)-ld*, *aac(6')lb-cr*, *rmtB*	*qnrB1*, *qnrS1*, *qepA*, *mdfA*, *mphA*, *ermB*, *msrE*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA14*, *dfrA17*, *fosA*, *sul1*, *sul2*, *catA1*, *cmlA1*, *floR*, *sat2*, *tetA*, *tetD*
E. cloacae complex	42	OXA-48 (40)	OXA-48 + NDM-1 (2)	45 (3), 51, 66 (5), 90, 93 (3), 104 (4), 106, 108 (17), 135, 145, 268, 269, 279 (3)	FIB, FII, HI1, L/M	bla_{TEM-1}, bla_{OXA-1}, bla_{SHV} (5,12), bla_{CTX-M} (9,15,82), bla_{ACT} (7,14,15,16)	*strA*, *strB*, *aadA2*, *aadA3*, *aadA12*, *aac(3)-lla*, *ant(2″)-la*, *aac(6')-llc*, *aac(6')lb-cr*	*qnrA1*, *qnrB1*, *qnrS1*, *ereA*, *mphA*, *dfrA12*, *dfrA14*, *dfrA16*, *dfrA18*, *fosA*, *sul1*, *sul2*, *catA1*, *catA2*, *tetA*, *tetD*
K. oxytoca	13	OXA-48 (13)		27 (4), 36, 95, 168, 176 (6)	FII, HI2, L/M	bla_{TEM-1}, bla_{OXY} (1,2,5,6)	*strA*, *strB*, *aac(3)-lla*	*qnrA1*, *mphA*, *dfrA18*, *sul1*, *tetD*, *tetK*
Citrobacter spp.	11	OXA-48 (10)	OXA-181 + NDM-1 (1)	ND	A/C, FIB, FII, L/M	bla_{TEM-1}, bla_{CMY-48}, bla_{MAL-1}		*qnrB12*, *dfrA7*, *sul1*, *sul2*, *tetD*
Other *Enterobacter* spp.	5	OXA-48 (5)		ND	H, L/M, N	bla_{TEM-1}, $bla_{CTX-M-14}$, bla_{ACT-37}, bla_{SHV-12}	*strA*, *strB*, *aadA1*, *aadA2*, *aph(6)-ld*, *aac(6')-llc*, *aph(3')-Vib*	*qnrA1*, *ereA*, *dfrA18*, *floR*, *tetA*, *sul1*, *sul2*, *sul3*
S. marcescens	2	OXA-48 (2)		ND	L/M	bla_{CTX-M} (14,82)	*strA*, *strB*, *aph(3')-Vib*	
P. aeruginosa	1	OXA-181 (1)		773		bla_{PAO}, bla_{OXA-50}	*aac(3)-Ie*, *aph(3")-IIb*, *aadA1*	*catB7*, *dfrB5*, *sul1*

ND, not determined.

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obtained ST was ST108 (17 isolates from two centres over 15 months); the remaining 12 STs were represented by ≤5 isolates. Multiple plasmid replicon types were identified including IncL/M, IncN, IncHI1 and several IncF replicons. Plasmid mapping revealed that 42 of 47 (89%) isolates had DNA exhibiting >99% sequence identity to pOXA-48a. These comprised 40 *E. cloacae* and single isolates of *E. aerogenes* and *E. hormaechei*. Thirteen STs were identified among the 40 *E. cloacae* isolates, but predominantly ST108 with 17 representatives. Most were submitted (16 of 17) from laboratory 1 and 14 of 16 were obtained from hospital A, over a 3 month period. Fifteen of these 16 (94%) harboured pOXA-48a sequences. In total, over half (27 of 48) of the *Enterobacter* spp. isolates with OXA-48-like enzymes were submitted by laboratory 1 over a 15 month period—26 of which were obtained from hospital A, with these comprising 25 *E. cloacae* and 1 *E. hormaechei*. All 26 isolates produced OXA-48 and the 25 *E. cloacae* isolates represented 5 STs.

K. oxytoca

Thirteen non-duplicate *K. oxytoca* isolates, sent from seven laboratories across four UK regions, were sequenced, with individual laboratories submitting between one and four isolates. All harboured the classical bla_{OXA-48} variant. Five STs were identified, namely ST176 (six isolates from five centres in four regions), ST27 (four isolates from one centre in 1 month) and single representatives of ST36, ST95 and ST168. Multiple plasmid replicons were identified including IncL/M, IncHI2 and several IncF replicons. Plasmid mapping analyses revealed that all isolates shared >99% sequence identity to plasmid pOXA-48a.

Other species

The remaining 14 isolates that were sent for WGS comprised: *Citrobacter freundii* ($n = 9$), *Citrobacter koseri* ($n = 2$), *S. marcescens* ($n = 2$) and *P. aeruginosa* ($n = 1$). All harboured classical bla_{OXA-48} except for one *C. freundii* isolate, which harboured $bla_{OXA-181} + bla_{NDM-1}$, and the *P. aeruginosa* isolate that had $bla_{OXA-181}$. The *P. aeruginosa* isolate was previously found to belong to ST773 with $bla_{OXA-181}$ encoded within Tn*2013*.[21] Plasmid replicon types included IncL/M, several IncF replicons and IncA/C. Within the nine *C. freundii* isolates eight STs were identified. Five of these were obtained from inpatients in hospital A, three of which harboured pOXA-48a sequences. In the remaining isolates, two *C. koseri* isolates that produced OXA-48 shared >99% identity to plasmid pOXA-48a and one OXA-181-producing *C. freundii* isolate shared >99% identity to pKP3-A.

Antibiotic susceptibility

MIC distributions for OXA-48-like-positive isolates are shown in Table 3. Ninety-nine percent (724 of 728) of isolates with available susceptibility data were resistant or non-susceptible to ertapenem; however, 42% (312 of 735) and 54% (400 of 735) remained susceptible to imipenem and meropenem, respectively, based on EUCAST breakpoints. In all but eight cases, MICs of meropenem were above the EUCAST screening cut-off concentration of 0.125 mg/L.[22] For the remaining eight cases the imipenem MICs were also below the EUCAST screening cut-off of 1 mg/L, but were above the ertapenem MIC cut-off of 0.125 mg/L. All eight isolates were *E. coli* and the analysis of seven of these by WGS indicated that six different STs and two OXA-48-like variants [bla_{OXA-48} (five) and bla_{OXA181} (two)] were represented. Piperacillin/tazobactam resistance (MIC >16 mg/L) was observed in 732 of 734 of tested isolates (data not shown). All the isolates that co-produced either NDM or VIM enzymes were non-susceptible to all three carbapenems. Non-susceptibility to ceftazidime and cefotaxime was observed in 69% and 89% of isolates. Non-susceptibility to the aminoglycosides amikacin, gentamicin and tobramycin was observed in 26%, 55% and 61% of isolates, respectively. Almost all (28 of 34) isolates that co-produced another carbapenemase were non-susceptible to all three aminoglycosides and all were resistant to ciprofloxacin. Most (91%; 659 of 722) isolates were susceptible to colistin.

Colistin resistance was observed in 63 isolates, submitted from 32 laboratories over 6 years, the majority (54 of 63) of which were *K. pneumoniae* and had MICs in the range of 4 to >32 mg/L. Sequencing of 22 of 54 colistin-resistant *K. pneumoniae* isolates identified 10 STs, 6 of which were represented by a single isolate and the 4 remaining STs were as follows: ST14 (6), ST101 (4), ST147 (2) and ST231 (4). These 22 were submitted from 14 laboratories across five regions. For 10 isolates colistin MICs were >32 mg/L; these comprised 7 *K. pneumoniae* and 1 *E. coli* along with 2 *S. marcescens* with inherent resistance. These were referred from 10 laboratories across four regions. Non-susceptibility to ciprofloxacin and tigecycline was observed in 63% and 32% of isolates, respectively.

Discussion

This report reviews all isolates producing an OXA-48-like carbapenemase and referred to PHE's AMRHAI Reference Unit from laboratories across the UK between November 2007 and December 2014. Over this study period, 741 OXA-48-like-positive isolates were obtained from 536 patients across all UK regions.

The majority of isolates were from clinical specimens, predominantly urines. All isolates were resistant to at least two classes of antibiotics and most were non-susceptible at EUCAST breakpoints to at least one of the three carbapenems tested. A high rate of susceptibility was maintained only to colistin (91%), with amikacin (74%) and tigecycline (68%) next in rank order. High levels of resistance to the third-generation cephalosporins, ceftazidime and cefotaxime, could be attributed to the co-carriage of ESBL/AmpC enzymes in 76% of sequenced isolates. There was huge variation in susceptibility to other antibiotics tested in this study, attributable to the presence of a plethora of other resistance genes, as identified in the WGS analyses (Table 2), sometimes including other carbapenemase genes—5% (34 of 741) of isolates with OXA-48-like enzymes also harbouring either a bla_{NDM} (33 of 741) or bla_{VIM} (1 of 741) allele. It follows that there can be no 'standard' antibiotic regimen for the treatment of infections caused by OXA-48-like producers without additional susceptibility testing and/or resistance gene data, although ceftazidime/avibactam shows promise based on *in vitro* data.[23] Although colistin, tigecycline and amikacin retained the highest levels of susceptibility their individual indications may make them unsuitable for the treatment of some infections. For example, tigecycline cannot be used for the treatment of urinary tract infections[24] and colistin use has been associated with both neurotoxicity and nephrotoxicity.[25]

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Table 3. MIC distributions for OXA-48-like-positive isolates ($n = 741$)

Carbepenemase gene(s)	Isolates	Antibiotic (range tested, mg/L)	EUCAST breakpoints ≤S/>R	Number of isolates with MIC (mg/L)												
				≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	NA	%S
OXA-48-like	Enterobacteriaceae	ertapenem	≤0.5/>1			4	15	53	124	147	351[a]				7	<1
	other spp.[c]	(0.125–16)													6	NA
NDM + OXA-48-like	Enterobacteriaceae										33[a]					0
VIM + OXA-48-like	Enterobacteriaceae										1[a]					0
OXA-48-like	Enterobacteriaceae	imipenem	≤2/>8		5	28	92	186	180	98	47	22	20	17[a]	6	45
	other spp.[c]	(0.06–128)				1			1			1	1	2[a]		17
NDM + OXA-48-like	Enterobacteriaceae								1	4	6	3	7	12[a]		0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	meropenem	≤2/>8	7[b]	38	105	138	110	69	35	81	112[a]			6	57
	other spp.[c]	(0.06–32)		1[b]			1				1	3[a]				33
NDM + OXA-48-like	Enterobacteriaceae									1	4	28[a]				0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	ceftazidime	≤1/>4	14[b]	57	69	86	31	28	26	23	47	83	231[a]	6	33
	other spp.[c]	(0.125–256)		2[b]				2	2							33
NDM + OXA-48-like	Enterobacteriaceae													33[a]		0
VIM + OXA-48-like	Enterobacteriaceae													1[a]		0
OXA-48-like	Enterobacteriaceae	cefotaxime	≤1/>2	1[b]	3	28	48	40	45	42	23	22	33	405[a]	11	12
	other spp.[c]	(0.125–256)		1[b]											5	100
NDM + OXA-48-like	Enterobacteriaceae													33[a]		0
VIM + OXA-48-like	Enterobacteriaceae													1[a]		0
OXA-48-like	Enterobacteriaceae	amikacin	≤8/>16			26[b]	138	186	124	58	47	30	86[a]		6	77
	other spp.[c]	(0.5–64)					2		1			1	2[a]			50
NDM + OXA-48-like	Enterobacteriaceae							2	2	2			27[a]			18
VIM + OXA-48-like	Enterobacteriaceae												1[a]			0
OXA-48-like	Enterobacteriaceae	gentamicin	≤2/>4	3[b]	55	175	85	10	11	10	22	324[a]			6	47
	other spp.[c]	(0.125–32)			1			1	1	2	1					33
NDM + OXA-48-like	Enterobacteriaceae					1						32[a]				3
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	tobramycin	≤2/>4	1[b]	17	119	114	29	36	59	87	230[a]			9	40
	other spp.[c]	(0.125–32)					1	1		1		3[a]				33
NDM + OXA-48-like	Enterobacteriaceae										5	28[a]				0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	ciprofloxacin	≤0.5/>1	191[b]	45	33	24	11	37	343[a]					17	39
	other spp.[c]	(0.125–8)		1[b]			1			4[a]						17
NDM + OXA-48-like	Enterobacteriaceae									31[a]					2	0
VIM + OXA-48-like	Enterobacteriaceae									1[a]						0

Continued

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OXA-48-like	Enterobacterales other spp.[c]	colistin (0.5–32)	≤2/>2		397[b]	204	20	11	16	13	21[a]		19	91
					2[b]	2	1	1						83
NDM + OXA-48-like	Enterobacterales				19[b]	12	1	1						97
VIM + OXA-48-like	Enterobacterales					1								100
OXA-48-like	Enterobacterales other spp.[c]	tigecycline (0.25–16)	≤1/>2		185[b]	140	144	118	65	24	6[a]		19	69
					1[b]			1					4	50
NDM + OXA-48-like	Enterobacterales				8[b]	3	6	12	3		1[a]			52
VIM + OXA-48-like	Enterobacterales									1				0

S, susceptible; R, resistant; NA, not available.
Broken vertical lines indicate intermediate breakpoints and the continuous vertical lines indicate resistant breakpoints.
[a]MIC greater than or equal to indicated value.
[b]MIC less than or equal to indicated value.
[c]Other spp. comprise *P. aeruginosa* and *Shewanella* spp.

At the time of writing, there are 14 known OXA-48-like variants, 11 of them CHDLs, and WGS analysis of 351 non-duplicate UK isolates identified 5 of the CHDL types. OXA-48 was by far the most common variant, found in two-thirds (235 of 351) of isolates. The earliest OXA-48-positive isolate identified in the UK was obtained in 2007 from a patient who had recently been hospitalized in Turkey; this isolate was shown here to be an ST147 *K. pneumoniae* carrying a plasmid with >99% identity to pOXA-48a. pOXA-48a has been implicated in early OXA-48 dissemination in Turkey.[15,26]

A travel history was available for only 24% of patients, of whom 42% had documented travel to 17 different countries, several of which have previously reported outbreaks involving bacteria with OXA-48-like enzymes.[2,4,5,27] All five patients with reported travel to Turkey and six of seven with travel to other Middle Eastern or North African countries whose isolates were analysed by WGS were found to carry organisms with bla_{OXA-48}, as repeatedly found in Turkey.[26,27] By contrast, both OXA-181 and OXA-232 have been associated with the Indian subcontinent,[12,28] and were found in 12 of 13 sequenced isolates from patients reporting travel to India, Pakistan or Sri Lanka. These data further underscore the role that international travel may play in carbapenemase dissemination. Notably four *K. pneumoniae* with OXA-48-like enzymes were from patients transferred to the UK for intensive care treatment of injuries received during the Libyan 'Emergency' of 2011.

Forty-four percent (154 of 351) of sequenced isolates and 153 of 235 of those with classical bla_{OXA-48} possessed DNA with >99% sequence identity to pOXA-48a, an IncL/M plasmid of ~62 kb first associated with bla_{OXA-48}, in Turkey and North Africa and now with multiple polyclonal and cross-species outbreaks.[15] pOXA-48a-like sequences were found here in multiple species and STs. Except for one isolate with $bla_{OXA-245}$ these all carried bla_{OXA-48}. The demonstration of both the broad range and success of this plasmid supports an earlier and much smaller analysis where we found IncL/M OXA-48 plasmids among several enterobacterial species and STs.[10] Of the remaining 197 isolates sequenced, 68 carried DNA with >99% identity to one of three plasmids: pOXA-232 (32 of 68), pOXA181 (30 of 68) or pKP3-A (6 of 68). pOXA-232 and pKP3-A are ColE-like plasmids, of ~6 and 7.6 kb, respectively, originally discovered in *E. coli* and *K. pneumoniae* isolates obtained from patients following hospitalization in India.[28,29] All of the 32 sequenced isolates harbouring $bla_{OXA-232}$ and 6 of 69 isolates with $bla_{OXA-181}$ contained sequences with >99% sequence identity to pOXA-232 and pKP3-A, respectively. Thirty further isolates with OXA-181 enzymes were shown to carry DNA with >99% sequence identity to pOXA181, an IncX3 plasmid of ~51.5 kb that was first found in an *E. coli* ST410 isolate obtained from the blood sample of a patient in China who had no history of travel to the Indian subcontinent.[30]

We found $bla_{OXA-48-like}$ genes in multiple species and STs and that some clones were particularly successful as a vehicle for $bla_{OXA-48-like}$ dissemination. In *K. pneumoniae* some STs (such as ST14 and ST147) were associated with multiple OXA-48-like plasmids, indicating the success of the ST, but not indicating expansion of a specific clone/plasmid pairing. Although *K. pneumoniae* ST395 has been associated with outbreaks in Europe and Morocco,[13] we found only two representatives among those sequenced and these harboured different OXA-48-like variants, bla_{OXA-48} and $bla_{OXA-181}$, in different genetic environments; one in a pKP3-A sequence and the other in an unidentified environment.

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Rather alarmingly, 16% of isolates were submitted from a single hospital, hospital A, representing at least six species and 33 STs. Within the 61 isolates from hospital A that were sequenced, most (54 of 61) harboured pOXA-48a sequences. This suggests the local spread of pOXA-48a among different genera, species and STs within this hospital and is indicative of the success of pOXA-48a in dissemination.

In contrast to the ST diversity among *K. pneumoniae* isolates with OXA-48-like enzymes ST38 accounted for almost half of all the sequenced *E. coli* isolates with OXA-48-like enzymes (53 of 114). ST38 has previously been associated with bla_{CTX-M} carriage in multiple countries.[31] Plasmid mapping could not establish a location for bla_{OXA-48} in most (50 of 53) of these isolates. In a previous study[10] the authors failed to obtain any plasmid transformants from ST38 isolates and, more recently, it was reported that bla_{OXA-48} and $bla_{OXA-244}$ can be chromosomally encoded in ST38 isolates.[32] This may apply here, but establishing this would require utilization of longer read sequencing techniques (e.g. PacBio and MinION).

In conclusion, this study has shown an increase in OXA-48-like enzymes in the UK over a 7 year period. We suggest that the accumulation of OXA-48-like carbapenemases within the UK is due to repeated import, coupled with both the dissemination of successful plasmids, notably pOXA-48a, and the spread of successful clones (e.g. *E. coli* ST38); the linkage to plasmid spread was particularly strong.

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Contaminated Handwashing Sinks as the Source of a Clonal Outbreak of KPC-2-Producing *Klebsiella oxytoca* on a Hematology Ward

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We investigated sinks as possible sources of a prolonged *Klebsiella pneumonia* carbapenemase (KPC)-producing *Klebsiella oxytoca* outbreak. Seven carbapenem-resistant *K. oxytoca* isolates were identified in sink drains in 4 patient rooms and in the medication room. Investigations for resistance genes and genetic relatedness of patient and environmental isolates revealed that all the isolates harbored the bla_{KPC-2} and bla_{TEM-1} genes and were genetically indistinguishable. We describe here a clonal outbreak caused by KPC-2-producing *K. oxytoca*, and handwashing sinks were a possible reservoir.

The prevalence of carbapenem-resistant *Enterobacteriaceae* has increased over the past 10 years (1). The production of *Klebsiella pneumonia* carbapenemases (KPCs) is one way of conferring resistance to carbapenems belonging to Ambler class A (2). Nosocomial infections due to KPC-producing *Enterobacteriaceae* are of increasing concern, especially in long-term care facilities and intensive care units (ICUs) (2). In 2011, a nosocomial outbreak of KPC-producing *Klebsiella oxytoca* occurred in a medical ICU at the Medical University of Graz in Graz, Austria. Five patients, all of whom survived, were affected (3).

From October 2011 to October 2013, KPC-producing *K. oxytoca* isolates with resistance patterns identical to that of the outbreak strain were identified from 10 more patients in the Division of Hematology ward. As *K. oxytoca* isolates were detected over a 2-year period, a reservoir in the environment was suspected. Nosocomial outbreaks with *K. oxytoca* have been reported previously (4–10). In two publications, handwashing sinks, especially sink drains, were identified as the source of the outbreaks (9, 10).

The aim of this study was to investigate sink drains as possible sources of the KPC-producing *K. oxytoca* isolates, the genetic relatedness of patient and environmental isolates, and the underlying mechanism of carbapenem resistance.

The outbreak occurred at the Division of Hematology at the Medical University of Graz, a tertiary care facility. This 28-bed ward has approximately 1,200 admissions annually, almost exclusively of patients with hematological malignancies. The index patient involved in the outbreak of KPC-producing *K. oxytoca* was in the medical ICU in December 2010 (3). The patient was considered colonized but not infected according to CDC criteria (11). The patient was then transferred to the hematology ward, where contact precautions were continued.

KPC-producing *K. oxytoca* isolates with resistance patterns identical to those of the outbreak strain were identified from 10 more patients in the Division of Hematology. Three patients were identified in late 2011 and 2012, and seven were detected from January to July 2013. Six patients developed an infection (Table 1). All the patients were admitted more than once during the outbreak period either for chemotherapy or because of side effects during chemotherapy.

Screening swabs from dry surfaces are taken and tested routinely 4 times per year by our infection control team and have never yielded *K. oxytoca*. In addition, in two publications dealing with nosocomial outbreaks of *K. oxytoca*, swabs from dry surfaces in the environment surrounding the patients did not yield any *K. oxytoca* isolates. In contrast, *K. oxytoca* was identified in sinks and drains (9, 10). Therefore, we chose to focus our investigation on sinks. Fifty-eight swabs were taken from handwashing sink drains, 23 from handwashing sink overflows (not all sinks had overflows), and 19 from shower drains in the Division of Hematology. Swabs from sink surfaces were taken from contaminated sinks in a second round of testing.

Swabs were seeded onto MacConkey agar and chromID Carba (both from bioMérieux, Marcy l'Étoile, France) and analyzed according to microbiological standards (12). The isolates were screened for the presence of resistance genes with the DNA microarray-based Check-MDR 103 kit (Check-Points, Wageningen, Netherlands) according to the manufacturer's protocol (http://www.check-points.com/support/manuals/). Sequencing of the detected carbapenemases and other beta-lactamase gene families was performed as previously described (13, 14). Repetitive sequence-based PCR (rep-PCR) was carried out with the DiversiLab system (bioMérieux, Nürtingen, Germany). In addition, all the isolates were tested using multilocus sequence typing (MLST), as described previously (15).

Eleven *K. oxytoca* isolates were found in the sink drains. Seven

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isolates showed multidrug resistance, including resistance to carbapenems, one was an extended-spectrum beta-lactamase (ESBL)-producing *K. oxytoca* isolate, and three isolates showed wild-type resistance. The 7 KPC-producing *K. oxytoca* isolates were found in rooms 23, 25, and 29 (double rooms housing 2 patients each with two sinks), room 37 (a single room), and 32A (a room in which medication is prepared by the nursing staff). Five KPC-producing *K. oxytoca* isolates were detected in sink drains (in rooms 23, 25, 29, 37, and 32A). One isolate was found in the sink overflow (room 37), and one was found in the shower drain (room 25). Swabs taken from sink surfaces in a second round did not yield any additional KPC-producing *K. oxytoca* isolates.

All the isolates from the patients and the seven isolates derived from sinks were multidrug resistant, exhibited susceptibility to amikacin, colistin, and fosfomycin only, and were found to be KPC producers. When microarray technology was used, bla_{KPC} and bla_{TEM} were detected in all the isolates and were identified as bla_{KPC-2} and bla_{TEM-1} by sequencing. rep-PCR revealed that all strains tested were indistinguishable with a similarity index of >97.5%. MLST yielded only one sequence type, ST4, for all the KPC-2-producing *K. oxytoca* isolates.

The starting point for this outbreak was a colonized patient from the ICU who later was transferred to the hematology ward. We hypothesize that in the case of this patient, KPC-2-producing *K. oxytoca* got into the sink most likely during personal hygiene activities or by the disposal of contaminated body fluids where it persisted. In the Division of Hematology, the water from the sink faucets directly hits the mesh that covers the sink drain. We hypothesize that some patients were colonized by contaminated aerosols when using the sinks for personal hygiene; 6 of 10 affected patients stayed in rooms with contaminated sinks, and 2 patients shared a room with a patient who later proved to be infected or colonized. We speculate that cross-contamination took place either by direct contact between the patients or through the hands of health care workers. The potential involvement of the health care workers is indicated by the fact that KPC-2-producing *K. oxytoca* was found in the sink of the room where medication is prepared by the health care staff.

As a consequence of the prolonged outbreak, the existing infection control measures (isolating colonized patients, enforcing hand hygiene measures, and cleaning the ward, particularly the sinks and equipment) were reinforced. An exchange of all the colonized sinks is under way. Since October 2013, no more KPC-producing *K. oxytoca* isolates have been identified.

In conclusion, a clonal relationship between environmental isolates and patient strains was determined and pointed to handwashing sinks as a possible reservoir for the prolonged nosocomial outbreak of KPC-2-producing *K. oxytoca*.

TABLE 1 Clinical data and epidemiological information

Patient no.	Patient age (yr) and gender[a]	Comorbidity	Date of first detection (day/mo/yr)	Site(s) of detection	Infection or colonization	Outcome	Patient stayed in a room with *K. oxytoca* in sink (room no.)
1	39, m	AML[b]	25/10/2011	Anal swab	Colonization	Alive	No
2	65, m	AML	28/12/2011	BAL[c]	Infection	Death due to KO[d] pneumonia	No
3	50, f	AML	13/11/2012	Blood, skin, stool, urine, abscess	Infection	Death due to KO abdominal wall abscess	Yes (23, 25)
4	51, f	AML	8/1/2013	Stool, BAL	Infection	Death due to AML relapse	No, but shared room with patient 5
5	60, f	AML	15/1/2013	Stool	Colonization	Alive	Yes (25)
6	61, m	B-cell non-Hodgkin lymphoma	9/4/2013	Anal swab, throat, BAL	Infection	Death due to KO pneumonia	Yes (25)
7	72, m	Myelodysplastic syndrome, secondary AML	14/05/2013	Stool	Colonization	Alive	Yes (23)
8	54, m	AML	17/5/2013	BAL, throat, anal swab	Infection	Death due to KO pneumonia	Yes (25)
9	89, m	B-cell non-Hodgkin lymphoma	31/7/2013	Catheter urine, throat	Colonization	Alive	No, but shared room with patient 8
10	56, m	AML	31/10/2013	Blood, throat, anal swab, skin, stool	Infection	Alive	Yes (25)

[a] m, male; f, female.
[b] AML, acute myeloid leukemia.
[c] BAL, bronchoalveolar lavage.
[d] KO, KPC-2- producing *K. oxytoca*.

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RESEARCH

Outbreak of Extended-Spectrum β-Lactamase–producing *Klebsiella oxytoca* Infections Associated with Contaminated Handwashing Sinks[1]

Christopher Lowe, Barbara Willey, Anna O'Shaughnessy, Wayne Lee, Ming Lum, Karen Pike, Cindy Larocque, Helen Dedier, Lorraine Dales, Christine Moore, Allison McGeer, and the Mount Sinai Hospital Infection Control Team

Klebsiella oxytoca is primarily a health care–associated pathogen acquired from environmental sources. During October 2006–March 2011, a total of 66 patients in a hospital in Toronto, Ontario, Canada, acquired class A extended-spectrum β-lactamase–producing *K. oxytoca* with 1 of 2 related pulsed-field gel electrophoresis patterns. New cases continued to occur despite reinforcement of infection control practices, prevalence screening, and contact precautions for colonized/infected patients. Cultures from handwashing sinks in the intensive care unit yielded *K. oxytoca* with identical pulsed-field gel electrophoresis patterns to cultures from the clinical cases. No infections occurred after implementation of sink cleaning 3×/day, sink drain modifications, and an antimicrobial stewardship program. In contrast, a cluster of 4 patients infected with *K. oxytoca* in a geographically distant medical ward without contaminated sinks was contained with implementation of active screening and contact precautions. Sinks should be considered potential reservoirs for clusters of infection caused by *K. oxytoca*.

Klebsiella oxytoca is an opportunistic pathogen that causes primarily hospital-acquired infections, most often involving immunocompromised patients or those requiring intensive care. Reported outbreaks have most frequently involved environmental sources (*1*–*4*). *K. oxytoca*, like other *Enterobacteriaceae*, may acquire extended-spectrum β-lactamases (ESBL) and carbapenemases (*1*,*5*); outbreaks of multidrug-resistant *K. oxytoca* infection pose an increasing risk to hospitalized patients.

We report an outbreak of infections caused by ESBL-producing *K. oxytoca* in the intensive care unit (ICU), step-down unit, and medical care unit at a hospital in Toronto, Ontario, Canada, during a 4-year period. Contributing to the ongoing difficulties in the containment of this outbreak has been the contamination of handwashing sinks in the ICU. We describe a retrospective review of all *K. oxytoca* isolates intermediate or resistant to third-generation cephalosporins identified from inpatients from April 1997 through December 2011, the investigation of the source of the *K. oxytoca* outbreak, and the interventions implemented to contain the outbreak.

Methods

The outbreak occurred at an acute tertiary-care facility in Toronto with 472 beds, including a 16 single-bed medical-surgical ICU, a 6-bed cardiac care unit , and two 4-bed step-down units. Outbreak cases of *K. oxytoca* were defined as hospital-acquired isolates with pulsed-field gel electrophoresis (PFGE) patterns belonging to 2 related clonal groups; all such isolates produced an Ambler class A ESBL. Isolates were considered hospital acquired if the first specimen (clinical culture or rectal swab) yielding resistant *K. oxytoca* was obtained ≥3 days after the admission date or if the specimen was obtained <3 days after admis-

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sion in a patient who had been hospitalized at the outbreak hospital within the previous 3 months. Patients were characterized as infected or colonized on the basis of National Healthcare Safety Network definitions (*6*).

Hospital infection-control guidelines provide that each patient colonized or infected with class A ESBL-producing *Enterobacteriaceae* be moved to a separate, single room with contact precautions in place. Risk factor–based admission rectal swab screening for ESBL-producing *Enterobacteriaceae* was initiated in 2004. High-risk populations are all patients admitted to an ICU and general medicine or surgical patients being transferred from an acute or long-term care facility or having a history of recent hospitalization or colonization/infection with a multidrug-resistant organism. Periodic prevalence screening is undertaken on medical and surgical wards, and potential clusters of clinical ESBL-producing isolates are investigated. ESBL colonized or infected patients are flagged in an electronic clinical system. Additional precautions are continued until weekly rectal swab specimens are negative over 4 weeks, at which point patients are placed in private rooms with ongoing periodic screening for 6 months.

Clinical specimens were processed by using conventional microbiological techniques. The VITEK 2 system (bioMérieux, Marcy l'Etoile, France) was used for identification and antimicrobial drug susceptibility testing of *K. oxytoca* isolates. Rectal screening swabs were plated on MacConkey agar with cefpodoxime (2 μg/mL). Tap water was cultured by vigorously swabbing the inside of each faucet with a cotton swab, turning the tap on and collecting 50 mL of water, vortexing the tube containing the water and the swab, centrifuging the sample twice at 3,500 × *g* for 15 minutes, and resuspending the resulting pellet of precipitated material in 3 mL of brain–heart infusion broth. Other environmental samples were obtained by inoculating premoistened cotton swabs (for dry surfaces) or by adding ≈0.25 mL of gel/liquid to 3 mL of brain–heart infusion broth. For sink cultures, cotton swabs were used to sample ≈10 cm^2 areas of the surface of the sink rim or basin. Drains were sampled by rotating swabs inserted 5–7 cm through the sink drain. Inoculated brain–heart infusion broth was incubated overnight at 37°C and then plated onto MacConkey agar with cefpodoxime. Clinical isolates intermediate or resistant to cefpodoxime (MIC ≥4 μg/mL) and colonies growing on the MacConkey agar with cefpodoxime underwent disk diffusion phenotypic confirmation (ceftriaxone, ceftazidime and aztreonam plus/minus clavulanic acid and cefoxitin) on Mueller-Hinton agar (*7*). PFGE was performed by using the restriction enzyme *Xba*I, with a run time of 20 h and switch times of 5 to 35 s at 12°C and 6 V/cm (CHEF-DR II System; Bio-Rad, Hercules, CA, USA); profiles were analyzed by using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium).

Results

Outbreak Description

Isolation of ESBL-producing *K. oxytoca* was uncommon in the 9 years before the outbreak; from January 1, 1997, through September 30, 2006, 10 clinical isolates (no bacteremias) and 6 colonized patients were identified. All but 1 colonized patient acquired the organism in the hospital, and 16/19 (84.2%) patients were previously or currently admitted to the ICU at the time of culture. PFGE of these isolates revealed that 5 (26.3%) isolates belonged to pattern A, 3 (15.8%) isolates belonged to pattern B, 3 isolates were closely related to each other but unrelated to isolates of pattern A or B, and 3 isolates had unique patterns. Two isolates were unavailable for typing. Only 1 case (April 2004) was identified between April 2003 and September 2006.

From October 2006 through March 2011, ESBL-producing *K. oxytoca* was isolated from 87 patients (Figure 1); 21 were not part of the outbreak. Eight of these nonoutbreak patients had isolates from clinical cultures or screening specimens obtained within 72 hours of first admission to the hospital, and each isolate had a unique pattern by PFGE. The remaining 13 had isolates first identified ≥3 days after admission (n = 11) or had been previously admitted to this hospital (n = 2), but each isolate had a unique PFGE pattern, with no temporal or geographic clustering. The remaining 66 patients were classified as outbreak case-patients.

All 66 outbreak case-patient isolates carried Ambler class A β-lactamases. Clinical *K. oxytoca* isolates were identified from 27 patients; among these, 24 patients had 25 hospital-acquired infections (9 urinary tract infections, 4 of them bacteremic; 8 asymptomatic bacteriurias; 4 soft tissue infections, 1 of them bacteremic; 3 primary bacteremias; and 1 pneumonia with bacteremia). Of the 9 bacteremias,

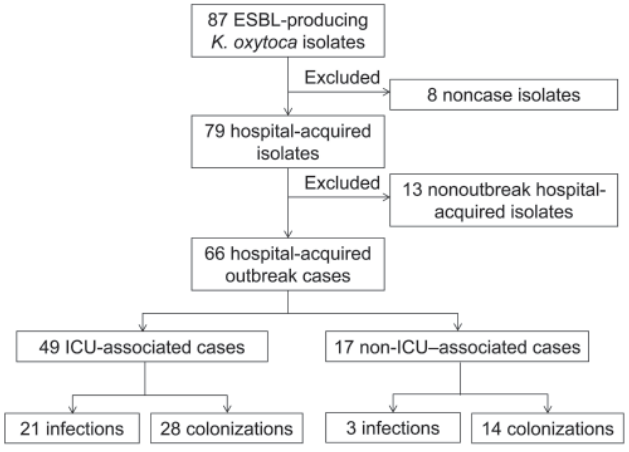

Figure 1. Flow of extended-spectrum β-lactamase (ESBL)–producing *Klebsiella oxytoca* infection and colonization in patients at a hospital in Toronto, Ontario, Canada, October 2006–March 2011. ICU, intensive care unit.

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8 were PFGE pattern A. On the basis of study definitions, 3 patients had clinical specimens (2 sputum samples and 1 bronchoalveolar lavage culture) that were not associated with infection. In 11 cases, clinical cultures were preceded by identified rectal colonization; median time to first identification of a clinical isolate after recognition of colonization was 10 days (mean 12.5 days, range 1–31 days). Of the remaining 16 cases with clinical isolates of *K. oxytoca*, 13 patients had a prior negative result from an ESBL rectal screening. Thirty-nine patients were identified as colonized by rectal swab screening but no subsequent clinical isolate was identified. Patients remained colonized for variable periods of time, with the proportion colonized still positive on repeat screening as follows: 7 days (30/49, 61.2%), 14 days (20/41, 48.7%), 21 days (15/38, 39.4%), 28 days (14/33, 42.4%), 2 months (6/22, 27.3%), and 3 months (4/17, 23.5%).

Figure 2 summarizes the occurrence of outbreak-related ESBL-producing *K. oxytoca* clinical isolates over time in the ICU, where most cases occurred (49/66, 74%). Cases with clinical isolates were identified regularly from October 2006 through December 2009; however, no clinical isolates have been recovered from patients in or exposed to the ICU since that time. The 6 outbreak cases in 2010 and 4 in 2011 were identified only by rectal swab screening. The number of newly identified cases on point-prevalence screens was 13/1,049 in 2008 (1.2%), 7/1,744 (0.4%) in 2009, 6/921 (0.7%) in 2010, and 1/754 (0.1%) in 2011 ($p = 0.01$). Because prevalence screens were performed more frequently when new cases were occurring, the total number of prevalence screens decreased in 2010 and 2011 compared with 2008 and 2009.

Seventeen case-patients who had not been admitted to the ICU were identified as colonized or infected with outbreak strains. Eleven of these were part of 3 clusters. In August 2010, a total of 4 patients (3 colonized, 1 infected) from whom isolates were identified that were indistinguishable by PFGE (pattern A) acquired the strain on a single medical ward. In the medical step-down unit, 3 patients were identified as becoming colonized during June–September 2010; an additional 4 patients became colonized in February and March of 2011 (all pattern A). The remaining cases were sporadically identified on the surgical step-down unit (n = 4) and in general surgery/gastroenterology units (n = 2).

Outbreak-specific Infection-control Interventions

At the onset of the outbreak (October 2006), the investigation encompassed a search for potential environmental sources. Samples for culture were obtained from potential reservoirs (e.g., shared equipment such as electrocardiogram and ultrasound machines, bronchoscopes and solutions used in endoscopy areas, glucometers, hand creams, lubricating gels, disinfectant swabs, blood gas machines, water baths, ice machines, mouthwashes, oral medications, and soaps), and prevalence screening was conducted to detect patient colonization in the ICU. No environmental sources were identified, no multidose vials or bags of parenteral fluids or oral medications were in use in the unit, and no procedures or exposures were identified that linked affected patients but not other patients. Two of 16 sinks had aerators, neither of which yielded *K. oxytoca* on culture. Tap water and sinks were not cultured at this time. All patients newly identified as colonized or infected had contact

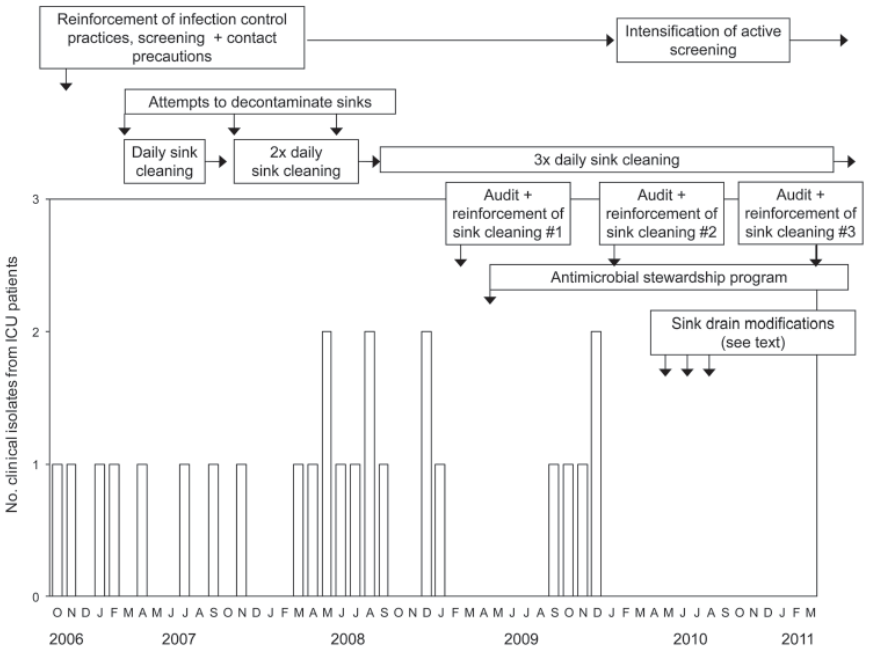

Figure 2. Nosocomial extended-spectrum β-lactamase–producing *Klebsiella oxytoca* clinical isolates from patients in the intensive care unit of a hospital in Toronto, Ontario, Canada, and the associated interventions implemented to contain the spread of the outbreak, October 2006–March 2011. ICU, intensive care unit.

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precautions applied. Hand hygiene practices, adherence to contact precautions, and appropriate management of water/liquid/gels were reinforced. These interventions had no apparent effect on the rate of new clinical infections; new patients acquired the organism after weeks without identified colonized patients in the ICU.

In April 2007, results of repeat screening of potential environmental sources were negative, as were samples of tap water; however, multiple handwashing sinks were found to be contaminated with the outbreak strains. Sinks in the ICU are foot pedal–operated, free-standing, porcelain hand hygiene sinks located just inside the door to each ICU room. Although intended only for hand hygiene, they were also used for disposal of fluids, including body fluids. When sinks were identified as a potential reservoir, use of the sinks for hand hygiene only was reinforced. Attempts were made to reduce or eradicate *K. oxytoca* contamination by cleaning sinks and leaving them unused for 48 hours with disinfectant standing in traps. When this process failed, routine daily sink disinfection was initiated; sink surfaces, including taps, rims of sinks, and basins, were cleaned with a 1:16 dilution of Virox (Virox Technologies Inc., Oakville, CA, USA), and ≈250 mL of the diluted solution was poured down the drain. Neither this daily cleaning, nor month-long trials of cleaning with bleach and with a foaming hydrogen peroxide product, resulted in reduced sink colonization rates. Sink cleaning was increased to 2×/day in late 2007 and 3×/day in August 2008. Adherence to cleaning standards, particularly frequency of cleaning, was variable. Regular reminders to cleaning staff were required, and the identification of new hospital-acquired cases usually resulted in recognition that adherence had decreased.

Figure 3 shows the overall rates of recovery for patients with outbreak-related ESBL-producing *K. oxytoca* infection associated with handwashing sinks in the ICU. ICU sink culture screens were performed on 29 separate occasions, yielding a total of 910 cultures. The average rate of sink contamination during the outbreak period was 16.4% (149/910). After implementation of 3×/day cleaning/disinfection of sinks (October–December 2008), the sink colonization rate decreased to 3.9% (3/77) during the quarter; the rate increased to 16.7% (71/424) the following quarter (January–March, 2009), when adherence to routine sink cleaning was noted to have decreased.

Many of the ICU sinks had old patented opening drains (a pipe connecting the sink basin to the sink trap), a design that allowed drainage from the overflow hole to mix with the regular drainage water, potentially impairing adequate drainage. During February–June 2010, all drains were changed, eliminating the connection with the overflow drain; the overflow holes were decommissioned; the strainers in the sink basin were replaced by strainers containing a larger number of smaller holes to reduce backsplash; and sink traps were replaced.

Investigation of the medical unit on which 4 patients acquired outbreak strains during summer 2010 failed to identify an environmental source; all sink cultures were negative. After initiation of standard contact precautions for the colonized patients, no additional colonized or infected patients were identified in that unit. In contrast, the outbreak strain of ESBL-producing *K. oxytoca* was recovered from sinks, but not other environmental sources, in the medical step-down unit during August 2010. The implementation of regular sink cleaning and contact precautions for colonized patients resulted in no cases being identified during September 2010–February 2011. When new cases were identified in 2011, the previously described sink modifications were implemented in the step-down unit.

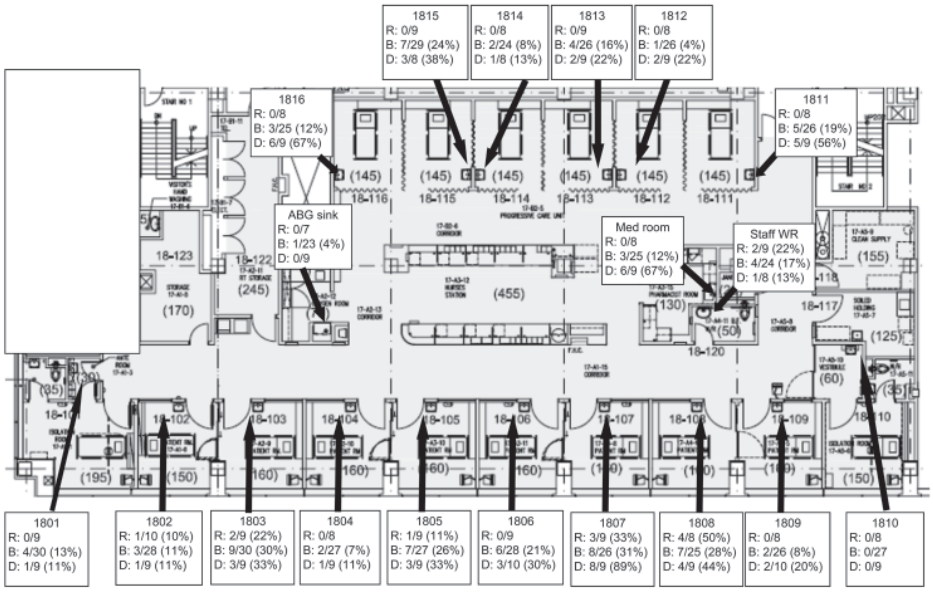

Figure 3. Locations of environmental screening for extended-spectrum β-lactamase–producing *Klebsiella oxytoca* in the intensive care unit sinks. Numbers indicate room numbers. R, sink rim; B, sink basin; D, sink drain; WR, washroom; med room, medication room (pharmacy); ABG, arterial blood gas room.

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Concomitant Infection-control Interventions

As part of an ongoing program to improve adherence to hand hygiene, routine observational hand hygiene audits started throughout the hospital in fall 2008. Compliance rates improved gradually over the course of the outbreak, from 59.4% in 2008 to 69.8% in 2011.

An antimicrobial stewardship program was initiated in only the ICU in February 2009. An audit and feedback program was instituted and run by an infectious diseases physician and a pharmacist. During the first year of the program, the mean antibacterial defined daily dose per 100 patient-days decreased 9.2% compared with the same time period in the previous year (*8*).

Discussion

Outbreaks of health care–associated infection caused by *K. oxytoca* have most often been associated with contamination of environmental reservoirs such as disinfectants (*9*), multidose vials or parenteral fluid bags (*10*,*11*), humidifiers (*2*), and ventilators (*3*). This outbreak suggests that handwashing sinks in high-intensity hospital care areas may be a reservoir for *K. oxytoca* and that person-to-person transmission may also occur.

Patients in this medical-surgical ICU continued to acquire the outbreak organism despite review of routine practices, hand hygiene education and auditing, screening to identify colonized patients, and implementation of contact precautions for colonized and infected patients. In contrast, this approach seemed to control transmission in the medical ward, where contamination in sinks was not found, and has been reported to be successful in the control of other outbreaks of ESBL-producing *Enterobacteriaceae* (*12*). In this hospital, transmission of the outbreak strains of *K. oxytoca* seemed to occur both from sinks and from colonized/infected patients. As the emergence of carbapenemase-producing organisms focuses attention on health care–associated infections due to *Enterobacteriaceae,* other reservoirs may also be recognized. Recently, a clone of *K. pneumoniae* possessing SHV-1 and CTX-M-15 ESBLs was implicated in a hospital-wide foodborne outbreak in Spain, in which the hospital kitchen and colonized food handlers were the presumed reservoirs (*13*).

During the outbreak reported here, patients who acquired ESBL-producing *K. oxytoca* colonization were followed up with routine rectal swab screening; 23.5% remained colonized after 3 months. Colonization with ESBL-producing *Enterobacteriaceae* can persist for months to years (*14*,*15*); data are insufficient to determine whether duration of carriage is different for different species or clones of *Enterobacteriaceae*. In 1 study, only 6.8% of colonized patients cleared carriage over 3 years of follow-up (*14*). In addition, colonized patients may have intermittently positive rectal screening results, which suggests carriage at concentrations below the limit of detection for rectal swab specimens (*16*).

At the hospital in this study, patients to whom contact precautions are applied remain under these guidelines for 1 month and in private rooms for 6 months. However, for resistant gram-negative bacteria of epidemiologic importance (e.g., carbapenemase-producing organisms), extending the duration of contact precautions until discharge may be warranted. Because of the long duration of colonization, hospitalization is also likely to be a risk factor for community-onset infection with multidrug-resistant *K. oxytoca*, as has recently been described in Athens, Greece (*17*). The existence of asymptomatic colonized patients compounds the difficulty of containing the spread of these organisms; containing outbreaks without active surveillance may not be possible (*18*).

The outbreak-associated clones of *K. oxytoca* found in this study were ubiquitous in sinks in the ICU, cultured from 15/16 patient rooms as well as from other sinks (e.g., staff washrooms). Increased sink cleaning and auditing was associated with a decline in clinical isolates, but these measures proved difficult to sustain. Achieving persistent reductions in the degree of contamination in ICU sinks is difficult but has been a necessary intervention in outbreaks of *Pseudomonas aeruginosa* (*19*,*20*). In these outbreaks, structural changes, including renovation to sinks and plumbing or alteration of water temperature, reduced but did not eliminate the outbreak organism from sink drains. As in our experience, although the organisms could still be recovered after alterations to improve drainage and reduce splashing, these modifications were temporally associated with persistent declines in the rate of clinical infections. Persistence of *Pseudomonas* spp. in ICU sinks has been attributed to biofilm formation, which allows stable attachment to environmental surfaces and protection from disinfection (*20*,*21*). Biofilm formation has also been described for *K. oxytoca* on filtration membranes (*22*) and is probably a factor in the persistence of *K. oxytoca* in sinks in this outbreak.

This outbreak also emphasizes the challenges associated with limited space and sinks in older hospitals. Presumptively, these handwashing sinks became contaminated because they were used for the disposal of body fluids from colonized patients. While this is clearly unacceptable, nurses in the ICU are required to walk past several rooms (and out of isolation rooms) to reach the dirty utility room for disposal of body fluids, an activity that is also associated with risk. As we increasingly recognize the risks associated with hospital water and sinks, the design of ICUs becomes critical for protecting patients from these risks.

In conclusion, we describe an outbreak in which colonized sinks were a contributing reservoir for ESBL-producing class A *K. oxytoca.* A multifaceted approach including reinforcement of infection control policies (hand hygiene,

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contact precautions, isolation and admission/routine rectal screening, clear delineation between handwashing sinks and sinks for other purposes), intensified cleaning of sinks, structural changes to the sinks, and antimicrobial stewardship has reduced but not eliminated transmission of the outbreak strain. Although *K. oxytoca* is in the family *Enterobacteriaceae*, its epidemiology is not clearly defined, and it may be more likely than other *Enterobacteriaceae* to be associated with environmental reservoirs in hospitals. Sinks should be considered potential reservoirs when clusters of infection caused by *K. oxytoca* are investigated.

Dr Lowe is a resident in medical microbiology at the University of Toronto. His current research interests are focused on identifying optimal methods for infection control of multidrug-resistant gram-negative organisms.

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Genomic surveillance of *Escherichia coli* and *Klebsiella* spp. in hospital sink drains and patients

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Abstract

Escherichia coli and *Klebsiella* spp. are important human pathogens that cause a wide spectrum of clinical disease. In healthcare settings, sinks and other wastewater sites have been shown to be reservoirs of antimicrobial-resistant *E. coli* and *Klebsiella* spp., particularly in the context of outbreaks of resistant strains amongst patients. Without focusing exclusively on resistance markers or a clinical outbreak, we demonstrate that many hospital sink drains are abundantly and persistently colonized with diverse populations of *E. coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, including both antimicrobial-resistant and susceptible strains. Using whole-genome sequencing of 439 isolates, we show that environmental bacterial populations are largely structured by ward and sink, with only a handful of lineages, such as *E. coli* ST635, being widely distributed, suggesting different prevailing ecologies, which may vary as a result of different inputs and selection pressures. Whole-genome sequencing of 46 contemporaneous patient isolates identified one (2%; 95% CI 0.05–11%) *E. coli* urine infection-associated isolate with high similarity to a prior sink isolate, suggesting that sinks may contribute to up to 10% of infections caused by these organisms in patients on the ward over the same timeframe. Using metagenomics from 20 sink-timepoints, we show that sinks also harbour many clinically relevant antimicrobial resistance genes including $bla_{\text{CTX-M}}$, bla_{SHV} and *mcr*, and may act as niches for the exchange and amplification of these genes. Our study reinforces the potential role of sinks in contributing to Enterobacterales infection and antimicrobial resistance in hospital patients, something that could be amenable to intervention. This article contains data hosted by Microreact.

DATA SUMMARY

Raw sequencing data are available under NCBI SRA accessions PRJNA604910 and PRJNA604975 (cultured isolates), and ENA project PRJEB36775 (metagenomes). A supporting data repository containing metadata, phylogenies, Jupyter notebooks and Pavian reports is archived at Figshare (doi:10.6084/m9.figshare.12337283). Microreact projects are available for *E. coli*, *K. oxytoca* and *K. pneumoniae* cultures. The authors confirm all supporting data, code and protocols have been provided within the article or the supporting data repository.

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Abbreviations: AMR, antimicrobial resistance; ESBL, extended-spectrum beta-lactamase; PERMANOVA, permutational analysis of variance; SNP, single nucleotide polymorphism.
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Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Six supplementary tables and eight supplementary figures are available with the online version of this article.
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INTRODUCTION

Infections caused by Enterobacterales, including *Escherichia coli* and *Klebsiella* spp., are major causes of global morbidity, and particular antimicrobial-resistant strains (namely extended-spectrum beta-lactamase [ESBL] and carbapenemase producers) have been listed as critical priority pathogens for mitigation by the World Health Organization. In the UK, year-on-year increases have been observed in the number of *E. coli* and *Klebsiella* spp. bloodstream infections [1] for reasons that remain unclear. As well as causing invasive disease, these organisms are capable of colonizing a wide range of animal and environmental niches, and are frequently carried in the human gastrointestinal tract [2]. As such, they are also commonly found in human wastewater, and in wastewater-associated sites such as sewers and water-treatment infrastructure [3].

A significant proportion of Enterobacterales infections are healthcare-associated, prompting the UK government to introduce a target in 2016 to halve the number of healthcare-associated Gram-negative bloodstream infections by 2021 [4]. Wastewater sites in hospitals have been highlighted as reservoirs of drug-resistant Enterobacterales, with several studies reporting that ongoing transmission and outbreaks of human disease are associated with the contamination of, for example, sinks, by these organisms [5, 6]. More recently, several studies have shown reductions in colonization and/or invasive infection with Enterobacterales and other Gram-negative bacilli following the introduction of strategies to remove sinks and mitigate possible contamination from wastewater sources in patient rooms [7, 8]. Most of these studies however focus on the sampling and control of antimicrobial-resistant strains, often representing a more immediate clinical problem in an outbreak setting, rather than on the possibility that these sites may represent part of the wider endemic transmission network of both susceptible and resistant strains causing infection in patients.

Whole-genome sequencing of bacterial isolates is increasingly used as the most robust, high-resolution approach to characterizing relatedness between strains, and hence determining likely transmission [9]. However, the diversity of complex, polymicrobial environmental reservoirs is incompletely captured by sequencing small numbers of isolates, and this breadth of diversity can be more fully captured by using a metagenomic approach, which characterizes the genetic complement of a whole sample [10]. Combining both approaches has been shown to improve our understanding of species and antimicrobial resistance (AMR) gene diversity within environmental, wastewater and river samples [11, 12] and of transmission in a sink-associated outbreak of *Sphingomonas koreensis* (also a Gram-negative bacillus) in the NIH Clinical Centre in the USA [13].

In order to investigate the prevalence of contamination of healthcare sinks by strains of *E. coli* and *Klebsiella* spp., including those resistant to third-generation cephalosporins and carbapenems, we sampled all sink sites using p-trap (U-bend) aspirates across several wards and timepoints in a single UK hospital in 2017. We used a combination of whole-genome sequencing of cultured isolates from all sink samples and metagenomic sequencing of a subset of sink samples to facilitate a high-resolution assessment of the genetic diversity present in these niches. To determine whether sinks were a reservoir of Enterobacterales strains causing infection in patients over similar timeframes, we simultaneously retrieved relevant isolates from culture-positive specimens taken from patients admitted to the same ward locations, and used genomics to identify the degree of genetic relatedness.

Impact Statement

Escherichia coli and *Klebsiella* spp. cause a wide range of bacterial infections, including bloodstream, urine and lung infections. Previous studies have shown that sink drains in hospitals may be part of transmission chains in outbreaks of antimicrobial-resistant *E. coli* and *Klebsiella* spp., leading to colonization and clinical disease in patients. We show that even in non-outbreak settings, contamination of sink drains by these bacteria is common across hospital wards, and that many antimicrobial resistance genes can be found and potentially exchanged in these sink drain sites. Our findings demonstrate that the colonization of handwashing sink drains by these bacteria in hospitals is likely contributing to some infections in patients, and that additional work is needed to further quantify this risk, and to consider appropriate mitigating interventions.

METHODS

Ward-based sink sampling

We sampled three units (acute admissions [AA], adult critical care [ACC], adult general medicine [female only] [GM]) within a single hospital (John Radcliffe Hospital, Oxford, UK) four times on rotation every 3 weeks over 3 months, March–May 2017. Units were chosen to capture different patient populations, admission turnaround times and wastewater plumbing infrastructure. The haematology ward (on a separate hospital site) was also sampled on a single day (12 May 2017) subsequent to a small cluster of patient cases of $bla_{\text{OXA-48}}$ carbapenemase-associated Enterobacterales bloodstream infections described previously [14]. Ward and sink/wastewater layouts were obtained from estates, and each sink/drain site was assigned a unique site identifier (Table S1).

On each day of sampling, autoclaved tubing cut to 10 inches was used to aspirate from sink p-traps via a sterile 50 ml syringe. Up to 50 ml of fluid was aspirated where possible. Then, 100 µl of tenfold dilutions (10^{-2}, 10^{-3}, 10^{-4}) of each sink p-trap aspirate were plated onto CHROMagar Orientation media (Becton Dickinson, Franklin Lakes, NJ, USA), with no disc, cefpodoxime (10 µg), ertapenem (10 µg) (Thermo Scientific Oxoid, Basingstoke, UK) applied in a triangular fashion to each plate. Cultures were incubated at 37 °C for

~18 h. Growth of Enterobacterales (presence/absence) and density (sparse/dense/confluent) in all zones was recorded (i.e. no antibiotic, in the presence of cefpodoxime, and in the presence of ertapenem). Up to four distinct colonies of each of presumptive *E. coli* and *Klebsiella* spp. were sub-cultured on CHROMagar Orientation to confirm purity and species identification. Species identification of sub-cultured colonies was confirmed by MALDI-ToF (MALDI Biotyper, Bruker, Billerica, MA, USA). Stocks of sub-cultured isolates were stored at −80 °C in 400 µl of nutrient broth +10% glycerol prior to DNA extraction for sequencing. Aspirates from sink p-traps were then centrifuged at 4000 r.p.m. for 10 min at 4 °C, and supernatants removed; pellets were stored at −80 °C.

Patient isolate sampling

For AA, ACC and GM wards, a pseudo-anonymized, prospective feed was set-up to try and enable real-time capture of isolates from all samples culture-positive for *E. coli*, *K. pneumoniae* and *K. oxytoca* from patients admitted to any of these wards during the study time period, and were processed routinely through the clinical microbiology laboratory in the John Radcliffe Hospital in accordance with local standard operating procedures for clinical sample types, and compliant with national standards for microbiology investigations [15]. These typically involve selective culture steps and species identification using MALDI-ToF (MALDI BioTyper, Bruker, Billerica, MA, USA).

Pseudo-anonymized extracts of all patient culture results and admission/discharge data covering the study period were obtained after the study was finished through the Infections in Oxfordshire Research Database to enable an evaluation of (i) baseline sampling denominators, (ii) the extent of relevant clinical isolate capture and (iii) the temporal and spatial overlap of any genetically related sequenced isolates from patients and sequenced isolates/metagenomes from sinks.

Isolate sequencing and p-trap aspirate metagenomics

All isolates confirmed as *E. coli*, *K. pneumoniae* and *K. oxytoca* from patients and p-trap aspirates were extracted for sequencing using the QuickGene DNA extraction kit (Autogen, MA, USA) following manufacturer instructions, plus an additional mechanical lysis step prior to chemical lysis (FastPrep, MP Biomedicals, CA, USA; 6 m/s for two 40 s cycles).

For metagenomics, DNA was extracted from a subset of stored pellets (n=20) using the MoBio PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions, and including a mechanical lysis step of two 40 s cycles at 6 m/s in lysing matrix E and final elution in buffer CDT-1 (Autogen, MA, USA). In total, 45 ng of *Thermus thermophilus* DNA (reference strain HB27, ATCC BAA-163 [DSMZ, Germany]) was added to each sample in the PowerBead tube at the start of the experiment, prior to the addition of solution C1 as an internal control and normalization marker [11]. Sink aspirates were selected for metagenomic sequencing to enable evaluation of (i) microbiome differences within and between wards, (ii) longitudinal change in microbiota composition, and (iii) whether culture-negative sinks harboured the bacterial species being studied, i.e. indicating limited sensitivity of culture-based approaches.

Short-read sequencing (single isolate and metagenomics) was performed on the Illumina HiSeq 4000, pooling 192 isolate extracts and six metagenomes per lane, and generating 150 bp paired-end reads. Median metagenomic sequencing throughput was 547 Mb (IQR 499Mb to 942Mb) per sample.

Computational methods

Cultured isolate informatics. Of the isolates sent for sequencing, 439/446 (98%) sink and 46/46 (100%) patient isolates were successfully sequenced and classified with Kraken/MiniKraken [16] as Enterobacterales, and used for subsequent analysis. Isolate consensus sequences were constructed by read mapping and consensus inference with respective *E. coli*, *K. oxytoca* and *K. pneumoniae* reference genomes AE014075.1, NC_018106.1 and CP000647.1 using Snippy 4.4.0 [17]. Isolate genomes were assembled using Shovill 1.0.4 [18]. Recombination-adjusted phylogenetic reconstruction was performed using runListCompare 0.3.8 [19] wrapping IQ-TREE 1.6.11 [20] and ClonalFrameML 1.12 [21]. Final core-genome alignments included 218/219 *E. coli* isolates, 165/167 *K. oxytoca* isolates and 98/99 *K. pneumoniae* isolates, all of which satisfied the runListCompare filtering criteria of perACGT_cutoff >=70%, varsite_keep >=0.8 and seq_keep >=0.7. Overall, 100 SNP core-genome clusters were defined by single linkage clustering of runListCompare pairwise distance matrices. Trees were midpoint rooted prior to visualization. See supporting data repository for runListCompare configuration. Read-based MASH trees were constructed using MASH 2.2.2 [22] and RapidNJ 2.3.2 using 21 mers, a sketch size of 10 000 and a minimum abundance threshold of 10 *k*-mers. Nearest neighbours between sink and clinical isolates were identified in both Core SNP and MASH space. Assembly-based core and accessory genome partitioning was performed using PopPUNK 1.1.7 [23]. Resistance genotyping and phenotype prediction in cultured isolates was performed using ResPipe [11] and ARIBA 2.14.4 [24] with the CARD 3.0.3 database [25]. Tree comparisons (tanglegrams) were generated using the R package Dendextend 1.5.0 [26].

Metagenome informatics. Metagenomic sequences were analysed for taxonomic and antimicrobial resistance gene presence using ResPipe [11] and Kraken2 [27] with CARD database version 3.0.3. Large resistance gene families were clustered to facilitate visualization of resistance profiles (methodology documented in supporting data repository). A metagenomic assembly of the *mcr-4* gene was generated with MEGAHIT 1.2.9 [28], to which reads were aligned with Minimap2 2.17-r941 [29] and consensus inferred using Kindel [30]. Metagenomic summary statistics were generated using Pavian [31].

Data analysis was performed with the SciPy ecosystem [32] and JupyterLab [33]. Matplotlib [34], Bokeh and Microreact [35] were used for visualization.

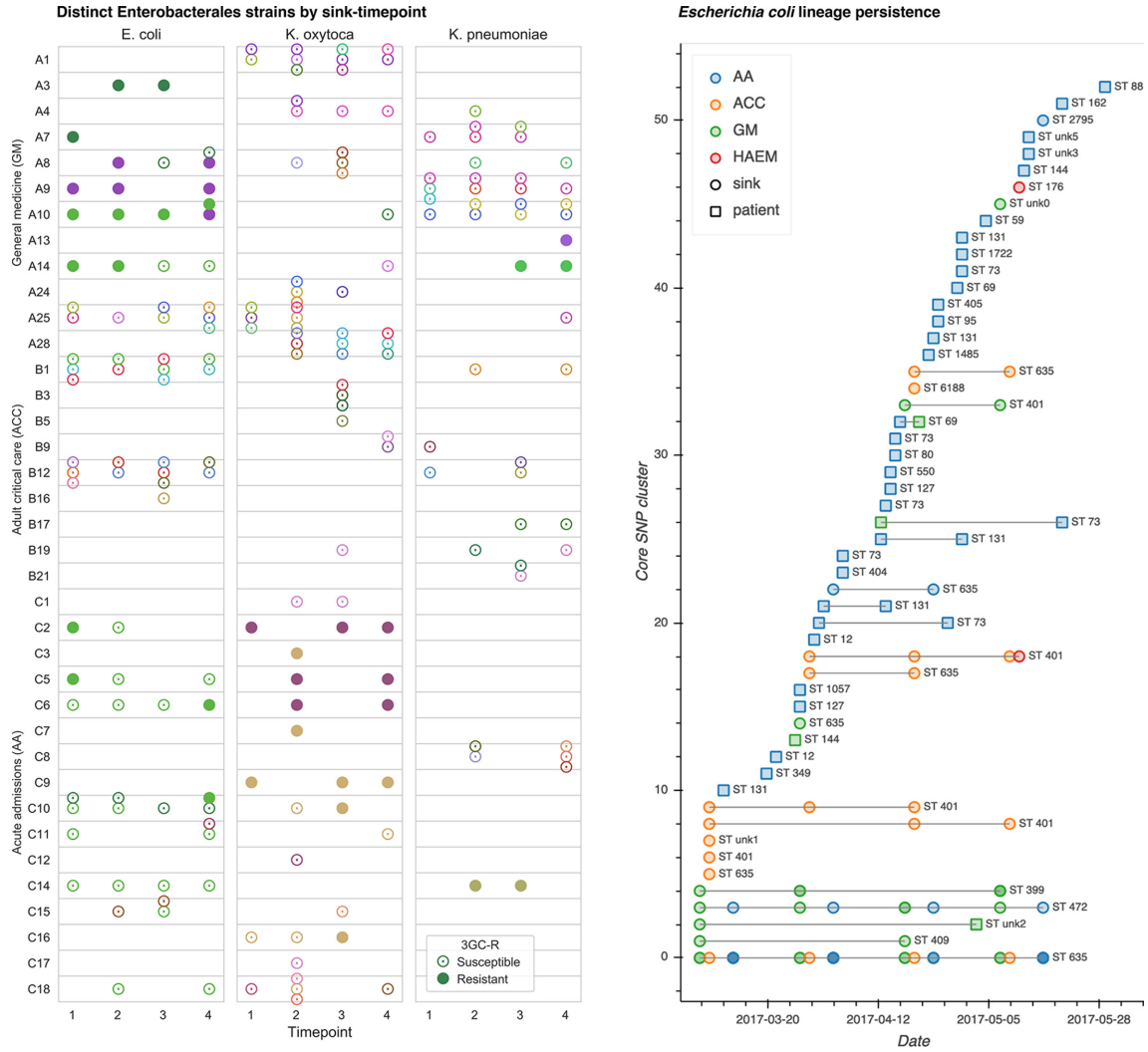

Fig. 1. Cluster distribution and persistence. Left: strain-distinct cultured isolates of *E. coli*, *K. oxytoca* and *K. pneumoniae* from sink drain aspirates sampled over 12 weeks across three hospital wards. Different colours indicate distinct strains (defined by 100 SNP clusters), and cefpodoxime-resistant and/or selected ESBL-positive isolates are indicated by filled markers. Right: persistence of sink and contemporaneous patient *E. coli* strains throughout the sampling period.

RESULTS

Diverse, often antimicrobial-resistant Enterobacterales lineages are frequent—and often persistent—colonizers of hospital sink drains

In total, 439 Enterobacterales isolates comprising *E. coli* (*n*=180), *K. oxytoca* (*n*=166) and *K. pneumoniae* (*n*=93) were cultured and successfully sequenced at one or more timepoints from 12/20 (60%), 9/23 (43%) and 16/23 (70%) sinks sampled four times over 12 weeks (March–May 2017) in general medicine (GM), adult critical care (ACC) and acute admissions (AA) wards, respectively (97/264 [37%] sink-timepoints culture-positive overall; Fig. 1). A further 30 isolates of *E. coli* (*n*=13), *K. oxytoca* (*n*=13) and *K. pneumoniae* (*n*=4) were cultured from 11/59 (19%) sinks in a haematology ward, sampled at a single timepoint only during this period (Fig. S1, available in the online version of this article). See Table S1 for surveyed sink descriptions. Species distributions (by culture) were relatively even across the general medicine ward, while the adult critical care unit was enriched for *E. coli*, and the acute admissions ward was depleted in *K. pneumoniae* (Table S2).

Analysis of whole-genome sequences from cultured Enterobacterales revealed widespread and sustained colonization of sinks by multiple sequence types (STs) of these species (Figs 1 and S1) In total, eight known and four novel *E. coli* STs were represented, of which STs 635 (*n*=109, 61%), 401 (*n*=25, 14%) and 472 (*n*=18, 10%) accounted for 84% of sequenced isolates (152/180). *Klebsiella* spp. STs were more varied: 15 known and six novel *K. oxytoca* STs were represented, of which the most frequent was ST177 (33/166, 20%), while there were 18 known and one novel *K. pneumoniae* STs, the most frequent being ST872 (24/93, 26%).

Table 1. Spatiotemporal distribution of 100 core SNP lineages of cultured *E. coli* (*n*=53), *K. oxytoca* (*n*=51) and *K. pneumoniae* (*n*=30) (a) overall, and (b) occurring in >1 isolate in sinks on wards that were repeatedly sampled

(a)		*E. coli*	*K. oxytoca*	*K. pneumoniae*	Total
Lineages with >1 isolate					
	Patient	28	1	3	32
	Sink	3	37	12	52
Lineages with >1 isolate					
Single timepoint	Same sink	5	6	3	14
	Different sinks; same ward	0	1	0	1
Multiple timepoints	Same sink	7	1	7	15
	Different sinks; same ward	1	4	4	9
	Different wards	3	1	0	4
Patients	Patient and sink (single timepoint)	1*	0	0	1
	Same patient	1	0	1	2
	Different patients	4	0	0	4
Total		53	51	30	134
(b)		***E. coli***	***K. oxytoca***	***K. pneumoniae***	**Total**
Sink lineages with >1 isolate (excludes haematology ward)					
Single timepoint	Same sink	5	4	3	12
	Different sinks; same ward	0	0	0	0
Multiple timepoints	Same sink	8	1	7	16
	Different sinks; same ward	1	4	4	9
	Different wards	2	1	0	3
Total		16	10	14	40

*Lineage has three isolates; all taken from the same ward; two from the same sink at the same timepointand one from a patient 2 months later.

Across all locations and sink-timepoints, sequenced isolates comprised 20, 50 and 26 distinct lineages (defined as differing by <=100 recombination-adjusted core SNPs; see Methods) of *E. coli*, *K. oxytoca* and *K. pneumoniae,* respectively (Fig. 1, Table 1). Numerous sinks were colonized with multiple lineages (Figs 1, S1 and S2), reflecting significant diversity within species in sink niches. Of the 37 longitudinally sampled culture-positive sinks from which sequences were obtained, 31 (84%) grew isolates of the same lineage across multiple timepoints, highlighting persistent background colonization illustrated for *E. coli* and *Klebsiella* spp. in respective Figs 1 and S3. Isolates resistant to third-generation cephalosporins were cultured at 16 sink-timepoints across 12 distinct sinks, with resistant and susceptible cultures of the same lineage co-occurring in 11/16 (69%) sink-timepoints, suggestive of gain and/or loss of genes conferring cephalosporin resistance in this setting. No carbapenem-resistant isolates were cultured.

Enterobacterales can be highly abundant in sink drains, representing dominant populations in some wards

Deep metagenomic Illumina sequencing was performed for 20 sink-timepoints on p-trap aspirates from seven sinks on the three wards at two timepoints, and all four timepoints for a single sink unit in the adult critical care ward (median throughput 547 Mb per sample; IQR 499Mb to 942Mb). The three most abundant bacterial genera were *Klebsiella*, *Escherichia* and *Citrobacter*, all common healthcare-associated pathogens (Fig. 2). Sink drains in the general medicine ward were the most abundantly colonized by Enterobacterales (Fig. 2), to which more than 50% of reads were assigned, and were markedly less diverse than those in adult critical care and acute admissions wards, which had a dominance of *Klebsiella* spp., mirroring the culture results. 90% of species-level classifications in the sink-timepoints from the general medicine ward came from a median of

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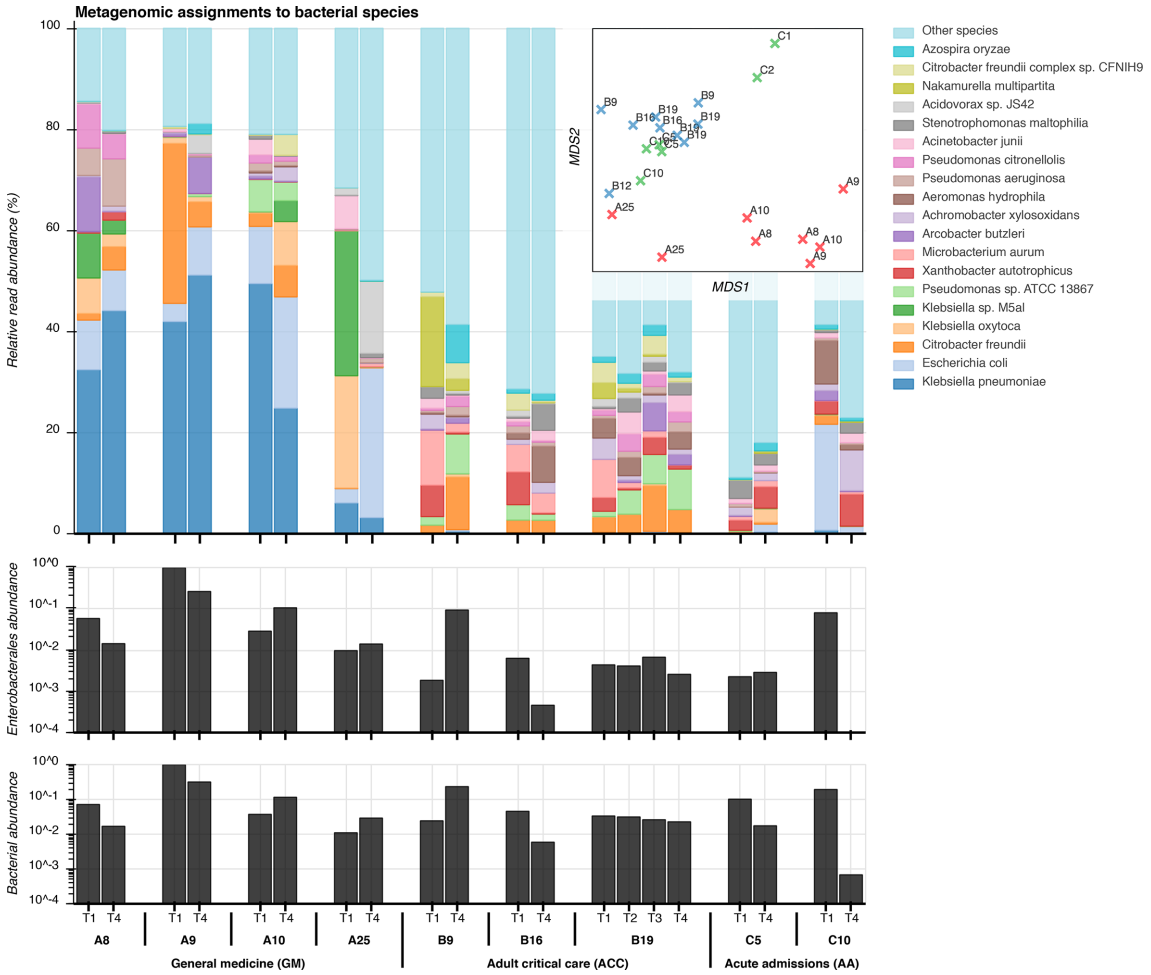

Fig. 2. Taxonomic composition of sink microbiota from metagenomic sequencing. Top: relative abundance of the 20 most abundant bacterial species among sink drain aspirates (Kraken), inset with a corresponding multidimensional scaling (MDS) projection of pairwise distances between samples. Centre: spike-normalized relative abundance of species classifications at or below the order Enterobacterales among sink-timepoints. Bottom: spike-normalized relative abundance of Kraken classifications at or below the superkingdom bacteria.

just 21 bacterial species, compared with medians of 310 and 450 species in the adult critical care and acute admissions wards, respectively. Microbial composition varied markedly between sampling timepoints for individual sinks, but sinks within wards exhibited more similar taxonomic profiles than those between wards (Fig. 2), suggesting distinct ward-based wastewater ecologies. Total metagenomic sequence content was hierarchically structured by ward and by sink (Fig. S4). Staff room sink A25 exhibited distinctive taxonomic and *k*-mer profiles from patient room sinks in the general medicine ward (Figs 2 and S4).

Sinks with high metagenomic abundance of the three Enterobacterales species reliably yielded corresponding cultures. The area beneath the receiver operating characteristic (ROC) curve for culture-based detection of these species was 0.93 (Fig. S5). When the relative metagenomic abundance of a species was above 0.1, 1 and 10%, one or more cultures of the same organism were obtained in 58% (18/31), 76% (16/21) and 89% (8/9) of sinks, respectively. Conversely, culture detection therefore failed in 42% (13/31), 24% (5/21) and 11% (1/9) of cases where an Enterobacterales species was present at or above respective thresholds of 0.1, 1 and 10% metagenomic abundance. A single sink-timepoint (first sample from A8; general medicine) failed to culture any Enterobacterales, but yielded 4, 5 and 16% relative metagenomic abundances for the three study species. Thus metagenomic sequencing suggests that persistence may be even more widespread than indicated by culture alone.

Most environmental Enterobacterales appear to cluster within specific sinks and wards, except for *E. coli* ST635, which is widely distributed

The 96 distinct 100 core SNP lineages found in sinks exhibited structure at the ward and sink level. Three were found in multiple wards (*E. coli* STs 472 and 635; *K. oxytoca* ST 146). Overall, 93 (97%) lineages were only ever found in a single ward, and of the 39 lineages cultured twice or more, only 11

(28%) were cultured from different sinks (Table 1). Further, of the 35 lineages cultured twice or more on wards which were repeatedly sampled (i.e. where it was theoretically possible for a lineage to be observed at different timepoints), 12 (34%) were only seen at the same sink-timepoint, 13 (37%) were seen in the same sink at different timepoints, and 10 (29%) in different sinks at different timepoints. This structure was reflected in the recombination-adjusted core-genome species phylogenies (Fig. 3). The main exception to ward and sink-based clustering was *E. coli* ST635, which comprised more than half of isolates sequenced from sinks, and was found in 13/20 (65%) *E. coli*-positive sinks.

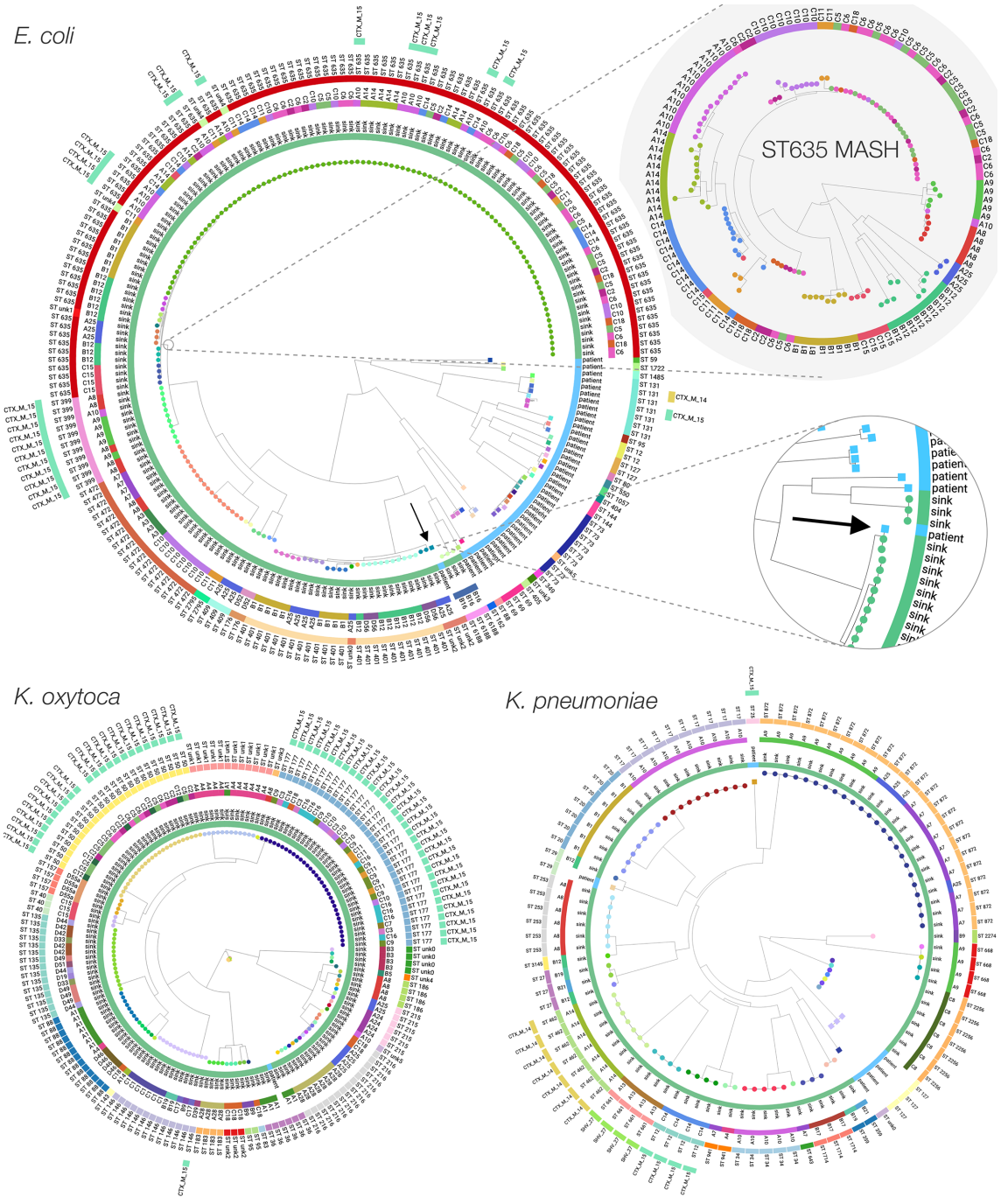

Fig. 3. Maximum-likelihood phylogenies of *E. coli*, *K. oxytoca* and *K. pneumoniae* cultured from sink drain aspirates sampled over 12 weeks across three wards, with two enlargements corresponding to an *E. coli* ST635 neighbour-joining MASH subtree whose tips are coloured by sink, and genetic overlap between a sink culture and a urine culture from a patient with ward contact during the study. Tip colours indicate strains, with rings inside-to-out denoting: patient/sink, sink designation, sequence type, and ESBL genotype.

However, there was sink-level clustering even within *E. coli* ST635, more clearly shown in neighbour-joining trees constructed from pairwise read-based MASH distances, representing both core and accessory genomic content (e.g. *E. coli* ST635 enlargement in Fig. 3; colours indicate distinct sinks). Although pairwise correlations between core and accessory genomic distances were high (Table S3), incorporating accessory content yielded additional resolution beyond core SNP distances (Fig. S6). Permutational analysis of variance (PERMANOVA) using pairwise core SNP and read-based MASH distances supported significant grouping of isolates from all three species by ward and by sink ($P<0.001$), most conclusively for *K. pneumoniae* (Table S4).

Overall diversity among patient *E. coli* isolates was greater than among sink isolates, and included known high-risk clinical lineages

From March to May 2017, 1384 relevant clinical samples from 719 patients were submitted to the microbiology laboratory for processing (AA *n*=779, ACC *n*=365, GM *n*=240), of which 397/1384 (29%) were positive for micro-organisms, and 115 were culture-positive for one of the study organisms (*E. coli* [*n*=104], *K. oxytoca* [*n*=2], *K. pneumoniae* [*n*=9]). In total, 46/115 (40%) of these isolates were retrieved for sequencing, including 21/24 isolates from bloodstream infections, 3/6 from respiratory samples and 22/79 from urine samples; no isolates were retrieved for sequencing in six cases where study organisms were cultured from other clinical specimens (fluid, tissue, joint aspirates).

Among 39 sequenced *E. coli* patient isolates, 21 STs were represented, including known high-risk lineages (23/39 [59%] isolates) not seen in sinks: namely ST73 (*n*=8), ST131 (*n*=7), ST69 (*n*=3), ST12 (*n*=2), ST127 (*n*=2) and ST95 (*n*=1). The single sequenced *K. oxytoca* isolate was ST36, and the six *K. pneumoniae* isolates came from four STs, including two high-risk lineages, ST25 and ST29. Across the three species, there were 34, 1 and 4 distinct lineages, respectively (Table 1).

Genetic similarity of sequenced patient and environmental isolates

As well as being diverse, the 39 clinical *E. coli* isolates were phylogenetically distinct from most sink isolates, which largely came from just four sequence types (ST635, ST401, ST472 and ST399). The exception was an *E. coli* isolated from urine taken on the general medicine ward, which was 17 and 19 core SNPs from two nearest neighbour isolates from sink A25 in the same ward, sampled 58 days prior to the clinical sample (Fig. 1; right; cluster 4). A read-based MASH distance of 7×10^{-6} between this pair of isolates indicated very high total genomic (chromosome+accessory) similarity. A records search for admissions of this patient prior to commencing sink sampling revealed four inpatient admissions (of 0 [day case], 1, 2 and 5 nights' duration), of which three included time on the acute admissions ward. There were no prior admissions onto the general medicine ward (in which their positive urine specimen was taken), and their 11-night spell on the general medicine ward commenced with a 7 h episode in acute admissions. The positive clinical specimen was taken 10 days after the patient's admission onto the general medicine ward, indicating a large duration of exposure to a ward environment shown to be harbouring a very similar strain of *E. coli* to the patient's urine culture. The next most closely related *E. coli* clinical isolate was 3688 core SNPs from its nearest sink neighbour (read-based MASH distance 0.009), reflecting the otherwise large evolutionary distances separating the cultured clinical and environmental *E. coli* (Fig. 3).

Unlike *E. coli*, the small numbers of *Klebsiella* spp. patient isolates were not phylogenetically segregated from environmental isolates, but the closest patient and sink isolates differed by 2558 core SNPs, indicating a lack of observed overlap over these timeframes.

Analysis of protein coding gene function using the Clusters of Orthologous Groups of proteins (COG) ontology highlighted functional differences between sink and patient isolates (Fig. S7). Accounting for the majority of clinical isolates, differences were most apparent among *E. coli*, in which sink and patient isolates were almost linearly separable in the first principle component (PC1; Fig. S7; right). Variable loadings for PC1 indicated COGs explaining the variation between sink and patient groups. The COG with highest loading was insertion sequence IS1R, highly abundant in sink *E. coli* isolates and possibly associated with the acquisition and expression of adaptive genes. Other highly explanatory COGs included genes involved in mobility, adhesion and iron metabolism, predicting functional adaptations of *E. coli* to the built environment of the sink (see supporting data repository).

Antimicrobial resistance genes are prevalent and spatially structured with respect to sink location

The presence of 571 clustered CARD antimicrobial resistance genes in cultured isolates was supported by >=75% exactly matching read coverage reported by ResPipe (Fig. 4). Among these were known transmissible genes of clinical concern including beta lactamases (e.g. bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{SHV}), aminoglycoside resistance genes (*aac(3)*, *aac(6)* families) and quinolone resistance genes (*qnr* family). Some of these, including *cmlA* and *qacH*, were widely seen in sink metagenomes but less frequently in cultured isolates, consistent with a background resistance reservoir that may pose a risk in different populations to those cultured (of either same or different species). Spatial structure with respect to sink location was evident among both cultured isolates and metagenomes, although resistance repertoires of isolates frequently clustered across ward boundaries, in keeping with findings of our prior core-genome analysis. Resistance genes detected in cultured sink isolates were also abundant within sink metagenomes at one or more timepoints. Sink drain metagenomes yielded 673 CARD genes exceeding 75% coverage, and after clustering large gene families represented by many similar sequences (see Methods for detailed description), only five genes abundant in one or more cultured isolates were not detected in at least one metagenome. Notably, these five genes (*gadW*, *len-26*, *tet(B)*, *mgrA* and *sat-2*) were all seen in isolates

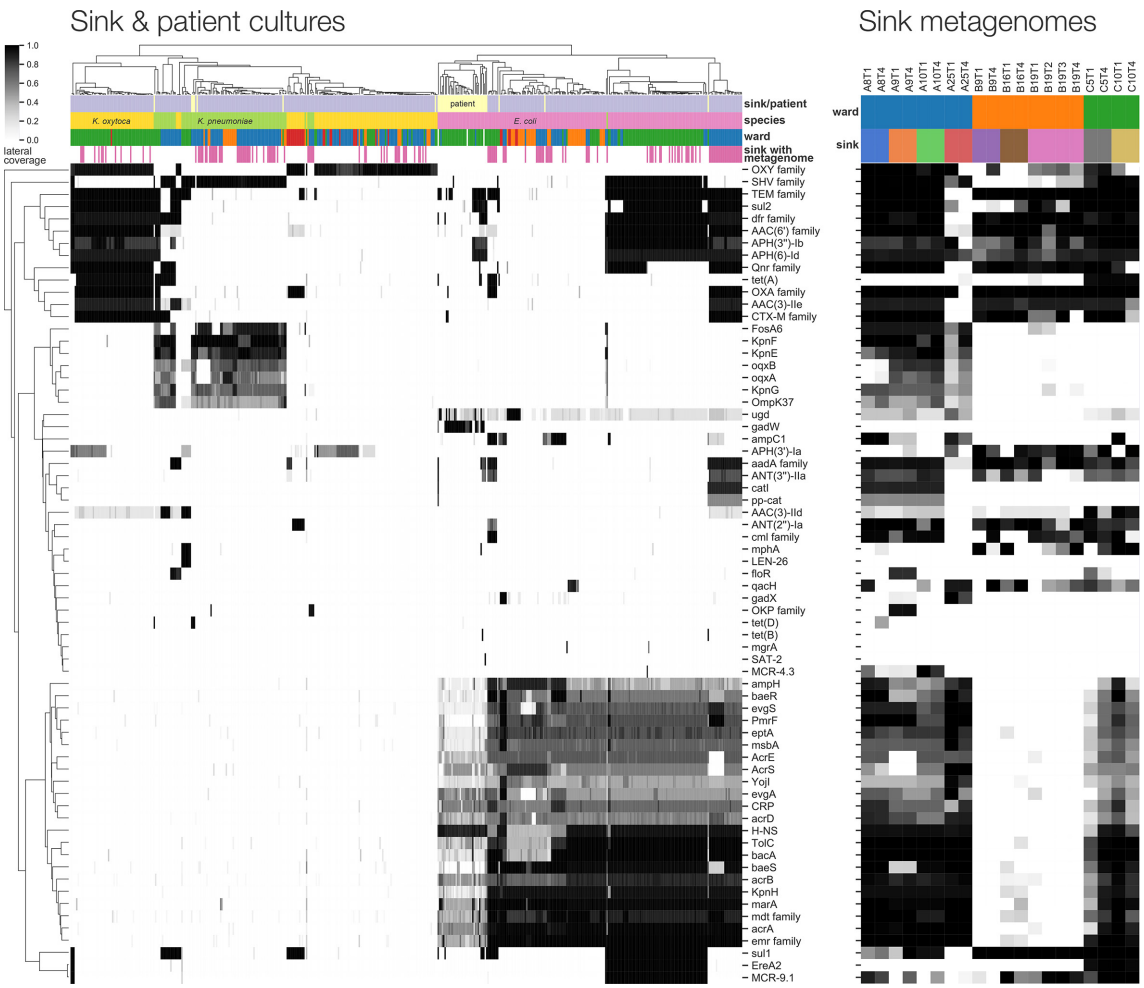

Fig. 4. Antimicrobial resistance gene content of cultured isolates and sink drain metagenomes. Left: lateral coverage of ResPipe/CARD genes within sink drain and clinical isolates. Displayed genes attained >=75% lateral coverage in one or more isolates. Right: corresponding lateral coverage of the same genes in sink drain aspirate metagenomes.

from sinks not subject to metagenomic sequencing, showing that resistance genes cultured from sink drains were highly contained in corresponding metagenomes.

Third-generation cephalosporin-resistant phenotypes in Enterobacterales sink isolates could be explained by the presence of major ESBL genes $bla_{\text{SHV-27}}$, $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$, detected by ARIBA/CARD in 4, 8 and 87 isolates, respectively. $bla_{\text{CTX-M-15}}$ was identified in two distinct lineages of *E. coli* ST635 and ST399, restricted to three bay sinks (A8, A9, A10) in three adjacent rooms of the general medicine ward. $bla_{\text{CTX-M-15}}$-positive *K. oxytoca* ST50 and ST177 were identified in 10 sinks (C2-3, C5-6, C7, C9-12, C16) on the acute admissions ward. $bla_{\text{SHV-27}}$, $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ were observed in *K. pneumoniae* from sinks A13, A14 (general medicine) and C14 (acute admissions), respectively; one *K. pneumoniae* patient isolate was also $bla_{\text{CTX-M-15}}$-positive. These findings suggest sink-associated isolates, such as *E. coli*, may represent reservoirs of clinically relevant resistance genes.

Surprisingly, the colistin resistance gene *mcr-4* was detected in the metagenomes—yet not cultured genomes—of three sinks in adjacent bays of the general medicine ward (A8-10). Assembly of the sink A10 metagenome generated a 5.4 kbp plasmid sequence containing an *mcr-4 gene* with 98.8% overall identity at 94% query coverage to an 8.7 kbp pMCR-4.2 plasmid previously reported in pigs from Italy, Spain and Belgium [36]. This *mcr* variant has been previously reported in European *Acinetobacter*, *Enterobacter*, *Salmonella* and *Escherichia* spp. but not to our knowledge in the UK. Screening all metagenomes for assembled *mcr-4* produced alignments in two sinks on the ward (A8, A9) across a total of six sink-timepoints, with coverage and abundance suggesting low and declining prevalence of this gene over time (Table S5). An *mcr*-positive *E. coli* (reported as *mcr4.3*) was cultured from sink C5 (acute admissions; second timepoint) according to both ARIBA/CARD and ResPipe/CARD. Metagenomic sequencing was performed for the first and the fourth but not the second timepoint aspirate for this particular sink.

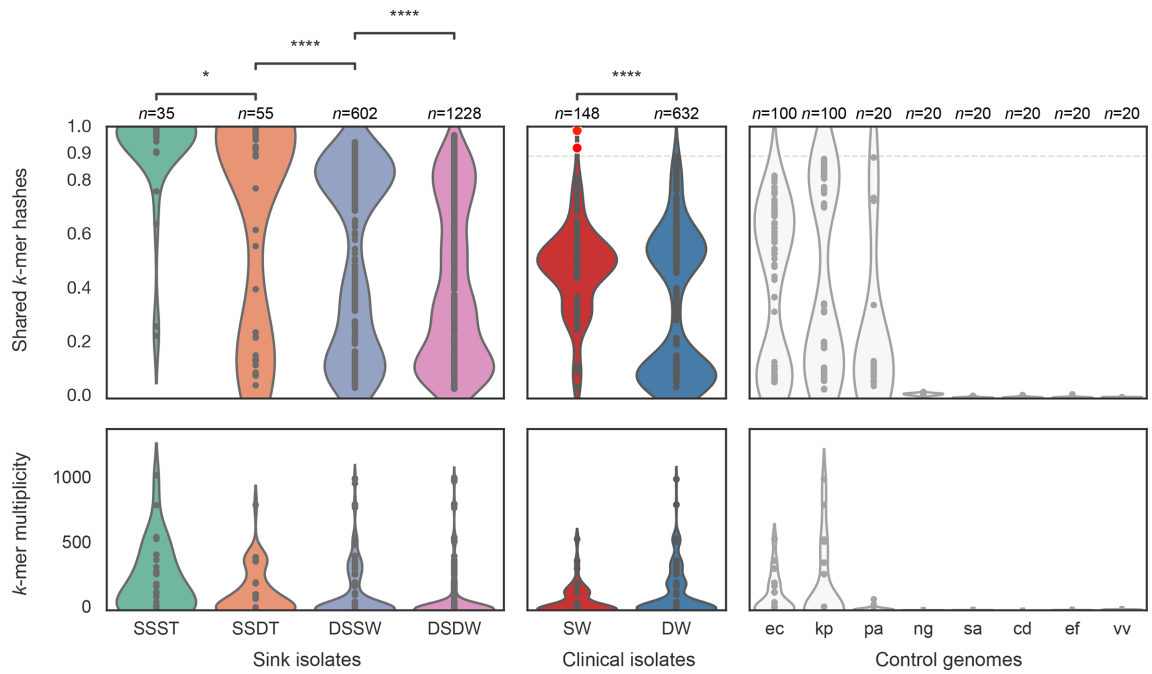

Fig. 5. Metagenomic containment of sink (left) and patient (centre) cultured lineage-representative genome assemblies, and control genomes (right). Shared *k*-mer hashes and median *k*-mer multiplicity values are as reported by MASH Screen. SSST=same sink and same timepoint; SSDT=same sink at different timepoints (shared hashes Mann–Whitney P=0.013 vs. SSST); DSSW=different sinks of the same ward (P<0.0001 vs. SSDT); DSDW=sinks on a different ward (P<0.0001 vs. DSSW). SW=lineage-representative assemblies of clinical isolates in the same ward; DW=lineage-representative assemblies of clinical isolates from a different ward (P<0.0001 vs. SW). Control genomes comprised *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *N. gonorrhoeae*, *S. aureus*, *C. difficile*, *E. faecalis* and *V. vulnificus*, shown abbreviated with binomial initials. A case of within-ward sink-patient overlap is highlighted with red markers, corresponding to high strain containment in the metagenomes of sink A25 timepoints 1 and 4.

Another *mcr* gene, *mcr-9* was more widespread, and detected with complete coverage in 77 cultured isolates across 11 distinct sinks, predominantly but not exclusively in *E. coli* (73/77 occurrences). Plasmid replicon typing highlighted differences in plasmid presence between sink and clinical isolates, revealing 29 and 11 mutually exclusive replicon types to sink and patient isolates, respectively, and 15 types that were detected independently in both sink and patient isolates (Fig. S8, Table S6).

Metagenomic screening suggests that clinical isolates may be more widely present in the environmental reservoir than observed from culture-based comparisons

Sink metagenomes were individually screened for *k*-mer containment of (i) lineage-representative sink isolate genome assemblies, (ii) lineage-representative patient isolate genome assemblies and (iii) core genomes of selected control organisms, including five clinical core genomes each from pathogenic strains of *E. coli* and *Klebsiella* spp. [37], together with NCBI canonical reference sequences for several pathogen species expected to be absent from sink drain microbiota (Fig. 5; left). This demonstrated similar sink, ward and temporal structure to that of culture, particularly underlying similarities in the microbiota of nearby sinks, as well as flux between sampling timepoints. Lineage-representative sink culture assemblies from the same sink and timepoint as the screened sink metagenome were the best contained, sharing the most *k*-mer hashes. Assembled isolates originating from the same sink at a different timepoint to the screened metagenome shared significantly fewer *k*-mer hashes than same sink/same timepoint comparisons (median [IQR]: 93% [22.6%–100.0%] vs. 99.6% [97.7–100.0%], Mann–Whitney P=0.013). The containment of lineage-representative assemblies from different sinks in the same ward as the metagenome was significantly lower still (49.2% [13.8%–84.8%] vs. 93.3%[22.6%–100.0%], P<0.0001), and so in turn were the remaining comparisons of cultures grown from different sinks in different wards to the screened metagenome (33.5% [12.6%–71.1%] vs. 49.2% [13.8%–84.8%], P<0.0001).

Among control genomes, *k*-mer hashes shared between sink metagenomes and the core genomes of *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Clostridioides difficile*, *Enterococcus faecalis* and *Vibrio vulnificus* did not exceed 3%. Reference genomes of *E. coli*, *K. pneumoniae* and *Pseudomonas aeruginosa* were abundant and highly contained by many metagenomes, but none exceeded 90% shared *k*-mer hashes (Fig. 5; right).

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In contrast, screening for lineage-representative patient assemblies in sink metagenomes revealed significantly greater containment in terms of shared *k*-mer hashes (median [IQR]: 49% [37%–55%] vs. 32% [8%–56%], Mann–Whitney $P<0.0001$) between patient and environmental Enterobacterales collected from the same ward than from different wards (Fig. 5; centre), showing genetic similarity between clinical isolates from patients and uncultured isolates in sink niches in a given ward setting. Indeed, the only lineage-representative patient isolate with greater than 90% sink metagenome *k*-mer containment was the *E. coli* urine culture described in the aforementioned case of sink-patient overlap, of which 99.5 and 93.0% of *k*-mers were contained within the respective A25T1 (first timepoint) and A25T4 (fourth timepoint) sink metagenomes (Fig. 5; centre; red markers). Interestingly, this particular lineage was cultured at timepoint 1 but not timepoint 4 in sink A25, suggesting that culture may have failed to detect persistence of this lineage detected by metagenomics.

DISCUSSION

In this study, we have demonstrated that hospital sink drains are widely—and in many cases abundantly—contaminated with key Enterobacterales species causing healthcare-associated infections, and are potential reservoirs of multiple resistance genes encoding resistance to important clinical antimicrobials. Populations of antimicrobial-resistant and susceptible *E. coli* and *Klebsiella* spp. may be persistent colonizers of sinks, and different wards may have markedly different sink ecosystems, perhaps reflecting different and potentially modifiable infrastructures, selection pressures and contributing sources. Ward and sink-level genetic structure was especially evident within the accessory genome, and reflected in the observed repertoires of resistance genes. Nevertheless, a handful of key Enterobacterales lineages persisted across multiple wards throughout the duration of the sampling period, and sinks across ward settings were common reservoirs of important clinical resistance genes, including those encoding resistance to beta-lactams, sulphonamides, aminoglycosides and quinolones. Characterizing these highly diverse reservoirs is difficult, and we have shown that combination approaches utilizing metagenomics and sequencing of cultured isolates are complementary in understanding the diversity of species, lineages and the resistance genes present within these niches. For example, metagenomics highlighted several cases of abundant *mcr*-4 in sink drain aspirates from which cultured Enterobacterales isolates did not carry the gene. Furthermore, in the case of an *E. coli* lineage overlapping between sink and patient, metagenomics supported a hypothesis of persistent colonization throughout the sampling period, whereas culture detected this lineage only once.

Colonization patterns of sink niches differed markedly between the two genera investigated. *E. coli* strains have evolved to colonise and adapt to multiple niches, including some which have adopted pathogenic lifestyles, and appear to have different distributions in humans, domesticated and wild animals and the environment. There is however no absolute correlation between phylogenetic lineage and any given niche, and overlaps are observed. Interestingly, in our study, more than half of the *E. coli* sink isolates cultured were ST635, which has been recently described as a highly adapted, resistance- and virulence gene-enriched wastewater-associated strain thought to be globally distributed, but is also found in humans, animals and other environments [38]. Of note, it has been observed in association with several clinically relevant transmissible resistance genes, including ESBLs, carbapenemases and rRNA methylases, and was one of only two *E. coli* STs in our study that harboured an ESBL ($bla_{\text{CTX-M-15}}$). We observed presence/absence of $bla_{\text{CTX-M-15}}$ across closely related ST635 isolates, suggesting that this gene may be frequently lost/gained in sinks. Also notable in the context of ST635 was the ability of read-based *k*-mer composition to resolve fine-grained structure between the populations of different sinks, beyond that observed in the core-only SNP phylogeny. Other common *E. coli* sink lineages were ST399 and ST472, which to date have predominantly been seen in humans/animals, rather than the environment.

The phylogenetic distribution of sink isolates of *K. pneumoniae* appeared to mirror that seen in a global collection of isolates [39], providing little evidence that a particular lineage was predominating in, or particularly adapted to, the wastewater environment. Studies of the population structure of unselected *K. oxytoca* are limited, but again we observed a diverse population amongst sink isolates, with a deep branch separating two distinct groups as previously described. Interestingly, two *K. oxytoca* lineages associated with $bla_{\text{CTX-M-15}}$ were widely distributed amongst sinks in the acute admissions ward; outbreaks of ESBL- and carbapenem-associated *K. oxytoca* in association with contaminated handwashing sinks have been described in other settings [40].

Genomic overlap with sink isolates was identified in 1/46 (2%; 95% CI: 0.05–11%) of all sequenced isolates causing clinical infections over the same timeframe, with a temporal association consistent with acquisition from a sink source (i.e. sink isolate observed first), and following 10 days of patient exposure to a ward environment wherein the overlapping strain was previously cultured. We may have significantly underestimated the degree of overlap between these two compartments for several reasons. Firstly, we have shown the diversity in sink niches is substantial, and with a culture-based approach agnostic to any selective marker, even sequencing 444 isolates from 48 sinks will have limited ability to capture the underlying diversity for complete comparison of sink-patient pairs at the isolate-level. Supporting this, screening the metagenomes of a subset of 20 sinks using patient isolates suggests that overlap between these reservoirs may be more common than observed at the isolate-level. Second, clinical isolates represent the tip of the iceberg of any transmission chain, with the majority of transmission events likely occurring between gastrointestinal tract (asymptomatic carriage) and the wastewater environment. Nonetheless, in the context of understanding how sinks may be contributing to infection caused by *Klebsiella* spp. and *E. coli*, focusing on clinical isolates seems appropriate. Third, the interval between sampling dates for our observed patient-sink isolate-pair was

58 days, suggesting that the timeframe between acquisition from the environment and infection may be long, and may not be adequately captured with a study timeframe spanning 3 months. Refining estimates of the risk of patient infection associated with colonized sink sites ideally requires larger studies, over longer timeframes, and which ideally also capture transmission events resulting in gastrointestinal colonisation as an intermediate step to invasive infection.

In addition, a major study limitation is the fact that only 46/107 patient isolates could be successfully retrieved (due to the high turnover of samples in our high-volume service laboratory), and *Klebsiella* spp. cultures were especially limited. The risks of transmission and possibly sink-associated infection could be more clearly defined by more extensive sampling over a greater timeframe, and thorough investigation into the exchange of resistance-associated mobile genetic elements by, for example, using long-read sequencing to reconstruct plasmid sequences [41, 42]. Doing so would require a considerable increment in resource. Characterizing microbial diversity present on sink strainers would also be of benefit, as the risks of droplet-mediated dispersal from sink drains have been shown to be most significant when the sink drain is located immediately below the tap, and if the organisms migrate from the sink trap onto the strainer [43, 44]. However, given the different sink structures across wards, the p-trap was the only site that could be consistently sampled (since ACC had horizontally draining sinks without strainers). Characterizing factors that might be associated with greater predominance of Enterobacterales and drug-resistant Enterobacterales, such as sink usage, ward-level antimicrobial usage and patient populations, would also be of interest.

In conclusion, without conditioning on the presence of resistance markers, we have demonstrated that colonization of ward sink drains with diverse and abundant populations of Enterobacterales, including drug-resistant lineages, is common and persistent. The evidence linking contaminated, unmitigated wastewater reservoirs (including sink drains) in healthcare settings with outbreaks of colonization/disease with drug-resistant Gram-negative bacilli in patients seems clear [5, 45], but no study to our knowledge has focused on the potential risk posed by Enterobacterales in sinks in general. Screening of sinks is not carried out in the absence of observed outbreaks, making it difficult to quantify wider patient-associated risk from the studies available. We demonstrate that contaminated sinks may be contributing to a proportion of healthcare-associated infections caused by Enterobacterales, and further work to investigate how to reduce the risk posed by this hospital environmental reservoir is warranted.

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Author contributions
This study was conceptualized by N.S., A.S.W., A.J.M., B.C., K.K.C.; funding for the study was provided through grants to N.S., D.W.C., T.E.A.P. and A.S.W., K.K.C., N.S. and A.J.M. collected samples; K.K.C. performed laboratory culture and DNA extractions with help from G.R. Data processing and curation were performed by B.C., K.K.C., N.S., H.S.G., S.L. and T.P.Q. A.P., G.P., J.M., M.B., M.H. and C.G. facilitated ward-sink sampling and M.A., K.J., T.P.Q. and M.M. collected the clinical microbiology samples. Data analysis and visualization was performed by B.C. supervised by N.S., A.S.W. and T.E.A.P. B.C. and N.S. drafted the manuscript with input from A.S.W. All authors reviewed and approved the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The Infections in Oxfordshire Research Database (IORD) has generic Research Ethics Committee, Health Research Authority and Confidentiality Advisory Group approvals (14/SC/1069, ECC5-017(A)/2009).

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Outbreak of *Stenotrophomonas maltophilia* on an intensive care unit

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SUMMARY

Background: *Stenotrophomonas maltophilia* causes opportunistic infections and remains a problem pathogen on intensive care unit (ICU) due to its multidrug resistance.
Aim: An outbreak of *S. maltophilia* on ICU is described in order to highlight the risk from contaminated devices for supply of drinking water.
Methods: The outbreak was investigated by a combination of epidemiology, environmental sampling and molecular typing.
Findings: From 2009 to 2011 isolates of *S. maltophilia* from 23 patients were found to belong to only two genotypes by contrast with isolates from 52 other patients during this period, which represented distinct strains. The monthly incidence for all *S. maltophilia* strains ranged from 0 to 11% and for the two outbreak strains from 0 to 9%. Admission and weekly pharyngeal screening on ICU showed that the outbreak strains were acquired on ICU (range: 3–90 days). The majority of isolates (74%) were from the respiratory tract. Only two of 12 (17%) colonized intubated patients developed pneumonia. Environmental sampling found the two outbreak strains in two sinks and in the drinking water of the cooling unit in the ICU kitchen. *S. maltophilia* had formed a biofilm in the flexible tube from the carbon filter to the chiller and from the latter to the tap at the kitchen sink. This cooled water was used for providing drinking water and mouth care to ICU patients. The outbreak strains disappeared after removal of the water-cooler and the monthly incidence fell to <2% of ICU admissions.
Conclusion: This outbreak report highlights the risk from biofilms in devices that supply drinking water for ICU patients.
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Introduction

Stenotrophomonas maltophilia is one of the nine most common organisms from respiratory specimens of intensive care unit (ICU) patients in Europe. Surveillance on European ICUs showed that 4% of 1409 bronchial lavage specimens grew *S. maltophilia* on quantitative culture.[1] In this surveillance report *S. maltophilia* occurred as frequently as *Acinetobacter baumannii* and was associated with late onset pneumonia.

As *S. maltophilia* is intrinsically resistant to carbapenems, aminoglycosides and polymyxins and as *in vitro* susceptibility testing is unreliable, the antimicrobial choice is limited to co-trimoxazole, ticarcillin–clavulanic acid, aztreonam plus co-amoxiclav and ceftazidime in combination with other agents. These beta-lactams often appear active against isolates of *S. maltophilia* on Iso-Sensitest agar but will appear less active if tested on Mueller–Hinton agar.[2] Its antimicrobial multi-

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resistance makes *Stenotrophomonas* spp. a problem in ICU. Though the majority of patients remain colonized, few debilitated patients develop pneumonias.[3]

We observed on the ICU of a district general hospital a high isolation rate from respiratory specimens, which showed on genotyping two predominant strains. When admission screening by throat swabs was introduced in 2010, this allowed us to assess the acquisition dynamics. This report describes the results of the investigation on these two outbreak strains.

Methods

Epidemiological investigations

Cases were defined as ICU patients from whom *S. maltophilia* genotypes designated FR04 and FR06 were isolated. Admission and weekly screening by throat swab was introduced in March 2010 after recognizing the cluster of these two predominant *S. maltophilia* outbreak strains. As admission screening showed that the outbreak strains were acquired in ICU, a questionnaire was applied to cases covering risk factors such as bed space, mechanical ventilation, surgery, bronchoscopy and Acute Physiology and Chronic Health Evaluation (APACHE) II score. During this 27-month period environmental inspection and sampling was performed on three occasions. In October 2011 the plugholes and faucets of 12 handwash basins in ICU were swabbed. Environmental swabs were collected from the ventilator sockets for the circuit tubes, water for suctioning, chlorhexidine mouth gel, bronchoscopes and laryngoscopes. In December 2011 preflush water from the faucets of all 12 handwash basins, three sluice basins, the cold water and drinking water tap in the ICU kitchen were collected for culture.

Microbiological investigations

Throat swabs were streaked on to cysteine-lactose-electrolyte-deficient (CLED) plates (E&O Diagnostics, Bonnybridge, UK), to which a meropenem disc was added on to the inoculum. Non-lactose fermenters growing around the meropenem disc were identified biochemically (API, bioMérieux, Craponne, France) until July 2011, and from then onwards by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis (Bruker, Billerica, MA, USA). Sensitivity testing was only performed for co-trimoxazole using the BSAC (British Society for Antimicrobial Chemotherapy, Birmingham, UK) disc diffusion methodology.

Tap water was collected into sterile 500 mL containers and filtered through a filtration device (Nalgene, Nalge Nunc, Rochester, UK) on to a 0.45 μm pore size membrane (Millipore, Bedford, MA, USA) within 30 min after collection. The membrane was imprinted on to CLED agar and then three imipenem discs were distributed over the plate. The PVC tubings before and after the carbon filter were cut into 0.5-cm-long pieces and cultured in cooked meat broth (Oxoid, Basingstoke, UK) and then subcultured on CLED plates with three imipenem discs. Any non-lactose fermenting colonies growing in the imipenem inhibition zones were identified by MALDI-TOF analysis and, if *S. maltophilia*, sent for typing by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA. Electrophoresis was carried out at 6 V/cm for 30 h at 12 °C with ramping times of 5—35 s.

All *S. maltophilia* isolates from ICU patients were genotyped by PFGE at the Health Protection Agency Antimicrobial Resistance and Healthcare Associated Infections Reference Unit in Colindale, London.

Investigations on source

Drinking water was supplied by an under-sink water-cooler in the ICU kitchen, which was connected to the mains water supply. A carbon filter was installed between mains and water-cooler via a flexible polyvinylchloride (PVC) tubing (Figure 1), from where a plastic tube led to a special drinking water tap at the kitchen sink. Five hundred millilitres from this tap, 200 mL mains water from the tubing to the carbonfilter and 200 mL of filtered water after the filter were collected on disassembly of the chilling unit and filtered on to a membrane for isolation of *S. maltophilia*.

Results

From August 2009 to November 2011, 23 patients acquired the outbreak strains designated FR04 and FR06 (Figure 2). This resulted in a prevalence of 1.6% among 1411 ICU patients during this period. These 23 representatives of the two outbreak strains accounted for 31% of all 75 *S. maltophilia* isolates. The other 52 *S. maltophilia* strains had unique genotypes and were unrelated to the outbreak strains. The monthly incidence of all *S. maltophilia* cases varied from 0 to 10%, and from 0 to 7% for the outbreak strains (Figure 3). The incidence of these two outbreak strains peaked during the summer months, particularly during June to October 2011, when seven new cases occurred (Figure 2).

Admission and weekly screening by throat swab for culture of *S. maltophilia* was introduced in March 2010 and revealed that none of the outbreak strains was present on admission by contrast with unique genotypes, which were detected on admission. The median duration from ICU admission to first isolation of *S. maltophilia* was 14 days (range: 3—90 days). After the first isolation of *S. maltophilia*, patients remained

Figure 1. Under-sink water-cooler with an upstream carbon filter and blue flexible tubes from filter to chilling unit and from outlet of cooler to tap.

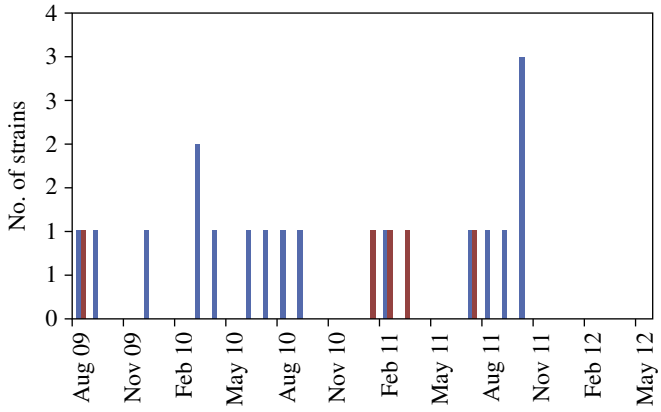

Figure 2. Incidence of outbreak strains in intensive care unit. Blue: FR04; red: FR06.

throat-carriers for up to 4 weeks as could be shown by weekly screening during their ICU stay.

The mean age of patients was 61 years (SD: 17), 74% had recent surgery and 56% were intubated and ventilated. The outbreak strains were isolated in 17 of 23 cases (74%) from respiratory samples such as throat screening swabs and endotracheal aspirates or sputums. Two of 12 (17%) colonized and ventilated patients developed pneumonia, which required treatment with co-trimoxazole. Eighty-two percent received a carbapenem before acquisition for 1–10 days (median: 5). The questionnaire on risk factors did not reveal any common exposure: acquisition was not associated with intubation, certain side-rooms nor bed spaces nor procedures. Only a few cases were overlapping, particularly during the summer months, but there were several months without any cases. This made cross-transmission less likely and suggested a point source for these outbreak strains. In October 2011 the outbreak strains FR04 and FR06 were found in the sinks of the sluice room and one bedside handwash basin. The water stream from the tap was not directed into the drain, which made splash-back unlikely. On review of nursing practices the nurses reported that they had discarded the water from tooth-brushing or patients' drinking water into handwash basins. They revealed also that they had used cooled water from the ICU kitchen from the special tap for cooled water for serving patients drinking water and mouth care. In December 2011 water was cultured for *S. maltophilia* from all 12 handwash basins in ICU, from the two taps in the sluice room and from the tap for the sink and the drinking water tap in the ICU kitchen. Only the narrow-diameter drinking water from the special kitchen tap grew *S. maltophilia* outbreak strains FR04 and FR06 (Figure 4).

This tap was supplied with water via an under-sink water-cooler (Figure 1). The mains water was passed through a carbon filter to remove the chlorine dioxide for better taste before entering into a water-cooler, from where plastic tubing led to the drinking water tap at the kitchen sink. On disassembly the sampled mains water before the carbon filter was free from *S. maltophilia*; however, the water collected after the filter from the unit grew >100 cfu *S. maltophilia* per 100 mL, which matched the two outbreak strains FR04 and FR06. Also the plastic tubing to the tap grew the outbreak strains in enrichment broth. The chilling unit and tubing was removed in December 2011 and chilled bottled water was used as drinking water. Since that time no more FR04 and FR06 genotypes have been found in ICU and the stenotrophomonas prevalence has fallen to <2% of admissions. This chilling unit was installed in 2009 and the carbon filter had been changed quarterly, but not the tubing.

Discussion

Although *S. maltophilia* is one of the nine most frequently sampled organisms from endotracheal aspirates, the pulmonary infection rate among colonized patients was low with 17% patients developing pneumonia with this strain. Laing *et al.* reported an infection rate of 64% among colonized ICU patients; however, their case definition for lung infection comprised physician's clinical judgement and repeated

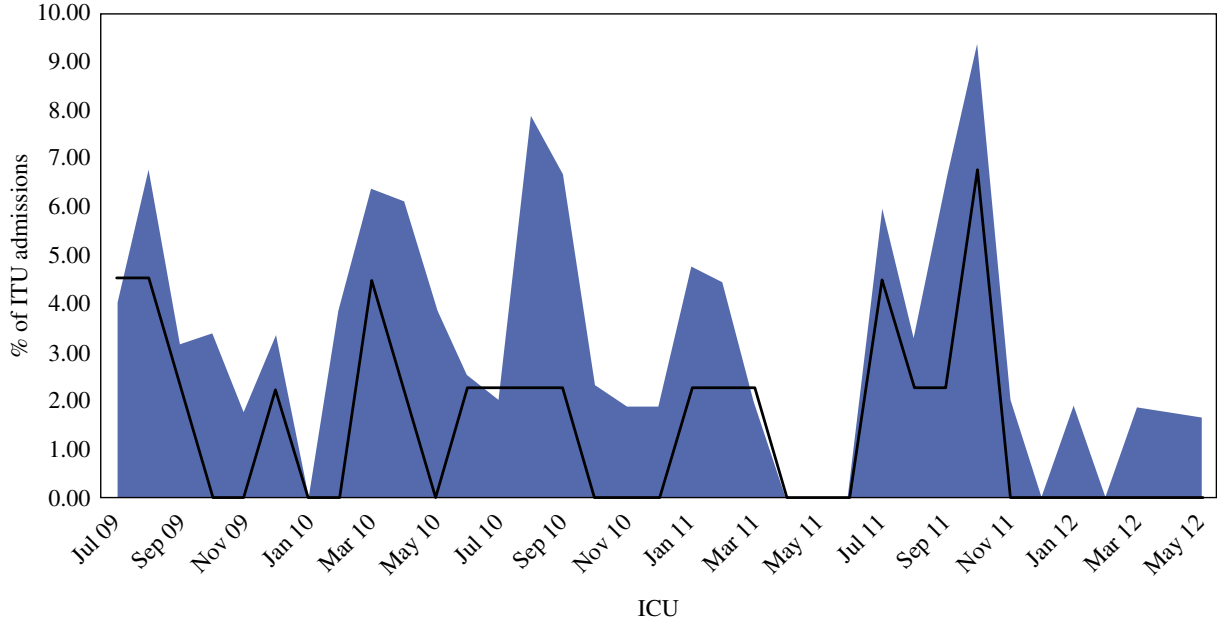

Figure 3. Incidence of outbreak strains and incidence of all *Stenotrophomonas maltophilia* strains on intensive care unit (ICU).

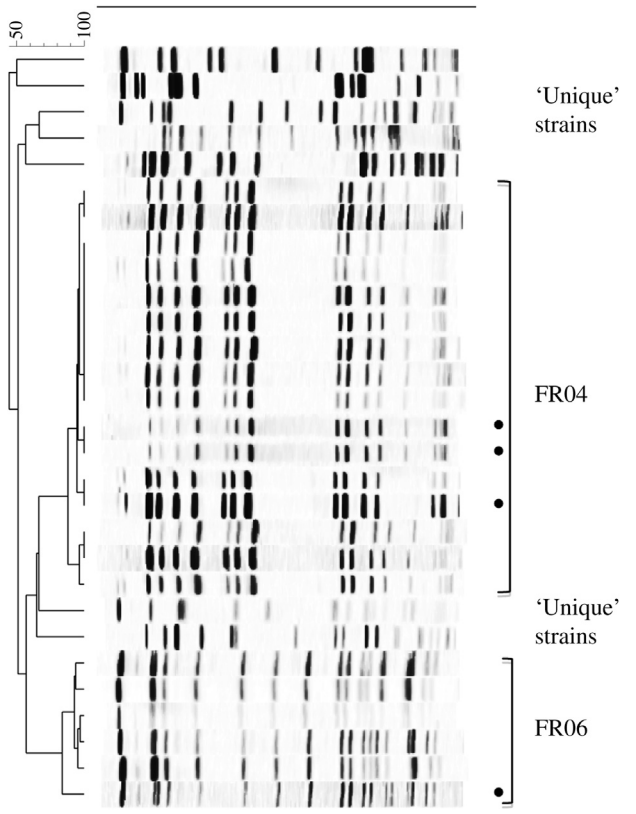

Figure 4. Dendrogram showing pulsed-field gel electrophoresis profiles of *Xba*I-digested genomic DNA from representative isolates of *Stenotrophomonas maltophilia* from patients and water over the period 2009–2011. Only one isolate is included per patient. Water/tap/sink isolates are marked with bullets.

growth.[4] A recent study found that patients with *S. maltophilia* in their respiratory tract and a high lung injury score had a significantly higher mortality than colonized patients with lower scores.[5]

This outbreak report illustrates that a water-cooler for drinking water was the source of *S. maltophilia* on ICU, because a carbon filter had not only removed the disinfectant chlorine dioxide before the water-cooler, but had also accumulated organics, which serve as nutrients for bacteria facilitating the growth of biofilms on downstream tubing. Carbon filters are installed at drinking water dispensers in order to improve the organoleptic quality of mains water by removing chlorine and other ions. Water in our hospital is treated with chlorine dioxide for the control of legionella. The British Standard BS EN 'Chemicals used for treatment of water intended for human consumption' (BS EN 12671:2009) permits on-site generation of chlorine dioxide up to concentrations of 0.5 mg/L, at which limit odour and taste of water change.[6] The absence of the disinfectant chlorine dioxide beyond the carbon filter allowed *S. maltophilia* to form a biofilm on the 6 mm diameter PVC tubes. Flexible pipes are known for colonization with waterborne bacteria and the addendum of HTM01-04 restricts the use of flexible hoses and tubes for water conveyance to few necessary installations, to which water-coolers do not belong.[7]

Drinking water-related outbreaks with opportunistic pathogens in hospitals have been reported previously. Wilkinson and Kerr found high rates of faecal carriage of *S. maltophilia* among patients with haematological malignancies and identified non-carbonated bottled water, which contained up to 49,000 cfu/mL of *S. maltophilia* and other non-fermenters.[8] *S. maltophilia* was able to grow in non-carbonated water over a range of pH and at refrigeration. In a bone marrow transplant unit a high prevalence of *Pseudomonas fluorescens* was detected in pharyngeal surveillance swabs.[9] The same strain was found in the water of one of the drinking water dispensers. *P. fluorescens* was thought to have formed a biofilm in the dispenser, because the water of the second dispenser was sterile, though it was supplied by the same batch of bottled still spring water. Other water-related outbreak reports found that the aerators of the faucets on a neonatal ICU were colonized with a biofilm of *S. maltophilia*, which contaminated the bathwater for the newborns.[10] Five neonates became colonized with *S. maltophilia* and one baby died from bacteraemia. On a haematology unit *S. maltophilia* formed biofilms on faucets and two neutropenic patients developed severe soft tissue infections with the same strain as on the faucets.[11]

In the UK the addendum of HTM 04-01 stipulates control measures for the prevention of *Pseudomonas aeruginosa* growth in distal pipework, where immunosuppressed patients are accommodated.[7] Measures such as 6-monthly sampling of preflush water will detect biofilms in faucets, shower heads and in the peripheral pipes. Remedial measures comprise design such as avoidance of dead legs, blind ends, flow straighteners and unnecessary thermostatic mixing valves and removal of under-used outlets and flexible tubes. Maintenance foresees regular flushing and descaling of taps and showers in augmented care areas. These measures will reduce the risks from taps and pipes; however, the dilemma of providing safe drinking water that is free from non-fermenters has not been fully addressed. The public water supply is monitored for *E. coli*, coliforms, *C. perfringens* and enterococci, but not for *Pseudomonas aeruginosa* and other non-fermenters.[12] According to the European Council Directive 98/83/EC, bottled water is also controlled for *P. aeruginosa*, but not for other non-fermenters, which may be included in the permitted total viable count of 100/mL at 22 °C.[13] The safest drinking water supply is boiled or distilled water. Water filters can be fitted to taps, but they require regular change of cartridges. If bottled or filtered water is offered to immunosuppressed patients for drinking, regular monitoring for non-fermenters is advisable. Chlorine dioxide treatment of hospital water has been used successfully to prevent legionella, but will also reduce the planktonic burden of other water-borne bacteria. Chlorine dioxide levels up to 0.5 mg/L are permitted in drinking water and, if the organoleptic quality is acceptable, this may be another method of providing pseudomonas-free drinking water, provided that no carbon filters are attached to the dispensers.

In augmented care areas it is advisable that any water additions that have narrow-diameter tubing or carbon filters attached are regularly controlled for growth of non-fermenting bacteria.

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Conflict of interest statement
None declared.

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Major Article

Stenotrophomonas *maltophilia* outbreak originating from a pull-out faucet in a pediatric intensive care unit in Turkey: Insights from clinical records and molecular typing

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Key Words:
Stenotrophomonas *maltophilia* bacteremia
Immunocompromised Patients
Faucet aerator
Genotype
Outbreak-investigation

Background: Nosocomial Stenotrophomonas *maltophilia-related* cases are rising and pose a threat to immunocompromised patients. Twelve patients from our pediatric intensive care unit (PICU) presented with S *maltophilia-associated* bloodstream infection.
Methods: This outbreak investigation includes 12 patients from PICU between the ages of 2 months and 4 years (mean 16 months, 7 male). To identify the origin, samples from all possible sources throughout the hospital were collected and ran through DNA isolation and Pulse Field Gel Electrophoresis.
Results: 120 samples were collected during the outbreak. 31 samples (26%) were positive for *S maltophilia.* 30 *S maltophilia* isolates were analyzed, 10 different genotypes were identified. Clustering isolates were grouped into 3 different clusters (tolerance and optimization 1.0, cutoff 90%). The largest cluster was genotype 1, which included 19 isolates, those belong to patients' samples and a sample from a pull-out faucet inside the PICU. The Pull-out faucet was the origin of the bloodstream infection.
Discussion: Pull-out faucets allow biofilm production, due its structure. Pulse Field Gel Electrophoresis identifies the transmission dynamics of the outbreak, with its high discriminatory power.
Conclusions: Water sources should be monitored on a regular basis. Pull-out faucets enable bacterial overgrowth; therefore, we recommend water surveillance during outbreak investigations.
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BACKGROUND

Stenotrophomonas maltophilia is the third most common non-fermenting gram-negative rod, which infects humans after *Pseudomonas aeruginosa* and *Acinetobacter spp.*[1,2] In the last few decades *S maltophilia* occurrences have multiplied, making it increasingly recognized as an important cause of nosocomial infections. Therefore, many outbreaks have been reported within the last few years in hospitals and intensive care units (ICUs).[3,4] A study conducted in a European center for pediatric hematology and oncology reported an incidence of 2.3% of *S maltophilia* infections among pediatric patients admitted to the ICU.[5]

S maltophilia presents with minimal invasiveness in immunocompetent individuals, but it can lead to severe even fatal infections in immunocompromised patients. Its high resistance to many antimicrobial agents may result in unsuccessful treatments and a significant mortality rate in patients.[6–8]

METHODS

This is an outbreak investigation, and the project was deemed IRB-exempt.

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Table 1
Demographics and lab findings of the patients

	Patients (n) (%)
Male	*7 (58.3%)*
*Age in months**	*11 (2-48)*
Positive culture after 'n' days in PICU admission	*9.5 (48 h-28 days)*
Known congenital disorder	*10 (83.3%)*
Cardiovascular disease	*6 (50%)*
*Peak C-reactive protein****	*49.9 (5.23-200.89)*
*White blood cell count**	*12.05 (5.5-22.76)*
*Neutrophil count**	*7.01 (2.01-18.61)*
Immunosuppressed	*9 (75%)*
Central venous Catheter presence	*8 (66.6%)*

NOTE. * Median values are calculated ** Fifthpercentile ***peak CRP at the time of BSI.

Subjects

In the PICU of Istanbul Medipol University Mega Hospital, between August 6, 2021, and December 2, 2021, 12 patients between the ages of 2 months and 4 years (mean 16 months, 7 males, 5 female) were diagnosed with bloodstream infection (BSI) due to *S maltophilia*. All patients were positive ≥48 hours of their PICU admission and therefore fulfilled the criteria for hospital-acquired BSI.[9] Each patient was reviewed for their history of known disorders, immunosuppression status, presence of any type on intravenous instruments, peak C-reactive protein, white blood cells, and neutrophil levels at the time of BSI.

As a part of the investigation process environmental samples had been swabbed from all possible sources, especially from damp environments, (such as faucets, pull out-faucets, wash trays, water hoses, water reservoirs of humidifiers, ventilators, nebulizers, continuous positive airway pressure machines, nasal cannulas, oxygen masks, tracheostomy tubes, central lines, medicine pumps, nasogastric and gastrostomy tubes, feeding bottles, baby pacifiers, inflatable cuffs, temperature probes, heart monitors, pulse oximeters, hospitals bed head surfaces and sheets, shelving and care trolleys, foley, indwelling catheters). In addition, patient surveillance samples were obtained from endotracheal aspirates, rectal and skin swabs.

Antimicrobial sensitivity tests for Stenotrophomonas maltophilia

Antimicrobial sensitivity tests were conducted on 30 strains of *S maltophilia* using the standard disk diffusion method (Bioanalyze). The tests were performed with 5 different antibiotics: ceftazidime (5 μg), minocycline (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), and trimethoprim/sulfamethoxazole (1.25 μg/23 μg). These tests were performed at the Medical Microbiology Research Laboratory of Recep Tayyip Erdogan University, Faculty of Medicine. The results were evaluated according to the EUCAST 2022 (v. 12.0) (European Committee on Antimicrobial Susceptibility Testing) criteria.[10]

DNA isolation and integron gene cassette screening

Total DNA isolation was performed using the boiling method.[11] Integron gene cassettes and resistance genes were screened by protein chain reaction from all bacterial strains using sequences described previously.[12–17]

Pulse field gel electrophoresis (PFGE) protocol

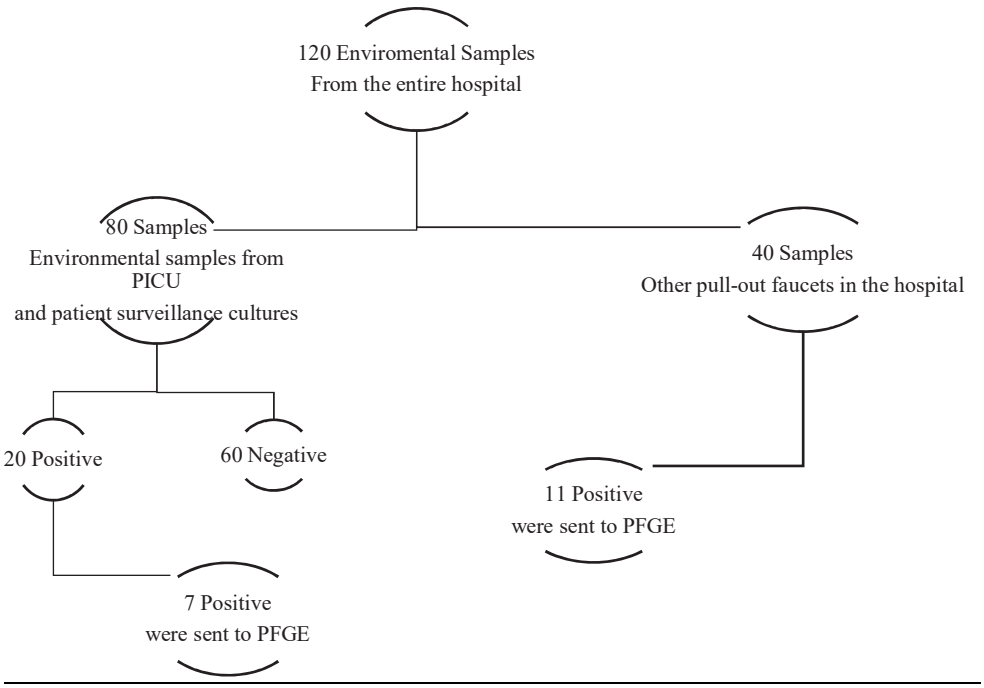
All 30 *S maltophilia* isolates were typed by PFGE according to a protocol published by Durmaz et al with modifications. All strains recovered from patient clinical samples and environmental sources were taken for PFGE study from Medipol Mega hospital in İstanbul. Genotypes were grouped for this outbreak investigation.

Results

Patients' demographics and lab findings are given in Table 1.

A total of 120 samples were collected from the hospital during the outbreak (80 samples from the PICU and 40 samples from the other pull-out faucets in the hospital) and 31 samples (26%) were positive for *S maltophilia*. In addition to 18 environmental isolates, 12 BSI isolates were analyzed with PFGE (Table 2). Epi-curve for the outbreak is given in Figure 2.

Table 2
Flowchart of the collected environmental samples



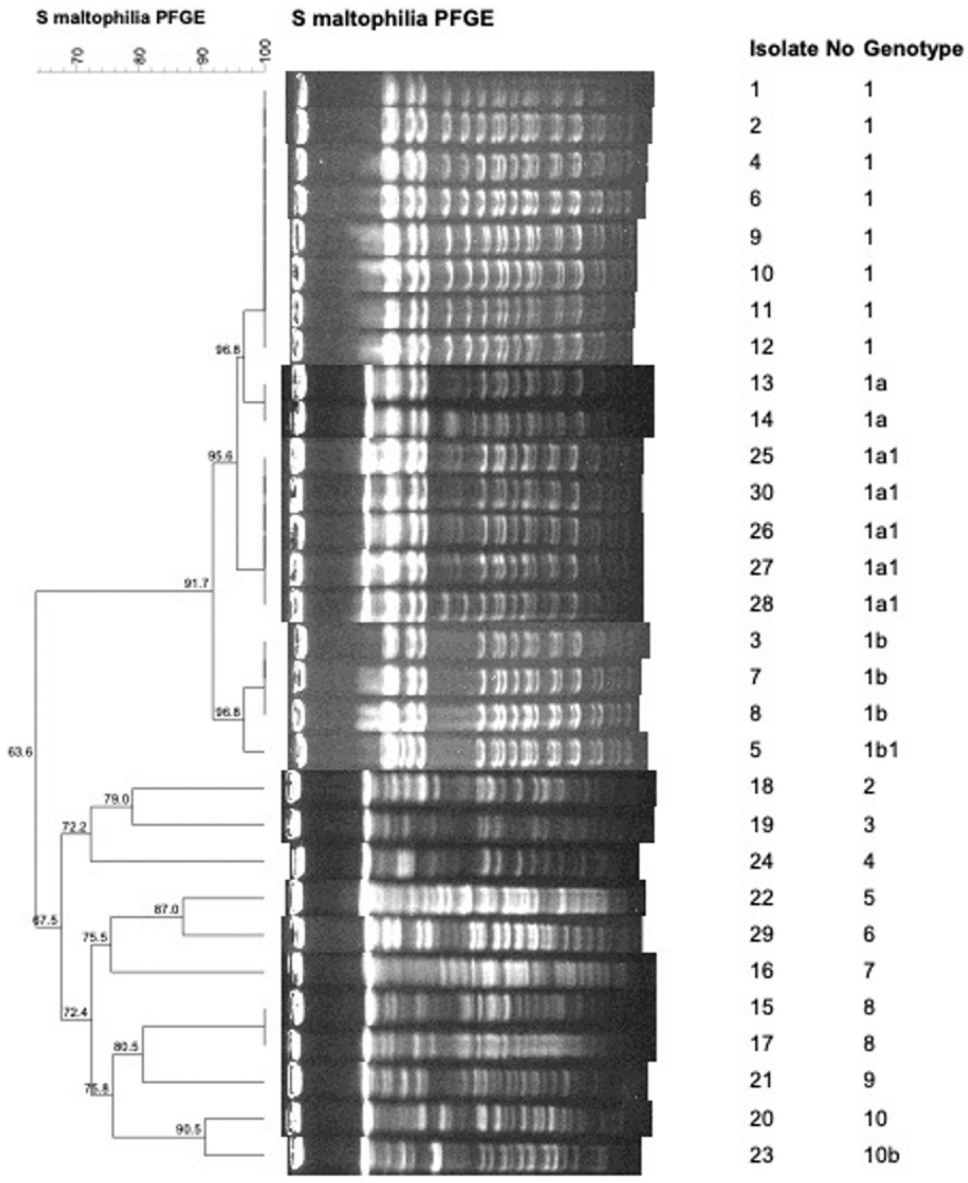


Fig. 1. The PFGE results of 8 environmental and 12 bloodstream infection isolates.

Among the 30 *S maltophilia* isolates, 10 different genotypes were identified, and the isolates showing clustering were grouped into 3 different clusters (tolerance 1.0, optimization 1.0, cutoff 90%). The largest cluster was genotype 1, which included 19 isolates (Fig 1). It is likely that the isolates within genotype 1 were associated with an outbreak. Isolate numbers 1, 2, 4, 9, 10, 11, 12, 13, 14, 25, 26, and 30 are isolates recovered from patients' blood cultures. Isolate numbers 3, 5, 7, 8, 27, and 28 were environmental isolates. Isolate number 6 was recovered from the pull-out faucet inside the PICU. In Figure 3 the rooms that patients stayed during the outbreak is represented with star sign. Overall, in the PICU, there were 6 faucets (shown with a tap sign) and a pull-out faucet (isolate no: 6, represented with a star sign), which were located in the medicine preparation room. Only the pull-out faucet was positive for *S maltophilia* . When the contaminated pull-out faucet was replaced in December 2021, the outbreak ceased, which clarified the origin. The other clusters were genotype 8 and genotype 10, each containing 2 isolates. 23 out of the 30 *S maltophilia* isolates were included in any of the clusters, with a clustering rate of 76.6%. According to the protein chain reaction results, no gene cassettes were found. All isolates recovered from blood cultures were susceptible to ceftazidime and trimethoprim/sulfamethoxazole. We treated all our patients with trimethoprim/sulfamethoxazole.

DISCUSSION

S maltophilia is frequently recognized as a pathogen that thrives in immunocompromised hosts because of its low virulence and consequent opportunistic nature. According to genomic diversity

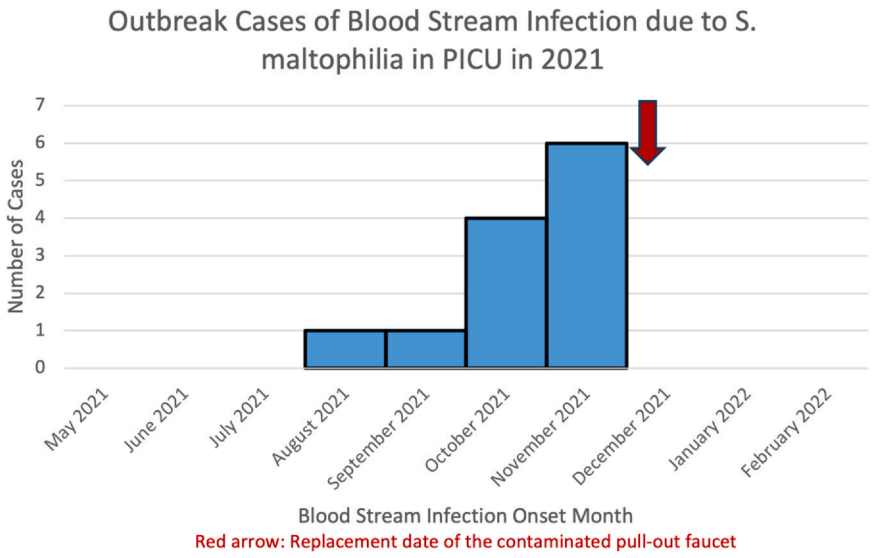


Fig. 2. The epicurve of the outbreak.

among isolates associated with nosocomial cases, there is a strong suggestion of multiple independent environmental sources of transmission.[18,19] *S maltophilia* can attach to and survive on abiotic surfaces within clinical settings via its charged cell wall surface and biofilm production, such as central venous catheters, disinfectant, and handwashing solutions, solutions for hemodialysis, endoscopes, inspiration and expiration circuits of ventilators, nebulizers, tap water, and showerheads.[20–22] Moreover, patient-to-patient transmission has also been reported.[17,18]

E. Sakhnini et al reported 2 infants in a neonatal ICU had *S maltophilia* BSI originating from faucets in the room of one of the patients.[23] Another study from the Netherlands reported 1 *S maltophilia* BSI, which was cultured from tap water from 3 outlets in the neonatal ICU.[24] Data by Weber et al demonstrated that 2 patients were colonized by strains of *S maltophilia* contaminating the faucet aerators in the sink.[25]

WHO has classified *S maltophilia* as one of the leading multidrug-resistant organisms in hospital settings.[19] The risk factors of *S maltophilia* BSI include underlying malignancy (especially hematologic malignancy), organ transplantation, HIV infection, underlying cardiovascular disease, prolonged hospitalization, ICU admission, indwelling catheters (vascular, urinary, and biliary), corticosteroid or immunosuppressive therapy, and prior antibiotic treatment with carbapenem.[21,26–29] Analyzing our patients' profiles, 75% of them were immunocompromised, 50% had underlying cardiovascular disease, and 66.6% had central venous catheters.

We employed various methodologies to assess the contributing factors and identify the transmission patterns. The infection control team observed PICU-staff through the surveillance cameras for 24/7 and weekly meetings were done to give feedback. Our investigation revealed that a pull-out faucet within the PICU and the pipeline attached to it was completely covered with biofilm, which grew *S maltophilia* . Flexible polymeric pipe materials are commonly used as shower hoses or connections to faucets in the last meters of building plumbing. These tend to leach high concentrations of carbon, which may encourage bacterial growth.[30]

There were few theories on how the bacteria located in the pull-out faucet could have caused the outbreak. The theories include a lack of appropriate hand hygiene, incompatible use of medical gloves, asepsis, and cleaning techniques, and not following the isolation rules properly. After pinpointing the outbreak, the hospital's infection control team arranged mandatory training and replaced the pull-out faucet with standard faucets. Training on isolation procedures was provided to resident doctors, whereas new nurses entering the field received training on bedside and mock scenarios. Auxiliary staff were also trained on cleaning procedures.

Our outbreak investigation had several strengths, including access to comprehensive medical records and evaluation of the relevant risk factors. Differentiating true BSI from pseudo-outbreaks due to contamination is crucial for accurate analysis. To address this issue, we used peak C-reactive protein levels as an indicator of true BSI. This approach helped us distinguish genuine infections from contamination-related incidents, thereby enhancing the reliability of our findings. To identify the transmission dynamics of the outbreak, we employed PFGE, which has high discriminatory power. Previous studies conducted in newborns and PICUs have successfully used molecular typing to identify the cross-transmission of *S maltophilia.* [31,32] Furthermore, we took extensive measures to explore all possible sources of contamination by collecting samples from various environmental sources. This comprehensive approach allowed us to thoroughly investigate potential reservoirs and contributing factors within the PICU.

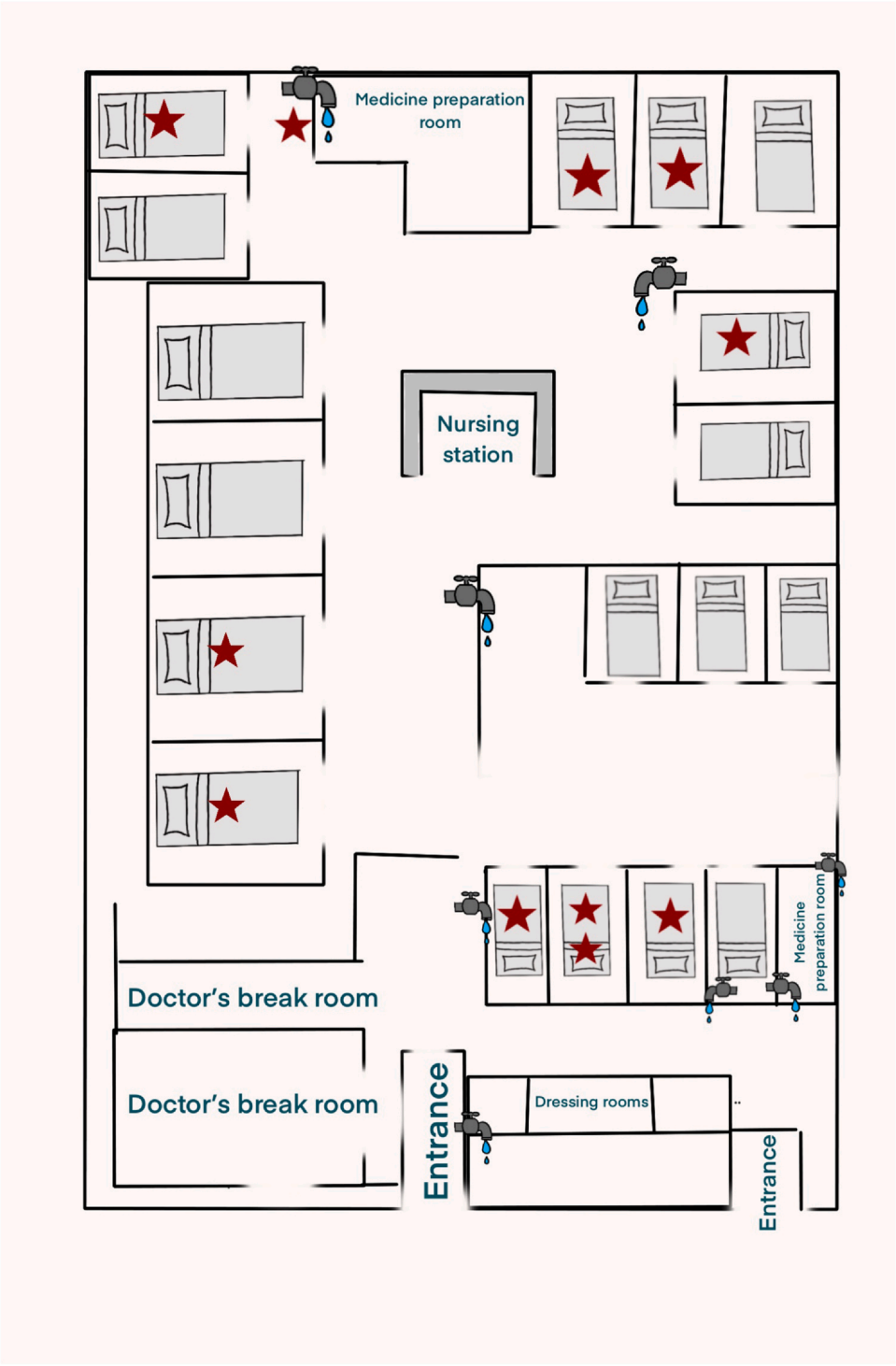


Fig. 3. The map of the pediatric intensive care unit during the outbreak.

CONCLUSIONS

Our outbreak investigation highlights a significant positive link between waterborne *S maltophilia* prevalence rates in PICU patients and tap water supply contamination. The molecular correlation between pull-out faucet originating *S maltophilia* and clinical strains isolated from vulnerable patients was a remarkable finding. Even though pull-out faucets allow easier access to water, it is susceptible to biofilm formation. Therefore, we do not recommend the use of pull-out faucets inside the PICU. Our research highlights the importance of mandating the use of sterile water in PICUs, training hospital staff to spot breaks in sterilization techniques, and including water surveillance during outbreak investigations.

Acknowledgment

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Role of environmental cleaning in controlling an outbreak of *Acinetobacter baumannii* on a neurosurgical intensive care unit

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KEYWORDS
Acinetobacter baumaii;
Outbreak control;
Environmental cleaning

Summary An outbreak of *Acinetobacter baumannii* colonization and infection occurred in 19 patients over a 14-month period during 1998–1999 on a neurosurgical intensive care unit. During efforts to control the outbreak a significant correlation was observed between the number of environmental isolates of *A. baumannii* obtained during each monthly screening and the number of patients with *A. baumannii* colonization/infection in the same calendar month ($P = 0.004$). Use of 1000 ppm hypochlorite solution and the introduction of new cleaning protocols reduced the number of environmental isolates. Failure to maintain low levels of environmental contamination with *A. baumannii* resulted in increases in patient colonization. This study showed that high standards of cleaning play an integral role in controlling outbreaks of *A. baumannii* in the intensive care unit setting.
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Introduction

Outbreaks of *Acinetobacter baumannii* colonization and infection on intensive care units are a common problem, particularly in Europe[1–4] and North America.[5–8] A propensity to tolerate drying[9,10] and resistance to multiple classes of antibiotics[1,4,7] are key factors in enabling the organism to survive and spread in the nosocomial environment. Although the importance of cleaning in controlling epidemics of *A. baumannnii* has been emphasized in previous studies[7] little is known about the optimum approach to environmental cleanliness in an endemic situation. We report an outbreak of *A. baumannii* colonization and infection on a neurosurgical intensive care unit (NICU) where the intensity of environmental cleaning closely

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correlated with the number of positive environmental sites and, subsequently, the number of affected patients.

Materials and methods

Description of the outbreak

During one month of 1998 seven cases of colonization and infection with *A. baumannii* were noted on the NICU of a 1000 bed tertiary-care teaching hospital. This is a seven-bedded unit, five in an open-plan area and two in single rooms. The ward environment was screened revealing that 27 of 51 (53%) sampled sites were contaminated with an indistinguishable strain of *A. baumannii*, as determined by pulsed-field gel electrophoresis (PFGE). The unit was then closed and existing patients moved to an adjacent intensive care unit for eight days whilst the ward environment was thoroughly cleaned using detergent and water. However, after re-opening, patient cases continued and re-sampling of the environment again revealed significant contamination with *A. baumannii*. An environmental cleaning policy using 1000 ppm sodium hypochlorite solution was instigated and monthly environmental sampling of the same 51 sites continued. Over the next 13 months a further 12 cases of infection/colonization were identified. Patient's details are listed in Table I.

Ward databases were also analysed to provide information on bed occupancy rates and mortality during the period of the outbreak. During this period a number of issues with cleaning protocols were also identified and addressed. These included problems with recruiting and retaining cleaning staff, proper training and supervision, failure to follow agreed cleaning procedures, clear designation of cleaning responsibilities between nursing and cleaning staff, and failure to adequately dry cleaning equipment, e.g. mop heads between use.

Isolate origin and identification

All oxidase-negative, non-lactose fermenting, Gram-negative bacilli obtained from clinical samples were identified as *A. baumannii* using API 20NE (bioMérieux, Marcy l'Etoile, France). After isolation they were stored at −70 °C in 15% glycerol broth.

Antibiotic susceptibility testing

Antibiotic susceptibility to eight antimicrobial agents (ampicillin, cefradine, gentamicin, ciprofloxacin, cefotaxime, aztreonam, piperacillin-tazobactam, and meropenem) was performed on Isosensitest agar (Oxoid Ltd., Hampshire, United Kingdom) using Stokes' method.

Environmental screening

Environmental sampling was carried out on the NICU after the identification of the first seven cases. A total of 51 sites were sampled from the patients' immediate environment, particularly sites where dust accumulated. These included

Table I Details of cases involved in the outbreak

Case no.	Age	Sex	Diagnosis	Sources
1	53	F	Subarachnoid haemorrhage	Sputum
2	68	F	Subarachnoid haemorrhage	Cerebrospinal fluid
3	18	M	Road-traffic accident, head injury	Blood, cerebrospinal fluid, central line tip
4	15	M	Assault, facial fractures	Sputum, central line tip
5	39	M	Road-traffic accident, head injury	Wound
6	21	M	Road-traffic accident, head injury	Wound
7	39	M	Assault, head injury	Wound, sputum
8	53	M	Cervical spine injury	Wound, sputum, drain tip
9	25	F	Road-traffic accident	Blood, extradural haematoma
10	43	M	Subarachnoid haemorrhage	Sputum
11	55	M	Subarachnoid haemorrhage	Sputum
12	37	M	Subarachnoid haemorrhage	Sputum
13	39	M	Road-traffic accident, head injury	Sputum
14	69	F	Subarachnoid haemorrhage	Sputum
15	53	M	Subarachnoid haemorrhage	Sputum
16	26	M	Road-traffic accident, head injury	Sputum, central line tip
17	16	F	Road-traffic accident, head injury	Central line tip
18	50	M	Road-traffic accident, head injury	Wound
19	13	F	Road-traffic accident, head injury	Sputum, central line tip

patient monitors, bed frames, X-ray illuminators, curtain rails, and equipment trolleys. Ventilation grilles from the air-conditioning unit were also targeted. The same 51 sites were sampled monthly with sterile swabs, moistened in 1/4 strength Ringers solution, which were then applied directly onto Columbia agar containing 5% horse blood supplemented with 6 mg/L vancomycin. Plates were incubated overnight at 37 °C in air. All oxidase-negative Gram-negative coccobacilli were confirmed as *A. baumannii* using API 20NE.

PFGE

The method used was that of Gautom[11] with modifications as follows. Each strain was grown in 5 mL tryptone soya broth for 5 h at 37 °C on an orbital shaker at 100 rpm. Point six millilitres of culture was pelleted in an Eppendorf tube using a low-speed centrifuge, washed with 1 mL SE buffer (75 mm NaCl; 25 mM EDTA, pH 8.5), and resuspended in 0.3 mL EC lysis buffer (6 mM Tris pH 8.0; 1 M NaCl; 100 mM EDTA Na_2 0.5 polyoxyethelene 20 cetyl ether (Brij 58); 0.5% N-lauryl sarcosine; 0.2% deoxycholic acid). Point three millilitres of 2% low melting point agarose in SE buffer was added, the mixture dispensed into block moulds, and left to set at 4 °C for 20 min. Blocks were then placed into 1 mL EC lysis buffer including 1 mg/mL proteinase K and incubated overnight in a 55 °C water-bath. Blocks were then removed and washed four times in 5 mL TE buffer (10 mM Tris; 1 mM EDTA, pH 8.0) whilst placed on a roller mill. Blocks were then stored at 4 °C until use. Before digest each block was washed in 5 mL TE buffer and a 2 mm slice placed in a 0.5 mL Eppendorf with 10 U/50 mL *Sma*I (Boehringer Mannheim, Penzberg, Germany). These were incubated at 30 °C for 6 h in a water-bath. Blocks were loaded into wells of a 1.2% agarose gel in 0.5 × TBE buffer and sealed with 1% low melting point agarose. Gels were placed into a PFGE tank containing 2.5 L 0.5 × TBE buffer and subject to PFGE using pulses 5–20 s for 18 h at 14 °C. Gels were stained using ethidium bromide, visualized by UV light and photographed using a gel documentation system (VDU Image Master, Pharmacia, Uppsala, Sweden).

Statistical analysis

Statistical analysis was performed using Pearson's correlation coefficient.[12]

Results

The 19 colonized/infected patients (13 males) had a median age of 39 years. Twenty-seven isolates of *A. baumannii* were obtained from these patients over a 14 month period. They were cultured from a variety of sites, including sputum ($N = 12$), central line tips ($N = 5$), wounds ($N = 5$), blood ($N = 2$), CSF ($N = 2$), and drain tips ($N = 1$). All the clinical and environmental isolates had the same antibiogram (susceptible to ciprofloxacin, gentamicin, meropenem, resistant to ampicillin, aztreonam, cefotaxime, cefradine, and piperacillin-tazobactam) and PFGE profile. The relationship between the monthly total of clinical isolates and environmental isolates is shown in Figure 1. There was a significant correlation between the number of environmental isolates obtained during each monthly screening and the number of patients with *A. baumannii* colonization/infection in the same calendar month ($P = 0.004$). There was no significant correlation between the number of patient isolates per calendar month and bed occupancy or mortalities during the same period ($P > 0.05$).

Discussion

Environmental cleanliness is a key factor in the control of hospital-acquired infection. Guidelines for the control of nosocomial pathogens, such as methicillin-resistant *Staphylococcus aureus*, recommend high standards of ward cleaning.[13] Such recommendations are, however, based more on strong rationale and circumstantial evidence than on the significant findings of definitive scientific

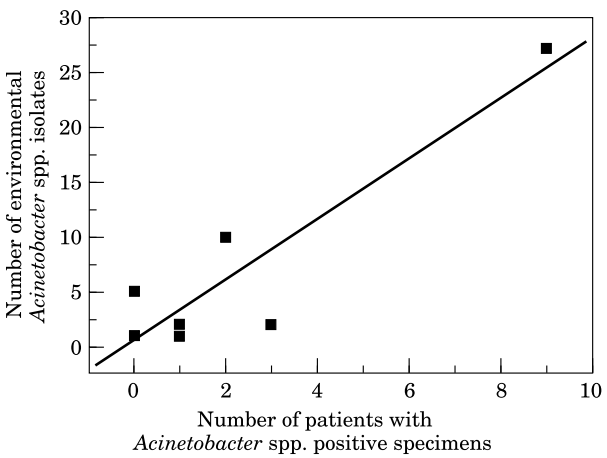

Figure 1 Graph showing relationship between number of patient isolations and environmental isolations of the outbreak strain of *Acinetobacter baumannii* obtained during the same calendar month.

studies. Attempts to reduce infection rates by enhancing environmental cleaning have generally proved unsuccessful.[14–16] As expected, floor cleaning with either detergent or disinfectant was found not to affect nosocomial infection rates.[14] Also, Dharan and colleagues recently observed that the incidence of nosocomial infections did not change during a four months trial of different cleaning agents.[16] A quaternary ammonium compound was inadequate for disinfecting bathrooms and toilets but an active oxygen-based compound was satisfactory. The detergent being examined was actually contaminated and so environmental surface counts of microbes increased post cleaning. In contrast, two recent studies have suggested that hypochlorite-based environmental cleaning may be associated with a reduced incidence of hospital-acquired *Clostridium difficile* infection.[17,18]

While *Acinetobacter* spp. do not form spores, their persistent survival when desiccated is at least partly analogous to that seen with *C. difficile*.[10] We did not measure *A. baumannii* hand contamination rates for the staff working in the NICU in the present study. Elsewhere, during an outbreak of gentamicin-resistant *A. baumannii* infection on an intensive care unit, 11% of staff hand samples versus 3% of environmental samples yielded the outbreak strain.[19] Larson[20] found that *Acinetobacter* spp. were the most frequent Gram-negative species persistently contaminating the hands of healthcare workers, both for frequent and less regular hand washers.[20] The frequency of *C. difficile* positive hand cultures in healthcare workers is known to correlate with the intensity of *C. difficile* environmental contamination.[17,21] Thus, it is plausible that the significant correlation observed in the present study between environmental contamination with *A. baumannii* and recovery of the bacterium from patients is explainable by concomitant increased hand carriage of this bacterium.

Over a period of several months this study showed a significant correlation between the number of positive environmental sites and the number of patients positive for *A. baumannii* colonization and/or infection in the same calendar month. There was no significant correlation with either bed occupancy or mortality during the same period, suggesting that rises in the number of colonized patients was not related to increased throughput or significant changes in patient population.

A number of cleaning issues were also addressed in order to control the outbreak. Responsibility for cleaning environmental infrastructure, e.g. floors, curtain rails, was delegated to ward cleaning staff whereas responsibility for cleaning patient equipment, e.g. monitors, bed frames, trolleys, was delegated to nursing staff. Additional cleaning staff were employed to ensure all bed spaces could be cleaned between patient admissions. Cleaning of areas inaccessible from ground level, e.g. ceiling ventilation grills, was instigated on a three-monthly basis. Additional storage trolleys were purchased to allow decanting and cleaning of stored medical equipment on a monthly basis. However, given the complexity of the cleaning protocols introduced, there were occasions when these new procedures were not followed. On one occasion the number of positive environmental sites increased shortly after additional cleaning input had been withdrawn because of recruitment and retention problems with ward cleaning staff.

This outbreak study has shown that levels of environmental contamination with *A. baumannii* correlate with patient colonization and infection. Failure to follow strict cleaning protocols, often because of problems recruiting, retaining and training cleaning staff, appeared to exacerbate the problem. Healthcare planners need to be aware that hospital cleaning is an invaluable in controlling outbreaks and further studies regarding the cost-effectiveness of cleaning are urgently required.

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Stenotrophomonas maltophilia Associated Factors and Outcomes in a Neonatal Intensive Care Unit

A Retrospective Matched Case-control Study

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Background: *Stenotrophomonas maltophilia* is a multi-drug-resistant, hospital-acquired Gram-negative bacillus associated with significant morbidity and mortality. The objective of this study is to identify risk factors and outcomes associated with *S. maltophilia* isolation in a high-risk neonatal population.

Methods: This was a retrospective matched case-control study. Cases were matched 1:2 for years of neonatal intensive care unit admission, completed weeks' gestational age and birth weight in 250-gram incremental categories.

Results: A total of 15 cases and 35 controls were included in the analyses. Risk factors for *S. maltophilia* isolation included days of antibiotics (24 vs. 18, $P = 0.036$), days of broad-spectrum antibiotics (19 vs. 12 days, $P = 0.027$), days of meropenem (9 vs. 6 days, $P = 0.018$) and any meropenem exposure (100% vs. 22%, $P = 0.005$). Other risk factors were any corticosteroid exposure (66.7% vs. 20%, $P = 0.001$), days of total parenteral nutrition (55 vs. 31 days, $P = 0.017$) and days of invasive mechanical ventilation (28 vs. 7, $P = 0.015$). *S. maltophilia* isolation was associated with increased length of neonatal intensive care unit stay (134 vs. 69 days, $P < 0.001$) and mortality (33.3% vs. 0%, $P = 0.001$).

Conclusions: Antibiotic stewardship efforts should be made to decrease the risk of *S. maltophilia* isolation and associated mortality. Carbapenem overuse should be specifically addressed with institutional policies and unit-based guidelines. Additional neonatal studies are needed to confirm these findings and explore other possible risk factors.

Key Words: *Stenotrophomonas maltophilia*, neonatal infection, risk factors, carbapenems, antimicrobial stewardship

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Stenotrophomonas maltophilia is an aerobic, nonfermenting, Gram-negative bacillus typically associated with opportunistic infections.[1–3] Although *S. maltophilia* is usually found in the environment in soil and water, the World Health Organization classifies it as one of the most significant multidrug-resistant organisms isolated in hospitalized patients.[1,4] *S. maltophilia* is most frequently found in the respiratory tract and can cause chronic respiratory infections.[1,3,5] The most common clinical manifestations of *S. maltophilia* infection are pneumonia and bacteremia followed by urinary tract infection, peritonitis, ophthalmological infection and meningitis.[2] Other manifestations include endocarditis and bone and joint, skin and soft tissue and gastrointestinal infections.[2–4]

S. maltophilia is associated with increased length of hospital stay and significant morbidity and mortality.[1] The mortality rate in adults is about 37%; data in children are more variable, with mortality ranging from 10% to 60%.[4] Neonatal mortality data are limited. One study from a neonatal intensive care unit (NICU) in Turkey reported a mortality rate of 17%.[3] Neither outcomes nor risk factors have been adequately studied in the neonatal population, as the majority of clinical studies focus on the adult and, to a lesser extent, pediatric populations.[3] Known risk factors that may apply to neonates include indwelling devices, chronic respiratory disease, immunocompromised state, exposure to antibiotics and long-term NICU stay.[1] Our urban medical center houses a 29-bed Level IIIB NICU and serves a diverse and clinically complex neonatal patient population. From 2010 to 2019, our unit experienced an increase in *S. maltophilia* infections, prompting a retrospective investigation. The objective of this study is to determine what potential risk factors and outcomes are associated with neonatal *S. maltophilia* isolation in our critically ill neonatal population.

METHODS

This is a retrospective, age-matched case-control study of neonates admitted to the NICU at the University Hospital at Downstate between January 1, 2008, and April 1, 2020. The study was approved by the Institutional Review Board at University Hospital at Downstate as a minimal-risk study with a waiver of informed consent. Microbiology records between January 1, 2008 and April 1, 2020 were manually reviewed for *S. maltophilia* cultured from any site. Admission and medical records of those neonates with positive cultures were then reviewed, and neonates were included as cases in the study if their NICU admission was at least 72 hours. Cases were then stratified by year of admission.

Within each year of admission, controls were selected randomly, then individually matched 2:1 to cases first for gestational age (GA) and then for birth weight by incremental 250-gram categories. Controls were identified by manual review of NICU admission records if the desired year of admission was before the installation of our electronic medical record system in 2011. Upon successful matching of admission year, GA and birth weight, medical records were reviewed to confirm an admission of at least 72 hours. Those neonates who met matching and inclusion criteria were then removed from the pool of controls for subsequent cases.

Medical charts of all neonates were reviewed for birth weight, GA, race, sex, *S. maltophilia* infection risk factors and adverse outcomes. The presence of premature rupture of

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membranes (more than 24 hours before delivery), maternal chorioamnionitis and Apgar scores <5 at 5 minutes of life were extracted from delivery history records. All data for analysis of risk factors for infection were recorded before time of collection of the first positive *S. maltophilia* culture in cases or until discharge in controls. Neutropenia was defined as an absolute neutrophil count less than 1.5×10^9/L. An antibiotic course was defined as the uninterrupted administration of 1 or more antibiotics for at least 48 hours. A prolonged antibiotic course was defined as the uninterrupted administration of 1 or more antibiotics for more than 5 days in duration. Antibiotic days were defined as days of exposure rounded to the nearest half-day at the end of the dosing interval. Third- and fourth-generation cephalosporins, piperacillin/tazobactam, meropenem, amikacin, vancomycin and linezolid were classified as broad-spectrum antibiotics.

Data were not normally distributed and thus analyzed nonparametrically. Categorical values were counted, then percentages and odds ratios were calculated. The χ^2 test was used for between-group comparisons of categorical variables. Fisher exact test was used for comparisons of categorical variables if any value was expected to be <5 and for all comparisons between tracheal isolates and other isolates. Medians and interquartile ranges (IQR) were calculated for continuous variables and then analyzed with the Mann–Whitney *U* test. An alpha of 0.05 was deemed statistically significant.

RESULTS

A total of 21 individual neonates were identified as *S. maltophilia* cases; all were included in the study as they were admitted to the NICU for at least 72 hours and matched 1:2 to 42 controls. Six cases and 7 controls were ultimately excluded from analyses due to incomplete data, for example, delivery history was unknown, total parenteral nutrition (TPN) or antibiotic administration data was incomplete, or duration of mechanical ventilation and records of invasive procedures were unclear. Neonates were predominantly Black or African American (98%) with a median GA at birth of 26 (25–28) weeks. All neonates included received mechanical ventilation, antibiotics and TPN during their admission (cases before their culture and controls at any point before discharge). Baseline characteristics were not different between the 15 cases and 35 controls that were included in the analysis (Table 1).

Potential risk factors for infection are presented in Table 2. Total days of TPN were significantly higher in cases compared with controls (55 vs. 31 days, $P = 0.017$). Cases were exposed to more days of invasive mechanical ventilation compared with controls (28 vs. 7 days, $P = 0.015$). Cases had more occurrences of any corticosteroid exposure versus controls (66.7% vs. 20%; $P = 0.001$) and were 8 times more likely to receive corticosteroids than controls. Calculated odds ratios are summarized in Table 3. The total number of antibiotic days was higher in cases than in controls (24 vs. 18 days, $P = 0.036$); total number of broad-spectrum antibiotic

TABLE 1. Baseline Characteristics

	Case (N = 15)	Control (N = 35)	Significance (*P* value)
Black or African-American race, N (%)	35 (100)	14 (93.3)	0.300
Female sex, N (%)	10 (66.7)	25 (71.4)	0.736
Birth weight (g), median (IQR)	675 (523–998)	794 (765–920)	0.294
GA (wk), median (IQR)	26 (24.9–27.1)	26.3 (25.6–28.9)	0.509
Premature rupture of membranes, N (%)	2 (13.3)	10 (28.6)	0.298
Maternal chorioamnionitis, N (%)	0 (0)	6 (17.1)	0.659
Apgar score <5 at 5 min of life, N (%)	1 (6.7)	1 (2.9)	0.514

TABLE 2. Potential Risk Factors for *S. maltophilia* Infection

	Cases (N = 15)	Controls (N = 35)	Significance (*P* value)
Length of stay (days), median (IQR)	55 (36–84)	69 (60–96.5)	0.075
Postmenstrual age (weeks), median (IQR)	33 (31–41)	37.1 (36.4–39.6)	0.062
TPN days, median (IQR)	55 (34.5–59)	31 (24–50.5)	**0.017**
Mechanical ventilation days, median (IQR)	55 (34.5–84)	39 (18.5–67)	0.087
Any invasive mechanical ventilation, N (%)	15 (100)	29 (82.9)	0.160
Invasive mechanical ventilation days, median (IQR)	28 (21–49)	7 (1–26.5)	**0.015**
Any CVC, N (%)	15 (100)	31 (88.6)	0.302
CVC days, median (IQR)	25 (20.8–31.5)	15 (11–31)	0.180
Any intubation, N (%)	15 (100)	30 (85.7)	0.305
Any tracheostomy, N (%)	1 (6.7)	2 (5.7)	1.000
Any neutropenia, N (%)	3 (20)	1 (2.9)	0.075
Chronic lung disease, N (%)	8 (53.3)	11 (31.4)	0.144
Any corticosteroids, N (%)	10 (66.7)	7 (20)	**0.001**
Antibiotic days, median (IQR)	24 (19.3–41.3)	18 (8–29.8)	**0.036**
Antibiotic courses, median (IQR)	3 (3–4.5)	3 (2–4)	0.111
Antibiotic courses >5 days, median (IQR)	2 (1–3)	2 (1–3)	0.390
Broad-spectrum antibiotic days, median (IQR)	19 (12.3–39.8)	12 (3.5–25.3)	**0.027**
3rd or 4th generation cephalosporin days, median (IQR)	0 (0–3.8)	0 (0–3.25)	0.772
Piperacillin/tazobactam days, median (IQR)	0 (0–2)	0 (0–0)	0.222
Vancomycin days, median (IQR)	13 (8–26.8)	9.5 (3–18.5)	0.187
Meropenem days, median (IQR)	9 (7.3–18.5)	6 (0–11)	**0.018**
Any meropenem, N (%)	15 (100)	22 (62.9)	**0.005**
Meropenem course >5 days, N (%)	14 (93.3)	17 (48.6)	**0.004**

CVC indicates central venous catheter.
All data were recorded prior to S. maltophilia isolation in cases and prior to discharge among controls.
Bold values indicate statistical significance.

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TABLE 3. Odds Ratios of Risk Factors for *S. maltophilia* Infection

	Odds Ratio	95% Confidence Interval	
		Lower	Upper
Black or African-American race	1.071	0.936	1.227
Female sex	1.250	0.341	4.587
Any invasive mechanical ventilation	0.829	0.713	0.963
Any CVC	0.886	0.786	0.998
Any intubation	0.857	0.749	0.981
Any tracheostomy	1.179	0.099	14.081
Any neutropenia	8.500	0.805	89.746
Chronic lung disease	2.494	0.721	8.619
Any corticosteroids	**8.000***	**2.062**	**31.041**
Any meropenem	0.657	0.517	0.835
Meropenem course >5 days	3.667	0.974	13.806

*Statistically significant.
CVC indicates central venous catheter.
Bold values indicate statistical significance.

days was higher in cases compared with controls (19 vs. 12 days, $P = 0.027$). Total days of third- or fourth-generation cephalosporins, piperacillin/tazobactam and vancomycin were similar between cases and controls; total days of meropenem were higher in cases compared with controls (9 vs. 6 days; $P = 0.018$). Additionally, 100% of neonates were exposed to meropenem before isolation of *S. maltophilia* compared with 62.9% of controls ($P = 0.005$). Postnatal age and postmenstrual age at time of culture collection (cases) or discharge (controls) were not different.

Adverse outcomes associated with *S. maltophilia* are shown in Table 4. *S. maltophilia* isolation was associated with increased length of NICU stay (134 vs. 69 days, $P < 0.001$) and mortality (33.3% vs. 0%; $P = 0.001$) Mortality among infants with peritoneal infection was 100% (3/3). Mortality among infants with polymicrobial infection was 37.5%. We also compared the incidence of any culture-proven sepsis (other than *S. maltophilia*) during NICU stay to assess sepsis as a confounding risk factor for mortality which was not different between cases and controls (46.7% vs. 31.4%, $P = 0.304$). Incidence of necrotizing enterocolitis (NEC) was also analyzed as a potential confounder for mortality and length of stay which was significantly higher in cases versus controls (40% vs. 8.6%, $P = 0.015$). *S. maltophilia* was most frequently isolated in the trachea, with 9 isolates; 3 cases were isolated from the peritoneum, 2 from the conjunctiva and 1 from the blood (Table 5). Most cases occurred between 2014 and 2019; see Figure, Supplemental Digital Content 1, http://links.lww.com/INF/F683. Time to *S. maltophilia* culture positivity, susceptibility of *S. maltophilia* to levofloxacin and sulfamethoxazole-trimethoprim (TMP-SMX) and presence of polymicrobial infection are presented in Table 5.

TABLE 4. Adverse Outcomes

	Cases (N = 15)	Controls (N = 35)	Significance (*P* value)
Total length of NICU stay (days), median (IQR)	134 (113.5–231)	69 (60–96.5)	**< 0.001**
Mortality, N/total patients (%)	5/15 (33.3)	0/35 (0)	**0.001**
Blood	0/1 (0)		
Conjunctiva	0/2 (0)		
Peritoneum	**3/3 (100)**		
Trachea	2/9 (22.2)		

Bold values indicate statistical significance.

TABLE 5. *S. maltophilia* Culture Results

	Cases (N = 15)
Site of isolation, N (%)	
Blood	1 (6.7)
Conjunctiva	2 (13.3)
Peritoneum	3 (20)
Trachea	9 (60)
Time to positivity (h), median (IQR)	72 (56.8-91)
Levofloxacin susceptibility, N (%)	15 (100)
TMP-SMX initial susceptibility, N (%)	14 (93.3)
Development of R to levofloxacin, N (%)	4 (26.7)
Development of R to TMP-SMX, N (%)	1 (6.7)
Polymicrobial, N (%)	8 (53.3)
Enterococcus faecium	1 (6.7)
Klebsiella aerogenes	1 (6.7)
Klebsiella pneumoniae	4 (26.7)
Staphylococcus epidermidis	3 (20)

R, resistance based on institutional susceptibility testing.

We also compared potential risk factors in the cases of *S. maltophilia* isolated in the trachea to cases isolated from all other sites (peritoneum, conjunctiva and blood), as the assessment of tracheal isolates as colonization versus infection was limited. As shown in Table, Supplemental Digital Content 2, http://links.lww.com/INF/F684, tracheal isolates were associated with greater postnatal age (78 vs. 36 days, $P = 0.018$), more days of TPN (58 vs. 34 days, $P = 0.013$), more days of mechanical ventilation (64 vs. 34.5 days, $P = 0.046$) and more antibiotic courses (4 vs. 3 courses, $P = 0.029$) before culture collection.

DISCUSSION

Limited data exist to assess potential risk factors for *S. maltophilia* infection in critically ill neonates. A retrospective matched case-control study in Turkey by Mutlu et al[3] in 2011 was the first to assess risk factors and clinical outcomes associated with *S. maltophilia* infection in neonates. They compared 23 cases with 45 matched controls and, in agreement with our study, reported that meropenem exposure, corticosteroid exposure, duration of TPN and duration of invasive mechanical ventilation were associated with *S. maltophilia* infection.[3] Postnatal age (ie, length of NICU stay) at the time of first positive culture (cases) or at discharge (controls) did not affect risk for *S. maltophilia* isolation in our study as has been previously reported in the literature of all age groups.[2,3,6,7] The extended admissions among extremely preterm neonates may have affected the analysis of this risk factor; it is unclear at what duration of hospital stay infection risk increases.

The majority of antibiotics used in the neonatal population, including ampicillin and gentamicin, have been linked to *S. maltophilia* isolation in children and adults.[3,6] Exposure to broad-spectrum antibiotics, including carbapenems, third- and fourth-generation cephalosporins, vancomycin and piperacillin/tazobactam, is a commonly reported risk factor for *S. maltophilia* colonization and infection.[3,6,8] We therefore analyzed days of all broad-spectrum antibiotics as well as days of third- and fourth-generation cephalosporins, piperacillin/tazobactam, vancomycin and meropenem individually. Days of amikacin and linezolid were not analyzed individually as only 4 patients were exposed. Adult, pediatric and neonatal studies of *S. maltophilia* infection risk factors show that carbapenem exposure in particular increases the risk of *S. maltophilia* infection and mortality.[2,4,9] Mutlu et al[3] reported that 78% of neonates with *S. maltophilia* infection had been exposed to a carbapenem. In our study, 100% of neonates were exposed to meropenem before isolation of *S. maltophilia* compared to 62.9%

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of controls ($P = 0.005$). More days of meropenem was also associated with *S. maltophilia* infection (9 vs. 6 days, $P = 0.018$). We did not find that days of any other broad-spectrum antibiotic were associated with infection. While total broad-spectrum antibiotic days were significantly greater in cases, this may have been driven by meropenem specifically. We also compared exposure to prolonged meropenem courses to further elucidate how meropenem was used and to investigate the influence of prolonged courses versus total days of meropenem exposure on *S. maltophilia* infection. Among cases, exposure to prolonged meropenem courses was signifcantly higher than among controls (93.3% vs. 48.6%, $P = 0.004$).

NEC could have contributed to adverse outcomes otherwise attributable to *S. maltophilia*. However, the higher incidence among cases also illustrates the association between NEC and antibiotic days and broad-spectrum antibiotic exposure.[10] Isolation of *S. maltophilia* was associated with an almost 2-fold longer total length of NICU stay. The mortality rate of 33.3% is higher than that reported by Mutlu et al[3] (17%) and comparable to rates reported in adults (37%) and children (10%–60%) with *S. maltophilia* infection.[4] Peritoneal *S. maltophilia* infection was associated with 100% mortality (3/3 cases). These infants had undergone abdominal surgery to repair a perforated gut secondary to NEC. Pediatric studies report higher mortality with polymicrobial infections.[1] In our study, 8 cases (53.3%) were polymicrobial with a mortality rate of 37.5%. The 4 polymicrobial tracheal cultures grew *Klebsiella aerogenes* (1), *Staphylococcus epidermidis* (1) and *Klebsiella pneumoniae* (2). The other 4 polymicrobial cases were nonrespiratory infections, which is inconsistent with neonatal and pediatric literature.[9,11] The 2 polymicrobial conjunctival cultures grew *K. pneumoniae* (1) and *S. epidermidis* (1). Two of the 3 peritoneal cultures were polymicrobial; 1 grew *K. pneumoniae* and *Enterococcus faecium*; the other grew *S. epidermidis.*

S. maltophilia is intrinsically resistant to beta-lactams, carbapenems and aminoglycosides.[1] We tested the isolates against drugs of choice, TMP-SMX and levofloxacin. All 15 isolates were initially susceptible to levofloxacin and 14 to TMP-SMX. Resistance to levofloxacin developed during treatment after 8 days in 3 cases. In 1 case, levofloxacin resistance developed after 16 days followed by TMP-SMX resistance after 2 months, which may have contributed to this neonate's death. Otherwise, susceptibilities did not appear to be associated with mortality or length of stay, as has been previously reported in the literature.[1]

The trachea was the most common site of isolation with 60% (9/15) of isolates. Mutlu et al[3] similarly found that the most common site of infection was the respiratory tract. In contrast with our study, they excluded 54% of tracheal aspiration samples before analysis, identifying these samples as colonization versus infection. Neonates with *S. maltophilia* in that study were notably larger and older than those reported in our study, with a mean birth weight of 2.18 kg and GA of 33.9 weeks.[3] Colonization was suspected in 4 neonates and infection suspected in 2 neonates with *S. maltophilia* isolated in the trachea in our study by the pediatric infectious disease team based on review of chest radiographs, C-reactive protein, complete blood count with differential, culture data (polymorphonucleated white cells and presence of additional organisms) and susceptibility patterns of repeat isolates; categorization as colonization or infection was not described (or data were not available) in the medical records for the remaining 3 neonates. Tracheal cultures are typically performed in invasively mechanically ventilated, critically ill neonates; differentiating S. *maltophilia* infection from colonization is challenging. We attempted to determine if tracheal isolates were less likely to be infection by comparing risk factors for infection between tracheal isolates and all other isolates (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/F684); we did not find tracheal isolates were less associated with *S. maltophilia* infection risk factors, but these secondary analyses are extremely limited by potential confounders and by even smaller sample sizes. We might expect about half of our cases of *S. maltophilia* isolation in the trachea to be colonization according to Mutlu et al.[3] However, based on the documented management of these infants and overall mortality, most tracheal isolates in our study likely represent true infection.

There are several additional limitations in this study that have not yet been discussed. Confounders for infection risk and mortality were controlled for by matching.[7] However, 6 cases and 7 controls were removed from analysis after matching which may have skewed baseline comparisons and is a major limitation. Apgar score < 5 at 5 minutes of life was used as a baseline indicator of mortality risk in addition to birth weight and GA; all baseline characteristics were comparable between groups despite the exclusion of 6 cases and 7 controls after matching.[12] We compared the incidence of any other culture-proven sepsis (including after *S. maltophilia* isolation among cases) which was not different between cases and controls (46.7% vs. 31.4%, $P = 0.304$), but we could not compare the incidence of sepsis-related mortality due to limitations in our medical record documentation. Furthermore, underlying illness (eg, gastrointestinal, neurologic and cardiovascular diseases) and other contributors to mortality (eg, culture-negative infection) were not compared. We also did not assess cholestasis incidence or total days of corticosteroid exposure, which may be additional *S. maltophilia* risk factors.[3] Duration of central venous catheter access could not be collected for 7/15 cases and 14/35 controls due to documentation limitations; while we found no difference between cases and controls, this analysis should be interpreted cautiously. It should also be noted that the case-control study design limits the interpretation of our results to associations, as causality cannot be established. Despite these limitations, this study provides valuable insight on the topic as it is, to our knowledge, the only case-control study on *S. maltophilia* risk factors in neonates in the United States.

CONCLUSIONS

In accordance with the limited neonatal literature, meropenem exposure, days of TPN, days of invasive mechanical ventilation and corticosteroid exposure were significantly associated with *S. maltophilia* isolation at our institution.[3] Pediatric and adult data support our findings that days of antibiotic exposure, and especially days of broad-spectrum antibiotic exposure, increase risk.[2,8] Days of meropenem may be the most important potential risk factor for *S. maltophilia* isolation. Antibiotic stewardship efforts should be made to decrease the risk of S. *maltophilia* infection and associated mortality. Carbapenem overuse should be specifically addressed with institutional policies and unit-based guidelines. Broad-spectrum antibiotic restrictions (including meropenem) were implemented in our NICU in December 2023 in response to our study's findings. We intend to study the impact of these restrictions on the incidence of *S. maltophilia* colonization and infection in the future. Additional studies in extremely preterm neonates are needed to confirm these findings and to further explore the contribution of broad-spectrum antibiotics, particularly carbapenems, to *S. maltophilia* colonization and infection.

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Risk Factors for Infection and Mortality Associated With *Stenotrophomonas maltophilia* Bloodstream Infections in Children; Comparison With *Pseudomonas aeruginosa* Bloodstream Infections

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Introduction: The increasing incidence of *Stenotrophomonas maltophilia* (*S. maltophilia)* infections raises concern because of the high fatality/case ratio. This study aimed to evaluate the risk factors for infection and mortality associated with *S. maltophilia* bloodstream infections (BSIs) in children and compare them with *Pseudomonas aeruginosa* BSIs.

Methods: All BSIs caused by *S. maltophilia* (n:73) and *P. aeruginosa* (n:80) were enrolled in this study between January 2014 and December 2021 at the Medical School of Ege University.

Results: Previous Pediatric Intensive Care Unit (PICU) admission, prior glycopeptide, and carbapenem use were significantly more common in patients with *S. maltophilia* BSIs ($P = 0.044$, $P = 0.009$, and $P = 0.001$, respectively) than with *P. aeruginosa* BSIs. C-reactive protein (CRP) levels were significantly higher in *S. maltophilia* BSIs ($P = 0.002$). Multivariate analysis showed that prior carbapenem use was associated with *S. maltophilia* BSIs ($P = 0.014$, adjusted odds ratio [AOR]: 2.710; 95% confidence interval [CI]: 1.225–5.992). PICU admission because of BSI, prior carbapenem and glycopeptide use, neutropenia, and thrombocytopenia were significantly more common in patients with mortality because of *S. maltophilia* BSIs ($P < 0.001$, $P = 0.010$, $P = 0.007$, $P = 0.008$, $P = 0.004$, respectively), while only PICU admission because of BSI, and prior glycopeptide use were significant in multivariate analysis (AOR, 19.155; 95% CI: 2.337–157.018; $P = 0.006$ and AOR, 9.629; 95% CI: 1.053–88.013; $P = 0.045$, respectively).

Conclusion: Prior carbapenem use is a significant risk factor for developing *S. maltophilia* BSIs. PICU admission because of BSI and prior glycopeptide use are risk factors associated with the mortality rate in patients with *S. maltophilia* BSIs. Therefore, *S. maltophilia* should be considered in patients with these risk factors, and empirical treatment should include antibiotics for *S. maltophilia*.

Key Words: *Stenotrophomonas maltophilia*, Bloodstream infections, mortality, children, *Pseudomonas aeruginosa*

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Stenotrophomonas maltophilia (*S. maltophilia*) is a bacteria of rising concern that has been increasingly reported as a cause of healthcare-associated infection and significant morbidity and mortality.[1,2] It is a non-fermentative, aerobic, Gram-negative bacillus usually isolated from humid places such as water, soil and sewage.[1,2] It can form biofilms and colonize surfaces of medical devices.[3] The most common *S. maltophilia* infection types are bloodstream infections (BSIs) and pneumonia. Urinary tract, gastrointestinal, skin, and soft tissue infections, bone and joint infections, meningitis, and endocarditis caused by *S. maltophilia* have also been reported.[1,4,5]

Indwelling central venous catheters (CVC), previous use of broad-spectrum antibiotics, prolonged hospitalization, intensive care unit admission, malignancy, neutropenia, mechanical ventilation and malignancy have been described as possible risk factors for *S. maltophilia* BSIs.[1,4,5] *S. maltophilia* is intrinsically resistant to various antibiotics, including carbapenems which are commonly used to treat nosocomial infections.[1,6–8] Therefore, a delay in treatment can be life-threatening for the patients until culture results are available.

Patients with *S. maltophilia* BSIs usually have significant underlying diseases. Therefore, it is hard to estimate the attributable mortality directly caused by this pathogen. Reported crude mortality rates range from 21% to 69% in adult studies.[9,10] In a limited number of pediatric studies to evaluate the mortality caused by these infections, mortality rates vary between 10% and 60%, which were similar to adult studies.[5,11–13]

S. maltophilia infections are a therapeutic challenge because of limited treatment options. Infectious Diseases Society of America (IDSA) guidelines suggested trimethoprim-sulfamethoxazole (TMP/SMX) monotherapy as a first-line treatment.[14] Levofloxacin, minocycline and cefiderocol are other treatment options. Combination therapies can be used in treatment-resistant strains or patients unresponsive to treatment.[14]

This study aimed to evaluate the risk factors for infection and mortality associated with *S. maltophilia* BSIs in children and compare them with *Pseudomonas aeruginosa* (*P. aeruginosa*) BSIs.

MATERIALS AND METHODS

Study Design and Study Population

Ege University Children's Hospital is a 216-bed tertiary care facility in Izmir, Turkey, with bone marrow transplantation, hematology, oncology and Pediatric Intensive Care Units (PICU). A retrospective observational study was performed between January 2014 and December 2021. All patients aged under 18 years with *S. maltophilia* and *P. aeruginosa* BSIs were conducted in the study. Medical records of 73 patients with *S. maltophilia* BSIs and 80 with *P. aeruginosa* BSIs were evaluated retrospectively through medical records obtained from the Ege University Clinical Microbiology Laboratory. A standardized form was used to collect

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epidemiologic data, including demographic characteristics, risk factors such as underlying medical conditions, PICU stay before or because of infection, duration of PICU stay before or because of infection, presence of a central venous catheter, invasive procedures such as previous surgeries, laboratory findings including antimicrobial susceptibility, neutropenia and prognosis. Patients older than 18 years with positive blood cultures and/or without clinical symptoms compatible with infection were excluded.

Microbiologic Methods

The automated Matrix-Assisted Laser Desorption Ionization Time of Flight mass spectrometry (MALDI TOFF MS, Biomerieux, France) was used for species-level identification of isolated strains. Sensitivity testing for *S. maltophilia* was performed with E-test, and sensitivity testing for *P. aeruginosa* was performed with VITEK 2 (Biomerieux, France) automated microdilution method and evaluated according to the "European Committee for Antimicrobial Susceptibility Testing (EUCAST)"[15] criteria.

Definitions

Clinically significant *S. maltophilia* and *P. aeruginosa* BSIs were defined if the bacteria was isolated from one or more blood samples obtained from a peripheral vein and/or central venous catheter and the patient had clinical symptoms compatible with the infection. Infection that occurred 48 hours after admission was classified as nosocomial infection. Infections detected within the first 48 hours of admission in patients without previous healthcare contact were classified as community-acquired infections.[16]

Previous PICU admission was defined as being hospitalized in the PICU within 1 month before the BSI. Thrombocytopenia was defined as a blood platelet <150,000/mm^3, neutropenia was defined as absolute neutrophile count <1500/mm^3 and lymphopenia was defined as absolute lymphocyte count <1500/mm^3. Prior antibiotic treatment was defined as antibiotic therapy within 14 days before BSI. Prior glycopeptide use was defined as the use of teicoplanin or vancomycin, and prior carbapenem use was defined as the use of meropenem, ertapenem or imipenem. Previous corticosteroids, chemotherapy and immunosuppressive therapies were defined as the use of corticosteroids, chemotherapy and immunosuppressive therapy within 3 months before BSI. Mortality was defined as death within the first 14 days of BSI.

Statistical Analysis

Statistical analysis was performed with SPSS statistical package (version 25 for Windows). Numerical data were expressed as medians. Mann-Whitney *U* and Wilcoxon tests were used for intervariable analysis. Categorical variables were evaluated with the Chi-square test or the 2-tailed Fisher exact test and presented as percentages. A *P* value of <0.05 was considered statistically significant. A multivariate logistic regression analysis model was used to identify the independent risk factors for infection and mortality of *S. maltophilia* BSIs. The results of this regression analysis were reported as an AOR with a 95% confidence interval (CI). All variables with a *P* value <0.05 were included in the multivariate analysis.

Ethics

Necessitated permission was obtained from the Ethical Board of Ege University (ethical decision No 2112.1T/35).

RESULTS

Demographic and Clinical Data

We identified 73 *S. maltophilia* BSIs and 80 *P. aeruginosa* BSIs. The median age of the patients with *S. maltophilia* BSIs was 2.35 (IQR: 7.93) years, and 60.3% were male. The median age of the patients with *P. aeruginosa* BSIs was 3.07 (IQR: 6.78) years, and 57.5% were male. The most common underlying disease was bone marrow transplantation (21.9%), followed by cardiopathy (11%) and hematologic malignancy (9.6%) in patients with *S. maltophilia* BSIs. Five patients were previously healthy. The most common underlying disease in patients with *P. aeruginosa* BSIs was hematologic malignancy (21%), followed by solid organ malignancy (16.3%) and cardiopathy (13.8%). In *S. maltophilia* BSIs, 80.8% of episodes were nosocomial infections, and 95% were in *P. aeruginosa* BSIs. *S. maltophilia* infection was most commonly detected in the PICU (34.2%), followed by the bone marrow transplant unit (21.9%) and gastroenterology ward (8.2%). *P. aeruginosa* BSIs were most common in PICU (32.5%), followed by the oncology ward (16.3%) and hematology ward (12.5%). The comparison of demographic characteristics, clinical characteristics, and laboratory findings of the patients with *S. maltophilia* BSIs and *P. aeruginosa* BSIs are presented in Table 1.

Risk Factors for Infection

Age, the length of hospital stay before the onset of infection, and the total length of hospital stay showed no statistical difference between the patients with *S. maltophilia* BSIs and *P. aeruginosa* BSIs. Previous PICU admission was significantly more common in patients with *S. maltophilia* BSIs than in *P. aeruginosa* BSIs (57.5% vs. 41.2%; odds Ratio [OR], 1.930; 95% CI: 1.014–3.671; $P = 0.044$). Invasive procedures such as mechanical ventilation, tracheostomy, nasogastric tubes, urinary catheters or CVC did not show significant differences among groups. Steroid use and immune suppressive therapy were not significantly different. Chemotherapy use and malignancy were more common in patients with *P. aeruginosa* BSIs (42.5% vs. 17.8%; OR, 3.411; 95% CI: 1.618–7.191; $P = 0.001$ and 40% vs. 17.8% OR, 1.923; 95% CI: 1.180–3.134; $P = 0.003$, respectively). Prior glycopeptide and carbapenem use were significantly higher in patients with *S. maltophilia* BSIs. (41% vs. 21.2%; OR, 2.544; 95% CI: 1.250–5.180; $P = 0.009$ and 52% vs. 23.7%; OR, 3.429; 95% CI: 1.719–6.840; $P = 0.001$, respectively). The attributable mortality rate was not significantly different among groups. There was no significant difference in leukocyte count, absolute neutrophil or lymphocyte count, and platelet levels between *S. maltophilia* BSIs and *P. aeruginosa* BSIs. C-reactive protein (CRP) significantly showed higher levels in patients with *S. maltophilia* BSIs (Median, 31 vs. 9.8, $P = 0.002$). Multivariate analysis showed that only prior carbapenem use was associated with *S. maltophilia* BSIs (adjusted odds ratio [AOR], 2.710; 95% CI: 1.225–5.992; $P = 0.014$) (Table 2).

Risk Factors for Mortality

PICU admission because of BSI was significantly associated with mortality in patients with both *S. maltophilia* and *P. aeruginosa* BSIs (OR, 1.663; 95% CI: 1.130–2.449: $P < 0.001$ and OR, 6.875; 95% CI: 1.762–26.824; $P = 0.002$, respectively). PICU admission before infection was a risk factor for mortality in patients with *P. aeruginosa* BSIs (OR, 8.438; 95% CI: 1.686–42.223; $P = 0.003$), while it was insignificant in patients with *S. maltophilia* BSIs.

The total length of hospital stay, PICU stay before infection, and length of hospital stay before the infection did not associate with mortality in both *S. maltophilia* and *P. aeruginosa* BSIs. Steroid, chemotherapy, and immune suppressive therapy use were similar among survivors and non-survivors in both *S. maltophilia* and *P. aeruginosa* BSIs. Invasive procedures such as mechanical ventilation, indwelling CVCs, nasogastric tubes, or urinary catheters were not associated with mortality in patients with *S. maltophilia* BSIs ($P > 0.05$). Mechanical ventilation and CVCs were significantly

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TABLE 1. Comparison of Demographic Features, Clinical Features and Laboratory Results of Patients With *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* Bloodstream Infections

	Stenotrophomonas maltophilia n = 73 (47.7%)	*Pseudomonas aeruginosa* n = 80 (52.3%)	*P*	Odds ratio (95% CI)
Age, years, median (IQR)	2.35 (7.93)	3.07 (6.78)	0.649	
Length of hospital stay before the onset of infection, days, median (IQR)	23 (54.5)	15 (39.5)	0.213	
Length of hospital stay after the onset of infection, days, median (IQR)	22 (58.0)	19 (30.75)	0.664	
Total length of hospital stay, days, median (IQR)	62 (97)	43 (64.5)	0.133	
Negative culture time, days, median (IQR)	4 (6.5)	4 (3)	0.043	
Neutropenia, n, (%) (ANC<1500/mm^3)	19 (26)	29 (36.2)	0.173	
Prior PICU admission, n, (%)	42 (57.5)	33 (41.2)	**0.044**	**1.930 (1.014–3.671)**
Mechanical ventilator, n, (%)	27 (36.9)	26 (32.5)	0.560	
Tracheostomy, n, (%)	9 (12.3)	10 (12.5)	0.974	
Nasogastric tube, n, (%)	36 (49.3)	41 (51.2)	0.811	
Urinary catheter, n, (%)	25 (34.2)	24 (30)	0.574	
Central venous catheter, n, (%)	60 (82.1)	58 (72.5)	0.154	
Total parenteral nutrition, n, (%)	26 (35.6)	26 (32.5)	0.684	
History of surgery, n, (%)	26 (35.6)	23 (28.7)	0.363	
Steroid use, n, (%)	23 (31.5)	22 (27.5)	0.587	
Chemotherapy, n, (%)	13 (17.8)	34 (42.5)	**0.001**	**3.411 (1.618–7.191)**
Immunosuppressive therapy, n, (%)	20 (27.3)	25 (31.2)	0.601	
Prior glycopeptide use, n, (%)	30 (41)	17 (21.2)	**0.009**	**2.544 (1.250–5.180)**
Prior carbapenem use, n, (%)	38 (52)	19 (23.7)	**0.001**	**3.429 (1.719–6.840)**
Malignancy, n, (%)	13 (17.8)	32 (40)	**0.003**	**1.923 (1.180–3.134)**
PICU admission, n, (%)	19 (26)	21 (26.2)	0.975	
14-day mortality, n, (%)	10 (13.6)	11 (13.7)	0.364	
30-day mortality, n, (%)	17 (23.2)	14 (17.5)	0.374	
Laboratory results, median (IQR)				
Leukocyte/mm^3	6150 (7755)	8260 (13,650)	0.972	
ANC/mm^3	3000 (4907)	4830 (9490)	0.872	
ALC/ mm^3	1750 (2855)	1490 (2870)	0.501	
Hemoglobin (g/dL)	8.6 (2.35)	9.4 (2.30)	0.252	
Hematocrit (%)	27.1 (6.65)	28 (6.23)	0.375	
Mean corpuscular volume (fL)	83.9 (6.10)	83.3 (9.23)	0.350	
Platelet/mm^3	157,000 (221,500)	131,000 (244,000)	0.734	
C-reactive protein (mg/dL)	31 (85.9)	9.8 (43.2)	**0.002**	

ANC indicates absolute neutrophil count; ALC, absolute lymphocyte count; IQR, interquartile range; PICU, pediatric intensive care unit.
Bold lines represent statistically significant results.

associated with mortality in patients with *P. aeruginosa* BSIs (OR, 4.605; 95% CI: 1.209–17.539; $P = 0.018$ and OR, 1.234; 95% CI: 1.090–1.398; $P = 0.028$, respectively). However, these risk factors were not associated with mortality in patients with *S. maltophilia* BSIs. Neutropenia and thrombocytopenia were the risk factors for mortality in patients with *S. maltophilia* BSIs (OR, 5.765; 95% CI: 1.416–23.506; $P = 0.008$ and OR, 8.600; 95% CI: 1.672–44.236; $P = 0,004$, respectively). The higher levels of CRP were significantly associated with mortality in patients with *S. maltophilia* BSIs (Median, 100 vs. 29, $P = 0.010$). Among the patients with *P. aeruginosa* BSIs, thrombocytopenia was not a risk factor for mortality; however, platelets were significantly lower in non-survivors (median, 36,000 vs. 143,000, $P = 0.007$). Neutropenia at the onset of infection was a risk factor for mortality in patients with *P. aeruginosa* BSIs (OR, 3.739; 95% CI: 0.990–14.118; $P = 0.042$). Prior carbapenem use was significantly higher in non-survivors in patients with *S. maltophilia* BSIs (OR, 10.552; 95% CI: 1.261–88.309; $P = 0.010$), while insignificant in patients with *P. aeruginosa* BSIs. Prior glycopeptide use was significantly higher in non-survivors in both groups (OR, 7.455; 95% CI: 1.455–38.191; $P = 0.007$ and OR, 3.889; 95% CI: 1.018–14.871; $P = 0.037$, respectively). A comparison of risk factors for mortality because of *S. maltophilia* and *P. aeruginosa* BSIs are presented in Table 3. Multivariate analysis showed PICU admission because of BSI and prior glycopeptide use were independently associated with mortality in patients with *S. maltophilia* BSIs (AOR, 19.155; 95% CI: 2.337–157.018; $P = 0.006$ and AOR, 9.629; 95% CI: 1.053–88.013; $P = 0.045$, respectively). Multivariate analysis results for risk factors associated with mortality are presented in Table 4.

Treatment

The susceptibility rate of *S. maltophilia* was highest for TMP/SMX (88.6%), followed by levofloxacin (81.4%) and ceftazidime (68.6%). Ceftazidime was the most commonly administered antibiotic (39.7%), followed by TMP/SMX (30.1%) and levofloxacin (8.6%) because of culture results. Eight of 10 deceased patients did not receive proper empiric treatment for *S. maltophilia.* The most commonly administered empiric antibiotics were glycopeptides and carbapenems. Despite appropriate empiric treatment, mortality occurred in 2 patients; 1 with a liver transplant and 1 with

TABLE 2. Risk Factors for *S. Maltophilia* Bloodstream Infections Compared With *P. aeruginosa* Bloodstream Infections; Multivariate Analysis

Risk Factors	AOR (95% CI)	*P*
Previous PICU admission	1.614 (0.822–3.117)	0.165
Prior carbapenem use	2.710 (1.225–5.992)	**0.014**
Prior glycopeptide use	1.444 (0.628–3.320)	0.387

AOR indicates adjusted odds ratio; PICU, pediatric intensive care unit.
Bold lines represent statistically significant results.

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TABLE 3. Comparison of Risk Factors for Mortality Because of *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* BSI

Risk Factors	Patients With *S. maltophilia* BSI				Patients With *P. aeruginosa* BSI			
	Non-survivor n = 10	**Survivor n = 63**	***P***	**Odds ratio (95% CI)**	**Non-survivor n = 11**	**Survivor n = 69**	***P***	**Odds ratio (95% CI)**
Previous PICU admission, n, (%)	5 (11.9)	37 (88.1)	0.604		9 (27.3)	24 (72.7)	**0.003**	**8.438 (1.686–42.223)**
PICU admission because of bacteremia, n, (%)	8 (42.1)	11 (57.9)	**<0.001**	**1.663 (1.130–2.449)**	7 (33.3)	14 (66.7)	**0.002**	**6.875 (1.762–26.824)**
Length of PICU stay before the onset of infection, days, median (IQR)	3 (11.75)	3 (10)	0.640		15 (23.25)	5.5 (16.25)	0.091	
Length of hospital stay before the onset of infection, days, median (IQR)	44 (56.75)	18 (45)	0.131		73.5 (139)	29.5 (45.25)	0.352	
Total length of hospital stay, days, median (IQRmm)	59 (63.5)	65.5 (103)	0.710		82.5 (145.5)	69 (120.5)	0.716	
Negative culture time, days, median (IQR)	3 (3)	4 (7)	0.431		3.5 (2.5)	4.5 (4)	0.316	
Mechanical ventilation, n, (%)	5 (18.5)	22 (81.5)	0.359		7 (26.9)	19 (73.1)	**0.018**	**4.605 (1.209–17.539)**
Central Venous Catheter, n, (%)	9 (15)	51 (85)	0.487		11 (10)	47 (81)	**0.028**	**1.234 (1.090–1.398)**
Steroid, n, (%)	5 (21.7)	18 (78.3)	0.175		5 (22.7)	17 (77.3)	0.151	
Chemotherapy, n, (%)	2 (15.4)	11 (84.6)	0.845		7 (20.6)	27 (79.4)	0.127	
Immune suppressive treatment, n, (%)	4 (20)	16 (80)	0.336		6 (24)	19 (76)	0.073	
History of surgery, n, (%)	4 (15.4)	22 (84.6)	0.755		1 (4.3)	22 (95.7)	0.121	
Total parenteral nutrition, n, (%)	4 (15.4)	22 (84.6)	0.755		6 (23.1)	20 (76.9)	0.093	
Bone marrow transplantation, n, (%)	3 (18.8)	13 (81.2)	0.506		1 (11.1)	8 (88.9)	0.807	
Malignancy, n, (%)	1 (7.7)	12 (92.3)	0.487		6 (18.8)	26 (81.2)	0.289	
Prior carbapenem use, n, (%)	9 (23.7)	29 (76.3)	**0.010**	**10.552 (1.261–88.309)**	5 (26.3)	14 (73.7)	0.073	
Prior glycopeptide use,n,(%)	8 (26.7)	22 (73.3)	**0.007**	**7.455 (1.455–38.191)**	5 (29.4)	12 (70.6)	**0.037**	**3.889 (1.018–14.871)**
Neutropenia (ANC<1500/mm^3), n, (%)	6 (31.6)	13 (68.4)	**0.008**	**5.769 (1.416–23.506)**	7 (24.1)	22 (75.9)	**0.042**	**3.739 (0.990–14.118)**
Lymphopenia (ALC<1500/mm^3), n, (%)	7 (21.2)	26 (78.8)	0.318		8 (20)	32 (80)	0.114	
Thrombocytopenia, n, (%) (platelet<150,000/mm^3)	8 (28.6)	20 (71.4)	**0.004**	**8.600 (1.672–44.236)**	8 (19.5)	33 (80.5)	0.125	
Laboratory findings, median (IQR)								
Leukocyte/mm^3	4815 (19,885)	6240 (6040)	0.716		80 (14,170)	8640 (12,928)	0.064	
ANC/mm^3	3310 (16,867)	3000 (4390)	0.763		1 (9910)	4925 (9110)	0.047	
ALC/ mm^3	920 (3175)	1950 (2800)	0.479		10 (3030)	1715 (2866)	0.048	
Hemoglobin (g/dL)	8.6 (2.43)	8.6 (2.3)	0.514		9.8 (2.30)	9.2 (2.28)	0.539	
Platelet/mm^3	42,500 (84,250)	165,000 (226,000)	**0.004**		36,000 (176,200)	143,000 (240,000)	**0.007**	
C-reactive protein (mg/L)	100 (283)	29 (76)	**0.010**		9.4 (26)	9.9 (45.3)	0.685	

ANC indicates absolute neutrophil count; ALC, absolute lymphocyte count; BSI, bloodstream infections; PICU, pediatric intensive care unit.
Bold lines represent statistically significant results.

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TABLE 4. Risk Factors for Mortality of *S. maltophilia* Bloodstream Infections; Multivariate Analysis

Risk Factors	AOR (95% CI)	*P*
PICU admission because of BSI	19.155 (2.337–157.018)	**0.006**
Prior carbapenem use	2.640 (0.211–33.026)	0.452
Prior glycopeptide use	9.629 (1.053–88.013)	**0.045**
Neutropenia	3.032 (0.335–27.424)	0.324
Thrombocytopenia	2.209 (0.226–21.590)	0.496

95% CI indicates 95% confidence intervals; AOR, adjusted odds ratio; BSI, bloodstream infection; PICU, pediatric intensive care unit.
Bold lines represent statistically significant results.

a metabolic disorder. Of deceased patients with *P. aeruginosa* BSIs, 8 were receiving appropriate empirical treatment. In 3 patients, *P. aeruginosa* was carbapenem resistant. Carbapenem resistance did not show any significant difference between survivors and nonsurvivors in patients with *P. aeruginosa* BSIs ($P = 0.187$). Empirical treatment of 10 patients with attributable mortality because of *S. maltophilia* BSIs and 11 patients with attributable mortality because of *P. aeruginosa* BSIs were summarized in Table 5. Five of the 10 patients who died because of *S. maltophilia* BSI did not receive proper antibiotic treatment because they died before the culture results were obtained.

DISCUSSION

This study included 73 *S. maltophilia* BSIs and 80 *P. aeruginosa* BSIs. Previous PICU admission and prior glycopeptide use were more common in *S. maltophilia* BSIs than in *P. aeruginosa* BSIs. Multivariate analysis revealed prior carbapenem use was significantly associated with *S. maltophilia* BSIs. PICU admission because of bacteremia, neutropenia, thrombocytopenia and higher levels of CRP at the onset of infection, prior glycopeptide and carbapenem use were risk factors for patient mortality because of *S. maltophilia* BSIs. Multivariate analysis revealed PICU admission because of infection and prior glycopeptide use increased mortality risk in patients with *S. maltophilia* BSIs. To our knowledge, this study is the largest one that evaluated *S. maltophilia* BSIs in pediatric patients in the literature and was the first study that compared with *P. aeruginosa* BSIs.

S. maltophilia is a nosocomial pathogen that forms biofilms on surfaces of devices such as mechanical ventilators and CVC.[3] Previous studies reported that *S. maltophilia* BSIs were more common in patients with an immunocompromised status, such as immunosuppression, organ transplantation and malignancies.[10,17] In our study, immunocompromised status did not significantly associate with *S. maltophilia* BSIs. In previous studies, well-defined risk factors for developing *S. maltophilia* BSIs were prolonged hospital stay, previous PICU admission, CVCs, mechanical ventilation, nasogastric tubes and urinary catheters.[5,6,11,17–19] Most of our patients with *S. maltophilia* BSIs (82%) had a CVC; however, the presence of CVC did not increase the risk for *S. maltophilia* BSIs compared with *P. aeruginosa* BSIs in our study. When laboratory parameters were evaluated, univariate analysis showed that CRP was significantly higher in patients with *S. maltophilia* BSIs than in those with *P. aeruginosa* BSIs. However, no statistically significant difference was found in the multivariate analysis. Although insignificant in the multivariate analysis, it should be kept in mind that high CRP may indicate *S. maltophilia* BSIs. Consistent with previous studies, our study identified PICU admission before BSI as a risk factor for *S. maltophilia* BSIs. Therefore, *S. maltophilia* should be considered in patients with previous PICU admission while initiating empirical antibiotic treatment.

The mortality rate among children with *S. maltophilia* infections ranges between 10% and 60%.[12,13] A recent study by Latzer et al[1] among critically ill pediatric patients determined that the crude mortality was 42% and attributable mortality was 18%. Alsuhaibani et al[5] stated that 33.8% of their patients died within 7 days of infection. In our study, attributable mortality was 13.7%, which was less than in previous studies.[4,5,13] Our heterogeneous study group can explain this difference, which consisted of both critically ill and stable patients.

The risk factors of attributable mortality associated with *S. maltophilia* BSIs are prior use of broad-spectrum antibiotics, neutropenia, mechanical ventilation, prior PICU stay, and malignancy.[8,20] Alsuhaibani et al[5] demonstrated that previous PICU admission and neutropenia were risk factors for mortality in children with *S. maltophilia* BSIs. Senol et al[10] compared patients with *S. maltophilia* BSIs to control groups and stated that neutropenia and previous antibiotic use were associated with increased mortality rates. In our study, PICU admission, neutropenia, thrombocytopenia, and higher levels of CRP at the onset of infection were significantly associated with mortality in patients with *S. maltophilia* BSIs. Consistent with previous studies, our study showed that neutropenia was associated with higher mortality.[5,10] This study demonstrated thrombocytopenia, and higher CRP levels increased the risk for mortality with a limit of statistical significance revealed by multivariate analysis. Unlike previous studies, mechanical ventilation and the presence of a central venous catheter were not associated with mortality in patients with *S. maltophilia* BSIs in our study. However, mechanical ventilation and catheter presence were significantly higher in the patients with mortality because of *P. aeruginosa* BSIs. There was no significant difference between the 2 groups regarding mechanical ventilation and catheter use. The most significant difference was a higher hematologic and oncologic malignancy rate in patients with *P. aeruginosa* BSIs. The underlying disease was hematologic or oncologic malignancy in 54.5% of patients who died because of *P. aeruginosa* BSIs. Of the 11 deceased patients, ten died while they were already treated in the PICU, which the severity of their underlying condition can explain.

Several studies determined that previous use of antibiotics, particularly carbapenems, was associated with increased frequency and mortality of these infections.[4,6,11,19] Consistent with previous studies, we showed a higher previous carbapenem and glycopeptide use in deceased patients with *S. maltophilia* BSIs than in *P. aeruginosa* BSIs. Glycopeptide use was a risk factor for mortality in both groups, and carbapenem use was a risk factor for mortality in patients with *S. maltophilia* BSIs. Multivariate analysis revealed only glycopeptide use was independently associated with mortality in patients with *S. maltophilia* BSIs. These findings remind the importance of rational antibiotic use in preventing these infections and reducing the mortality associated with these infections.

S. maltophilia infections cause therapeutic challenges for clinicians because of intrinsical resistance to many antibiotics primarily used to treat nosocomial infections.[5,8,19,21] TMP/SMX, quinolones, minocycline, and ceftazidime are the most commonly used antibiotics in these infections.[2,5,11] The first-line recommended treatment is TMP/SMX for *Stenotrophomonas* spp. caused infections.[14,19,22] IDSA guidelines suggest TMP/SMX as monotherapy or in combination with levofloxacin in more severe or unresponsive cases.[14] Metan et al[19] showed that carbapenem and glycopeptide administration, the presence of a CVC, and longer hospitalization increased the risk for *S. maltophilia* infections, and patients with these risk factors should receive TMP/SMX empirically. Przygonska et al[23] determined that empirical TMP/SMX treatment in patients with hematologic malignancies and bone marrow transplants lowered mortality because of *S. maltophilia* infections. The most common empirical antibiotic

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TABLE 5. Characteristics of Patients With Attributable Mortality Because of *S. maltophilia* and *P. aeruginosa* Bloodstream Infections

		Patients With *S. maltophilia* BSIs					Patients With *P. aeruginosa* BSIs				
Patient No:	Underlying Disease	PICU Admission Because of Infection	Empiric Antibiotic Treatment	Appropriate Treatment	Death (days)	Patient No:	Underlying Disease	PICU Admission Because of Infection	Empiric Antibiotic Treatment	Appropriate Treatment	Death (days)
1	Aplastic anemia, Bone Marrow Transplant	Yes	Vancomycin Meropenem Amikacin Colistin	No	2	1	Hematologic Malignancy	PICU stay	Vancomycin piperacillin-tazobactam Amikacin	Yes	12
2	SCID, Bone Marrow Transplant	Yes	Vancomycin piperacillin-tazobactam Colistin	No	2	2	Aplastic anemia	PICU stay	Vancomycin Meropenem	No (Carbapenem resistant)	13
3	Chronic Granulomatous Disease, Bone Marrow Transplant	Yes	Vancomycin Meropenem	No	2	3	Hematologic Malignancy	PICU stay	piperacillin-tazobactam	Yes	7
4	Duschennes Muscular Distrophy	PICU stay	Vancomycin Meropenem Amikacin	No	3	4	Solid Organ Malignancy	PICU stay	Vancomycin Meropenem Amikacin	Yes	12
5	Liver transplant	PICU stay	Teicoplanin Meropenem Trimethoprim-sulfamethoxazole	Yes	2	5	Mitochondrial cytopathy	PICU stay	Ciprofloxacin Amikacin	Yes	7
6	Mitochondrial complex deficiency	PICU stay	Ceftazidime Trimethoprim-sulfamethoxazole	Yes	6	6	Hematologic Malignancy	PICU stay	Vancomycin Meropenem	No (Carbapenem resistant)	1
7	SCID, Bone Marrow Transplant	Yes	Linezolid Meropenem	No	4	7	Hematologic Malignancy	PICU stay	piperacillin-tazobactam Amikacin	No (Carbapenem resistant)	10
8	Hirschprung Disease	Yes	Vancomycin Meropenem	No	3	8	Solid Organ Malignancy	Yes	Vancomycin Meropenem Colistin	Yes	12
9	Chronic renal failure	Yes	Meropenem	No	4	9	Chronic Neurological Disorder	PICU stay	Vancomycin Meropenem Colistin	Yes	1
10	Aortic stenosis	PICU stay	Vancomycin Meropenem	No	1	10	Congenital Heart Disease	PICU stay	Teicoplanin Meropenem	Yes	5
						11	Congenital Heart Disease	PICU stay	Vancomycin Meropenem	Yes	1

BSI indicates bloodstream infection; PICU, pediatric intensive care unit; SCID, severe combined immunodeficiency.

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treatment in our study was TMP/SMX. Combination therapies with TMP/SMX and ciprofloxacin were also initiated in 28.7% of the patients. Only 20% of deceased patients received appropriate empiric antibiotics for *S. maltophilia* BSIs. It was observed that the remaining 8 deceased patients had been receiving carbapenems and glycopeptides. In these patients, death usually occurred within 2–3 days while the bacterial culture results were pending. This result shows us the importance of *S. maltophilia* infections and expanding empirical treatment in patients with risk factors. The most commonly administered empirical antibiotics were carbapenems and piperacillin-tazobactam in deceased patients with *P. aeruginosa* BSIs. *P. aeruginosa* was carbapenem resistant in 3 of the 11 deceased patients, and they were not receiving appropriate treatment. However, carbapenem resistance did not show any significant difference between survivors and non-survivors in patients with *P. aeruginosa* BSIs.

The limitations of our study are its retrospective nature; therefore, some clinical data may have been missed. It is a single-center study; resistance patterns and risk factors may differ between centers. Despite the highest number of pediatric patients in the literature, the low number of patients is another limitation of this study.

In conclusion, our study showed that prior carbapenem use is a risk factor for developing *S. maltophilia* BSIs in children. PICU admission because of infection and prior glycopeptide use are risk factors for mortality in these patients. Among deceased patients, 8 of 10 patients could not receive appropriate therapy for *S. maltophilia*. Therefore, we suggest *S. maltophilia* should be considered in patients with well-defined risk factors, such as prior PICU admission, PICU admission because of bacteremia, neutropenia, thrombocytopenia and higher levels of CRP at the onset of infection, prior glycopeptide and carbapenem use. This study emphasizes the importance of rational antibiotic use in reducing these infections and the mortality caused by these infections. More extensive prospective studies are needed to confirm these risk factors associated with *S. maltophilia* infection and mortality in children.

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Molecular epidemiology of multiresistant *Escherichia coli* isolates from community-onset urinary tract infections in Cornwall, England

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***Objectives*: To study the clonality of gentamicin-resistant, extended-spectrum β-lactamase (ESBL)-negative and ESBL-producing *Escherichia coli* isolated from community-onset urinary tract infections (UTIs) in Cornwall.**

***Methods*: Isolates were identified by API, susceptibilities were determined by local disc testing, and MICs were determined at the reference laboratory, both interpreted using BSAC guidelines. *bla*$_{CTX-M}$ genes were sought by PCR, and isolates were compared by PFGE.**

***Results*: In the years 2004 and 2005, 69 *E. coli* were submitted by Truro (Cornwall) laboratory for reference laboratory testing: these included 14 gentamicin-resistant, ESBL-negative isolates; 45 with group 1 CTX-M enzymes; seven with group 9 CTX-M enzymes; and three with non-CTX-M ESBLs. By PFGE, nine gentamicin-resistant, ESBL-negative *E. coli* were distinct (<85% similarity) from all the ESBL producers, but three were related to producers of group 1 CTX-M enzymes, and two isolates were related to a non-CTX-M ESBL producer. An outbreak strain was identified, represented by 11 gentamicin-resistant and one gentamicin-susceptible isolates, all with group 1 CTX-M enzymes, and two gentamicin-resistant, ESBL-negative isolates. This was distinct by PFGE from nationally distributed CTX-M-producing strains. Five of nine patients infected with this strain had been on the same ward in a local hospital; four presented with community-onset UTIs; one inpatient developed a hospital-acquired bacteraemia. Of the other four patients presenting with community-onset UTIs, three were admitted to different hospitals and the fourth had only attended an outpatient clinic.**

***Conclusions*: Community-onset, ESBL-producing and non-producing *E. coli* were diverse. Two ESBL-negative isolates were closely related to a local CTX-M-producing outbreak strain, suggesting gain or loss of a *bla*$_{CTX-M}$-carrying plasmid. An outbreak strain was linked with prior hospital admission and appeared not to represent genuine community acquisition.**

Keywords: cephalosporins, ESBLs, resistance, Enterobacteriaceae

Introduction

Multiresistant *Escherichia coli* strains from community-onset infections are an increasing public health concern, especially those with CTX-M extended-spectrum β-lactamases (ESBLs).[1] In the UK, ~90% of CTX-M-producing *E. coli* produce group 1 enzymes (mainly CTX-M-15) and PFGE analysis of a national collection has defined five major strains.[1] The local epidemiology of *E. coli* producing CTX-M enzymes may, however, differ from this national picture with, for example, dominance of different strains, or higher prevalence of those with group 9 CTX-M enzymes.

In Cornwall (the south-west peninsula of England), concern was raised over the emergence of gentamicin-resistant *E. coli* isolates recovered from urines of patients attending general practice. Gentamicin is rarely prescribed in such settings. Several, but not all of these resistant isolates were ESBL producers. We therefore studied the clonality of gentamicin-resistant, ESBL-negative isolates from urines of patients attending general practitioners (GPs) in Cornwall and compared them with

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ESBL-producing *E. coli*, many also gentamicin-resistant and also isolated from GP urines.

Materials and methods

Bacterial isolates and initial susceptibility testing

Most *E. coli* isolates included in this study were associated with community-onset urinary tract infections (UTIs) in patients attending GPs' surgeries in Cornwall during 2004 and 2005. One isolate was from a case of hospital-acquired bacteraemia (onset >48 h after admission).

The isolates were identified as *E. coli* (API system; bioMerieux, Basingstoke, UK), and their antibiotic susceptibilities were determined by disc testing using BSAC criteria[2] in the Department of Clinical Microbiology, Royal Cornwall Hospital. Fourteen gentamicin-resistant, ESBL-negative isolates and 55 ESBL-producing isolates, variably gentamicin-resistant, were referred to the Health Protection Agency's Centre for Infections for further investigation. Multiple isolates were referred from some patients.

Investigation in the reference laboratories

MICs were determined and interpreted using BSAC methodology,[2] and $bla_{CTX\text{-}M}$ genes were sought by multiplex PCR.[3] Selected isolates were compared by PFGE of *Xba*I-digested genomic DNA, and banding patterns were analysed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Investigation of an outbreak strain

The clinical notes for selected patients were analysed to determine any obvious common factors associated with acquisition of a PFGE-defined outbreak strain.

Results and discussion

Reference investigations and recognition of a 'Cornish outbreak' strain

During 2004–2005, the reference laboratories received 69 *E. coli* isolates from Truro (Cornwall) laboratory for inclusion in this study. These included: 14 gentamicin-resistant, ESBL-negative isolates; 45 with group 1 CTX-M enzymes; seven with group 9 CTX-M enzymes and three with non-CTX-M ESBLs.

All 14 ESBL-negative isolates, and 16 ESBL-producing isolates dating from 2004, were compared by PFGE (Figure 1). Nine gentamicin-resistant, ESBL-negative isolates were distinct (<85% similarity) from the ESBL producers, but three were related to isolates with CTX-M group 1 enzymes, and two isolates from one patient were related to an isolate with a non-CTX-M ESBL.

One major outbreak strain was identified, and comprised 11 gentamicin-resistant and one gentamicin-susceptible CTX-M group 1 producers and two gentamicin-resistant, ESBL-negative isolates (12 of these are shown in Figure 1); the 14 isolates were from nine patients. This 'Cornish outbreak' strain was distinct by PFGE from five nationally distributed CTX-M-producing strains, including the UK epidemic strain A (Figure 1),[1] which accounted for only three of the 69 *E. coli* isolates referred from Truro laboratory.

Geometric mean MICs for the 'Cornish outbreak' strain are shown in Table 1. CTX-M ESBL-producing isolates of this strain were resistant to penicillins, all cephalosporins tested (but with potentiation by clavulanate), and also to ciprofloxacin, gentamicin and tobramycin. Isolates of the outbreak strain were susceptible to piperacillin/tazobactam, amikacin and carbapenems.

Two gentamicin-resistant, ESBL-negative isolates (unrelated by PFGE and distinct from the 'Cornish outbreak' strain) were highly resistant to the veterinary aminoglycoside apramycin (MICs > 512 mg/L), which is consistent with AAC(3)-IV activity;[4] the range of apramycin MICs for all other isolates was 2–32 mg/L.

Analysis of case notes

Recognition of the 'Cornish outbreak' strain was unexpected and prompted analysis of clinical notes for the nine patients (5 male and 4 female) affected to determine any obvious common factors that might be associated with its acquisition. The average age of these patients was 80 years (range, 57–93 years).

One patient developed a hospital-acquired bacteraemia (defined as onset >48 h after admission) with the 'Cornish outbreak' strain while an inpatient on ward G of a local hospital (RCH) in March to May 2004 during a cluster of infections on this ward caused by ESBL-producing *E. coli*. This blood culture isolate was the only saved representative from the ward cluster. The other eight patients infected by the 'Cornish outbreak' strain had presented at various GP surgeries, separated by up to 30 miles. The strain was isolated from urine in eight patients, from sputum in three, from a peg site in one, and two patients were widely colonized. Despite the community-onset of their UTIs, seven of these eight patients had prior, and in some instances multiple, hospital inpatient admissions, and multiple transfers between wards. The eighth only attended an outpatient clinic. The average time after hospital discharge before presentation with a UTI at their GP's surgery was 39 days (range 6–89 days). Four of the eight GP patients had been inpatients on ward G of RCH during March–May 2004. Another had been in other wards at RCH during this period, though not on ward G. Two other patients had been on ward L of a second hospital (CRH), but there was no temporal overlap; one had been an inpatient from December 2003 to March 2004; the other was transferred from ward G of RCH in April 2004 and was discharged in May 2004. Six of the nine affected patients died. Mortality was not directly attributable to the ESBL-producing strain in five cases, but the sixth died with ESBL-producing *E. coli* septicaemia, implying causality.

These data suggest that the 'Cornish outbreak' strain may have caused the cluster of infections on ward G at RCH during March–May 2004, although only one isolate was available for testing. The alarming finding of a CTX-M-producing *E. coli* strain presenting in the community, but that could be traced to a hospital source led to a rapid review of susceptibility testing of all 'coliforms' in the clinical laboratory, as advocated previously,[1,5] and methodology for detecting ESBLs was in place within two months of these findings.

In summary, most gentamicin-resistant, ESBL-negative isolates from community-onset UTIs in Cornwall were diverse and were distinct from ESBL-producing *E. coli* from community-onset UTIs. However, two ESBL-negative, gentamicin-resistant isolates were closely related to a local CTX-M-producing and gentamicin-resistant outbreak strain, suggesting gain or loss of

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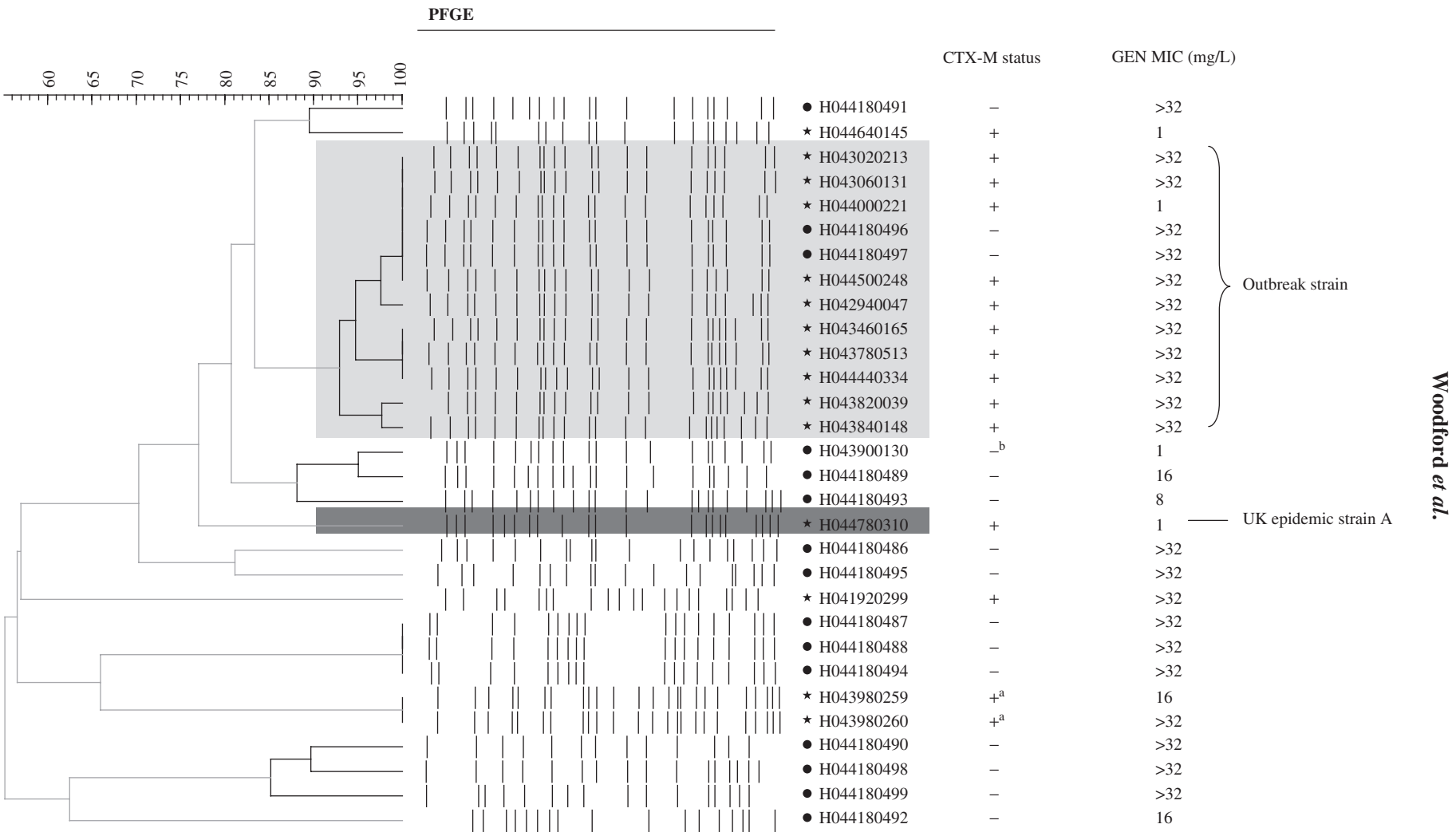

Figure 1. Dendrogram (UPGMA, Dice) showing the relatedness of PFGE banding patterns for 30 *E. coli* isolates from Cornwall. Bold lines indicate clusters with >85% similarity. Symbols: +, CTX-M group 1 producer; +[a], CTX-M group 9 producer; –[b], ESBL (non-CTX-M) producer; –, non-ESBL producer. Isolates of the local outbreak strain and UK epidemic strain A are shaded light grey and dark grey, respectively.

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Table 1. Susceptibilities of the 'Cornish outbreak' CTX-M-producing *E. coli* strain compared with UK epidemic strain A[1]

	Geometric mean MIC (mg/L)	
Antibiotic	'Cornish outbreak' strain (n = 12)[a]	strain A[1]
AMP	64	64
AMC	17	16
CTX	71.8	64
CTX + CLA	0.1	≤0.06
CAZ	26.9	8
CAZ + CLA	0.4	0.25
FEP	56.4	2
FEP + CLA	0.1	≤0.06
FOX	10.7	16
PIP	64	64
TZP	4.2	16
IPM	0.2	0.125
MEM	0.1	≤0.06
ETP	0.1	≤0.125
CIP	8	17.5
GEN	24	1.1
AMK	2.6	9
TOB	10.7	16
MIN	3.5	8.2

AMP, ampicillin; AMC, co-amoxiclav; CTX, cefotaxime; CLA, clavulanate; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; PIP, piperacillin; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; MIN, minocycline.

[a]Two non-CTX-M-producing isolates of the outbreak strain were excluded from this analysis.

the $bla_{\text{CTX-M}}$ gene or a plasmid carrying it. Most of the patients infected by this outbreak strain had hospital admissions in the three months prior to onset of their UTIs. This has been identified previously as a risk factor for infections caused by ESBL producers in non-hospitalized patients.[6,7] Old age (>60 years), as seen in patients described here, is also a known risk factor.[6]

Although manifesting in the community, many of the UTIs of these Cornish patients appeared to have their origins in local hospitals and did not represent genuine community acquisition. This study highlights weaknesses in the concept of a clear distinction between hospital- and community-acquired infections caused by CTX-M-producing and other multiresistant *E. coli*. Clearly, acquisition in hospital may be followed by a period of colonization, which may be prolonged in some instances, prior to infection. Screening for faecal colonization at discharge, especially during on-going hospital clusters with ESBL-producing strains, would identify those patients most at risk of developing subsequent community-onset infections with multiresistant strains.

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Transparency declarations

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(/) (https://nhsnss.org/)

National Infection Prevention and Control Manual

Care Home Infection Prevention and Control Manual (CH IPCM)

Home (/) > Care Home Infection Prevention and Control Manual (CH IPCM) >

When an organisation uses products or adopts practices that differ from those stated in this Care Home Infection Prevention and Control Manual, that individual organisation is responsible for ensuring safe systems of work including the completion of risk assessments approved through local governance procedures.

Use of this manual online is advised as printed versions are uncontrolled.

The ARHAI Scotland Transmission Based Precautions (TBPs) literature review is currently ongoing and so the information may be subject to change.

View latest news and updates (/) for the NIPCM and CHIPCM
Last updated: 21 August 2024

What is the Care Home Infection Prevention and Control Manual (CH IPCM)?

The Care Home Infection Prevention and Control Manual (CH IPCM), referred to as 'the manual' throughout, was first published in 2021. It is evidence-based and is intended to be used by all those involved in care home provision in Scotland.
The manual is context specific and has been co-produced with national and local stakeholders. The content of the manual is completely aligned to the evidence based National Infection Prevention and Control Manual (NIPCM) (/) which was first published in 2012, by the Chief Nursing Officer (CNO (2012)1 (http://www.sehd.scot.nhs.uk/cmo/CNO(2012)01.pdf)).
The manual currently contains

- Chapter 1 – Standard Infection Control Precautions (SICPs) (/infection-prevention-and-control-manual-for-older-people-and-adult-care-homes/#a2821)

- Chapter 2 – Transmission Based Precautions (TBPs) (/infection-prevention-and-control-manual-for-older-people-and-adult-care-homes/#a2926)

The manual is a practice guide for use in care homes. When used, it can help reduce the risk of infections and ensure the safety of residents, as well as staff and visitors in the care home environment. It is the Scottish Government expectation that care home settings apply guidance contained within this manual to achieve the aims.
The manual aims to:

- make it easy for staff to apply effective infection prevention and control (IPC) precautions
- help reduce the risk of infection
- reduce variation, promote standardisation, and optimise IPC practices throughout care home settings
- improve the application of staff knowledge and skills in IPC
- help align practice, monitoring, quality improvement and scrutiny

The manual should be adopted for all IPC practices and procedures within care home settings.

Is the manual based on scientific literature?

The recommendations for practice made in the manual are fully aligned to the NIPCM and are based on real-time reviews of the current scientific literature (for example Medical Journals) and best practice. Any major changes identified in the scientific literature may lead to a change being made to the content, and so, it is recommended that the online version is always accessed and used locally.

What's in the appendices?

The appendices (/appendices/) can be used as practical implementation of the manual and contain graphical representations (for example diagrams and charts) that can be used along with the contents of the manual.
Many of the appendices can be printed off as posters for local use throughout the care home.

Are there any other IPC materials that can be used?

There are links throughout the manual to additional resources and the resources page (https://www.nipcm.scot.nhs.uk/resources/) links to IPC campaign materials, education, training links and posters.
In addition you may find it useful to read the literature reviews and SBARs (/resources/literature-reviews/) for the manual.

How can I find out what the scientific and medical words mean?

A glossary (/glossary/) section has been provided.

Does the manual content work on mobile devices?

Yes, all content including appendices work on mobile devices for example laptops and smartphones.

Responsibilities for the CH IPCM

Responsibilities for content of the manual

ARHAI Scotland to ensure:

- that the content of this manual remains evidence based or where evidence is lacking, content is based on consensus of expert opinion

Stakeholders of ARHAI Scotland programme working groups to ensure:

- full participation in the working groups including full engagement with the consultation process outlined in the Terms of Reference associated with each working group

Responsibilities for the adoption and implementation of this manual

The manual should be used by:

- care home organisations
- care home staff including permanent, agency and where required external contractors
- health protection teams
- infection prevention and control teams
- professionals providing support
- individuals visiting the care home

Care home providers to ensure:

- the adoption and implementation of this manual in accordance with existing local governance processes
- that systems and resources are in place to facilitate implementation and compliance monitoring of IPC as specified in this manual in all care areas
  - compliance monitoring includes all staff (permanent, agency and where required external contractors)
- there is an organisational culture which promotes incident reporting and focuses on improving systemic failures that encourage safe IPC working practices including near misses
- there is a nominated lead with responsibility for IPC within the care home

Care home managers to ensure that all staff:

- are aware of and have access to this manual
- have completed appropriate IPC training relevant to their roles and that this is centrally recorded. Training may include resources developed by your organisation, your local NHS board, Health and Social Care Partnership, NHS Education for Scotland (NES) (https://learn.nes.nhs.scot/2482/infection-prevention-and-control-ipc-zone) or the Scottish Social Services Council (SSSC) (https://www.sssc.uk.com/)
- have adequate support and resources available to enable them to implement, monitor and take corrective action to ensure full compliance with this manual
- implement a robust risk assessment including detailing any deviations from the manual and the local mitigation measures that were undertaken and post-approval documented via local governance procedures
- with health concerns (including pregnancy) or those who have had an occupational exposure relating to IPC are timeously referred to the relevant agency, for example general practitioner (GP or doctor), occupational health or if required accident and emergency
- have undergone the required health checks or clearance (including those undertaking exposure prone procedures (EPPs))
- include IPC as an objective in their personal development plans (or equivalent)

Care home staff providing care to:

- understand, adopt and implement the principles of IPC as set out in this manual
- maintain IPC competence, skills, and knowledge through completing appropriate training relevant to their role as directed by their line manager. IPC training, NHS Education for Scotland (NES) (https://learn.nes.nhs.scot/2482/infection-prevention-and-control-ipc-zone) (https://www.nes.scot.nhs.uk/) or the Scottish Social Services Council (SSSC) (https://www.sssc.uk.com/)

- communicate the IPC practices to be taken to appropriate colleagues, residents, relatives and visitors without breaching confidentiality
- have up to date occupational immunisations/health checks/clearance requirements as appropriate
- report to line managers and document any gaps in IPC knowledge, resources, equipment and facilities or incidents that may result in transmission of infection including near misses for example sharps or PPE failures
- do not provide care while at risk of potentially transmitting infectious agents to others - if in any doubt they should consult with their line manager, occupational health department, local infection prevention and control team (IPCT) or local health protection team (HPT)
- contact the local HPT/IPCT if there is a suspected or actual HAI incident/outbreak (/chapter-3-healthcare-infection-incidents-outbreaks-and-data-exceedance/)

Local infection prevention and control teams (IPCTs) and health protection teams (HPTs) to:

- engage with care home staff to develop systems and processes that lead to sustainable and reliable improvements in relation to the application of IPC practice
- provide expert advice on the application of IPC and provide support when requested to develop individual or organisational risk assessments where deviations from the manual are necessary
- have epidemiological or surveillance systems capable of distinguishing resident case or cases requiring investigations and control
- provide local support and advice (when necessary and/or requested) and complete documentation when an incident/outbreak or data exceedance is reported

Chain of infection

In order for infection to occur several things have to happen. This is often referred to as the chain of infection. The six links in the chain are:

1. **infectious agent** or the microorganism which can cause disease

2. **reservoir** or source of infection where the microorganism can live and thrive. This may be a person, an animal, any object in the general environment, food or water

3. **portal of exit** from the reservoir. This describes the way the microorganism leaves the reservoir. For example, in the case of a person with flu, this would include coughing and sneezing. In the case of someone with gastroenteritis microorganisms would be transmitted in the faeces or vomit

4. **mode of transmission.** This describes how microorganisms are transmitted from one person or place to another. This could be via someone's hands, on an object, through the air or bodily fluid contact

5. **portal of entry.** This is how the infection enters another individual. This could be landing on a mucous membrane, being breathed in, entering via a wound, or a tube such as a catheter.

6. **susceptible host.** This describes the person who is vulnerable to infection.

Infection can be prevented by breaking the chain of infection.

Chain of infection diagram

The overall aim of Standard Infection Control Precautions (SICPs) (/infection-prevention-and-control-manual-for-older-people-and-adult-care-homes/#a2821), is to break the chain of infection.

The chain of infection diagram illustrates and gives examples of actions that can be taken to break it.
Select image for full size version.
(/media/1579/care_homes_chain_of_infection_graphic.jpg)
Use the NES SIPCEP Breaking the Chain of Infection (https://learn.nes.nhs.scot/3421/infection-prevention-and-control-ipc-zone/sipcep-foundation-layer/breaking-the-chain-of-infection) module to learn about breaking the chain of infection in care homes.

Hierarchy of controls

Reducing risk

The hierarchy of controls (HoC) (/appendices/appendix-17-hierarchy-of-controls/) is a system used to help prevent the transmission of infection. It details the most to least effective controls. You will note that PPE (/care-home-infection-prevention-and-control-manual-ch-ipcm/#a2884) is the last level of control in the hierarchy, used when all other controls have not reduced the risks sufficiently. To be effective, PPE must be used correctly which means putting it on and removing it correctly and safely (/care-home-infection-prevention-and-control-manual-ch-ipcm/#a2884).
See the Health and Safety Executive's (HSE) toolkit (https://www.hse.gov.uk/simple-health-safety/risk/index.htm) on managing risks and risk assessment at work.

The HoC principles can be broadly interpreted for care home settings and include:

- reducing hazards in the care home
- changing practice in the care home
- making the care home as safe as possible
- changing how we organise and work in the care home
- use of PPE

Examples of HoC principles

Here are some examples of how to apply the HoC principles in care home settings. These examples do not cover every situation where you might need to use HoC principles.

Reducing hazards in the care home

Measures such as vaccination, testing and isolation help to reduce the risk of infection. Not coming to work when ill, isolating while infectious and recognising and reporting infections promptly, all help to prevent infections spreading.

Changing practice in the care home

When faced with a particular risk, such as an outbreak, we may need to change what we do. This might include reducing communal activities, considering limiting visiting for a short period of time, or cleaning the care home environment more frequently. The local IPCT and/or HPT should always be contacted for advice and support in outbreak situations.

Making the care home as safe as possible

It is very unlikely that we will be able to change where we work but the care home setting should be made as safe as possible.
You can reduce opportunities for pathogens to survive in the care home by ensuring fixtures and fittings are in good repair and can be easily cleaned and following water safety guidelines.
Ventilation is also an effective measure to reduce the risk of some respiratory infections, by diluting and dispersing the pathogens which cause them. Consider opening windows and vents more than usual, even opening a small amount can be beneficial. Opening windows and doors may present security and safety

issues and so a local risk assessment should always be undertaken.

Changing how we organise and work in the care home

Changing the way, we organise and work in the care home can also help reduce risk. This might include reducing the number of people in a space at any one time and minimising the movement of staff between different settings as well as using administrative controls.
Administrative controls include local risk assessments, staff training, IPC audits, and providing clear signage and instructions throughout the care home.

Chapter 1: Standard Infection Control Precautions (SICPs)

The basic IPC measures that should be used in your care home are called **Standard Infection Control Precautions (SICPs).**

SICPs are used to reduce the risk of transmission of infectious agents from known and unknown sources of infection.

These should be used by **all staff, in all care settings, at all times, for all residents** whether infection is known to be present or not to ensure the safety of residents, staff and visitors in the care home.

SICPs should be part of everyday practice and applied **consistently** by everyone in the care home.
It is essential that optimal IPC measures are applied continuously as residents living in care homes are more vulnerable, therefore increasing their risk of acquiring infections which may then be serious and potentially life threatening. By applying optimum IPC precautions you will provide a safe environment and effective care.

There are 10 Standard Infection Control Precautions (SICPs)

1. resident placement/assessment for infection risk
2. hand hygiene
3. respiratory and cough hygiene
4. personal protective equipment
5. safe management of care equipment
6. safe management of care environment
7. safe management of linen
8. safe management of blood and body fluid spillage
9. safe disposal of waste
10. occupational safety: prevention and exposure management (including sharps)

1. Resident placement/assessment for infection risk

Before a resident is admitted to the care home it is important to risk assess for infection as part of resident's care plan, an IPC admission assessment should be undertaken by staff.
If you suspect or are aware that a resident has an infection, then details should be confirmed for the correct IPC precautions to be put in place for the safety of the resident and others.
Obtaining infection details may include appropriate clinical samples and/or screening to establish the causative organism which may be on advice from your local GP, IPCT or HPT.

Residents who may present a cross-infection risk include those with:

- diarrhoea

- vomiting, being sick
- unexplained rash
- fever or temperature of 37.8 C or higher
- respiratory symptoms such as coughing and sneezing
- known to have been previously positive with a Multi-drug Resistant Organisms (MDRO), for example Meticillin Resistant *Staphylococcus aureus* (MRSA), Carbapenemase Producing Enterobacterales (CPE)

Further information regarding general respiratory screening questions (/media/2147/2023-08-23-respiratory-screening-questions-aide.pdf) can be found within the resources section of the NIPCM.

> **Note:** If a resident requires isolation because of infection or in an outbreak situation, this should be individually risk assessed to ensure the safety and health and wellbeing needs of the resident. Isolation periods must be monitored on daily basis and be for the minimum period specified.

Resources

Appendix 11 (/appendices/appendix-11-best-practice-aide-memoire-for-optimal-patient-placement-and-respiratory-protective-equipment-rpe-for-infectious-agents-whilst-a-patient-is-in-hospital/) of the NIPCM gives you further information on the precautions required for different infections.

Read the **placement literature review** (/web-resources-container/patient-placement-isolation-and-cohorting-standard-infection-prevention-control-and-transmission-based-infection-control-precautions/) to understand the evidence base for resident placement.

2. Hand hygiene

> Please note that the term ‘alcohol-based hand rub (ABHR)’ has now been updated to ‘hand rub’. A hand rub (alcohol or non-alcohol based) can be used if it meets the required standards. Please see further information in the hand hygiene products literature review (/web-resources-container/sicp-literature-review-hand-hygiene-products/).

The most important thing you can do to prevent the spread of infection in a care home is to keep your hands clean. This is called **hand hygiene**.
Adherence with the following points is essential to ensure effective hand hygiene:

- expose forearms (known as bare below the elbows)
- remove all hand/wrist jewellery including any embedded jewellery (a single, plain metal finger ring or ring dosimeter (radiation ring) is permitted but should be removed (or manipulated) during hand hygiene). Bracelets or bangles such as the Kara which are worn for religious reasons should be able to be pushed higher up the arm and secured in place to enable effective hand hygiene which includes the wrists
- ensure fingernails are clean, short and that artificial nails or nail products are not worn
- cover all cuts or abrasions with a waterproof dressing

Hand washing should be extended to the forearms if there has been exposure of forearms to blood and/or body fluids.

Hand washing sinks should **only** be used for hand hygiene and should not be used for the disposal of other liquids.

To perform hand hygiene:

- hand rub should be available for staff as near to point of resident care as possible. Where this is not practical, personal hand rub dispensers should be used
- application of sufficient volume of hand rub to cover all surfaces of the hands is important to ensure effective hand hygiene
- manufacturer's instructions should be followed for the volume of hand rub required to provide adequate coverage for the hands. In the absence of manufacturer's instructions, volumes of approximately 3ml are recommended to ensure full coverage
- wall mounted or personal hand rub dispensers (cartridges/bottles) should not be refilled and should be replaced when empty or if outwith manufacturer expiry date

The World Health Organization's '4 moments for hand hygiene' should be used to highlight the key indications for hand hygiene:

1. before touching a resident
2. before clean/aseptic procedures. If hand rub cannot be used, then antimicrobial liquid soap should be used
3. after body fluid exposure risk
4. after touching a resident

Some additional examples of hand hygiene moments include, but are not limited to:

- after touching a resident's immediate surroundings
- before handling medication
- before preparing food
- before donning (putting on) and after doffing (taking off) PPE
- after visiting the toilet
- before putting on and after removing PPE
- between carrying out different care activities on the same resident
- after cleaning and disinfection procedures
- after handling used linen
- after handling waste

It is important that residents are routinely encouraged to perform hand hygiene and given assistance if required.

The **four moments for hand hygiene poster** (/media/1444/who-4-moments-residential-care.pdf) can be used in your care home to show staff when hand hygiene should be done and the reasons why.

Select image for full size version.
(https://www.nipcm.scot.nhs.uk/media/1444/who-4-moments-residential-care.pdf)

Hands should be washed with liquid soap and water if/when:

- they are visibly soiled or dirty
- they are potentially contaminated with blood, other body fluids or excretions
- caring for a resident with vomiting or diarrhoeal illness
- caring for a resident with a suspected or known gastro-intestinal infection such as norovirus or a spore forming organism such as *Clostridioides difficile*

> **Note:**
>
> Hands should be washed with warm/tepid water to mitigate the risk of dermatitis associated with repeated exposures to hot water and to maximise hand washing compliance. Compliance may be compromised where water is too hot or too cold.
>
> Hands should be dried thoroughly following hand washing using a soft, absorbent, disposable paper towel from a dispenser which is located close to the sink but beyond the risk of splash contamination.
>
> The use of antimicrobial hand wipes is only permitted where there is no access to running water. Staff should perform hand hygiene using hand rub immediately after using the hand wipes and perform hand hygiene with soap and water at the first available opportunity.
>
> In all other circumstances use hand rub for routine hand hygiene.

Skin care

- Hand rubs should contain emollients in their formulation.
- Pat hands dry after hand washing using disposable paper towels. Avoid rubbing which may lead to skin irritation/damage.
- Use an emollient hand cream during breaks and when off duty. These should be applied all over the hands including between the fingers and the back of the hands.
- Staff with skin problems should seek advice from the local occupational health department if available or their GP.
- Barrier creams should not be used in the workplace.

Do not use refillable containers or communal tubs of hand cream in the care home setting.

Resources

Read the hand hygiene literature reviews (/resources/literature-reviews/hand-hygiene/) to find out more about the evidence base for hand hygiene.

To make sure you clean your hands properly with soap and water you should follow the steps in the poster **‘How to hand wash step by step images (/appendices/appendix-1-best-practice-how-to-hand-wash)’**. This poster can be printed off and displayed throughout the care home to ensure that all staff and visitors are aware of and practice this hand hygiene method when required in the care home.

Select image for full size version (/appendices/appendix-1-best-practice-how-to-hand-wash)

To make sure you clean your hands properly with hand rub you should follow the steps in the poster **'How to hand rub step by step images** (/appendices/appendix-2-best-practice-how-to-hand-rub/)'. This poster can be printed off and displayed throughout the care home to ensure that all staff and visitors are aware of and practice this hand hygiene method when required in the care home.
Select image for full size version (/appendices/appendix-2-best-practice-how-to-hand-rub/)

3. Respiratory and cough hygiene

Infections can spread by coughing and sneezing, therefore it is very important that respiratory and cough hygiene is used by everyone including staff, residents and visitors.
Any resident displaying symptoms of respiratory illness should be encouraged to wear a surgical (for instance TYPE IIR FRSM) face mask where it is clinically safe and can be tolerated by the wearer, especially in communal areas.

What you need for respiratory and cough hygiene

• disposable tissues

• waste bin and waste bags

• hand hygiene products

If a resident has a cough, cold or other respiratory symptoms then they should be supported and encouraged to:

- cover their nose and mouth with a disposable tissue when sneezing, coughing, wiping and/or blowing the nose. If a disposable tissue is not available encourage the use of elbow/sleeve to cover the nose and mouth when coughing or sneezing
- put used tissues and face masks into a waste bin immediately after use
- wash their hands with soap and water after coughing, sneezing, using tissues, or after contact with respiratory secretions or objects contaminated by these secretions
- keep contaminated hands away from the eyes nose and mouth

Staff should:

- promote respiratory and cough hygiene and help residents who need assistance
- ensure that residents are provided with the necessary products for respiratory and cough hygiene including tissues, waste bag and hand hygiene products and make sure that products are in close vicinity for the resident to access
- use hand wipes followed by hand rub if there is no running water available or if hand hygiene facilities are out of reach then wash hands at the first available opportunity.

Read the **respiratory and cough hygiene literature review** (/web-resources-container/standard-infection-control-precautions-sicp-literature-review-cough-etiquetterespiratory-hygiene-in-the-hospital-setting/) to find out the evidence for respiratory and cough hygiene practice.

4. Personal Protective Equipment (PPE)

PPE products you might need in the care home:

• gloves

• aprons

• masks

• eye/face protection

See PPE for visitors

Deciding which PPE to use

Before doing any procedure or task staff should risk assess any likely exposure to blood and/or body fluids and ensure PPE is worn that provides adequate protection against the risks associated with the procedure or task being undertaken.
All PPE should be:

- located close to the point of use
- stored in a clean and dry area to prevent contamination until needed for use
- within expiry dates
- single-use only items unless specified by the manufacturer
- changed immediately after individual use and/or following completion of a procedure or task
- disposed of after use into the correct waste stream as per local risk assessed processes

Reusable PPE items, for example non-disposable goggles, face shields or visors must be cleaned/decontaminated once removed or placed within a designated container for subsequent cleaning/decontamination with decontamination schedules in place and responsibility assigned.

Read further information on best practice for PPE (/appendices/appendix-15-selection-of-personal-protective-equipment-ppe-by-health-and-care-workers-hcws-during-the-provision-of-care/) in Appendix 15.

Donning (putting on) personal protective equipment (PPE)

The order for putting on PPE is:

1. apron or gown
2. surgical mask
3. eye/face protection (where required)
4. gloves

Doffing (taking off) personal protective equipment (PPE)

It is important that PPE is removed in the correct order.
The order for taking off PPE is:

1. gloves
2. apron or gown

3. eye/face protection (if worn)
4. surgical mask

> **Note:**
>
> Always carry out hand hygiene immediately after taking off PPE.
>
> All PPE should be removed before leaving the area and disposed of as healthcare waste

Resources

A video demonstrating the order for donning and doffing PPE (https://vimeo.com/393951705) is available.

COVID-19 - the correct order for donning, doffing and disposal of PPE for HCWs in a primary care setting

NHS National Services Scotland

06:12

The correct order for donning, doffing and disposal of PPE for healthcare workers (https://vimeo.com/393951705) from NHS National Services Scotland (https://vimeo.com/nationalservicesscotland) on Vimeo (https://vimeo.com).

A poster showing the order for putting on and removing PPE (/appendices/appendix-6-best-practice-putting-on-and-removing-ppe/) is available to print.

Select image for full size version (/media/1808/nipcm-appendix6-march_2022.pdf)

Gloves

Gloves should be:

- worn when it is likely or anticipated that you will be exposed to blood, body fluids (including but not limited to secretions and/or excretions), non-intact skin, mucous membranes, lesions and/or vesicles, hazardous drugs, and chemicals for example cleaning agents
- single-use and should be donned (put on) immediately prior to exposure risk and should be doffed (taken off) immediately after each use or upon completion of a task
- appropriate for use, fit for purpose and well-fitting. The glove selection chart (/appendices/appendix-5-best-practice-gloves-use-and-selection/) can help you select the correct gloves
- changed if damaged or a perforation or puncture is suspected

> **Note:**
>
> Using gloves reduces the risk of contamination but does not remove all risk.
>
> Gloves should not be used instead of carrying out hand hygiene.
>
> Gloves should not be worn inappropriately in situations such as to go between residents, move around a care area or whilst at workstations (on the telephone or computer).
>
> Gloves should never be decontaminated or cleaned with hand rub or by washing with cleaning products.

Use the **glove selection chart** (/appendices/appendix-5-best-practice-gloves-use-and-selection/) to support you to select the correct glove type.
Select image for full size version
(/appendices/appendix-5-best-practice-gloves-use-and-selection/)

Aprons and gowns

Aprons should be:

- worn by care staff when there is a risk of clothing being contaminated with blood or other body fluids
- worn during direct care, for example oral hygiene, bed-making or when undertaking the decontamination of equipment
- changed between residents and following completion of a procedure or task

The choice of apron or gown is based on an individual risk assessment and anticipated level of blood/body fluid exposure. Routine sessional use of gowns/aprons is not permitted.

Eye/face protection

Eye/face protection should:

- be worn when there is an anticipated risk of splashing or spraying of blood or bodily fluids and always during Aerosol Generating Procedures (AGPs)

> **Note:**
>
> Eye/face protection should not be touched when worn or worn around the neck or on top of the head when not in use.
>
> Eye/face protection should be compatible with other items of PPE and worn in accordance with manufacturer's instructions.
>
> Prescription eyeglasses and contact lenses should not be considered a form of eye/face protection.

Fluid Resistant Type IIR surgical face masks (FRSM)

Fluid Resistant Type IIR surgical face masks should be:

- worn if splashing or spraying of blood, body fluids, secretions or excretions onto the respiratory mucosa (nose and mouth) is expected/likely. (As part of SICPs a full-face visor may be used as an alternative to fluid resistant Type IIR surgical face masks to protect against splash or spray)
- well-fitting, fully covering the mouth and nose and fit for purpose. Manufacturer's instructions should be followed to ensure effective fit/protection
- removed or changed:
  - at the end of a procedure/task
  - if the mask is damaged or there is a build up from moisture after prolonged use or from gross contamination with blood or body fluids
  - following specific manufacturers' instructions

If you are using droplet precautions, you should always wear a Type IIR surgical face mask as well as eye/face protection (droplet precautions will be discussed further in Chapter 2 Transmission Based Precautions (/infection-prevention-and-control-manual-for-older-people-and-adult-care-homes/#a2926)).

Transparent face masks

Transparent face masks may be used to aid communication with residents where required.
Transparent face masks should:

- meet the specification standards of the Transparent face mask technical specification (https://azuksappnpdsa01.blob.core.windows.net/datashare/Transparent-Mask-Specifications-December-2023.pdf)

and

- have been approved for use within health and social care settings
- only be worn in areas where Fluid Resistant Type IIR surgical face masks are used as personal protective equipment

Resources

Read the aerosol generating procedures literature review (/web-resources-container/tbp-literature-review-aerosol-generating-procedures/)and surgical face masks literature review (/web-resources-container/standard-infection-control-precautions-and-transmission-based-precautions-literature-review-surgical-face-masks/) for further information regarding the evidence base.

(/appendices/appendix-11-best-practice-aide-memoire-for-optimal-patient-placement-and-respiratory-protective-equipment-rpe-for-infectious-agents-whilst-a-patient-is-in-hospital/)

See appendix 11 (/appendices/appendix-11-best-practice-aide-memoire-for-optimal-patient-placement-and-respiratory-protective-equipment-rpe-for-infectious-agents-whilst-a-patient-is-in-hospital/) for further information.

PPE for visitors

At times, PPE may be offered to visitors to protect them from acquiring a transmissible infection. If a visitor declines to wear PPE when it is offered, then this should be respected, and the visit must not be refused. PPE use by visitors cannot be enforced and there is no expectation that staff monitor visitor PPE use. However, if staff or visitors identify the need for PPE, advice on correct use should be provided by staff. Table 1 below is a guide to the PPE which should be worn where appropriate and when the visitor chooses to do so

Table 1: PPE for use by visitors

IPC Precaution	Gloves	Apron/Gown	Face covering/mask	Eye/face protection
Standard Infection Control Precautions (SICPs)	Not required unless providing direct care which may expose the visitor to blood and/or body fluids, for instance toileting.	Not required unless providing care resulting in direct contact with the resident, their environment or blood and/or body fluid exposure, for instance toileting, bed bath.	Where splash/spray to nose/mouth is anticipated during direct care	Not required unless splash/spray to the eyes/face is anticipated during direct care.
Transmission Based Precautions (TBPs)	Not required unless providing direct care which may expose the visitor to blood and/or body fluids, for instance toileting.	Not required unless providing care resulting in direct contact with the resident, their environment or blood and/or body fluid exposure, for instance toileting, bed bath.	If within 2 metres of resident with suspected or known respiratory infection	If within 2 metres of resident with suspected or known respiratory infection

Read the PPE literature reviews (/resources/evidence-and-research/personal-protective-equipment-ppe/) to find out more information about the evidence base for PPE use.

5. Safe management of care equipment

Care equipment is easily contaminated with blood, other body fluids, secretions, excretions and infectious agents and this can spread infection.

Important words and what they mean

Routine cleaning is regular cleaning which is carried out on a scheduled basis, not on an unplanned basis and not in response to an outbreak. For routine cleaning general purpose detergent and water solution or detergent impregnated wipes are sufficient.

Cleaning is the removal of any dirt by use of an appropriate cleaning agent such as detergent.

Decontamination is removing, or killing pathogens on an item or surface to make it safe for handling, re-use or disposal, by cleaning, disinfection and/or sterilisation.

Disinfectant is a chemical used to reduce the number of infectious agents from an object or surface to a level that means they are not harmful to health.

Detergent is a chemical cleansing agent that can dissolve oils and remove dirt.

If the resident has a known infection or the equipment is contaminated with blood or body fluids, then a disinfection agent needs to be used.

Note:

Do not use household bleach as the required dilution cannot be guaranteed.

Do not refill bottles for cleaning products as there is a risk of contamination.

What you need for safe management of care equipment

- Cleaning/disinfectant products:
  - general purpose detergent and water solution/detergent impregnated wipes

    or

  - combined detergent/disinfectant solution at a dilution of 1,000 parts per million available chlorine (ppm available chlorine (av.cl.)

    or

  - a general purpose neutral detergent in a solution of warm water followed by disinfection solution of 1,000ppm av.cl.
- Paper towels/disposable cloths.

Types of equipment

There are three different types of care equipment that you will use in your care home and it is important that you know how to deal with each type.
You should follow manufacturers guidance for all equipment and products you use including those used for cleaning and decontamination.
Before using any **sterile equipment**, you should check that:

- the packaging is intact
- there are no obvious signs of packaging contamination
- the expiry date remains valid

1. **Single-use** - equipment which is used once on a single resident and then discarded.

Single-use equipment **must never be reused** even on the same resident. The packaging carries the symbol.

> **Note:**
>
> Needles and syringes are single-use devices. They should **never** be used for more than one resident or reused to draw up additional medication.
>
> **Never** give medications from a single-dose vial or intravenous (IV) bag to multiple residents.

2. Single individual use – equipment which can be reused by same resident for example a sling and decontaminated following use as per manufacturers instructions.

3. Reusable non-invasive equipment (often referred to as 'communal equipment') – equipment which can be reused on more than one resident following decontamination between each use for example commode, moving and handling equipment or bath hoist.

Cleaning or decontaminating reusable non-invasive equipment

Residents should be given their own reusable (communal) non-invasive equipment where possible. Reusable equipment should be checked frequently for cleanliness and signs of integrity. This will include mattresses and pillows which should be clean, have a waterproof covering which is in a good state of repair.
Pillows used on resident's beds may not require a waterproof cover if they are single resident use and are subject to regular checks/laundering. Resident pillows may require labelling where appropriate.
Reusable equipment should be cleaned or decontaminated:

- between individual use
- after blood and/or body fluid contamination
- as part of the regular scheduled cleaning schedules/process
- before inspection, servicing or repair

Staff should:

- follow the local cleaning protocol/schedule which should include responsibility for, frequency of and method of decontamination required
- use a general purpose detergent and water solution/detergent impregnated wipes

  or
  a combined detergent/disinfectant solution at a dilution of 1,000 parts per million available chlorine (ppm available chlorine (av.cl.)
  or
  a general purpose neutral detergent in a solution of warm water followed by disinfection solution of 1,000ppm av.cl;
- make up cleaning/disinfection solution following manufacturers guidance
- follow the manufacturer's contact time for the cleaning/disinfection solution
- rinse and dry reusable equipment then store it clean and dry

> **Note:** When an organisation use products or adopts practices that differ from those stated in this manual, that individual organisation is responsible for ensuring safe systems of work including the

completion of risk assessments approved through local governance procedures.

Resources

Read the **management of care equipment literature review** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-and-transmission-based-precautions-literature-review-management-of-care-equipment/) to find out more about why we do things this way for care equipment.

The **decontamination of non-invasive care equipment** (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/) poster can help staff decide how to clean equipment.

Select image for full size version

(http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/)

6 - Safe management of the care environment

There are many areas in care homes that become easily contaminated with microorganisms (germs) for example door handles, toilets, waste bins, surfaces.
Furniture and floorings in a poor state of repair can have microorganisms (germs) in hidden cracks or crevices.
To reduce the spread of infection, the environment should be kept clean and dry and where possible clear from clutter and equipment.
Non-essential items should be stored and displayed in such a way as to aid effective cleaning
Keeping a high standard of environmental cleanliness is important in the care home as the residents are often elderly and vulnerable to infections.
The care home environment should be:

- visibly clean, free from non-essential items and equipment to help make cleaning effective
- well maintained and in a good state of repair
- routinely cleaned in accordance with the specified cleaning schedules:
  - a fresh solution of general purpose neutral detergent in warm water is recommended for routine cleaning. This should be changed when dirty or at 15 minutes' intervals or when changing tasks
  - routine disinfection of the environment is not recommended. However, 1,000 parts per million available chlorine (ppm available chlorine (av.cl.) should be used routinely on sanitary fittings

Staff should:

- report any issues with the environment cleanliness or maintenance to the person in charge to ensure that the care environment is safe. The person in charge should then act on problems reported to them
- be aware of the environmental cleaning schedules and clear on their specific responsibilities

Cleaning schedules should include:

- staff responsibilities
- cleaning frequencies
- cleaning methods

Managing cleaning services

Cleaning services should be managed in a systematic way, and staff responsible for cleaning should be appropriately trained to carry out the tasks they are responsible for.

The **care home manager** is responsible for managing the cleaning service which has a number of **essential** elements outlined in the cleaning services diagram.
Select the **Care Homes Cleaning Specification** (https://www.nss.nhs.scot/publications/safe-management-of-the-care-environment-shfn-01-05/) for full size version of cleaning services diagram.
Select the diagram for full size version

Cleaning Services

(https://www.nss.nhs.scot/publications/safe-management-of-the-care-environment-shfn-01-05/)
An effective service will include all of the elements above.

Care Homes Cleaning Specification

The **Care Homes Cleaning Specification** (https://www.nss.nhs.scot/publications/safe-management-of-the-care-environment-shfn-01-05/) provides a guide to planning cleaning services. It has tools to help with the planning and recording of cleaning activities and with the management activities marked with a * in the diagram above. These include:

- a structure to identify all spaces within a care home and plan appropriate cleaning tasks and frequencies
- a set of weekly and monthly **cleaning templates** to be assigned to each space within a care home. These can be used to develop a schedule and to provide a method for recording all cleaning activity

The tools within the Cleaning Specification should be used by the care home manager in the planning, training of staff, delivery, and checking of standards of the cleaning services they provide.
Manufacturer's instructions and recommended contact times should be adhered to.

Cleaning schedule and record

Table 2 provides an example of a cleaning schedule and record. These tools are examples and designed to support local practice, however care homes can use their own tools if preferred. If a local tool is used, it should reflect the standards set out in the Care Homes Cleaning Specification (https://www.nss.nhs.scot/publications/safe-management-of-the-care-environment-shfn-01-05/).
Table 2: Example cleaning schedule residents room

Standard operating procedures (SOPs) for all cleaning tasks.

Each SOP outlines the correct equipment, safety considerations, method, and outcomes required for each task. Table 3 shows the important steps that must be taken during the cleaning of floors.
Table 3: Example cleaning SOP: Floors

Process for checking

A **process for checking** the cleanliness of the care environment, to ensure standards are being maintained and to identify areas for improvement.

Decontamination of soft furnishings

Decontamination of soft furnishings may require to be discussed with the local HPT/ICT. If the soft furnishing is heavily contaminated with blood or body fluids, it may have to be discarded. If it is safe to clean with standard detergent and disinfectant alone then follow appropriate procedure.
If the item cannot withstand chlorine releasing agents staff are advised to consult the manufacturer's instructions for a suitable alternative to use following or combined with detergent cleaning.

> **Note:** When an organisation adopts decontamination processes not recommended in the CH IPCM the care organisation is responsible for governance of and completion of local risk assessment(s) to ensure safe systems of work.

Read the **routine cleaning of the care environment** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-and-transmission-based-precautions-literature-review-safe-management-of-the-care-environment/) literature review to find out more about why we do things this way for the care environment.

7 - Safe management of linen

Examples of linen you may have in the care home includes:

- bed linen (bed sheets, duvet, duvet covers, pillowcases)
- blankets
- curtains
- hoist slings
- towels
- resident clothing

There are three categories of linen:

Clean – Linen washed and ready for use
Used – All used linen in the care setting not contaminated by blood or body fluids
Infectious – All linen used by a person known or suspected to be infectious and/or linen that is contaminated with blood or body fluids for example faeces
Used or infectious linen may also be categorised as heat-labile: usually personal clothing where the clothing may be damaged (shrinking/stretching) by washing at a higher than recommended temperature than the label advises and therefore, cannot be subject to thermal disinfection. If such linen needs to be washed at a higher temperature for example if soiled or resident has a known infection they or their relatives need to be advised that the clothing may be damaged.
All clean, used and infectious linen should be handled with care and attention paid to the potential spread of infection. Appropriate temperatures for processing all used and infectious linen should be adhered to achieve thermal disinfection.

Clean linen

- Should be stored in a clean, allocated area. This should be an enclosed cupboard but a trolley could be used as long as it is completely covered with a waterproof covering that is able to withstand cleaning.
- Perform hand hygiene, in accordance with Section 2 (/care-home-infection-prevention-and-control-manual-ch-ipcm/#a2828), prior to handling clean linen.

Used linen

Staff should:

- refer to Appendix 15 (/appendices/appendix-15-selection-of-personal-protective-equipment-ppe-by-health-and-care-workers-hcws-during-the-provision-of-care/) for decision making regarding PPE use
- check that linen is free from inappropriate items before placing into the laundry receptacle, for example used equipment, service user personal belongings
- make sure that a laundry trolley or container is available as close as possible to the point of use for immediate linen deposit

Staff should **not**:

- rinse, shake or sort linen on removal from beds or trolleys
- place used linen on the floor or any other surfaces for example on a locker or tabletop
- re-handle used linen once bagged
- overfill laundry receptacles or trolleys

Infectious linen

Staff should:

- wear disposable gloves and apron before handling infectious linen
- put infectious linen directly into a water soluble laundry bag and secure before putting into a clear plastic bag and placing into a laundry receptacle/trolley

If using external laundry services both used and infectious linen bags/receptacles should follow local procedure and arrangements. Store all used/infectious linen in a designated, safe, lockable area whilst awaiting uplift.
All linen that is deemed unfit for re-use, for example torn or heavily contaminated, should be categorised at the point of use and disposed of in the appropriate local healthcare waste stream.

Washing residents personal linen

Appendix 1 National Guidance for Safe Management of Linen in NHSScotland Health and Care Environments - For laundry services/distribution (https://www.nss.nhs.scot/publications/national-guidance-for-safe-management-of-linen-in-nhsscotland-health-and-care-environments-for-laundry-servicesdistribution-v22/) contains information that is particularly relevant and may be useful for residential care settings where domestic-type (household) washing machines may be in place for laundering resident's personal items and clothing.
Domestic-type washing machines are not typically programmed with the temperature settings required for thermal disinfection, therefore domestic-type machines may only be used for laundering personal items of clothing belonging to residents, such as those that are heat-labile.
Other types of used linen such as sheets should be reprocessed using a machine that is capable of a validated temperature disinfection stage.
If using a domestic type washing machine to launder resident's personal items:

- wash items using the highest temperature you can and following the washing instructions
- use your normal washing powder or detergent and follow the instructions on the correct amount to use
- tumble-dry (if possible) following the washing instructions
- iron according to washing instructions. If possible, use a hot steam iron.

It is considered best practise to launder a resident's personal items separately, that means not to mix items from multiple persons within a single load.
If visitors wish to take their relatives clothes home to be laundered, place laundry in an appropriate bag and provide them with a **washing clothes at home leaflet** (https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2639/documents/1_washing-clothes-home-english.pdf).
If the residents clothing is very soiled or infectious, staff may recommend that the clothing is washed in the care home's laundry service if available, otherwise, the item should be disposed of in the appropriate healthcare waste stream following discussion with the resident or their relative(s).

Read the **safe management of linen** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-sicp-literature-review-safe-management-of-linen-in-the-hospital-setting/) literature review to find out more about why we do things this way when dealing with linen.

8 - Blood and body fluid spillages

Spillages of blood and other body fluids may transmit blood borne viruses.

Important words and what they mean

A **blood borne virus** is a virus carried or transmitted by blood, for example Hepatitis B, Hepatitis C and HIV.

Body fluids are fluids produced by the body such as urine, faeces, vomit or diarrhoea. These body fluids may also contain blood.

Blood and body fluid spillages must be decontaminated:

- immediately by staff trained to undertake this safely
- using body fluid spill kits/equipment available

Responsibilities for the decontamination of blood and body fluid spillages should be clear within each area/care setting.

Resources

Read the **management of blood and body fluid spillages** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-sicp-literature-review-management-of-blood-and-body-fluid-spillages-in-the-hospital-setting/) literature review to find out more about why we do things this way for blood and body fluid spillages.

Use the poster **management of blood and body fluids** (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-9-best-practice-management-of-blood-and-body-fluid-spillages/) to help you when you clean up blood and body fluid spillages.

Select the image for full size (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-9-best-practice-management-of-blood-and-body-fluid-spillages/)

9 - Safe disposal of waste (including sharps)

Classification of waste

Waste regulations require the classification of waste based on hazardous characteristics.

- **Special (hazardous) waste**. Special waste includes a range of controlled wastes, defined by legislation, which contain dangerous or hazardous substances. Examples of special (hazardous) waste resulting from healthcare activities includes sharps, infectious or potentially infectious clinical waste and some pharmaceuticals or medicinal wastes.
- **Non-hazardous waste** is residual waste produced in both clinical and non-clinical settings which may include dry recyclates (glass, paper and plastics, metals, cardboard), food waste, packaging waste and furniture.

Segregation (separating) of waste

Waste bags in care homes should be colour coded to denote the different waste streams.
Different types of waste will be produced within care homes.
Some waste may be considered non-hazardous, for example paper hand towels, while other types of waste need special handling and disposal because of their hazardous properties for example, sharps and waste from service users who have or may have an infection.
SHTN 03-01 (https://www.nss.nhs.scot/media/5724/shtn-03-01-v8-dec-2024.pdf) contains a full colour-coded waste segregation guide however, the most frequently used waste streams are summarised below.

- **Black (non-hazardous)**

- residual waste remaining after all source segregated recyclates have been removed.

- **Orange (infectious)**
  - consists of infectious or potentially infectious substances or items.
  - orange lidded leak resistant receptacles may be used for solidified infectious liquids.
  - orange bags may be used for items such as PPE, spillage kits, swabs or dressings contaminated or likely to be contaminated with blood and/or body fluids including saliva.
  - orange lidded sharps box used for sharps disposal only

Local risk assessed processes for waste disposal should be followed and guidance from local contractors may apply.

Safe management of waste

Care home staff should ensure:

- all waste is managed according to relevant legislation and any local risk assessed processes
- the appropriate PPE is used when handling any special (hazardous) waste
- waste is disposed of as close to the point of use as possible, and segregated using the correct colour-coded waste bag or container compliant with UN and relevant industry standards
- liquid waste is disposed of via the toilet or macerator, or where this is not possible, solidified and placed in an appropriate rigid leak resistant receptacle
- clearly marked and secure containers for sharps disposal are available where sharps are used
- schedules are in place for the cleaning, emptying and uplifting of waste bins and waste does not accumulate in corridors, rooms, care areas or other publicly accessible areas
- waste bags are never overfilled and have been appropriately sealed, labelled and marked with the date and location before being stored for uplift.
- waste bags are securely sealed using a closure technique such as a ‘swan neck’
  - a ‘swan neck’ is a way of closing bag by tying in a loop and securing with a zip tie or tape to make a handle
- there is a dedicated area for the storage of waste that is secure and not accessible to residents or the public
- any PPE is removed, and hand hygiene is performed after handling waste

Read the **safe disposal of waste** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-literature-review-the-safe-disposal-of-waste/) literature review to find out more about why we do things this way when dealing with waste.

10. Occupational Safety: Prevention and Exposure Management (including sharps)

All care homes should have policies in place to ensure that staff are protected from occupational exposure to microorganisms (germs), particularly those that may be found in blood and body fluids.

Important words and what they mean

Occupational exposure is exposure of staff to blood or body fluids in the course of their work.

A **sharp** is a device or instrument such as needles, lancets and scalpels which are necessary for the exercise of specific healthcare activities and are able to cut, prick and/or have the potential to cause injury.

Safety device or safer sharp is a medical sharps device which has been designed to incorporate a feature or mechanism that minimises and/or prevents the risk of accidental injury. Other terms include (but are not limited to) safety devices, safety-engineered devices and safer needle devices.

The **Health and Safety (Sharp Instruments in Healthcare) Regulations (2013)** (https://www.legislation.gov.uk/uksi/2013/645/made#:~:text=The%20Health%20and%20Safety%20%28Sharp%20Instruments%20in%20Healthcare%29,event%20of%20injury%207%20Notification%20of%20injuries.%20) outline the regulatory requirements for employers and contractors in the healthcare sector in relation to:

- arrangements for the safe use and disposal of sharps
- provision of information and training to employees
- investigations and actions required in response to work related sharps injuries

Safe management of sharps in the care home

Sharps handling must be assessed, kept to a minimum and eliminated if possible with the use of approved safety devices.

Sharps safety

- **Always** dispose of needles and syringes as a single unit immediately at the point of use.
- **Always** assemble and label sharps containers correctly as per manufacturers instructions.
- **Always** use the temporary closure mechanisms on sharps containers in between use.
- **Always** follow manufacturers' instructions for safe use and disposal.

- **Never** re-sheath used needles or lancets.
- **Never** store sharps containers on the floor.
- **Never** allow access of sharps containers to residents or the public.
- **Never** fill sharps containers more than three-quarters full.

Significant occupational exposure

A significant occupational exposure is when someone is injured at work from using sharps or exposed to risk from blood or body fluids which may then result in a blood borne virus (BBV) or other infection. Examples of this would be:

- a percutaneous injury for example injuries from needles, instruments, bone fragments, or bites which break the skin
- exposure of broken skin for example abrasions, cuts, eczema

- exposure of mucous membranes including the eye from splashing and/or spraying of blood or bodily fluids

If you think or know you have had a significant occupational exposure you should:

- report this immediately to the designated person in your care home**, this is a legal requirement**
- follow the local agreed process for management of an occupational exposure incident and follow the management of occupational injuries flow chart

Resources

Read the **management of occupational exposure to Blood Borne Viruses (BBVs)** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-sicp-literature-review-occupational-exposure-management-including-sharps/) literature review to find out more about why we do things this way for occupational exposure.

The **management of occupational exposure incidents flowchart (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-10-best-practice-management-of-occupational-exposure-incidents/)** should be used within your care home so you know what to do for an occupational exposure.

Select the image for full size (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-10-best-practice-management-of-occupational-exposure-incidents/)

Transmission-based precautions (TBPs) definitions literature review update

Why are transmission descriptors (contact, droplet, airborne) being reviewed?

> The pandemic highlighted that the way in which respiratory transmission is currently described (droplet and airborne transmission) may not reflect what is happening in real life and so, there is a need to identify alternatives ways to describe respiratory transmission routes.
>
> Understanding how infectious agents are released into the air and the risks associated with particle size and distance from source will help inform this. Reviewing the evidence to understand if there is increased risk associated with certain medical procedures will also inform IPC practice.
>
> The World Health Organization (WHO) and Centers for Disease Control (CDC) have also reviewed transmission descriptors indicating a global shift in the way transmission routes are described. ARHAI Scotland were invited to meet with the WHO global IPC unit to discuss the topic and our literature review findings were well received.

What is likely to change in the CHIPCM?

> ARHAI are currently developing recommendations for practice. It is likely that ‘droplet transmission’ and ‘airborne transmission’ will be replaced with new definitions to describe respiratory transmission. This will mean changes throughout the CHIPCM to update the terminology including the addition of resources to support any guidance changes.

What might this mean for care home staff in practice?

It is too early to understand what might change in practice, but it is likely that there will be a need for care home staff to consider more factors when risk assessing what PPE to wear.

The goal of the CHIPCM is to provide care home staff in Scotland with guidance that is evidence based, up-to-date, effective, practical, and as a result, safe.

Update announcements

Use of the CHIPCM online is always advised to ensure access to up to date guidance. Updates to the CHIPCM content are communicated to stakeholders via the Care Home IPC Oversight and Advisory Group in addition to the news section of the NIPCM.

For further detail please use this link to the Transmission Based Precautions Definitions Literature Review (/resources/evidence-and-research/transmission-based-precautions-definitions/). (/resources/evidence-and-research/transmission-based-precautions-definitions/)

Transmission based precautions (TBPs)

In certain circumstances using standard infection control precautions (SICPs) won't be enough to stop an infection spreading and you will need to use some extra precautions. **These extra precautions are called Transmission Based Precautions or TBPs.**
Clinical judgement and decisions should be made by staff to determine the necessary IPC precautions required (the local IPCT and/or the HPT should be contacted for advice and support where required).
Clinical judgement and decisions should be based on the:

- suspected or known infectious agent
- transmission route of the infectious agent
- care setting and procedures undertaken
- severity of the illness caused

When TBPs should be used

TBPs should be used if a resident has a suspected or known infection or colonisation.

Important words and what they mean

Colonisation is the presence of microorganisms on a body surface (such as the skin, mouth, intestines or airway) that does not cause disease in the person or signs of infection.

Infection transmission routes

Infections can be transmitted or spread by:

- **contact** with microorganisms (germs) on hands or from contaminated equipment or environment
- **droplet infection** by inhaling infectious droplets, for example flu or COVID-19
- **aerosol infection** which are inhaled or directly contaminate a mucosal surface or conjunctivae (eyes, nose, and mouth)

Different transmission routes need different TBPs

TBPs are categorised by the route of transmission of infectious agents (some infectious agents can be transmitted by more than one route). **Appendix 11** (https://www.nipcm.scot.nhs.uk/appendices/appendix-11-best-practice-aide-memoire-for-patient-placement-considerations-and-respiratory-protective-equipment-rpe-or-fluid-resistant-surgical-facemasks-frsms-for-infectious-agents/) provides details of the type of precautions, optimal resident placement, isolation requirements and any respiratory precautions required. Application of TBPs may differ depending on the setting and the known or suspected infectious agent.

- **Contact precautions** are used to prevent infections that spread through direct contact with the resident or indirectly from the resident's immediate care environment and care equipment. This is the most common route of cross-infection transmission.
- **Droplet precautions** are used to prevent and control infections spread over short distances (at least 3 feet or 1 metre) via small droplets from the respiratory tract of one individual directly onto the mucosal surface of another person's mouth or nose or eyes. Droplets penetrate the respiratory system to above the alveolar level.
- **Airborne precautions** are used to prevent and control infections spread without necessarily having close contact via from the respiratory tract of one individual directly onto the surface of another person's mouth or nose or eyes. Aerosols penetrate the respiratory system to deep into the lung.

Different infections need different TBPs

You might have heard of some infections like norovirus, Meticillin-resistant *Staphylococcus aureus* (MRSA), *Clostridioides. difficile* (C.diff/CDI) and flu but there are lots of others.
You can find out more information about the infection the individual has and the precautions you should use in **Appendix 11** (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-11-best-practice-aide-memoire-for-optimal-patient-placement-and-respiratory-protective-equipment-rpe-for-infectious-agents-whilst-a-patient-is-in-hospital/) and/or **A-Z of pathogens** (http://www.nipcm.hps.scot.nhs.uk/a-z-pathogens/) in the NIPCM.
You can also contact your local IPCT or HPT for further advice if required.
Before using transmission based precautions you need to find out:

- what the suspected or known infection/colonisation is?
- how is it transmitted?
- how severe is the resident's illness?
- what is the care setting and required procedures?

There are different ways you can find out if a resident has an infection that needs TBPs to be put in place. You can get information about a resident's infection status from:

- their GP
- local health protection team
- local infection prevention and control team
- laboratory
- hospital or care homes staff from where the resident has been discharged or transferred

Local processes should be followed when obtaining this information.

Further information on transmission based precautions can be found in the definitions of (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/transmission-based-precautions-literature-review-definitions-of-transmission-based-precautions/)Transmission Based Precautions literature review. (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/transmission-based-precautions-literature-review-definitions-of-transmission-based-precautions/)

1. Resident placement/assessment for infection risk

All residents require to be regularly monitored for infection throughout their stay for the correct IPC precautions to be put in place to minimise the risk of infection being spread.
Residents may be an infection risk if they have:

- diarrhoea, vomiting, an unexplained rash, fever or respiratory symptoms
- a known (laboratory confirmed) or suspected infectious pathogen for which **appropriate** duration of precautions as outlined in A-Z of pathogens (https://www.nipcm.scot.nhs.uk/a-z-pathogens/)are not yet complete
- been previously positive with a Multi-drug Resistant Organism (MDRO) for example Meticillin-resistant Staphylococcus aureus (MRSA); Carbapenemase Producing Enterobacterales (CPE)*

*CPE should be considered if the resident meets **any** of the following criteria within the 12-month period before admission:

- been an inpatient in a hospital outside of Scotland
- received holiday dialysis outside of Scotland
- been a close contact of a person who has been colonised or infected with CPE.

See the CPE toolkit for non-acute settings (https://www.nss.nhs.scot/publications/toolkit-for-managing-carbapenemase-producing-enterobacteriaceae-cpe-in-scottish-non-acute-care-settings/) for further information and requirements.

Staff should do the following if any resident displays signs and/or symptoms of infection:

- obtain advice on the resident's clinical management from their GP and advice on appropriate IPC management from either the local HPT or IPCT
- make resident placement decisions based on advice received or sound judgement by clinical staff who are involved in the resident's management
- advise the ambulance service of the resident's infectious condition if transport to hospital is required

Residents should **not** be moved within the care home if they have signs and symptoms of infection unless essential.

Resident isolation requirements within the care home

Residents may require to be isolated in their own room because of a known or suspected infection. During this time it is important that:

- residents remain in their rooms whilst considered infectious and the door should remain closed. If it is not possible for safety reasons, for example the resident has dementia, an individual risk assessment should be done and any decisions or actions should be documented. The local HPT or IPCT should be contacted for advice
- suitable discrete signage is placed on the door advising others not to enter the room
- there should be as much consistency in staff allocation as possible to care for residents in isolation room areas as an additional IPC measure. This is known as 'staff cohorting'
- resident isolation requirements should remain under continuous review considering individual risk factors and the impact on the resident. The local HPT or IPCT should be contacted for advice in these circumstances

> **Note:** If a resident requires isolation because of infection or in an outbreak situation, this should be individually risk assessed to ensure the safety and health and wellbeing needs of the resident. Isolation periods must be monitored on daily basis and be for the minimum period specified.

Read the **patient placement, isolation and cohorting** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/patient-placement-isolation-and-cohorting-standard-infection-prevention-control-and-transmission-based-infection-control-precautions/) literature review to find out more about why we do things this way for resident placement for TBPs.

2. Safe management of care equipment in an isolation room

Cleaning and decontamination of care equipment is essential to reduce the spread of infection when infection is confirmed/suspected.
When dealing with the equipment used in the resident's isolation room staff should:

- use single use or dedicated reusable care equipment, for example commodes, for the resident in isolation where possible
- clean and decontaminate all care equipment after each use as per Appendix 7 (/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/)

Read the **management of care equipment** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-and-transmission-based-precautions-literature-review-management-of-care-equipment/) literature review to find out more about why we do things this way for patient care equipment for TBPs.

3 - Safe management of the care environment

Isolation room cleaning

Staff should:

- clean and decontaminate the isolation room at least daily or more frequently if advised to do so. If you have been advised to clean more than daily this should be documented in the environmental cleaning schedule
- clean frequently touched surfaces like door handles, bed frames and bedside cabinets at least twice daily
- use correct cleaning products which are either:

  a combined detergent/disinfectant solution at a dilution of 1,000 parts per million available chlorine (ppm available chlorine (av.cl.));
  or
  a general purpose neutral detergent in a solution of warm water followed by disinfection solution of 1,000ppm av.cl.
- follow manufacturers guidance and instructions on how to use the product and what the recommended contact time is for the product to work. This may include rinsing off the disinfection solution to prevent damage to surfaces.

Do not **refill spray containers** for cleaning products as there is a risk of contamination.

Terminal clean

> A **terminal clean** is carried out when the resident is no longer considered infectious and/or when the environment is cleaned/decontaminated to ensure it is safe for a new resident.

A terminal clean is carried out by:

- removing all healthcare waste and other disposable items from the room

- removing bedding, curtains (bagged before removal from the room) and then wash as infectious linen
- cleaning and decontaminating all reusable care equipment in the room (prior to removal from the room)

The room should then be decontaminated using either:

- a combined detergent disinfectant solution at a dilution (1,000ppm av.cl.) or
- a general purpose neutral detergent clean in a solution of warm water followed by disinfection solution of 1,000ppm av.cl.

The room should be cleaned from the highest to lowest point and from the least to most contaminated point.
Manufacturers' guidance and recommended product "contact time" should be followed for all cleaning/disinfection solutions.

(/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/) (/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/)Appendix 7 (/appendices/appendix-7-best-practice-decontamination-of-reusable-non-invasive-care-equipment/) is a poster flowchart for decontamination of reusable non-invasive care equipment that you may wish to print off and place in the care home.

> **Note:** When an organisation use products or adopts practices that differ from those stated in this manual, that individual organisation is responsible for ensuring safe systems of work including the completion of risk assessments approved through local governance procedures.

4. Personal Protective Equipment (PPE): Respiratory Protective Equipment (RPE)

In addition to PPE used for Standard Infection Control Precautions, appendix 15 (/appendices/appendix-15-selection-of-personal-protective-equipment-ppe-by-health-and-care-workers-hcws-during-the-provision-of-care/) of the NIPCM outlines what type of PPE and RPE you will need to wear for infections spread by different transmission routes.
AGPs (/care-home-infection-prevention-and-control-manual-ch-ipcm/#AGP)

Gloves

Gloves are a single-use item and should be donned immediately prior to exposure risk and should be doffed immediately after each use or upon completion of a task.
Gloves should:

- be worn when exposure to blood, body fluids, (including but not limited to secretions and/or excretions), non-intact skin, lesions and/or vesicles, mucous membranes, hazardous drugs, and chemicals for example cleaning agents, is anticipated/likely
- never be worn inappropriately in situations such as to go between residents, move around a care area, work at IT workstations
- be changed if a perforation or puncture is suspected or identified
- be appropriate for use, fit for purpose and well-fitting
- not be worn as a substitute to hand hygiene
- never be decontaminated with ABHR

Resources

For appropriate glove use and selection see the flowchart poster (https://www.nipcm.scot.nhs.uk/appendices/appendix-5-best-practice-gloves-use-and-selection/) which may be printed off and placed in the care home. (https://www.nipcm.scot.nhs.uk/appendices/appendix-5-best-practice-gloves-use-and-selection/)

Further information can be found in the Gloves literature review (https://www.nipcm.scot.nhs.uk/web-resources-container/sicp-literature-review-personal-protective-equipment-ppe-gloves/).

Aprons/gowns

An apron should be worn when caring for residents known or suspected to be colonised/infected with antibiotic resistant bacteria including contact with the resident's environment.
Plastic aprons should be used in care home settings for protection against contamination with blood and/or body fluids.
A fluid repellent gown should be used if excessive splashing or spraying is anticipated.
A full body fluid repellent gown should be worn when conducting AGPs on residents known or suspected to be infected with a respiratory infectious agent.
Further information can be found in the Aprons/Gowns literature review (https://www.nipcm.scot.nhs.uk/web-resources-container/standard-infection-control-precautions-sicp-literature-review-personal-protective-equipment-ppe-apronsgowns/).

Eye/face protection

Eye and face protection should be used in combination with:

- a fluid resistant Type IIR surgical mask when caring for symptomatic residents infected with **droplet** transmitted infectious agents
- a fluid resistant FFP3 respirator when caring for symptomatic residents infected with an **airborne** transmitted infectious agent

Eye/face protection should be worn:

- by all of those in the room if potentially infectious AGPs are conducted
- for the care of residents with novel infectious agents including pandemic influenza

Read Appendix 11 (/appendices/appendix-11-best-practice-aide-memoire-for-patient-placement-considerations-and-respiratory-protective-equipment-rpe-or-fluid-resistant-surgical-facemasks-frsms-for-infectious-agents/) for details of the type of precautions, optimal resident placement, isolation requirements and any respiratory precautions required.

Surgical masks

A Type IIR fluid resistant surgical mask (FRSM) should be worn when caring for a resident with a suspected/confirmed infectious agent spread by the droplet route.
FRSMs should be worn (where tolerated) by residents with suspected/confirmed infectious agents spread by the droplet or airborne routes, as a form of source control and should meet type II or IIR standards.
Read Appendix 11 (/appendices/appendix-11-best-practice-aide-memoire-for-patient-placement-considerations-and-respiratory-protective-equipment-rpe-or-fluid-resistant-surgical-facemasks-frsms-for-infectious-agents/) for details of the type of precautions, optimal resident placement, isolation requirements and any respiratory precautions required.

Respiratory Protective Equipment (RPE)

PPE should still be used in accordance with SICPs when using respiratory protective equipment (RPE). See Chapter 1.4 (/infection-prevention-and-control-manual-for-older-people-and-adult-care-homes/#a2884) for PPE use for SICPs.

The use of FFP3s is governed by health and safety regulations and they must be fit tested to the user to ensure the required protection is provided.

The Health and Safety Executive (HSE) provides information regarding fitting and fit checking of RPE (https://www.hse.gov.uk/respiratory-protective-equipment/basics.htm).

Respiratory Protective Equipment (RPE), for instance FFP3 and eye/face protection, should be considered when:

- a resident is admitted or develops a known/suspected infectious agent/disease spread wholly by the airborne route
- carrying out AGPs on residents with known/suspected infectious agent spread wholly or partly by the airborne or droplet route

FFP3 respirators

It is an HSE requirement that staff who need to wear an FFP3 respirator must be fit tested.

FFP3 respirators should **not** be worn by staff who are not trained in their use or who have not been fit tested.

All FFP3 respirators must be:

- fit tested (by a competent fit test operator) on all staff who may be required to wear a respirator to ensure an adequate seal/fit according to the manufacturers' guidance
- fit checked (according to the manufacturers' guidance) every time a respirator is donned to ensure an adequate seal has been achieved
- single-use (disposable) and fluid resistant
- compatible with other items of PPE and worn in accordance with manufacturer's instructions. Prescription eyeglasses and contact lenses should not be considered a form of eye/face protection
- put on before entry into the resident's room and removed in a safe area once outside the resident's room
- changed after each use

Signs that a change in respirator is required include:

- breathing becomes difficult
- the respirator is wet or moist
- the respirator is damaged
- the respirator is obviously contaminated with body fluids such as respiratory secretions

Resources

A poster (/media/1491/facial-hair-and-ffp3-respirators.png) containing information on compatibility of facial hair and FFP3 respirators can be used when fit testing and fit checking.

Further information regarding fitting and fit checking of respirators can be found from the Health and Safety Executive (http://www.hse.gov.uk/respiratory-protective-equipment/basics.htm)

Aerosol Generating Procedure (AGP)

An AGP is a medical procedure that can result in the release of airborne particles from the respiratory tract and is associated with an increased risk of transmission when treating someone who is suspected or known to be suffering from an infectious agent transmitted wholly or partly by the airborne or droplet route.

The most common AGPs undertaken in the Care Home Setting are Continuous Positive Airway Pressure Ventilation (CPAP) or Bi-level Positive Airway Pressure Ventilation (BiPAP) or tracheostomy procedures (insertion or removal) and open suctioning beyond the oro-pharynx.
The full list of medical procedures that have reported to be aerosol generating and are associated with an increased risk of respiratory transmission can be found in appendix 16 (/appendices/appendix-16-aerosol-generating-procedures-agps-and-post-agp-fallow-time-pagpft/).
A poster (/media/1898/2022-07-22-v4-ppe-when-undertaking-agps-poster.pdf) is also available on PPE when undertaking AGPs within health and social care settings.
Rooms should always be decontaminated following an AGP. Clearance of infectious particles after an AGP is dependent on the ventilation and air change within the room. In an isolation room with mechanical ventilation 10-12 air changes per hour (ACH) a minimum of 20 minutes is required; in a side room with 6 ACH this would be approximately one hour.
It is often difficult to calculate air changes in areas that have natural ventilation only, meaning no mechanical ventilation. Natural ventilation, particularly when reliant on open windows can vary depending on the climate. An air change rate in these circumstances has been agreed as 1-2 air changes/hour.
Rooms should always be decontaminated following the completion of an AGP. Regardless of the number of air changes, a period of 10 minutes should pass to allow larger droplets to settle before the room can be cleaned. Staff are required to wear the appropriate PPE until the fallow time (https://www.nipcm.scot.nhs.uk/appendices/appendix-17-aerosol-generating-procedures-agps-and-post-agp-fallow-time-pagpft/) has been met.

Resources

For further information on fallow times refer to Table 1 in Appendix 16. (/appendices/appendix-16-aerosol-generating-procedures-agps-and-post-agp-fallow-time-pagpft/)

Further information can be found in the literature reviews aerosol generating procedures, (/web-resources-container/tbp-literature-review-aerosol-generating-procedures/)Respiratory Protective Equipment (RPE) (https://www.nipcm.scot.nhs.uk/web-resources-container/transmission-based-precautions-literature-review-respiratory-protective-equipment-rpe/), Personal Protective Equipment (PPE) for Infectious Diseases of High Consequence (IDHC) (https://www.nipcm.scot.nhs.uk/web-resources-container/literature-review-personal-protective-equipment-ppe-for-infectious-diseases-of-high-consequence-idhc/)

Post AGP fallow time (PAGPFT)

Time is required after an AGP is performed to allow the aerosols still circulating to be removed/diluted. This is referred to as post AGP fallow time (PAGPFT) and is a function of the room ventilation air change rate.
The post aerosol generating procedure fallow time (PAGPFT) calculations are detailed in appendix 16 (/appendices/appendix-16-aerosol-generating-procedures-agps-and-post-agp-fallow-time-pagpft/). It is often difficult to calculate air changes in areas that have natural ventilation only.
If the area has zero air changes and no natural ventilation, then AGPs should not be undertaken in this area.
The duration of AGP is also required to calculate the PAGPFT and clinical staff are therefore reminded to note the start time of an AGP. It is presumed that the longer the AGP, the more aerosols are produced and therefore require a longer dilution time. During the PAGPFT staff should not enter this room without FFP3 masks. Other residents, other than the resident on which the AGP was undertaken, should not enter the room until the PAGPFT has elapsed and the surrounding area has been cleaned appropriately. As a minimum, regardless of air changes per hour (ACH), a period of 10 minutes should pass before rooms can be cleaned. This is to allow for the large droplets to settle. Staff should not enter rooms in which AGPs have been performed without airborne precautions for a minimum of 10 minutes from completion of AGP.
Airborne precautions may also be required for a further extended period of time based on the duration of the AGP and the number of air changes. Cleaning can be carried out after 10 minutes regardless of the extended time for airborne PPE.
Contact your local HPT/IPCT if further advice is required.

Read the RPE (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/transmission-based-precautions-literature-review-respiratory-protective-equipment-rpe/) literature review to find out more about why we do things this way for respiratory protective equipment.

Use of FFP3 respirators

- Where staff have concerns, they may choose to wear an FFP3 respirator rather than a fluid-resistant surgical mask (FRSM) when providing patient care, provided they are fit tested. This is a personal PPE risk assessment.

5. Infection prevention and control during care of the deceased

If a resident dies when in the care home SICPs and TBPs should still be applied. This is due to the ongoing risk of infectious transmission via contact although the risk is usually lower than for the living.
It is important that information on the infection status of the deceased is sought and communicated at each stage of handling and risk assessments performed.
Viewing, washing and/or dressing of the deceased see Appendix 12 - Application of infection prevention precautions in the deceased (http://www.nipcm.hps.scot.nhs.uk/appendices/appendix-12-mandatory-application-of-transmission-based-precautions-to-key-infections-in-the-deceased/) for guidance on the precautions required and what is permitted for certain types of infections.
Staff should advise relatives of the appropriate precautions to be taken when viewing and/or having physical contact with the deceased, including when this should be avoided.

Read the **infection prevention and control during care of the deceased** (http://www.nipcm.hps.scot.nhs.uk/web-resources-container/transmission-based-precautions-literature-review-infection-prevention-and-control-during-care-of-the-deceased/) literature review to find out more about why we do things this way when dealing with the deceased.

Care home appendices

These appendices from the NIPCM can be used in care homes.
Appendix 1 - How to hand wash (/appendices/appendix-1-how-to-hand-wash/)
Appendix 2 - How to hand rub (/appendices/appendix-2-how-to-hand-rub/)
Appendix 5 - Glove use and selection (/appendices/appendix-5-gloves-use-and-selection/)
Appendix 6 - Putting on and removing PPE (/appendices/appendix-6-putting-on-and-removing-ppe/)
Appendix 7 - Decontamination of reusable non-invasive care equipment (/appendices/appendix-7-decontamination-of-reusable-non-invasive-care-equipment/)
Appendix 8 - Management of linen at care level (/appendices/appendix-8-management-of-linen-at-care-level/)
Appendix 9 - Management of blood and body fluid spillages (/appendices/appendix-9-management-of-blood-and-body-fluid-spillages/)
Appendix 10 - Management of occupational exposure incidents (/appendices/appendix-10-management-of-occupational-exposure-incidents/)
Appendix 11 - Aide memoire for patient placement considerations and respiratory protective equipment (RPE) or fluid resistant surgical facemasks (FRSMs) for infectious agents (/appendices/appendix-11-aide-memoire-for-patient-placement-considerations-and-respiratory-protective-equipment-rpe-or-fluid-resistant-surgical-facemasks-frsms-for-infectious-agents/)
Appendix 12 - Application of infection control precautions in the deceased (/appendices/appendix-12-application-of-infection-control-precautions-in-the-deceased/)
Appendix 15 - Selection of personal protective equipment (PPE) by health and care workers (HCWs) during the provision of care (/appendices/appendix-15-selection-of-personal-protective-equipment-ppe-by-health-and-care-workers-hcws-during-the-provision-of-care/)

Appendix 16 - Aerosol generating procedures (AGPs) and post AGP fallow times (PAGPFT) (/appendices/appendix-16-aerosol-generating-procedures-agps-and-post-agp-fallow-time-pagpft/)
Appendix 17 - Hierarchy of controls (/appendices/appendix-17-hierarchy-of-controls/)

Care home resources

The resources section can be used as supporting tools for the Care Home Infection Prevention and Control Manual (CH IPCM).

- Aerosol Generating Procedures
  - Poster on PPE to wear when undertaking aerosol generating procedures (/resources/aerosol-generating-procedures-agps/)
- Care Home Infection Prevention and Control Resource Toolkit, (v3.0 April 2025) (/media/2441/2025-04-23-care-home-ipc-tookit-v30.pdf)
- HAI Compendium (/resources/hai-compendium/)
- Hand hygiene
  - How to hand rub (/appendices/appendix-2-best-practice-how-to-hand-rub/)
  - How to hand wash (/appendices/appendix-1-best-practice-how-to-hand-wash/)
  - 4 moments for hand hygiene (/media/1444/who-4-moments-residential-care.pdf)
  - Washroom poster (/media/1472/germs-have-you-washed-your-hands-of-them.pdf)
  - Information leaflet (/resources/hand-hygiene-wash-your-hands-of-them/hand-hygiene-information-leaflets-for-the-public/) for the public
- Gastro-intestinal illness
  - Care home IPC resource for gastro-intestinal illness (/resources/care-home-ipc-resource-for-gastro-intestinal-illness/)
- Information leaflets.
  These leaflets are also available in different languages National Infection Prevention and Control Manual: Information Leaflets - language translations (scot.nhs.uk) (/resources/information-leaflets-language-translations/)
  - Clostridiodes difficile (CDI) - Information for people who are getting care, their visitors, and anyone else who is in a care setting (/media/2373/2024-11-29-cdi-trifold-print.pdf)
  - Group A Streptoccocal infection - information for patients (/media/1767/1_igas-english.pdf)
  - Healthcare associated infection (HAI) - Information for the public (/media/1769/1_hai-leaflet-easyread-english.pdf)
  - Vancomycin-resistant enterococci VRE - Information for patients (/media/1760/1_vre-patient-leaflet-final-190708.pdf)
  - Washing clothes at home - Information for people in hospitals or care homes and their relatives (/media/1758/1_washing-clothes-home-english.pdf)
- Respiratory hygiene
  - Catch it, bin it, kill it poster (/resources/respiratory-hygiene-catch-it-bin-it-kill-it/)
- Respiratory illness
  - Care home IPC resource for respiratory illness (/resources/care-home-ipc-resource-for-respiratory-illness/)

- Scabies: Back to Basics Presentation - 23 October 2024 (/resources/scabies-back-to-basics-presentation-23-october-2024/)
- Transmission Based Precautions
  - Airborne precautions poster (/media/1778/2022-1-13-tbp-ic-airborne-poster_jan22.pdf)
  - Contact precautions poster (/media/1779/2022-1-13-tbp-ic-contact-posters_jan22.pdf)
  - Droplet precautions poster (/media/1780/2022-1-13-tbp-ic-droplet-posters_jan22.pdf)
- Waste Management: Back-to-Basics webinar - 12 December 2024 (/resources/care-home-ipc-waste-management-back-to-basics-webinar-12-december-2024/)
- World Hand Hygiene Day 5 May 2025 - It might be gloves. It's always hand hygiene (/resources/campaigns/hand-hygiene-day/world-hand-hygiene-day-5-may-2025-it-might-be-gloves-its-always-hand-hygiene/)

How to contact us

If you have any questions or feedback about the Care Home IPCM then you can contact us by email or telephone.
Email (https://www.nipcm.scot.nhs.uk/about/contact-us/)
Telephone: 0141 300 1175

References

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SCOTTISH HOSPITALS INQUIRY

Review of the Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen

Elizabeth University Hospital and Royal Hospital for Children,

Glasgow written by Professor PM Hawkey, Dr SG Agrawal and Dr LN Drumright ('HAD' Report)

Expert Report prepared for the Scottish Hospitals Inquiry

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1 Executive Summary

1.1 This review is based on the data made available to NSS and does not seek to replicate the analyses undertaken by Dr Drumright. The raw and final datasets and additional information (**A52617217 – Bundle 44, Vol 2, Document 1, page 12 - Prof Peter Hawkey, Dr Lydia Drumright & Dr Samir Agrawal - Draft Joint Response to HAD Questionnaire** and **A52609154 - Bundle 44, Vol 2, Document 46, page 706 - SHI Source Identification and Supporting Workings Request Dated April 11, 2025**) provided were helpful context in assessing the epidemiological aspects of the report. However, a number of the analytical methods and calculations are not clearly defined and there is not sufficient information to fully assess the analytical work undertaken.

1.2 The epidemiological analyses of bloodstream infections do not account for some of the nuances of environmental/water-related outbreak epidemiology including polymicrobial episodes of infection or poly-clonal and/or poly-strain outbreaks. Furthermore, the authors rely heavily on unvalidated outbreak definitions. This limits the ability to fully assess the potential for an environmental source for the infections.

1.3 In the paediatric population, the authors have not acknowledged a potentially important trend in bloodstream infection (BSI) infection rates after the move to Royal Hospital for Children (RHC) nor the impact of mitigations or controls implemented. In Section 7.2.10 (page 119), the authors note “policies and procedures for patient management over time could be decreasing incidence of bacteraemia attributable to environmentally relevant microorganisms” but have not considered whether this may have been related to the environmental controls implemented at the time.

1.4 The strength of the conclusion made by the authors in Section 8.1.5 (page 130) in relation to the *Aspergillus* spp. ventilation analyses that the “observed number of cases and variations over time do not show evidence of the built environment increasing infection rates” does not reflect the limitations of the data and the analyses undertaken. The limitations and caveats have not been fully acknowledged by the authors. There is also a period where there appears to be a potentially higher number of cases in the paediatric population following the move to RHC and, whilst the numbers are small to draw conclusions, this was not acknowledged by the authors in their commentary.

1.5 In conclusion, this review has identified significant epidemiological limitations and caveats that have not been fully acknowledged by the authors. In relation to whether there is evidence of “widespread contamination of the water system”, the authors conclude in Section 7.2.10 (page 119) that “taken together, these data provide little to no evidence for environment sources of bacteraemia in patients at QEUH”. The analysis

assessing whether there is evidence of an “increased rate of airborne infections in QEUH consistent with a failure of the ventilation system” concludes in Section 8.1.5 (page 130) “for both the paediatric and adult services, the observed number of cases and variations over time do not show evidence of the built environment increasing infection rates”. The strength of these conclusions has not been made in the context of the methodological issues, limitations and caveats of the data as described in this review. Furthermore, there are potentially signals in the paediatric population data that appear to have been overlooked by the authors, and in the absence of their consideration, their conclusions made cannot be regarded as fully supported by the evidence presented.

2 Introduction and Background

2.1 This review has been prepared to assist the Inquiry in its considerations of the NHS Greater Glasgow and Clyde (NHSGGC) commissioned ‘Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen Elizabeth University Hospital and Royal Hospital for Children, Glasgow’ (**A49396252 – Bundle 44, Volume 1 – Documents in respect of NHS GGC – Expert Report by Prof Peter Hawkey, Dr Samir Agrawal and Dr Lydia Drumright – The evidence of risk of infection from water and ventilation systems at QEUH and RHC – 24 July 2024 (“HAD Report”)**). The HAD report was admitted into evidence following a Procedural Hearing on 11 March 2025.

2.2 The preparation of this review has been supported by an expert statistician from the University of Strathclyde (Dr Kimberley Kavanagh) and the multi-disciplinary team within ARHAI Scotland. Dr Kavanagh did not have access to the data and supported this work by answering specific queries regarding the statistical methods described in the report.

2.3 The focus of this review is on the epidemiological aspects of the HAD report as described in Chapter 7 and 8. It does not seek to comment on content relating to the microbiological aspects (other than those associated with epidemiological interpretation) including water testing results and whole genome sequencing. The NHSGGC authored water testing reports and whole genome sequencing (WGS) reports were therefore out of scope of this review.

2.4 There were several issues that limited the scope of this review:

2.4.1 There was a significant delay in receiving the final Chapter 7 datasets from Dr Drumright. These were received on 7 May 2025 so there was limited time to consider these files in any detail.

2.4.2 There remained a lack of detail on the calculations undertaken by Dr Drumright. The details of the underlying arithmetic/working provided to the Inquiry were not comprehensive and appeared to be responses to questions posed by the Inquiry team. A plan of the analysis undertaken and, ideally, outputs from statistical packages including the code used would have improved the transparency of the report and greatly assisted the NSS review.

2.4.3 There is follow up information from Dr Drumright outstanding. Dr Drumright indicated that the clustering analysis was being reviewed and the supplementary data were pending. NSS has not had sight of any further supplementary information.

2.4.4 Chapter 8 was produced by Dr Agrawal and the final dataset and calculations for the *Aspergillus* spp. analysis have not been made available to NSS. This made a comprehensive assessment of Chapter 8 challenging and, therefore this review is high-level only. Review of Chapter 7: 'Water - analysis of infection rates and data from QEUH & RHC'

3 Review of Chapter 7: 'Water - analysis of infection rates and data from QEUH & RHC'

3.1 Inclusion criteria and definitions

3.1.1 There were some challenges in the interpretation of the report as no summary information relating to the included patients and infections was provided. The lack of descriptive information about the total number of admissions, bed days and patients make the report difficult to interpret. A high-level summary of the underlying data would have improved transparency and aided interpretation. Furthermore, there was no description of the demographics of cases e.g. age, sex, diagnosis. It is best practice to provide this important contextual information in any epidemiological report to aid those considering the findings.

3.1.2 The report lacks summary information in relation to the infections and microbiological data included in the report. The lack of detail regarding the number of episodes of infection, the distribution of the microorganisms identified and whether there were polymicrobial episodes of infection also limits the ability to interpret the report. The report predominantly provides data graphically or presented in line lists of cases of infection at patient level. Tables 5A to 8 (adult patients) and Tables 11A to 14 (paediatric patients) are essentially the underlying data for report. The authors have not provided a high-level summary of the distribution of microorganisms identified in the patient populations or in the sub-populations of interest e.g. Yorkhill Hospital/RHC and

QEUH/Other Hospitals. This makes it difficult to interpret the overall epidemiological picture within the populations. Such a distribution provides an opportunity to consider the variety of microorganisms causing infection and the presence of polymicrobial episodes of infection. These are useful indicators given the likely heterogeneity of the microorganisms isolated when there is an environmental source of infection.

3.1.3 A review of the microorganisms that were included across various reports previously considered by the Inquiry indicated that there were some minor differences in the microorganism lists. This may be explained in part by the differing time periods included in the reports as the lists of microorganisms are based on those identified during the period. In the additional information provided by Dr Drumright (**A52609154 – Bundle 44, Vol 2, Document 46, page 706 - SHI Source Identification and Supporting Workings Request Dated April 11, 2025**), it was noted that "organisms of environmental concern are not identified through calculation, these are based on biological knowledge and expertise and can be confirmed by thorough literature search and review of biology texts". Notably, the authors have included *Mycobacterium* spp. in the list of microorganisms that are not usually associated with an environmental source though no reference is provided to evidence this in the literature review undertaken by Professor Hawkey. Non-tuberculous Mycobacteria have been implicated in healthcare outbreaks related to water sources so justification for their exclusion from the environmental microorganism list is required.

3.1.4 The case definition includes microorganisms grown from blood culture (Section 7.2.1, page 65). However, Cryptococcus neoformans is often detected in blood using a serology/antigen test rather than by culturing the microorganism and this will have resulted in an under-estimate of the number of Cryptococcus neoformans cases included in the report.

3.1.5 The authors refer to microorganisms that have a potential environmental source in several ways when reporting their analyses. For the purpose of this review, we have assumed that the following all refer to the same microorganism group in the analysis: 'could be environmentally transmitted' (Figure 5-6), 'environmental concern' (Table 5A-C, Table 11A-F), 'environmental significance' (Figure 7-8), 'environmentally relevant' (Figure 9-10, 13-16, 19, 22), 'potential environmental concern' (Figure 18), 'relevance to environmental survival and transmission' (Figure 20).

3.1.6 The authors describe the episode case definition in Section 7.2.2 (page 67) as a "new case if the patient had not had a positive detection for that organism within the prior 14 days". This would suggest that the case definition is aligned with that used by ARHAI

Scotland where the 14-day episode period is applied to the last positive blood culture in any series of blood cultures **(A32938402 - Bundle 19, paper 9, page 53 – ARHAI Scotland - Protocol for National Enhanced Surveillance of Bacteraemia)**. However, a review of the data provided by Dr Drumright indicates that this was not the case and the 14-day period was applied to the first positive blood culture. The inconsistency between the description of the definition and the final datafile makes interpretation of the data challenging.

3.2 Numerators and denominators

3.2.1 The description of adult and paediatric populations described in the HAD report is not well defined. Some clarification was provided in the questionnaire where the authors noted the dataset requested was described as “paediatric oncology patients and adult haemato-oncology patients” **(A52617217 – Bundle 44, Vol 2, Document 1, page 14 - Prof Peter Hawkey, Dr Lydia Drumright & Dr Samir Agrawal - Draft Joint Response to HAD Questionnaire 1 Question 1Ae**). Further understanding was developed by review of the datafiles provided though there remain aspects that are not well defined. This prevents a clear understanding of the patient population and activity included in their analyses. Specific issues relating to this are described in paragraphs 17 to 22.

3.2.2 The choice of a bed days denominator is appropriate for both adult and paediatric populations due to the differential lengths of stay in these patients. The authors suggest in Section 7.2.1 (page 64) that bed days are deemed a better denominator for healthcare associated infection (HCAI) incidence as they also account for “percentage bed occupancy”. The authors suggest that higher bed days equate to higher person density on the ward. High person density is a recognised risk for transmission of HCAI however aggregated bed days do not provide a measure of this. This requires an understanding of the capacity, availability of single rooms and size/floor plate of the unit. Whilst bed days are a better denominator for this population, person density is not an appropriate justification for this choice.

3.2.3 Bed days provide a measure of time at risk and patients with longer lengths of stay contribute more to the denominator/time at risk. It is not defined in the report whether day case attendance activities have been accounted for in the denominator. However, additional information provided by the authors indicated that “the dataset consists of all inpatients and day case admissions with a length of stay greater than zero” for adult and paediatric patients (**A51838352 – Bundle 44, Volume 1, Document 1, page 5 – Documents in respect of NHS GGC – Expert Report by Prof Peter Hawkey, Dr**

Samir Agrawal and Dr Lydia Drumright – The evidence of risk of infection from water and ventilation systems at QEUH and RHC – 24 July 2024 ("HAD Report")). This would suggest that the denominator does not account for the contribution of day cases, where patients can spend time at risk in hospital receiving treatment. Such day cases are relevant and ideally should be included in the denominator. Where this has not been possible, this limitation should have been acknowledged by the authors as the denominator will underestimate the time at risk.

3.2.4 Review of adult population denominators:

3.2.4.1 The authors have stated in the additional information provided by Dr Drumright **(A52609154 - Bundle 44, Vol 2, Document 46, page 706 - SHI Source Identification and Supporting Workings Request Dated April 11, 2025**) that the adult denominator data was extracted by NHSGGC based on the consultant of the patient and mapped to the Sector in which the consultant is based.

3.2.4.2 The bed days include all admissions where the patient's consultant is a haemato-oncology consultant, irrespective of where the patient was admitted to and cared for. This means that the Sector bed days include bed days for care delivered outside of haemato-oncology specialty wards and across NHSGGC.

3.2.4.3 The allocation of bed days to QEUH and Other Hospitals used Sector and date ranges noted by the authors (Section 7.2.2, page 69) as "South sector became QEUH in May 2015 and remained part of QEUH through 2023 where data provided stops; BMT became part of QEUH for a period of approximately one and a half months across the months of June and July in 2015 and then from June 2018 onward". Review of the datafiles provided indicated that this method results in QEUH bed days including bed days from Other Hospitals and vice versa. Furthermore, the QEUH bed days include bed days for other specialties in the hospital.

3.2.4.4 The impact of this limitation cannot be determined as the bed days within an inpatient stay are calculated as a whole and do not take into account transfers within NHSGGC during a continuous stay. The NHSGGC raw datafile included admissions which did not start in a haemato-oncology ward and others that did not end a haemato-oncology ward. Bed days in haemato-oncology wards cannot be distinguished from those in other locations.

3.2.4.5 The choice of denominator, which does not define the patient cohort by location of care and includes locations within and outside of haemato-oncology services, may limit the conclusions that can be drawn about a water system that is located in the QEUH estate.

3.2.4.6 The inclusion of non-haemato-oncology bed days in the denominator is an important point of clarity as this increases the denominator and will result in a lower rate of infections than if only haemato-oncology bed days were included.

3.2.4.7 On review of the data provided by the authors for Figure 4 (Section 7.2.3, page 73), there appears to be some discrepancy with the Other Hospitals denominator. It is unclear why the North Sector and Bone Marrow Transplant (BMT) bed days were not included in the Other Hospital denominator for Quarter 1 2013 to Quarter 2 2014 and the North Sector bed days were not included in Quarter 3 2014. The authors do not provide an explanation for this change from the definition of Other Hospital bed days. This exclusion of bed days may result in an over-estimated rate of infections in the early part of the time period and may impact on any comparisons with other time periods or when fitting linear trend lines.

3.2.4.8 The authors note in the report that they had identified points during the time period when there were an "uncharacteristically low number of bed days" and noted it was not clear if the low number was an error or real (Section 7.2.3, page 69). The analyses were carried out using adjusted bed days for the months noted to be low. The authors did not appear to consider or adjust for months when the bed days were uncharacteristically high e.g. in South Sector during July 2019 when the bed days were more than double other months in the year. This may have resulted in a lower rate for some of the months with an uncharacteristically high number of bed day and may impact on the linear trend analyses.

3.2.5 Review of adult numerator data

3.2.5.1 The review of the adult BSI numerator data was more difficult with some inconsistencies between the report and the data provided. As with the bed days, the BSI have been assigned to Sector based on the consultant's Sector (rather than location of where the blood culture was obtained). The report states in Section 7.2.2 (page 69) that "for adult patients, data were examined both by consultant sector, BMT, North and South, and by months and years in which consultant section was assigned to either the QEUH or all other locations". This implies that the same method of attribution from Sector to the QEUH and Other Hospitals categories was applied to BSI. This does not appear to have universally been the case. For example, there are BSI categorised as BMT consultant Sector during 2016 and 2017 that have been assigned to QEUH. However, according to the methods in Section 7.2.2 (page 69) of the report, BMT consultant Sector BSI would be attributed to Other Hospitals as "BMT became part of QEUH for a period of approximately one and a half months across the months of June and July in 2015 and then from June 2018 onward".

3.2.5.2 On review of the data, the majority of BMT BSI in 2016 and 2017 had a location of 'General OP QEUH' recorded which may suggest that the authors have mapped the location of where the blood culture was taken to QEUH or Other Hospitals rather than using consultant Sector information.

3.2.5.3 This difference in the way the denominator (consultant Sector irrespective of location) and cases (based on location) have been defined at hospital level has implications for interpretation of the rates. Not all cases that could arise from the population (defined by the Sector denominator) will have been included in the BSI numerator (defined by location) and may artificially reduce the rate in some analyses.

3.2.6 Review of paediatric denominator data

3.2.6.1 Based on the review of the raw denominator datafile from NHSGGC, the admission data appears to have been extracted based on the patients' specialty. This was confirmed in follow up information provided by the authors (**A51838352 – Bundle 44, Volume 1 – Documents in respect of NHS GGC – Expert Report by Prof Peter Hawkey, Dr Samir Agrawal and Dr Lydia Drumright – The evidence of risk of infection from water and ventilation systems at QEUH and RHC – 24 July 2024 ("HAD Report")**). Similar to the adult denominator data, there are admissions that did not start in a haemato-oncology ward and some that did not end there when patients were transferred to other specialties. All of the admissions started and ended in RHC so bed days from other hospitals are not included.

3.2.6.2 As with the adult denominator, the inclusion of non-haemato-oncology bed days in the denominator is an important point of clarity. The rate described is the rate of BSI in patients under the care of a haemato-oncology specialty and is not based on the location of the patient. The inclusion of bed days from other locations will increase the denominator compared with e.g. the denominator used in the HPS report that used haemato-oncology bed days. This may potentially result in an increased denominator in the HAD report and therefore a reduced rate of infection than if only haemato-oncology bed days were included.

3.2.7 Review of paediatric numerator data

3.2.7.1 The criteria for extraction and identification of paediatric BSI is not clear and the raw dataset from NHSGGC indicates that several criteria were used to identify cases though this is not described fully by the authors in the report.

3.3 General analytical and statistical methods

3.3.1 The small number of cases included in many of these analyses, particularly at month level and in the BMT specific analysis (Section 7.2.6, page 92), limits the interpretation. Statistics such as p-values and confidence intervals are used routinely in epidemiology to account for the inherent randomness in data, the effects of which are greater the smaller the amount of data available. When small numbers are included in analyses, it is more difficult to determine whether the result has been obtained through chance.

3.3.2 The usefulness of statistics when the numbers are so small can be limited and it appears that the authors have in places, by necessity, described the data rather than undertaking any formal testing. This makes it challenging to draw conclusions about signals in data outside of normal variation. This applies to spikes in cases that have been identified from review of the graphs and appear to have been described rather than formally tested. However, language such as 'significant' has been used without evidence of statistical testing of significance (Section 7.2.3, page 74; Section 7.2.6, page 85). It would be helpful for the authors to acknowledge when their assessment is descriptive only.

3.3.3 For analyses where statistical testing has been undertaken, it would be best practice to include the outputs from the tests to support the statements being made about the data. Further reporting of p-values and confidence intervals would have made the analysis more transparent to readers and enabled an assessment on whether the results were significant.

3.3.4 The authors refer to rates being higher/lower over long periods of time where the conclusions drawn are unlikely to be true over the entire time period. As an example, the authors have noted the near equal distribution of *Acinetobacter* spp. across QEUH and Other Hospitals (Section 7.2.3, page 75). This is based on a small number of cases (n=28) over an extended period of time (July 2013 to August 2023). We would suggest that the number of cases is too small to draw any conclusions from this statement.

3.3.5 In Tables 10 (adults, page 85) and 16 (paediatrics, page 114), the authors have compared incidence rates of BSI in QEUH versus Other Hospitals and in QEUH/RHC versus Yorkhill Hospital, respectively. These comparisons are unadjusted and do not take account of differences between the comparators and any confounding that may result. This is a limitation that should have been acknowledged by the authors.

3.3.6 The authors have used regression models to fit linear trend lines to the incidence rates over time and compared the linear trends between QEUH and Other Hospitals (adults)

and QEUH/RHC versus Yorkhill Hospital (paediatrics). A number of methodological issues were identified:

3.3.7 Whilst fitting a line over a long period may be statistically acceptable, important points or signals in the data within the time period can be masked. In any linear trend analysis, clinical/epidemiological interpretation is required when deciding whether a longer-term linear trend describes the time period adequately.

3.3.8 If there is a need to assess shorter term trends, more than one line can be fitted and if there is a period of interest, fitting a line over a shorter period of time may be helpful to interpret the trend.

3.3.9 In the paediatric analyses, the linear trend line presented in Figure 22 (page 118) does not adequately describe the changing rates of infection during the overall time period and has possibly masked a signal in the data. The authors did not consider the patterns in the data immediately following the move to RHC and the period where the incidence of infection appeared to remain higher (untested) until later in 2019. These issues are discussed in paragraphs 38 to 41.

3.3.10 The authors have not provided any outputs or coefficients from the models to assist interpretation and enable full assessment of the results. We are unable to assess how well the line has fit the data.

3.3.11 Whilst no issues with the selection of the models or statistical regression modelling techniques used by the authors were identified, the other issues relating to the data underlying the models restrict the conclusions that can be drawn.

3.4 Clustering analysis methods

3.4.1 In Section 7.2.2 (page 67), the authors describe a method to identify “potential epidemiological” clusters of two or more cases of a single species of microorganism that had “potential to be environmentally transmitted” using four different categories of clusters based on location and time between cases. The authors have not provided a reference to support the use and validity of this method including the criteria for defining a cluster. This does not appear to be a recognised method for identifying outbreaks in healthcare particularly outbreaks with a potentially environmental source. Nor do the definitions align with the ARHAI Scotland National Infection Prevention and Control Manual (**A49799834 – Bundle 27, Volume 4 – Miscellaneous Documents - National Infection Prevention and Control Manual**). These guidance definitions are evidence based and recognised across the UK.

3.4.2 The cluster analysis undertaken in this report is based on a restrictive definition of a cluster that does not reflect the often polymicrobial and/or poly-strain epidemiology of outbreaks with an environmental source. The analysis is based on a single species cluster and the authors have not acknowledged the limitations of a definition restricted to one species. Evidence provided by Professor Mark Wilcox (**A50851931 - Transcript – Professor Mark Wilcox – 29.10.2024 – transcript column 125**) during the Glasgow III Hearings described the complexity of microbiological aspects of outbreaks with an environmental source. Traditional epidemiological methods and definitions require to be tailored to ensure that this complexity is reflected in analyses. Clusters of any microorganism in the environmental microorganism group and a description of heterogeneity of the clusters would have provided additional information to assess the possibility of an environmental source. The responses provided in the questionnaire response (**A52617217 – Bundle 44, Vol 2, Document 1, page 30 - Prof Peter Hawkey, Dr Lydia Drumright & Dr Samir Agrawal - Draft Joint Response to HAD Questionnaire 1 Question H17**) noted that Professor Hawkey would provide further evidence from the literature though NSS have not been sighted in this.

3.4.3 The cluster analysis presented in the report is based on a single ward location where the sample was taken. This does not take account of whether the patient had been exposed to a different location prior to the sample being taken. Outbreak/cluster investigations in healthcare require a wider approach due to movement of patients within the healthcare system. To fully assess location as a risk for developing a BSI with a potentially environmental source, an epidemiological timeline is required for each patient to describe their admission history and identify links in time and place. This review should also consider recent discharges and re-admissions. HCAI epidemiological case definitions, including the ARHAI Scotland Protocol for National Enhanced Surveillance of Bacteraemia, often include criteria to account for recent discharge from hospital (**A32938402 - Bundle 19, paper 9, page 53 – ARHAI Scotland - Protocol for National Enhanced Surveillance of Bacteraemia**). This is a time-consuming exercise and likely not possible for the authors of this report. However, an acknowledgement of this limitation is necessary, and the strength of any conclusions should have been drawn in this context. As Professor Mike Stevens described in his evidence (**A50866602 - Transcript – Professor Mike Stevens – 30.10.2024 – transcript column 59**), the Case Note Review developed detailed timelines for each patient enabling them to undertake a fuller assessment of each patient's journey in the healthcare system.

3.4.4 The authors have selected to use the incidence of clusters of two or more cases to assess the likelihood of environmental contamination. A single case of some of the unusual microorganisms identified may give an indication of an environmental source and would require investigation. A focus on clusters without consideration for single cases of some of the rarer microorganisms alongside a lack of information about how many of the patients had polymicrobial episodes of infection limits the interpretation in the context of environmental source outbreak epidemiology.

3.4.5 The methods for the cluster analysis are not described in the report nor were they provided in the follow up information from Dr Drumright. It is not clear how the date parameters in the definitions described in Table 3 (page 68) have been applied to identify clusters. There are outstanding questions: was the exact date of the blood culture used or was this based solely on the calendar month? If so, was the first blood culture in an episode used or all subsequent? What is the specific definition of a month e.g. 28 days or calendar month? In addition, there is a possible error with an overlapping time period in the 'possibly cluster' and 'unlikely to cluster' categories. It is not clear how cases in different wards 2 months apart would be categorised as both definitions include 2 months ('unlikely to cluster' if >= 2 months, 'possibly cluster' if <=2 months).

3.4.6 The follow up information from Dr Drumright indicated that the clustering analysis was being reviewed and the supplementary data were pending. NSS has not had sight of any follow up information. This makes assessing the results from the cluster analysis difficult.

3.4.7 The authors have not provided evidence for the methodology used for the development of a weighted summary score described in Section 7.2.2 (page 68). It is unclear if this is a validated method for assessing the impact of clustering. The authors have not provided justification for the weighting of scores and they seem to be arbitrary which limits the interpretation. Furthermore, the denominator issues noted previously will impact on the summary score rates that have been calculated and compared across QEUH/Other Hospitals and RHC/Yorkhill Hospital.

3.4.8 None of the datafiles provided by Dr Drumright include a variable that identifies the cluster status of the cases (and note that the supplementary data table is yet to be provided). It was possible to create a datafile from the tables included in the report (Tables 5A-8 for adults and Table 11A-14 for paediatrics). There is limited utility in using this datafile to assess the cluster analysis as there were several inconsistencies

between the data in the report and the final datafile provided by Dr Drumright as noted below:

3.4.9 For the adult population, the report includes 278 BSI cases of “environmental concern” listed in Tables 5A-8. The final dataset provided includes 446 BSI cases. A number of reasons for the discrepancies have been identified though there remain outstanding inconsistencies (that may have been explainable with additional information on methods from the authors).

3.4.10 The datafile from Dr Drumright includes 43 *Rothia* spp. categorised as environmental BSI. These are not included in the environmental list in Table 1 (page 65) of the report so it is assumed these were categorised as environmental in error and were excluded from the tables in the report.

3.4.11 There are 108 *Klebsiella pneumoniae* in the final datafile from Dr Drumright. These are not included in Tables 5A-8 and no explanation is given by the authors regarding their exclusion. It is also not possible to determine if these BSI were excluded from the weighted cluster summary measure.

3.4.12 There is an error in the calculation of the summary score in Table 10 (page 85). Application of the weighting as described in the methods should have resulted in a score of 175 for QEUH but it appears that a weighting of 2 has been applied to the 22 probable clustering BSI rather than 3 resulting in an underestimate of the score by 22.

3.4.13 For the paediatric population, the report includes 479 cases of BSI whereas the datafile includes 499 environmental BSI.

3.4.14 The datafile includes 7 Rothia mucilaginosa, which are not included in the environmental list in Table 1 (page 65), presumably in error.

3.4.15 Unlike the adult population, 85 Klebsiella pneumoniae cases have been included in the report and are described in Table 13 (page 108).

3.4.16 There are multiple minor differences between the tables presented in the report and the final datafile provided by Dr Drumright. The raw datafile from NHSGGC was not cross checked with Dr Drumright’s final datafile so it is not possible to describe if there are any discrepancies between the files.

3.4.17 On Page 114, the authors discuss the higher number of cases and number of cases that cluster in Table 16. It is not appropriate to compare the absolute number of cases as the time periods included are different (125 months in Yorkhill Hospital and 91 months in QEUH).

3.5 Review of Paediatric incidence analyses

3.5.1 The incidence of environmental BSI in the paediatric population is described in Section 7.2.8 (page 95) where the authors note that “incidence starts off at QEUH similar to that observed for the prior 10 years at Yorkhill, but then steadily declines from ~2018 onward”. They do not acknowledge the immediate decrease in rate for 6 months following the move to RHC. A decrease in infections with a potential environmental source may be expected when moving from an older estate to a newly built hospital. The rate of BSI with microorganisms with “no environmental relevance” did not decrease following the move providing further evidence that there may have initially been an effect on the rate of environmental BSI. This also supports that the decrease observed in the environmental microorganisms is not a result of a data artefact as it is assumed this would have occurred across all of BSI data. These observations are based on visual inspection of the data rather than formal statistical testing.

3.5.2 The authors do not consider or describe what appears to be a period of increased incidence from approximately June 2016 to November 2019. Before and after this time, the rates had fluctuated with multiple months with a rate of zero. During the period of increased incidence, there were no months where the rate returned to zero.

3.5.3 The authors note a decline in the environmental microorganism BSI rate from 2018 onwards though do not refer to any of the environmental controls or mitigations e.g. installation of filters, cleaning of drains and decanting of patients from affected wards as a hypothesis for this decrease.

3.5.4 As previously noted, the duration of time over which the linear trend lines are fitted can mask important trends within the time period. The linear trend line reported in Figure 22 (page 118) describes a significant reduction in environmentally relevant BSI though does not account for variation in rate over time or the implementation of mitigations and controls in the intervening period. A time series analysis that considers the rates of BSI in two segments after the move to RHC may have assisted in interpretation of differing trends including the period where the incidence of BSI appears to be higher. The rates post-move to RHC could have been segmented following the implementation of mitigations/controls. This would have provided some evidence of the effect of these on the rates and better described the changing trend over the time period.

3.5.5 An important caveat that must be acknowledged when making any comparisons between the rates of BSI in Yorkhill Hospital and RHC is that the built environments in the estates will be different. It cannot be assumed that the environment and the risk of

infection from an environmental source therein, will be equivalent in an old estate versus a state of the art new build hospital.

3.5.6 As HPS previously produced the Review of NHSGGC paediatric haemato-oncology data, there is an opportunity to consider some of the signals in the HPS data (**A43940545 - Bundle 7 – Written Reports Prepared by Health Protection Scotland (HPS), Health Facilities Scotland (HFS) and Antimicrobial Resistance and Healthcare Associated Infection (ARHAI)**). There are some considerations in relation to the different methods applied:

3.5.7 There are differences in the case definitions applied. The "environmental-enteric" group in the HPS report is better aligned with the environmental microorganism list in the HAD report as it includes *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Pantoea* spp., *Serratia* spp. though there remain some differences in the microorganisms included.

3.5.8 There are differences in the denominator used in the HPS report which includes haemato-oncology admissions and the HAD report which includes some bed days out with haemato-oncology specialties and wards.

3.5.9 The HPS report included a statistical process control (SPC) chart (Figure 6) describing the incidence of a the environmental-enteric group of microorganisms deduplicated at group level rather than at single species level (**A43940545 - Bundle 7 – Written Reports Prepared by Health Protection Scotland (HPS), Health Facilities Scotland (HFS) and Antimicrobial Resistance and Healthcare Associated Infection (ARHAI)**). This definition was applied to account for patients with polymicrobial infections being repeatedly counted in the case numbers. Of interest to the Inquiry is the higher rate of infections reported in the HAD report in May 2018 (Figure 18, page 95). On review, the HAD data and HPS data have the same number of single species BSI (n=10) but the HPS graph reports the number of episodes of BSI from any environmental-enteric microorganism. This reduced the number of episodes to 5 as there was one patient with a polymicrobial episode of BSI with 5 different microorganisms isolated.

3.5.10 Figure 6 in the HPS report describes the rate of environmental-enteric BSI and the rates, whilst noting the above differences, follow a similar pattern to that of the HAD report (Figure 22, page 118). There is an initial decline in the rate of infection followed by an increase. This period of increased incidence was not identified as outside of normal variation though caution is required due to the aforementioned issues regarding

small numbers and the limitations of a baseline calculated using Yorkhill Hospital infection rates.

4 Review of Chapter 8: 'Ventilation - analysis of infection rates and data from QEUH & RHC

4.1 The final data and the underlying arithmetic/workings for the *Aspergillus* spp. analysis were not available to NSS therefore a fully comprehensive review has not been possible.

4.2 The definition of an episode of infection is described by the de-deduplication section in 8.1: (page 124) "where the same patient had multiple positive tests for the same admission episode, this was classified as one case". As the final datafile was not available, it was not possible to assess how the de-duplication was applied nor how the laboratory results were linked to admission data. Case definition de-duplication criteria would usually have a date parameter e.g. 14 days for repeat blood cultures. This definition implies that a patient can have an episode of infection on each admission (if tested) irrespective of the time between samples. A description of the total number of patients and cases/episodes of infection would have assisted with interpretation to understand whether there were patients with multiple episodes and the impact this may have on the conclusion.

4.3 Section 8.1 (page 123) describes that cases were identified based on "either a haematology/haemato-oncology consultant or location". There is no further detail on how the location criterion has been applied and whether cases identified by samples obtained outside of haemato-oncology wards are included. This would present a similar issue to that identified in the review of Chapter 7 and may impact on the conclusions that can be drawn about a ventilation system located in the QEUH estate. Additionally, the location information is based on where the sample was taken and does not account for any prior admissions or exposure to the hospital environment. Prior exposure history is particularly important when considering the links between *Aspergillus* spp. infections and the environment due to the long incubation period which can vary widely from days to months.

4.4 The *Aspergillus* spp. analyses do not include a denominator that accounts for activity. This is likely to be important particularly during the period where services were moving between locations and where data are presented at ward level. The authors have not provided any justification for the lack of denominator in the analysis. Based on the earlier review of bed days data for Chapter 7, ward level bed days were not available to the authors as the total bed days for each continuous inpatient stay was provided

irrespective of whether a patient was transferred between wards. Therefore, bed days could not be allocated to individual wards. The lack of denominator in the analyses should have been acknowledged by the authors as a limitation.

4.5 The number of *Aspergillus* spp. cases are small in these analyses. As discussed previously, a small number of cases and the effects of random variation can impact on the ability to draw strong conclusions about the data. The authors do attempt to describe whether the number of cases has changed over time and have made some descriptive comparisons (Sections 8.1.1-8.1.4, pages 124-130). Whilst a small number of cases can make epidemiological analyses more challenging, there are other methods that can be used to assess potential links to the environment. Root cause analyses can be used to assess each patient and explore the possibility of links. An important aspect of such an exercise would be consideration of a timeline that describes location information over a longer period rather than a point in time where a sample was taken. This was the approach taken in the Case Note Review (A50866602 - Transcript – Professor Mike Stevens – 30.10.2024 – transcript column 23).

4.6 Review of adult data

4.6.1 The authors include a comparison of data from the adult Haemato-Oncology Unit at St Bartholomew's Hospital, London in Section 8.1.2 (page 126). There is insufficient information about the populations, *Aspergillus* spp. testing and case definitions to determine whether this is an appropriate comparison.

4.6.2 In Section 8.1.2 (page 126), the authors conclude "there is no indication of increased cases" in the "Adult BMT service" and the "Adult Haematology South service" including after the permanent move of these services to QEUH and that the case numbers in the "Adult Haematology North" service indicate "expected variation over time". The limitations of the analyses and caveats have not been fully acknowledged in the strength of this conclusion.

4.7 Review of paediatric data

4.7.1 The authors describe Figure 24 in Section 8.1.3 (page 128) and note "the number of cases with evidence of infection with A. spp. in the paediatric haematology and oncology service following the move to RHC in 2015 varied from 1 to 7 per annum from 2016- 2022, as compared to 0 to 5 from 2005-2014". Whilst not possible to do a formal statistical test and no denominator has been used, there is period with a potentially higher number of cases that is not acknowledged by the authors. The authors have focused on the range of case numbers pre- and post-move to RHC but do not note

that following the move there were 5 months between 2016 and 2020 where there were 4 or more cases identified. This is in contrast to before the move when there was only one month with 4 or more cases. Whilst it is not possible to draw a strong conclusion based on this data, it is worth acknowledging whilst considering the question relating to the ventilation system.

4.7.2 In Section 8.1.4 (page 130), the authors conclude that the analyses show low level variation that is expected over time. The limitations of the analyses and caveats have not been fully acknowledged in drawing this conclusion nor has the potential period where there was a higher number of cases.

From: Lydia N Drumright
To: [redacted]
Cc: [redacted]; [redacted]
Subject: Re: Request for Calculations/Workings - Re HAD Report
Date: 18 April 2025 05:26:49
Attachments: image001.png
image002.png
image003.png
image004.png

Dear [redacted],

This will take a lot more time than I could devote so far. I will be working on it over the weekend and will update you again on Sunday.

I am aware of the need to work rapidly, but at the same time these are very big requests with little notice.

Following up on our discussion regarding individual level data. I am not comfortable assuming that the novation agreement covers this. I would kindly request that you or someone from your firm contact the CLO so that they can give me authorization to release individual level data in a documented and transparent communication.

Thank you so much.

Kind regards,

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Scopus Author ID: 7801514223

From: [redacted]
Sent: Friday, April 11, 2025 7:52 AM
To: Lydia N Drumright [redacted]
Cc: [redacted]; [redacted]
Subject: Request for Calculations/Workings - Re HAD Report

Dear Dr Drumright,

I hope this message finds you well.

Following a detailed review of Chapter 7 of the HAD report, our expert, Mr Sid Mookerjee, has compiled a set of queries and clarifications regarding the data sources, tables, and methodologies referenced throughout the chapter. The Inquiry seeks clarity on several key points, including the exact underlying data tables used in many of the figures, the spreadsheet sources, and the methodologies for deriving specific findings.

While we appreciate that you may not be best placed to respond to every query, please feel free to indicate where another expert (Dr Agrawal or Prof Hawkey) would be better positioned to answer, and kindly name the expert where appropriate.

In order to assist us in fully understanding and evaluating the findings, we would be grateful if you could review the questions below and provide the relevant documentation by **Wednesday 16 April**, if at all possible, and by **Friday 18 April** at the latest:

Source Identification and Supporting Workings

For the following pages, could you please confirm the exact spreadsheet or data source used (including the period the dataset covers), and provide the working spreadsheets, tables, and any relevant methodological details:

1. **Page 60** - Data mentioned in relation to QEUH and comparable hospitals.
2. **Page 62** –
    - Organism detection data differentiated by patient ID.
    - Data used to calculate Bed Days per month (including the period that it covers).
    - Working spreadsheet(s) that demonstrate the process used to define a new case (14-day rule), monthly case counts, environmental organism classification, and calculation of incidence rates per 1,000 bed days.
3. **Page 64 -**
    - Historical (2005 – May 2015) vs. current paediatric hospital data (June 2015 – 2022) - (Yorkhill vs. RHC).
    - Data used in Figures 1 and 2.
4. **Page 68 –** Data used in Figure 4, please also clarify if it is fair to assume that references to ‘other hospitals’ prior to page 69, do indeed refer, and always refer to the combined data from Beatson and SGH?

5. **Page 65 –**If available, please also provide the tables and workings for Figures 4–22.

6. **Pages 64–69**
    - Data source(s) for references to “other hospitals in NHS GGC” and related figures.

Terminology Clarifications

- Page 60: Please provide an explanation of what the 'ticks' and 'crosses' along the Adult and Paediatric columns in Table 1 and 2, in order to clarify the intent.
- Pages 64–69: Please confirm whether "other hospitals in NHS GGC" consistently refers to Beatson and SGH across the report.

Figure Labels and Consistency

- Page 65: Could you confirm whether Figures 1 through 22 represent *incidence rates* (cases per activity/time point) as opposed to *incidence* (cases per time point)?
- As many figures currently lack data labels, the provision of numerical tables would significantly aid in replicating and verifying your findings.

If the data you have drawn upon appears in the documents contained within **Bundle 44 Vol 1**, please refer to them as **Bundle X and Page Y**. If the sources are different or are not clearly identifiable within that bundle, we would be grateful if you could supply the specific spreadsheet(s), workings, and documentation as requested.

We understand this is a substantial request and appreciate your time. As noted above, please feel free to direct any specific queries to the relevant expert where appropriate. Additionally, we may seek to arrange a short **Teams consultation** in around two weeks' time, if needed, to follow up on any points arising from your response.

Please let us know your availability in advance and also if you would like to discuss any of the above further.

We also look forward to receiving your completed questionnaires tomorrow.

Many thanks & regards,

[redacted]

[redacted]
Legal Support Officer | Scottish Hospitals Inquiry

email: [redacted] **website:** www.hospitalsinquiry.scot
@ScotHospInquiry | Scottish Hospitals Facebook | Scottish Hospitals Inquiry

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NHS National Services Scotland

Environmental Pathogens Surveillance Pilot Report

January 2025

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Introduction

The National Infection Prevention and Control Manual (NIPCM) - Appendix 13 specifies a nationally agreed minimum (non-exhaustive) list of environmental bacteria alert organisms for high-risk units and patient cohorts to enable timely and adequate alert response and investigation.[1] In a September 2024 update, additional organisms were added to this list, along with signposting to the water section of NIPCM Chapter 4, which was launched in July 2024. However, there is currently no national advice to support boards to develop systems to pro-actively detect and monitor for infections caused by environmental bacteria in high-risk units in NHS Scotland hospitals.

ARHAI Scotland were tasked with scoping the feasibility of a surveillance system for environmental pathogens, to assist NHS boards with identification and monitoring of organisms which may have an environmental source. A scoping exercise, using Electronic Communication of Surveillance in Scotland (ECOSS) data, concluded that it would be feasible to use the positive microbiology results data from ECOSS to undertake surveillance of environmental pathogens isolated from clinical samples at the ward/unit level, acknowledging a number of limitations (**Appendix 1.** Data Limitations and Caveats). This activity was completed in FY 2023/24 and recommended that the proof-of-concept surveillance system be piloted in real-time with NHS board(s) to evaluate the attributes of the system and further refine suitable triggers for early investigation in high-risk settings.

Aims and Objectives of Pilot

The aim of the pilot was to further scope the feasibility of an environmental pathogen surveillance system for use in NHS Scotland high-risk units.

Objectives of pilot:

- To identify suitable triggers for further investigation, for high-risk units and patient cohorts.
- To assess the environmental pathogens surveillance system against the key attributes of a surveillance system.

- To further refine the environmental pathogens surveillance system as required.

Development of Methodology

Proposed surveillance system

Data processing

R scripts were developed for all data extraction and processing (version 4.0.2; R Core Team 2020). Data were extracted from ECOSS using an Open Database Connectivity (ODBC) connection to the ECOSS live database. All available final positive microbiology results (Status 50) were extracted from ECOSS, from December 2016 to date of report generation. Unidentified organisms, viruses, and those from veterinary and agricultural laboratories, were excluded. Organisms were categorised as either ‘environmental’ (those considered to be exogenous and not typically part of normal human flora, and may be acquired from an environmental reservoir), ‘environmental enteric’ (those considered to be endogenous and part of the normal human gut flora but may also be acquired from an environmental reservoir) or ‘non-environmental’, according to a reference list compiled by ARHAI Scotland. The reference list classifies and groups organisms in a number of ways, including by genus, type, Gram stain category, environmental source/habitat, and with tags for key antimicrobial resistance markers. Any novel organisms in an extract were flagged during data processing and investigated and added to the reference list. The reference list was maintained by ARHAI and validated by consultant microbiologist for the duration of the pilot.

All environmental and environmental enteric bacteria were included in the surveillance and predominantly focused on bacteria associated with water sources. A summary table of the bacteria included in these categories is provide in **Appendix 2.** Organism categories Fungi were excluded from surveillance due to issues with the reliability of reporting of these results into ECOSS.

Results may be submitted to ECOSS from both NHS board diagnostic laboratories and national reference laboratories. Therefore, where possible, results were matched to allow details of the specimen type and location from the diagnostic laboratory result to be combined with details

of the organism species and antimicrobial resistance from the national reference laboratory result.

ECOSS specimen type groupings were used to identify clinical microbiology results for inclusion in analysis, and include: Normally Sterile Site; Lower Respiratory; Upper Respiratory; Wound; Urine; Faeces; Alimentary (excl. Faeces); Genital; Superficial; Unspecified non-viral (re-categorised as Other/Not Known). Blood cultures were distinguished from the other normally sterile sites to allow identification of bacteraemia episodes. Sputum and cough swab results were distinguished from the other lower respiratory tract specimens such as bronchoalveolar lavage and endotracheal aspirates for analysis since sputum and cough swabs will be more likely to include contaminants. The Other/Not known category were results where no specimen site was stated, or the specimen site was unclear.

Results were deduplicated at the 'patient', 'patient-species', and 'patient-species-specimen type' level, according to the episode definitions below.

- ***Patient level episodes:*** a patient with any environmental or environmental enteric bacteria isolated from any specimen type, who has not had any environmental or environmental enteric bacteria isolated from any specimen type within the previous 30 days.
    - Additional patient level episode definitions, including only environmental or environmental enteric bacteria, or only specific specimen types were also used, to allow for maximum flexibility in the data visualisation dashboards.

- ***'Patient-species' level episodes:***
    - Episode of bacteraemia: a patient with any specified bacteria species isolated from a blood culture, who has not had the same bacterial species isolated from a blood culture within the previous 14 days.
    - Episode of infection or colonisation: a patient with any specified bacteria species isolated from any specimen type, who has not had the same bacterial species isolated from any specimen type within the previous 30 days.

- ***'Patient-species-specimen type' level episodes:*** a patient with any specified bacteria species isolated from a specified specimen type group, who has not had the same

bacterial species isolated from the same specimen type group within the set specimen group deduplication period:

- Alimentary (excl. Faeces): 30 days
- Blood: 14 days
- Faeces: 30 days
- Genital: 30 days
- Lower Respiratory (excl. Sputum and Cough Swab): 30 days
- Normally Sterile Site (excl. Blood): 14 days
- Other/Not Known: 30 days
- Other Respiratory: 30 days
- Sputum and Cough Swab: 30 days
- Superficial: 30 days
- Upper Respiratory: 30 days
- Urine: 30 days
- Wound: 30 days

Note: when using ‘patient-species’ level episodes or ‘patient-species-specimen type’ level episodes, a patient may have results for different bacterial species or isolated from different specimen types during the same time period, therefore an individual patient may contribute more than one episode.

The hospital and unit of each episode was determined by the location of the patient at the time of specimen collection for the first result of the episode. The location code in ECOSS, as provided by the laboratory submitting the result to ECOSS, was used to identify the specific wards/units involved in the pilot. Any episodes where the first positive result was not recorded as taken in pilot unit were excluded from analysis since the episode started before the patient was exposed to that environment.

Triggers Reports

Triggers reports were developed in R markdown with the aim of providing early triggers for local investigation of a potential issue in the built environment. The candidate triggers were adapted from those proposed by Anthony *et al* (2013).[2] Environmental incidents can be complex given the environmental source may contain multiple different organisms.[3,4,5,6,7] For

example, environmental organisms can form biofilms in hospital water systems, which support the existence of a diverse range of environmental organisms.[8] This can lead to polymicrobial infections in patients and polymicrobial outbreaks.[3,9] Triggers to identify multiple episodes of different organism species were therefore included, rather than only identifying multiple episodes of the same organism species. Additionally, the majority of triggers include episodes of both infection and colonisation, as opposed to focusing on only sterile site isolates, in order to provide more sensitivity in detection to allow earlier opportunity for intervention. Finally, to align with Appendix 13 of the NIPCM, a trigger to identify any of the listed environmental alert bacteria was included.[1]

Note: At the time of the pilot, Appendix 13 listed *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia* or *Serratia marcescens*, as minimum alert organisms, and these were incorporated into an Appendix 13 specific trigger. However, in September 2024, this list was updated to also include *Chryseomonas indologenes*, *Cupriavidus pauculus*, all *Serratia* spp., and *Sphingomonas spp.,* and to link to NIPCM Chapter 4, which was launched in July 2024.

Candidate triggers are listed below.

A. Environmental Bacteria:

1. A single episode of bacteraemia with an environmental bacterial species.
2. A single episode of infection or colonisation with *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia* or *Serratia marcescens* (environmental alert bacteria as per Appendix 13 of the NIPCM before September 2024 update).[1]
3. Two or more episodes of infection or colonisation in a two-week period with the same environmental bacteria.
4. Three or more episodes of infection or colonisation in a two-week period with any environmental bacteria.

B. Environmental Enteric Bacteria:

5. A single episode of infection or colonisation with an extended-spectrum beta-lactamase (ESBL) producer, carbapenemase-producing Enterobacterales (CPE) or vancomycin-resistant Enterococcus (VRE).
6. Two or more episodes of bacteraemia in a two-week period with any environmental enteric bacteria excluding *Escherichia coli*.

C. Environmental and Environmental Enteric Bacteria:

7. Three or more episodes of infection or colonisation in a two-week period with any environmental or environmental enteric bacteria excluding *Escherichia coli*, unspeciated Coliform species, vancomycin-sensitive *Enterococcus faecalis* and vancomycin-sensitive *Enterococcus* species.

Results are deduplicated at the 'patient-species' level according to the episode definitions for an episode of bacteraemia and an episode of infection or colonisation described in the definitions above. It is important to note that an individual patient may have results for different bacterial species during the same time-period, and therefore may contribute more than one episode. Consequently, a trigger may be met due to multiple episodes from the same patient. An anonymised patient ID is included in trigger data so that the number of unique patients contributing to a trigger can be identified. It should also be noted that the same patient episode may contribute to more than one trigger.

Triggers reports included all triggers identified from the unit in the past year, with any triggers identified in the past month highlighted.

Data Visualisation Dashboards

Data visualisation dashboards for each pilot unit were developed in Tableau (version 2023.1) to enable further exploration of the available clinical microbiology data from ECOSS at the 'patient', 'patient-species' and 'patient-species-specimen type' level. Dashboards included epidemic curves and T-charts, displaying data from January 2020. Data filters could be applied to select subsets of the data, for example particular species or specimen types. Epidemic curves plot the number of episodes per month and allow the identification and tracking of outbreaks over time. T-charts are a type of statistical process control (SPC) chart most appropriate for rare events. T-charts plot each episode along the x-axis and the time between

episodes along the y-axis, meaning changes in the frequency of episodes can be visualised. T-charts and epidemic curves do not adjust for changes in underlying patient population or activity on the unit but provide a visual measure of the frequency and distribution of events occurring over time. These dashboards were demonstrated to the pilot sites alongside the triggers reports to further support interpretation of the underlying data in the triggers.

The methodology needs to be considered along with the caveats and limitations outlined in **Appendix 1.** Data Limitations and Caveats.

Recruitment to pilot

ARHAI Scotland aimed to recruit three pilot wards/units treating high-risk patient cohorts. A call for volunteers was communicated through the Data & Intelligence Oversight & Advisory Group. Three NHS boards were offered the opportunity to participate in the pilot. Two of the NHS boards (Board A and Board B) had asked for ARHAI Scotland support with potential environmental linked incidents and the pilot was agreed as provision of epidemiological support.

The three pilot sites from these two NHS boards consisted of two neonatal units (NNU) and one adult intensive care unit (ICU), as shown in **Table** 1**Table 1**.

Table *1:* Participating units and pilot periods

NHS Board	Unit type	Pilot period
Board A	Neonatal Unit	February to June 2024
Board B	Adult Intensive Care Unit	February to August 2024
Board B	Neonatal Unit	April to August 2024
Board C	Neonatal Unit	NA – system demonstration received but declined to take part in pilot

A third NHS board (Board C) received a demonstration of the system but declined to take part in the pilot as they did not feel that the methods proposed would enhance their existing surveillance systems and noted reservations about the methods being piloted. ARHAI provided the board with a written response highlighting that the purpose of the pilot was to take their feedback in a structured way to support refinement of the methods. As ARHAI were not able

to further explore their feedback in the pilot, the information provided when Board C declined to participate has not been included in this pilot report.

Pilot process

Throughout the pilot, ARHAI Scotland met monthly with key stakeholders from the infection prevention and control teams (IPCTs) for each pilot unit from Board A and Board B to review and discuss outputs from the surveillance system. Monthly meetings were agreed with key stakeholders to be the most appropriate frequency to balance timely sharing of data against resource requirements.

During meetings, ARHAI Scotland shared surveillance system data on the pilot using the triggers reports and dashboards, including interactive epidemic curves, timelines and T-charts. Monthly triggers reports, developed and generated by ARHAI Scotland, were emailed to the agreed key stakeholders, in the form of HTML files so that they could review these further and consider any triggers identified. Given the level of complexity in the dashboards, these were only shared during meetings with ARHAI Scotland, to minimise the risk of misinterpretation of the data included. Feedback on the surveillance system received during meetings was used to further develop and refine the data processing, triggers reports and dashboards.

Participating sites were asked to continue to report as per Chapter 3 of the NIPCM.

Feedback and evaluation

Following the pilot, ARHAI Scotland sent a feedback questionnaire (**Appendix 3**Appendix 3. ARHAI Scotland Environmental Pathogens Surveillance Survey 2024) to the key stakeholders from the IPCTs who had been involved in the pilot for each pilot unit. The questionnaire was intended to assess the usefulness of the triggers reports and the appropriateness of the candidate triggers included. ARHAI Scotland further evaluated the surveillance system against the priority attributes of a surveillance system.

A comparison was undertaken between the triggers reports and incidents including potential environmental bacteria submitted to the Outbreak Reporting Tool (ORT) from the pilot units between January and August 2024. January to August 2024 was chosen to cover the full pilot

periods of both Board A and Board B, plus one month prior, to reflect the focus in the triggers reports on data from the past month.

Assessment and Evaluation

Summary of questionnaire responses

A total of three responses were received from Board A and Board B, with at least one response from each. Responses provided additional information on usefulness, efficiency and acceptability of the triggers in identifying potential environmental incidents for further investigation.

Surveillance system attributes

This section summarises the evaluation of the proposed surveillance system against the priority attributes of a surveillance system.

Usefulness

The triggers reports were broadly accepted by representatives from Boards A and B as useful for detecting trends and areas of concern for action, with one respondent highlighting the appeal of the visual nature of the trigger reports in combination with the dashboard. In contrast, one respondent indicated that although reports were interesting from an academic perspective, they were not providing any new information that the current incident management team (IMT) monitoring environmental incidents in the high risk-unit were not already aware of. This likely reflects the already heightened awareness due to an ongoing incident.

Where respondents were asked about the relevance of each individual trigger when applied to either an ICU or NNU, all triggers were deemed appropriate for both scenarios. The trigger concerning a single episode of bacteraemia with an environmental bacterial species merited further comment, with one respondent indicating that although notification of these incidents was of benefit for monitoring, a rating system to identify the more serious environmental bacteria species could help separate the triggers into more and less urgent.

It was additionally noted that although the trigger concerning a single episode of infection or colonisation with *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas*

maltophilia or *Serratia marcescens* was relevant for both high-risk settings, it may not always be easy to determine whether triggers for *Pseudomonas aeruginosa* in adults were arising due to infections, or colonisations. Due to the difficulty in defining colonisations in adults, a filter was added to remove all *Pseudomonas aeruginosa* specimens from the dashboard so these could be excluded to aid in interpretation.

Efficiency

The proposed environmental pathogens surveillance system employs passive surveillance using existing clinical specimens captured in ECOSS, compared to more resource-intense active data collection. Positive clinical microbiology results are obtained from ECOSS and a preprepared script is run to output triggers reports and dashboard. The process is currently adapted to the unit level and suited for use in the limited number of units participating in the pilot. As location data in ECOSS is not consistently recorded, detailed mapping of unit level data in ECOSS was required. When asked whether any existing local infection prevention and control (IPC) surveillance systems available in their NHS board could be used to identify the triggers included in the report, all respondents from Boards A and B indicated that it would be possible. However, two respondents indicated that this would require additional time and resource for implementation and continuous monitoring. As the triggers reports need to be manually updated and checked by ARHAI, this process would not be efficient if scaled up to include more units and is not a solution for providing all high-risk units in Scotland with timely triggers reports.

Sensitivity

Between January and August 2024, a total of 60 triggers were identified across the three pilot units, including a total of 77 unique patients. During this time period, 14 ORT incidents with potential environmental bacteria were submitted across the pilot units, with a total of 32 patient cases.

Although a one-to-one match with triggers and outbreak reports in the ORT was not expected due to differences in reporting criteria, the majority of ORT incidents could be matched to at least one trigger. There were 13 incidents that could be associated with triggers, with only one ORT incident involving a single patient infection with an enteric environmental organism in a NNU that did not meet the criteria for any of the triggers but met ORT (NIPCM Chapter 3) reporting criteria. As patients included in triggers reports are anonymised and no patient

identifiable information is submitted with the ORT, it was not possible to definitively match specific episodes to incidents reported within the ORT.

There were several triggers which did not result in an ORT submission. The ORT is designed for the reporting of healthcare infection incidents and outbreaks in line with Chapter 3 of the NIPCM, and the minimum list of alert organisms in Appendix 13. There may be reasons why triggers identified in this pilot were not reported via the ORT, including that they did not meet the requirements for reporting as described in Chapter 3 of the NIPCM. For example, where investigation by local IPCTs has determined that an incident is not healthcare-associated to the unit/hospital e.g. identified within 48 hours of admission or mother-to-neonate transmission. In addition, the organism list included in Appendix 13 and Chapter 4 is not exhaustive, and many potentially environmental bacteria, such as *Paenibacillus* spp. and *Roseomonas* spp., are not named though were included in the environmental organism list in this pilot (**Appendix 2**). Incidents involving organisms included in Appendix 13 are more likely to be reported than those that are not. The retrospective matching of the 60 triggers to ORT incidents was complex due to patients featuring across multiple triggers (not mutually exclusive); organisms featuring across multiple triggers (not mutually exclusive); and multiple/mixed organism ORT incidents that were open for extended periods of time and where the dates the organisms were isolated were not available to match (this information is not required or consistently provided in the ORT submission).

Overall, the proposed environmental pathogens surveillance system was sensitive in detecting potential environmental organism related incidents as reported via the ORT. However, further work is required to understand the links between the triggers and Chapter 3 reporting.

Timeliness

For data included in the pilot wards/units there was a median of five days lag between specimen collection and data being received in ECOSS. This lag in availability of confirmed results impacts on the timeliness of identification of and response to triggers.

On discussion with the pilot wards/units, monthly sharing of data was considered the most appropriate frequency for the pilot. This balanced the resource demands of producing and reviewing the pilot data and meeting ARHAI to discuss the results.

For triggers to be useful to identify potential areas for investigation, they must be timely. A lag of 4-5 weeks does not support timely investigation though this could be reduced if triggers could be reproduced using NHS boards' local surveillance systems rather than the use of national datasets.

Representativeness

All episodes of environmental and environmental enteric bacteria reported to ECOSS from the pilot wards/units were included in the surveillance system, and so it can be considered to be representative for these wards/units. However, as detailed in **Appendix 1.** Data Limitations and Caveats, there are a number of data considerations that limit representativeness. Reference laboratory specimens do not always include details of the original ward/unit location of the specimen; if these could not be matched with diagnostic laboratory specimens which detailed the location, they were excluded. Furthermore, due to limitations of ECOSS data, the pilot did not include fungi. Specimens taken from patients while they were not in the unit will not be included. As this is a laboratory-based surveillance system, no additional clinical information was collected including length of time of the patient in the unit prior to the sample being taken. It is also not possible to distinguish infection from colonisation or contamination, nor to distinguish community-associated from healthcare-associated episodes. The surveillance data is therefore not comparable with nosocomial data from other surveillance systems.

The environmental pathogens surveillance system is designed to be highly localised and for high-risk settings/patient cohorts, and is not intended to be representative of any wider patient population or higher level surveillance (i.e. board level). Triggers data cannot be used for benchmarking or comparisons with other high-risk units. No denominator data has been included in this surveillance, so no rates can be calculated, which further restricts comparisons. Variation in unit size, screening and testing practices, patient population, and unit environment (for example layout and staffing), all affect the data and comparability. In particular, triggers would likely need to be adapted if different patient cohorts were being investigated, even within the scope of high-risk units. Additionally, the boards included in the pilot may have altered their practice and vigilance during the pilot (e.g. by increasing swabbing in pilot high-risk units) due to perceived risk of environmental hazards.

Data quality

Environmental pathogen surveillance uses unvalidated ECOSS data as submitted by Scottish NHS laboratories and processed by the ECOSS Team. Between 2019 and 2020, five NHS boards went through the ECOSS Development Rollout Improvement Programme (EDRIP), aimed at improving the quality and completeness of data held within ECOSS. Activities related to this, along with the removal of filters in ECOSS between 2019 and 2022, have resulted in an increase in the number of reports recorded for organisms and specimen sites that have not historically been subject to national reporting (e.g. wound, sputum and cough swabs). This must be taken into consideration when interpreting results over time. Blood culture data in ECOSS are considered to be reliable, however reporting from other specimen types may be less complete, particularly from NHS boards that have not been through EDRIP. Some NHS boards are affected by issues with tagging of key antimicrobial resistance markers in ECOSS, which may affect the completeness of data captured by triggers 5 and 7 for these areas. Additionally, confirmation of carbapenemase-producers (included in trigger 5) is carried out by the reference laboratory, and therefore may be excluded from reports where location data cannot be obtained from a matched diagnostic laboratory specimen.

Positive results in ECOSS indicate the presence of the bacteria in the specimen, but it is not possible to differentiate colonisation or contamination from infection when using these data.

Differences in screening and testing practices over time and between wards/units will impact on the number of cases and organism species identified. Improvements in methods used to detect and identify organisms may contribute to apparent increases of particular species over time requiring specialist microbiological testing expertise to interpret. Therefore, data may not be comparable over longer time periods or between wards/units.

Reference laboratory reports do not always include unit location data, or specimen type details. Where possible, these reports were matched with original diagnostic laboratory reports to obtain this data. Where this was not possible, unmatched reports may result in unspeciated results being included in unit data, or reports being excluded from analyses focussing on specific specimen types.

Whilst ECOSS data can be used to effectively highlight triggers for further investigation, local interpretation and investigation is required to ascertain whether these triggers are incidents/outbreaks of concern and for artefactual changes to be ruled out e.g. changes to speciation or testing.

Further details regarding these data quality limitations can be found in **Appendix 1.** Data Limitations and Caveats

Acceptability

Key stakeholders from the IPCTs from both NHS boards involved in the pilot were willing to participate in the surveillance of environmental pathogens, with regards to reviewing the triggers reports shared with them and considering the triggers highlighted. Although overall positive feedback on the usefulness and sensitivity of the triggers reports was received from both Board A and Board B, two respondents raised concerns around the local surveillance resource required if considering identifying triggers using existing local IPC surveillance systems.

Limitations of Pilot

- The two NHS boards that took part in the pilot were approached to be part of the pilot after asking ARHAI Scotland for support with potential environmental linked incidents. As such, these NHS boards may not be representative of all NHS boards in their feedback to the pilot. Due to perceived risk of environmental hazards, these boards may have altered their practices resulting in a higher number of samples in ECOSS i.e. increased swabbing in high-risk units and changes to screening.
- An environmental pathogen isolated from a patient who is being treated within a unit does not necessarily indicate that this unit is the source of the organism and should only be used as a prompt for further investigation.
- The trigger definitions used have been adapted from those proposed by Anthony *et al* (2013) .[2] These triggers were developed for neonatal units, and so further adaptations may be required to ensure they are relevant to other high-risk settings.

- Changes in screening/testing practices and ward activity may impact on the types of organisms seen and number of episodes. This must be considered when comparing triggers data over time.
- This was a pilot at the local level and the triggers reports and dashboard are not suitable for larger scale surveillance including national surveillance. The current triggers are specific to the unit level, and T-charts used in the dashboard are designed for rare events.

Conclusion and Recommendations

The pilot included three units across two NHS boards, and seven candidate triggers for further investigation were developed. Feedback from boards was positive and suggested triggers were useful for detecting trends and areas of concern for action. The ORT incidents reported during the pilot were successfully identified in the triggers reports, with the exception of one incident. The links between the candidate triggers and Chapter 3 NIPCM reporting was more difficult to define in the pilot. Further work is required to describe the links and would support the review of Chapter 3 of the NIPCM. The triggers were designed for NNU and ITU settings and while sensitivity was suitable for the NNU it was suggested some of the triggers may be too sensitive for adult ITUs. Further work to refine the triggers is necessary including consideration for other types of high-risk units.

This phase of the pilot identified that there are some limitations with the availability of data in ECOSS and manual mapping is required to identify unit locations for reference laboratory results. Resource requirements of manual updates and checking required by ARHAI Scotland has indicated that this surveillance system could not be scaled up to include all high-risk units in Scotland. Furthermore, ARHAI provision of monthly trigger reports using ECOSS data is not timely enough to prompt local investigations. Theoretically, the triggers could be incorporated into existing IPC surveillance systems, however, concerns were raised around the local surveillance resource required to do this. The development of a future national IPC surveillance solution may support this in future if alert organism lists can be managed nationally.

The system developed has successfully been used to support boards with ongoing incidents with potential environmental source. This does limit the interpretation of the pilot as these areas already had heightened vigilance to these types of organisms. Future work to include units without ongoing incidents in the pilot is required to determine the feasibility of the system as a routine environmental organism surveillance system.

Finally, this surveillance system was designed to be highly localised, and the triggers reports and dashboards developed are not suitable as a national surveillance system or for benchmarking. National surveillance of priority environmental pathogens could be considered with stakeholder input as part of the national surveillance review. However, national surveillance would not be available to unit level.

In conclusion, further work is necessary to refine the triggers ensuring there is a balance between the resource implications of multiple triggers and associated investigations alongside identifying potential risk to patients in high-risk units.

ARHAI Scotland recommend the following:

- Continue with the pilot in 2025/26 to further refine the triggers ensuring setting-specific consideration.
- Recruit sites without ongoing incidents that are potentially linked to the environment to ensure triggers are developed in the context of ongoing surveillance.
- Consider the expansion of the pilot to include a wider range of high-risk settings (following completion of the development of ICU and NNU triggers).
- Further describe how the triggers identified are linked to Chapter 3 reporting, informing both the refinement of triggers and the development of Chapter 3.
- Work with NHS boards to explore how triggers could be reproduced using local surveillance systems such as ICNet.
- Ensure that flexible alert organism surveillance and Chapter 3 reporting requirements are included in the outline business case for the national IPC system.
- Consider national surveillance of environmental Gram-negative bacteraemia as part of the national surveillance review inclusive of board stakeholder engagement.
- Continue to make the triggers reports and dashboards available for outbreak support if required.

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Appendix 1. Data Limitations and Caveats

- Data are derived from the ECOSS database and as such are based on the data submitted by Scottish NHS laboratories. Not all results held within ECOSS are validated. Additionally, we are aware that data submissions are incomplete for some NHS boards and therefore cannot be certain that all relevant isolates have been captured in ECOSS.
- Improvements in methods used to detect and identify bacteria may contribute to apparent increases in incidence or prevalence of particular species over time. Laboratory diagnostic methods (e.g. MALDI-TOF, VITEK®) and subsequent reporting to ECOSS have changed over the years which may affect the numbers and types of organisms reported.
- Changes in screening practices in wards/units over time may affect the numbers and types of organisms reported into ECOSS. This is particularly relevant for results reported from specimen types commonly used for screening, such as rectal swabs.
- Activities related to the ECOSS Development Rollout Improvement Programme (EDRIP) and the removal of filters within ECOSS, which took place at different times between 2019 and 2022 for different NHS boards, have resulted in an increase in the number of reports recorded for organisms and specimen sites that have not historically been subject to national reporting (e.g. wound, sputum and cough swabs). This must be taken into consideration when interpreting results over time. Additionally, when interpreting results for NHS boards that have not been through EDRIP (AA, BR, FF, GJ, GGC, HG, LO and WI), it should be noted that reporting for specimen sites other than bloods may be incomplete. Reporting of blood specimens into ECOSS has not been significantly impacted by EDRIP or filter changes and is considered to be reliably reported for environmental bacteria for all NHS boards across time.
- Environmental fungi are not captured in this surveillance system as there are limitations around the reliability of fungal isolate reporting within ECOSS, with fewer results than expected received into ECOSS due to electronic data transfer issues between UK mycology reference laboratories and ECOSS.
- Positive results in ECOSS indicate the presence of bacteria in the specimen only. It is not possible to differentiate colonisation or contamination from infection when using

these data. Certain specimen types, for example sputum and faeces samples, are more likely to represent colonisation than other specimen types such as blood specimens.

- Ward location, where the patient was at time of specimen collection, has been determined from the requesting location or unit location raw fields in ECOSS. There may be inconsistencies in how wards are named, or changes in naming over time. Local knowledge of ward names may be needed to accurately identify ward locations.
- It is not possible to accurately identify the specialty of ward locations from the ECOSS data. ARHAI Scotland would need to work with NHS boards to reliably identify high-risk locations such as intensive care units and haemato-oncology wards.
- Reports from reference laboratories do not always include details of the original ward/unit location that the specimen was taken in, and instead may use the location details of the diagnostic laboratory submitting the specimen to the reference laboratory. Therefore reference laboratory reports cannot always be included in ward/unit data, or may be included in data for the incorrect ward/unit. Where possible, reference laboratory reports have been matched with the original diagnostic laboratory report to retain location information, however this is not possible for all reference laboratory reports. Consequently, ward/unit data may include unspeciated results, even where a subsequent reference laboratory result has identified the species of organism. This is particularly relevant to mycobacterium species.
- Results from reference laboratories do not always include specimen type details. Where possible, reference laboratory results have been matched with the original diagnostic laboratory result retain specimen type information, however this is not possible for all reference laboratory reports. Therefore, unmatched reference laboratory reports may be excluded from analyses focussing on specific specimen types such as blood cultures.
- ‘Patient – species’ level deduplication may count the same patient as an episode more than once if they have isolated multiple different species within the same time-period. Consequently, a trigger may be met due to multiple episodes from the same patient. Likewise, ‘patient – species – specimen type’ level episodes may count the same patient more than once if they have isolated multiple different species or have isolates from multiple different specimen type groups within the same time period. To estimate the

number of individual patients infected or colonised with environmental bacteria, the patient level episode data in the dashboards should be used.

Appendix 2. Organism categories

High-level classification of environmental and environmental enteric bacteria used for pilot.

Note that this list is not exhaustive. Unless species are listed individually, all species within the genus are included in the classification.

Environmental bacteria	Environmental enteric bacteria
Environmental bacteria are those considered to be exogenous and not typically part of normal human flora and may be acquired from an environmental reservoir. This includes, but is not limited to, organisms such as *Stenotrophomonas maltophilia*, *Serratia marsescens* and *Acinetobacter bauminii*.	**Environmental enteric bacteria** are those considered to be endogenous and part of the normal human gut flora, however, they may also be acquired from an environmental reservoir. This includes, but is not limited to, organisms such as *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Enterococcus faecium*.
Gram-negative *Achromobacter* species; *Acidovorax* species; *Acinetobacter* species; *Aeromonas* species; *Alcaligenes* species; *Anaeroarcus* species; *Aureimonas* species; *Brevundimonas* species; *Burkholderia* species; *Buttiauxella* species; *Cedecea* species; *Chromobacterium* species; *Chryseobacterium* species; *Chryseomonas* species; *Comamonas* species; *Cronobacter* species; *Cupriavidus* species; *Delftia* species; *Edwardsiella* species; *Elizabethkingia* species; *Erwinia* species; *Ewingella* species; *Flavobacterium* species; *Herbaspirillum* species; *Kalamiella* species; *Kosakonia* species; *Legionella* species; *Luteibacter* species; *Luteimonas* species; *Methylobacterium* species; *Myroides* species; *Ochrobactrum* species; *Olivibacter* species; *Pandoraea* species; *Photobacterium* species; *Providencia* species; *Pseudocitrobacter* species; *Pseudomonas* species;	***Gram-negative*** *Citrobacter* species; *Cloacibacillus* species; *Coliform* species; *Enterobacter* species; *Enterobacteriaceae* species; *Escherichia* species; *Hafnia* species; *Klebsiella* species; *Kluyvera* species; *Leclercia* species; *Lelliottia* species; *Morganella* species; *Pantoea* species; *Plesiomonas* species; *Proteus* species; *Shigella* species. ***Gram-positive*** *Enterococcus* species; *Eubacterium* species; *Levilactobacillus* species.

Pseudoxanthomonas species; *Psychrobacter* species; *Rahnella* species; *Ralstonia* species; *Raoultella* species; *Rhizobium* species; *Roseomonas* species; *Serratia* species; *Shewanella* species; *Sphingobacterium* species; *Sphingomonas* species; *Stenotrophomonas* species; *Variovorax* species; *Vibrio* species. ***Gram-positive*** *Alicyclobacillus* species; *Aneurinibacillus* species; *Arthrobacter* species; *Brachybacterium* species; *Brevibacillus* species; *Brevibacterium* species; *Carnobacterium* species; *Cellulosimicrobium* species; *Clavibacter* species; *Deinococcus* species; *Dietzia* species; *Erysipelothrix* species; *Exiguobacterium* species; *Gordonia* species; *Janibacter* species; *Leifsonia* species; *Lysinibacillus* species; *Microbacterium* species; *Niallia* species; *Nocardia* species; *Paenibacillus* species; *Patulibacter* species; *Priestia megaterium*; *Pseudoclavibacter* species; *Pseudoglutamicibacter* species; *Rhodococcus* species; *Solibacillus* species; *Sporosarcina* species; *Streptomyces* species; *Terrisporobacter* species; *Tsukamurella* species; *Vagococcus* species; *Virgibacillus* species. ***Gram-variable*** Non-Tuberculosis *Mycobacterium* species (NTM).	

Appendix 3. ARHAI Scotland Environmental Pathogens Surveillance Survey 2024

ARHAI Scotland have been tasked with scoping the feasibility of a surveillance system for environmental pathogens, to assist NHS boards with identification and monitoring of incidents/outbreaks which may have an environmental source. As part of this ask, ARHAI Scotland have undertaken a proof-of-concept pilot to test potential triggers using patients' clinical/screening results from laboratory data at unit level. Environmental pathogen trigger reports have been developed to identify and monitor key environmental pathogens in selected high-risk units in NHS Scotland hospitals.

As a pilot site, we would be grateful if you could please take the time to complete this short questionnaire to provide feedback on the usefulness of the environmental pathogen trigger reports and how they were used by your NHS board during this pilot.

NHS board: ……

Pilot unit: ……

Job role: ……..

1. **How useful have you found the triggers reports?**

2. **How have you used the triggers reports and who did you share them with?**

3. **In your opinion, are the environmental pathogen trigger criteria (as outlined in the table below) appropriate for the patient population in this high-risk unit?** *Please provide detail in the table below:*

Trigger criteria for further investigation	**Appropriate for NNU**	**Appropriate for ITU**	**Comments**
A single episode of bacteraemia with an environmental bacterial species.	Yes/No	Yes/No	
A single episode of infection or colonisation with *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia* or *Serratia*	Yes/No	Yes/No	

marcescens (environmental alert bacteria as per Appendix 13 of the National Infection Prevention and Control Manual).			
Two or more episodes of infection or colonisation in a two-week period with the same environmental bacteria.	Yes/No	Yes/No	
Three or more episodes of infection or colonisation in a two-week period with any environmental bacteria.	Yes/No	Yes/No	
A single episode of infection or colonisation with an ESBL producer, CPE or VRE.	Yes/No	Yes/No	
Two or more episodes of bacteraemia in a two-week period with any environmental enteric bacteria excluding *Escherichia coli*.	Yes/No	Yes/No	
Three or more episodes of infection or colonisation in a two-week period with any environmental or environmental enteric bacteria excluding *Escherichia coli*, unspeciated *Coliform* species, vancomycin-sensitive *Enterococcus faecalis* and vancomycin-sensitive *Enterococcus* species.	Yes/No	Yes/No	

4. **Are there any additional environmental pathogen trigger criteria that you would consider appropriate to initiate an investigation?**

   Please provide detail in box below:

5. **Could any existing local IPC surveillance systems you have in your NHS board (e.g. ICNet) be used to identify the triggers included in the report?** *Please provide detail in box below:*

6. **Is there anything that could further improve the triggers reports?**

   Please provide detail in box below:

SCOTTISH HOSPITALS INQUIRY

Review of the Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen

Elizabeth University Hospital and Royal Hospital for Children,

Glasgow written by Professor PM Hawkey, Dr SG Agrawal and Dr LN Drumright ('HAD' Report)

Expert Report prepared for the Scottish Hospitals Inquiry

Date: 16 May 2025

Dr Sara Mumford, MB. BS., MSc., FRCPath., SFFMLM.

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1. Summary

1.1. This report has been prepared at the request of the Scottish Hospitals Inquiry and reviews the report of Professor Hawkey and colleagues (herein referred to as the HAD Report), on its methodology and conclusions from a Microbiology and IPC perspective.

1.2. The Inquiry has asked a series of questions in order to assist in this review and has asked that two further documents, *Initial bullet point thoughts on executive summary for report.docx* and *Glasgow Hospitals Case Note Review D3 - notes and commentary on environmental bacteria.docx* are also considered.

1.3. This report does not address the aspergillus infections seen at QEUH and RHC. This subject is dealt with separately through a joint report with Sid Mookerjee.

2. Executive summary and Introduction

2.1. The issues in the water system at QEUH/RHC which would cause the system to become microbiologically unsafe have been well documented[1] [2]. From the time of occupation in 2015, *Legionella sp* counts in the water were out of specification and microbial contamination was able to spread through the system and form biofilms due to poor temperature control. The first recognised infection with an environmental gram-negative, *Cupriavidus pauculus* BSI in a paediatric haemato-oncology patient, which had a confirmed link to the water system occurred in 2016. By 2018/19 it was known that there was extensive microbial contamination and biofilm formation in water tanks, expansion vessels, tap fillings, showerheads and drain components. The extensive biofilm meant that where remedial works were undertaken at the outlets (replacement of taps and pipework, silver hydrogen peroxide dosing to pipes and drains, etc), recontamination occurred within a month.

2.2. Biofilms are complex structures in which microorganisms (single or multiple species) are encased in extracellular polymeric substances. As they develop they become more difficult to remove and the bacteria within them are protected from the action of antimicrobials. Physical interactions, co-adhesion and metabolic cooperation amongst bacterial cells promote multispecies biofilms. As the biofilms mature they release the bacteria held within, into the surrounding environment, which can then spread through the water system and settle elsewhere creating new biofilm. On reaching a certain thickness, parts of a biofilm may break off and settle in other parts of the system.

[1] Bundle 21, Volume 1, Paper 5, page 180.
[2] Bundle 21, Volume 1, Paper 6, page 354.

2.3. The risk that this level of contamination presented to the Schiehallion cohort of patients is complex to quantify. In relative terms, the paediatric haemato-oncology patients are the most vulnerable patients in a hospital, by the severity of their disease, the profound nature of their immunosuppression, their length of stay in hospital and the frequent courses of antimicrobials which may destroy the natural defences of their microbiota. The additional avoidable risk associated with a contaminated water and drainage system which could contaminate the environment through aerosol and droplet/splash spread combined with insufficient ventilation with low air changes, lack of filtration and potential recirculation of contaminated air through the use of thermal wheels has contributed in great part to the high number of blood stream infections with environmental organisms seen in this cohort of patients. Therefore, I would assess the risk of infection prior to the extensive remedial work on 2A/2B in individual patients as a high probability of occurring combined with a potentially catastrophic outcome.

2.4. The HAD Report fails to consider any of the earlier reports in relation to the environmental infection risk or other available information and reports based on the Case Note Review only.

2.5. The outbreak definition proposed in the paper is at odds with established IPC practice in the UK. The primary approach of any potential outbreak investigation is to protect patients in a timely manner.

2.6. The conclusions drawn by the authors do not consider the environmental challenges, preferring to speculate based on experience elsewhere such as the assumption that only 20% of cases are related to the environment in any way.

2.7. The authors also take a view on carbapenem usage which is at odds with the data presented to them.

2.8. The statistical analysis will be reviewed in detail in a separate paper by Sid Mookerjee. However, I do not agree with the approach taken, particularly the comparison of heterogenous patient groups and to conclude that there is no increase in infection at QEUH and not considering the remedial work undertaken to avoid an increased environmental infection rate.

2.9. For the Schiehallion cohort the authors have failed to follow their own stated recommendation of comparing rates of infection with external peer groups to ensure that an accurate comparison is made.

2.10. Overall, the HAD Report does not take into account available data which would enable the authors to come to a more accurate conclusion on the infection risk at QEUH/RHC and its underlying causes.

3. Chapter 2

3.1. Section 2.1 of the HAD Report attempts to describe the manner, or family tree, in which bacteria are grouped. This is difficult to describe in a few paragraphs as entire textbooks have been devoted to the subject and it is unclear therefore why this section has been included.

3.2. One comment is included that suggests that *Escherichia coli 0157* is more likely to cause infection than other strains in 'normal people'. I presume that this is in reference to the low infectious dose (around 100 bacteria) of *E. coli 0157* required to cause infection which results in a higher virulence. *E. coli* 0157 is an unusual finding, which prompts public health and outbreak investigation due to the danger posed by its ability to produce a shiga-like toxin resulting in severe infection and sequelae, one of which (haemolytic uraemic syndrome) is a notifiable disease in both England and Scotland. In 2021 there were 365 cases, 0.65/100 000 population seen in England[3] and 137 cases in Scotland[4]. *E. coli* in general is very commonly seen causing infections such as urinary tract infection (most often in otherwise fit and well individuals) and blood stream infections. In England the incidence of *E. coli* blood stream infection was 68.5 cases per 100 000 population in 2022-2023[5] and in Scotland, 79.1 per 100 000 population in 2023[6] . I would disagree with the impression given here that it is seen more frequently than other strains which is misleading, and the correct context would have been helpful.

3.3. In section 2.2 the authors discuss what is meant by an outbreak. For all practical purposes when managing healthcare associated outbreaks, the definition of an outbreak is that which is found in the Scottish Infection Prevention and Control Manual section on healthcare associated incidents[7] and includes:

[3] Shiga toxin-producing *Escherichia coli* (STEC) data :2021 UKHSA - https://www.gov.uk/government/publications/escherichia-coli-e-coli-o157-annual-totals/shiga-toxin-producing-escherichia-coli-stec-data-2021.

[4] Shiga toxin-producing *Escherichia coli* (STEC). HPS https://publichealthscotland.scot/population-health/health-protection/infectious-diseases/shiga-toxin-producing-escherichia-coli-stec/data-and-surveillance/.

[5] Annual epidemiological commentary: Gram negative, MRSA, MSSA bacteraemia and *C. difficile* infections up to and including financial year 2022 to 2023, UKHSA. https://www.gov.uk/government/statistics/mrsa-mssa-and-e-coli-bacteraemia-and-c-difficile-infection-annual-epidemiological-commentary/annual-epidemiological-commentary-gram-negative-mrsa-mssa-bacteraemia-and-c-difficile-infections-up-to-and-including-financial-year-2022-to-2023#epidemiological-analysis-of-gram-negative-organisms.

[6] ARHAI Scotland 2023 report https://www.nss.nhs.scot/media/5564/arhai-annual-report-2023.pdf

[7] Bundle 27, Volume 4, Document 16, page 176 - National Infection Prevention and Control Manual: Chapter 3 - Healthcare Infection Incidents, Outbreaks and Data Exceedance (scot.nhs.uk) accessed 10.04.2025.

- *An exceptional infection episode* - A single case of any serious illness which has major implications for others (patients, staff and/or visitors), the organisation or wider public health.

- *A healthcare associated infection outbreak* - Two or more linked cases with the same infectious agent associated with the same healthcare setting over a specified time period; or a higher-than-expected number of cases of HAI in a given healthcare area over a specified time period.

- *A healthcare infection exposure incident* - Exposure of patients, staff, public to a possible infectious agent as a result of a healthcare system failure or a near miss e.g., ventilation, water or decontamination incidents.

- *A healthcare infection data exceedance* - A greater than expected rate of infection compared with the usual background rate for that healthcare location.
- *A healthcare infection near miss incident* **–** an incident which had the potential to expose patients to an infectious agent but did not e.g. decontamination failure
- *A healthcare infection incident should be suspected if there is* – a single case of an infection for which there have previously been no cases in the facility (e.g. infection with a multidrug-resistant organism (MDRO) with unusual resistance patterns or a post-procedure infection with an unusual organism

3.4. The English national IPC manual is not as mature as the Scottish version, being first published in 2022 and does not yet have a section on outbreaks and incidents, however the definitions above would be widely accepted by IPC professionals in the rest of the UK.

3.5. In order to identify a potential outbreak all that is needed is the identification of two infections by the same organism which are related in time and place. The authors of the HAD Report suggest that identifying the source of the organism, the route of transmission and the portal of entry into the patient are needed in order to define an outbreak. In practice this information will often be identified during the investigation, not prior to, but is sometimes difficult to identify and should not be a barrier to identifying an IPC incident. The portal of entry and exact transmission route may not be identified and the experience, training and expertise of the IPC professionals leading the

investigation will enable them to make educated assumptions on how transmission has occurred.

3.6. Chapter 3 of the NIPCM discusses the importance of making an early and effective response to an actual or potential healthcare incident, outbreak, or data exceedance. In practice, surveillance systems are used to aid detection of incidents and outbreaks. Risk based approaches are utilised for particularly vulnerable groups such as those we are immunosuppressed, cystic fibrosis patients and renal dialysis patients.

3.7. Tools for the initial assessment of incidents (HIIAT) are available within the manual and these were widely used during the incidents seen at QEUH/RHC since its opening in 2015[8] and reporting to Antimicrobial Resistance and Healthcare Associated Infection Scotland (ARHAI) is expected as part of this process.

3.8. The NIPCM describes a three-pronged investigation looking at epidemiology, microbiology and the potential exposure to the infectious agent (environmental investigation). Review of IPC practice is also recommended and, in my experience, would be a routine part of any incident investigation involving infection.

3.9. The epidemiological investigation includes establishing a case definition and a working hypothesis regarding source and transmission. This approach allows investigation and preventative measures to be put in place simultaneously without waiting for confirmation of a definitive match of infections with potential primary sources.

3.10. In many circumstances, the identification of the primary source of infection can also be difficult to identify. If environmental surface contamination with the organism in question is found, for example, the original source of that contamination may no longer be available for testing. It is very unusual for contemporaneous (to the time of transmission) environmental samples to be available and interventions such as cleaning may have happened before the outbreak is recognised. If the source is another patient who is colonised and not recognised (eg with MRSA) then that patient may have been discharged prior to the recognition of the outbreak.

3.11. Whilst having the ability to perform WGS on isolates from an outbreak will be helpful to link cases, it is not a technique that is widely available to all hospitals except as a retrospective test and is therefore an unrealistic expectation for many outbreak investigations. The HAD Report refers to sub-species typing by whatever method (including WGS) as strain level typing.

3.12. The author's proscriptive approach to outbreak identification is not compatible with widely recognised infection prevention and control practice which is to control and

[8] For example, Problem Assessment Group Minutes - Bundle 2, Document 17, page 44-45.

prevent without waiting for results of further tests, in order to maintain patient safety and prevent further harm.

3.13. The authors suggest that infection in neutropenic patients commonly arises as a result of translocation of the gut by commensal bacteria in the patient's intestines. This is well recognised by all clinicians who care for this group of patients. It is also well recognised that for organisms such as *Pseudomonas aeruginosa* the level of gut colonisation of hospitalised patients, particularly those who are neutropenic, is higher than for the general population rising from 3-17% in the general population to 20-60% of neutropenic patients [9]. This rise in colonisation/carriage suggests that organisms are acquired from the hospital environment for instance through ingestion, contact with water or other contamination of surfaces[10]. Also see para 4.2.

3.14. This increase in colonisation by contact with the hospital environment could potentially be extrapolated to other, less pathogenic, environmental organisms, however little research has been carried out on these rarer organisms and the body of evidence to support this theory or that there is evidence of any level of gut colonisation with the rarer environmental organisms in this patient population does not exist. Whilst the authors of the HAD Report argue that the gut is the source for BSI in neutropenic patients, the published evidence of gut colonisation by the rarer of the organisms which caused BSI at QEUH/RHC is weak and other sources must therefore be considered.

3.15. The single roomed environment at QEUH/RHC would make it unlikely that direct patient to patient spread occurred, assuming that staff were observing standard IPC precautions. The reduction of infections through the use of single rooms is by the reduction of person to person spread and also by limiting the spread of infection by reducing person-surface-person contacts[11]. Studies have also shown that combining single room accommodation with good ventilation can reduce infection risk and mortality[12] [13].

[9] Markou P and Apidianakis Y. Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer. Frontiers in Cellular and Infection Microbiology, 2014, vol 3 article 115.
[10] Virieux M et al. Assessing the role of environment in *Pseudomonas aeruginosa* healthcare-associated bloodstream infections: a one-year prospective survey. Journal of Hospital Infection, 2025-02-01, Volume 156, Pages 26-33.
[11] Zimring C, Joseph A, Choudhary R. The Role of the Physical Environment in the Hospital of the 21st Century: A Once-in-a-lifetime Opportunity. Report to The Center for Health Design, California; 2004.
[12] McManus, A. T. et al. (1994). A decade of reduced gram-negative infections and mortality associated with improved isolation of burned patients. *Archives of Surgery, 129*(12), 1306-1309.
[13] Passweg, J. R., et al. (1998). Influence of protective isolation on outcome of allogeneic bone marrow transplantation for leukemia. *Bone Marrow Transplantation, 21*(12), 1231-1238.

3.16. On reviewing the published literature, Ulrich et al[14] found that the design of the physical environment impacts healthcare associated infection rates by affecting the three main transmission routes: air, contact and water. They found that the literature supports various design measures for controlling and preventing infection including single patient rooms in order to enable separation or isolation of patients from the time of admission, allowing patients to be tested for infection and reduce airborne transmission.

3.17. A further advantage of single-bedded patient rooms is the ability to clean with newer methods of cleaning such as hydrogen peroxide vapour (HPV) or UV-C light decontamination. French et al[15] found that the conventional methods of cleaning such as disinfectant and steam did not effectively decontaminate surfaces with 66% of surface swabs remaining positive for MRSA post-cleaning, whereas following the use of HPV, only 1.2% of swabs remained positive. As noted in my previous report for the inquiry[16], HPV was not in routine use at QEUH/RHC and the potential contamination of surfaces by environmental organisms was not recognised adequately.

3.18. The authors do not appear to have taken into account the views of the clinical teams on the Schiehallion Unit who were concerned about the rapid and persistent rise in cases of blood stream infection (BSI) due to environmental organisms. These clinicians are highly experienced in the care of neutropenic patients and had also worked at Yorkhill previously, but the PAG and IMT minutes throughout 2017-2019 do not reflect that gut translocation was a concern or considered as an explanation for the majority of infections at the time.

3.19. Where gut translocation is recognised as the most likely cause of infection, a PAG may be held in order to discuss and confirm that this is the case (particularly if a more unusual organism is involved), but this would not proceed to IMT and a heightened level of concern.

3.20. In addition, Ms Harvey-Wood (clinical scientist in microbiology) who specialised in paediatric microbiology, for both Yorkhill and RHC, expressed concern about the increased number of unusual environmental organisms seen in blood cultures and also the fact that multiple organisms were being found in the same blood culture on a more frequent basis, which again was an unusual finding[17]. Polymicrobial BSI are associated

[14] Roger S Ulrich et al. HERD: Health Environments Research & Design Journal Volume 1, Issue 3. Apr 2008.
[15] French GL et al Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. Journal of Hospital Infection (2004) **57**, 31-37.
[16] Bundle 21, Volume 1, Document 4, para 9.16-9.18.
[17] Transcript, Dr Kathleen Harvey Wood, col 35-36.

with increased mortality[18] [19] and are often more complex to treat due to the different antimicrobial sensitivities of the organisms[20].

3.21. The HAD Report note at the end of section 2.2, that *Cupriavidus spp*. and *Burkholderia spp*. can be found at low levels in Glasgow tap water, perhaps suggesting that contamination of the hospital system was inevitable. An explanation of wholesome water and why it is acceptable to have low levels of bacteria in tap water is found in Dr Walker's expert report for the Inquiry[21]. The finding of low levels of these two bacteria would not prevent the water being classed as wholesome and compliant with the Public Water Supplies (Scotland) Regulations 2014 SSI 2014/364 regulation 4(2)(a) "water intended for human consumption purposes must not contain any microorganism …at a concentration or value which would …constitute a potential danger to human health".

3.22. On entry into QEUH, mains water is put through an ultrafiltration plant, which removes organisms and fungi, and then to filtered water storage tanks. This is done to protect vulnerable patients and means that bacteria should not be able to enter the system through the mains water, however, it is known that there was a period of time in 2015 when the ultrafiltration plant was bypassed inappropriately[22], allowing bacteria and debris to enter, proliferate and colonise the hospital water system[23], creating a risk for neutropenic and immunocompromised patients coming into contact with the water.

4. Chapter 3

4.1. Chapter 3 is, in part, a textbook-style review of the bacteria causing infections included in the Case Note Review[24]. It appears to be attempting to make the case for the infections to be caused by commensal gut organisms which are then selected out by antimicrobial treatment, rather than a direct environmental source of infection.

4.2. However, it is known that many of these organisms including *Pseudomonas aeruginosa*[25] and others have higher carriage rates in hospitalised patients and evidence suggests that they may be acquiring them from the hospital environment and

[18] Pavlaki, M et al. Polymicrobial bloodstream infections: Epidemiology and impact on mortality. Journal of Global Antimicrobial Resistance 2013 Vol 1, Issue 4 p 207-212.
[19] Pittet D et al. Association of secondary and polymicrobial nosocomial blood stream infections with higher mortality. Eur J Clin Microbiol Infect Dis 1993 Nov 12(11):813-9.
[20] Lin J-N et al. Characteristics and Outcomes of Polymicrobial Bloodstream Infections in the Emergency Department: A Matched Case–control Study. Acad Emerg Med • October 2010, Vol. 17, No. 10.
[21] Bundle 21, Volume 1, Document 5, page 180.
[22] Bundle 6 (2023), Document 29, page 122.
[23] Bundle 21, Volume 1, Document 5, page 180.
[24] Bundle 6, Document 38, page 795.
[25] Markou P and Apidianakis Y. Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer. Frontiers in Cellular and Infection Microbiology, 2014, vol 3 article 115.

is not only dependent on antimicrobial use[26] [27] [28]. Bacteria can be carried on the skin or in the naso-pharynx and acquisition does not always result in intestinal carriage. However, colonisation of the patient by organisms from the hospital environment may be a precursor to what may appear to be endogenous infection. In the context of infection prevention and control is important to understand the full aetiology of an infection so that environmental risks can be recognised and mitigated as early as possible. The HAD Report does not appear to take this into account.

4.3. The HAD Report places emphasis on antimicrobial resistance amongst these organisms. However, no concerns were raised through IMTs for blood stream infections on the Schiehallion Unit at RHC regarding spread of multi-resistant organisms, suggesting that this was not a factor in these infections, save for those where the bacteria are inherently resistant such as *Stenotrophomonas maltophilia.* This is discussed further at paragraph 5.9.

4.4. In my experience it is widely accepted that colonisation by bacteria often precedes infection[29] [30] [31] [32] and strategies may be employed in individual patients to reduce risk of infection. For example, where patients are colonised with Meticillin resistant *Staphylococcus aureus* (MRSA), efforts are made to decontaminate the patient using antibacterial washes and nasal antimicrobial ointment prior to surgical procedures in order to protect them from surgical site infection with MRSA. Whilst skin carriage alone is not harmful to the patient, as soon as a surgical wound of other breach of the skin's integrity is present, the risk of infection is increased.

4.5. Alternatively, some infections arise without prior colonisation, for example wound infections where a traumatic wound becomes contaminated (as opposed to colonised) with the infectious agent immediately it occurs.

[26] Petignat C et al. Exogenous Sources of *Pseudomonas aeruginosa* in Intensive Care Unit Patients Implementation of Infection Control Measures and Follow-Up With Molecular Typing. Infect Control Hosp epidemiol 2006: 2007: 953-957.
[27] Venier A-G et al. Risk factors for Pseudomonas aeruginosa acquisition in intensive care units: a prospective multicentre study JHI 88 (2014) 103-108.
[28] Buchler AC et al. Outbreak investigations of contact patients and the hospital environment after detection of carbapenemase-producing Pseudomonas aeruginosa on general hospital wards. Journal of Hospital Infection 159 (2025) 11e19.
[29] Bonten MJ and Weinstein RA. The role of colonisation in the pathogenesis of nosocomial infections. Infect Control Hosp Epidemiol 1996 Mar;17(3):1993-200.
[30] Tishendorf et al. Risk of infection following colonisation with carbapenem-resistant Enterobacteriaceae: A systematic review Am J Infect Control. 2016 May 01;44(5) 539-543.
[31] Niederman M. Gram negative colonisation of the respiratory tract: pathogenesis and clinical consequences. Seminars in respiratory infections Volume 5; Issue 3 (1990).
[32] Latibeaudiere R. Surveillance cultures growing Carbapenem Resistant *Acinetobacter baumanii* Predict the development of clinical Infections: A Retrospective Cohort Study. Clinical Infectious Diseases, Vol 60, Issue 3, 1 Feb 2015, pages 415-422.

4.6. An example where both mechanisms may occur is infection of a central venous cannula where bacteria are transferred to the cannula through poor hygiene/infection prevention techniques, contact with contaminated water or surfaces, or from the bacteria colonising the patient's skin.

4.7. Mechanisms of transmission from the environment to individuals is described in my previous report[33].

4.8. The colonisation stage allows the number of bacteria (bacterial load) to increase to the point that it can cause infection by whatever mechanism is involved. So, whilst it could be argued that the infection is secondary to colonisation, the colonising organisms have to be first acquired whether from food, the environment, from other individuals or another source. The intermediate step of colonisation does not preclude an environmental source for the infection and hence Key Question 4 for the Inquiry remains valid.

5. Chapter 4

5.1. In this and previous chapters the authors place a lot of importance on colonisation of the patients in the home environment which, they opine, subsequently led to blood stream infections caused by translocation from the gut.

5.2. It should be remembered that the Schiehallion cohort of patients had not significantly changed and nor had the senior medical team caring for them, but nevertheless concerns were raised that there were an increased number of blood stream infections occurring which the clinical team identified as unusual following the move to RHC.

5.3. It is notable that Mookerjee, in his expert report to the Inquiry[34] found that the rate of BSI with environmental organisms was higher (for certain years) of that of comparator units in the UK. Given that the children concerned had frequent day and inpatient admissions to the Schiehallion Unit, the likelihood of the hospital environment being implicated in the infections cannot be dismissed as easily as the authors appear to wish to do. If the home environment was the issue then the infections would not be seen as unusual as this would have been a constant. The Case Note Review included only infections, with environmental gram negatives, which arose after attendance or admission to QEUH/RHC. Therefore, it does not seem reasonable that the authors make an assumption that only 20% or less of BSI are related to the hospital environment without any knowledge of the specific cases.

[33] Bundle 21, Volume 1, Document 4 para 9.21-9.32.
[34] Bundle 21, Volume 1, Document 1, page 3.

5.4. It was notable (and to an extent expected[35]) that the number of BSI temporarily reduced on the initial transfer of the Schiehallion Unit to RHC. This would be the hoped-for outcome of moving to a brand-new facility. This is noted and shown in the analysis by HPS and NSS[36] using SPC charts to compare the rate of infections with the expected rate and demonstrating a change in the trend in blood culture incidence for environmental organisms, which reduced around the time of the move to RHC in 2015, but increased above the centreline with 9 of 12 data points for 2017 above the centreline, including one point above the upper warning limit, and in 2018 the data points indicated two exceedances of the upper confidence limit. These are significant findings and reflect a real and sustained increase in the rate of blood stream infections due to environmental bacteria in the Schiehallion cohort of patients when compared with the equivalent patient cohort at Yorkhill prior to the move.

5.5. A further HPS analysis[37] reviewed data from 2013 to 2019 and showed the same step change in incidence of BSI caused by environmental organisms, followed by a significant reduction in cases from October 2018 to February 2019 when the Schiehallion Unit moved to wards 6A and 4B, followed by another significant increase in incidence with 6 of 7 data points above the centreline for the remainder of 2019.

5.6. The authors insist that a route of transmission and portal of entry of infection must be identified in order to support the probability of any site acting as the source. In my experience this is often challenging to do. The water system has been identified as a key source but that does not necessarily mean that direct water to patient transmission has taken place. Various other indirect routes of transmission are also identifiable and most Schiehallion patients will have central venous cannulas, breaching the skin defences, which give additional patient portals of entry. This is explained in my earlier report to the Inquiry[38].

5.7. A great deal of reliance is placed in the HAD Report on the lack of finding indistinguishable isolates of BSI causing organisms and the environment. The environmental sampling of the water system was not consistent, nor were all samples cultured for environmental organisms other than *Legionella spp* and *Pseudomonas*[39]. Nor were there water samples contemporaneous or from the patient's own room environment for BSI cases in the vast majority of cases. Where samples were cultured

[35] Bundle 18, Volume 2, Document 130, page 1566.
[36] Bundle 7, Document 5, page 205.
[37] Bundle 7, Document 6, page 214.
[38] Bundle 21, Volume 1, Document 4, paragraph 9.27-9.32.
[39] This is not surprising as routine water samples are not examined for other organisms unless specifically requested.

for environmental organisms, multiple strains of the same organism were often identified. It is not clear how many of the different strains were followed up and stored. Not all of the clinical isolates were subjected to WGS. For these reasons it cannot be argued that because the WGS evidence does not exist, that cases of BSI were not related to the hospital environment.

5.8. The authors criticise the exclusion of *E. coli* from the CNR. Whilst it is understood that the organism can, on occasion, contaminate surfaces in healthcare facilities and patient to patient transmission can occur via this mechanism, it is not a recognised environmental organism and is a constant in the human gut flora. The Terms of Reference for the CNR inclusion criteria were such that *E. coli* would be excluded as it is not an environmental organism.

5.9. In the paper, the number of *Stenotrophomonas spp* infections is, in part, attributed to the shortage of Piperacillin-tazobactam between May and September 2017. The authors suggest that this shortage resulted in an increase in meropenem usage which selects out resistant organisms such as *Stenotrophomonas spp.* However, the data supplied by NHSGGC[40] clearly shows that the use of Piperacillin-tazobactam increased during this period in paediatric haemato-oncology patients, peaking in August 2017 and the usage of Meropenem remained reasonably stable, dipping from June to November. Usage was no lower than the preceding 6-12 months in adult patients in BMT, North Sector or South Sector (sectors as defined in the paper).

5.10. Documents provided by NHS GGC[41] [42] examine the prescribing data for three antibiotics/antibiotic group: Carbapenems (including Meropenem), Piperacillin/Tazobactam and aminoglycosides. We know from other reviews [43] [44] [45] that the highest numbers of environmental gram-negative infections seen in the Schiehallion cohort occurred in 2017 and up to October 2018. The antimicrobial data shows that there was a clear upward step change in piperacillin/tazobactam prescribing in 2017 with Carbapenem prescribing remaining constant, as shown by mean prescribing and change point analysis, but in 2018 the opposite is seen, with an upward step change in Meropenem usage and a reduction in Piperacillin/tazobactam.

[40] Public Inquiry Data v11 (20240126) - This document contains datasets relied upon by the HAD group, spanning January 2010 to December 2022, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.
[41] Bundle 44, Volume 1, Document 7, page 248.
[42] Bundle 44, Volume 1, Document 8, page 297.
[43] Bundle 21, Volume 1, Document 1, page 3.
[44] Bundle 7, Document 5, page 205.
[45] Bundle 7, Document 6, page 214.

5.11. In my opinion, the increase in Meropenem usage would not have been seen if there had been a concern that a high number of Meropenem resistant infections with environmental gram negatives was being seen, as the Meropenem would have been ineffective against these organisms.

5.12. In addition, it is worth noting that although the authors put great emphasis on *Stenotrophomonas maltophilia*, the maximum number of blood stream infections in any quarter was three and this was not the main driver of the overall environmental gram-negative blood stream infections.

5.13. The authors dismiss the concept of an outbreak of infection at QEUH/RHC on the basis of a lack of WGS evidence, despite the environmental specimen collection being inconsistent and mostly non-contemporaneous to the clinical infections. However, there is no doubt that there was a significant increase in the number of infections seen[46] (a data exceedance) due to a number of different environmental organisms, some in clusters, over a prolonged period of time and the investigation of the built environment uncovered issues in the water system including poor management, contamination, taps with flow straighteners, extensive biofilm etc, and ventilation issues such as the lack of particulate (HEPA) filtration, lack of positive pressure and 2.5 air changes per hour compared with the national guidance of 10 ach [47]. All of these factors significantly increased the risk associated with the built environment for immunosuppressed patients such as those on the Schiehallion Unit.

5.14. The newly refurbished Schiehallion Unit, which has had the built environment faults corrected, has seen a reduction in blood stream infections caused by environmental organisms[48] to levels similar to peer Units in England. This is further evidence that the environment was contributing to the infections seen.

6. Chapter 5

6.1. In my experience, it is widely accepted that no water system can be sterile but that water supply and management of the water system must be managed on the basis of risk.

6.2. For example, much emphasis in water management is placed on *Legionella spp* and in particular *Legionella pneumophilia* serogroup1 due to its ability to infect previously healthy individuals by droplet spread from contaminated water. This affects all patients and potentially some staff in the hospital environment.

[46] Bundle 21, volume 1, Document 15.
[47] Bundle 13, Volume 3, Document 32, page 951.
[48] Bundle 21, Document 10, page 767.

6.3. For other organisms, such as *Pseudomonas aeruginosa* and other environmental organisms, the risk is much greater for certain groups of patients who are susceptible to opportunistic infections. Immunosuppressed children (particularly those undergoing haemopoietic stem cell transplant (HSCT)) are at greatest risk together with children with ineffective defence against infection such as those with cystic fibrosis[49] [50], neonates and ITU patients. The same groups apply for adults with BMT patients having the greatest risk but the risk for each group is lower than that for children.

6.4. Acquisition of environmental bacteria will often result in skin or mucosal (naso-pharynx) rather than enteric carriage. This is especially observed in cystic fibrosis patients but is also the case in others due to the body's natural defences of mucosal cilia, gastric acid and the enteric defence of the gut microbiota resulting in colonisation resistance.

6.5. Patients who have indwelling vascular access lines, abdominal feeding tubes, urinary catheters etc are all at higher risk of infection as their medical devices breach the skin and/or mucosal barriers.

6.6. Not all haematological conditions confer the same risk, for example hereditary spherocytosis which does not require immunosuppressive treatment but can result in splenectomy which then confers an increased risk of some vaccine-preventable diseases. These patients are cared for by haematologists who also care for patients with haematological malignancies.

6.7. The authors of the HAD Report do not appear to consider these differences in vulnerability in their approach. Not all patients are at equal risk and it must be remembered that the adult BMT patients moved back to the Beatson in July 2015 and did not return until July 2018 by which time a number of changes had been made including all water outlets on the ward had point of use filters, the ventilation in the BMT rooms had been upgraded to include HEPA filtration and the chlorine dioxide dosing system was installed soon after, in November 2018.

6.8. This group were the most vulnerable of the adult patients, but infections did arise in adults associated with environmental organisms and Problem Assessment Group meetings were held for suspected outbreaks in adults including *Mycobacterial*

[49] Mainz JG et al. Cystic Fibrosis Upper Airways Primary Colonization with Pseudomonas aeruginosa: Eradicated by Sinonasal Antibiotic Inhalation. American Journal of Respiratory and Critical Medicine; 2011. Vol 184, Issue 9 pages 1089-90.
[50] Sabine MP et al. Development of the Nasopharyngeal Microbiota in Infants with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine. 2016 Volume 193, Issue 5, pages471-P8.

abscessus in adult cystic fibrosis patients[51], Aspergillus in adult ITU[52], *Acinetobacter* infections in adults on neurosurgical ITU[53] and *Pseudomonas* on 10D[54].

6.9. Children are most vulnerable to environmental infections in hospitals because they have a comparatively immature immune system. Risk increases with severity of underlying illness, young age, nutritional status and extrinsic factors such as use of invasive devices (central venous catheter, urinary catheter, mechanical ventilation) and length of stay. Whilst adults can and do acquire environmental infections, their vulnerability is less than that seen in children.

6.10. The authors of the HAD report cite a paper by Couchoud et al[55] to conclude that endogenous infection is a more significant source of BSI than environmental sources or cross infection from another patient. As discussed at paras 3.13 and 4.2, environmental acquisition in hospital leading to increased incidence of gut colonisation/carriage is well recognised and the root cause of the source of an infection is much more complex than this might suggest. Environmental acquisition of colonisation of the oro-pharynx may also result in significant infection and blood stream infection.

6.11. The authors identify that 'when a water system is a source of environmental contamination and when there is spread to vulnerable patients it is generally not the whole system that has a high bacterial load'.

6.12. Evidence suggests that the water system at QEUH/RHC was indeed contaminated throughout and the authors conclusion at 5.3.2 is incorrect in this case.

6.13. There is evidence submitted to the Inquiry such as Dr Walker's expert report[56] and the draft Storrar and Rankin[57] HPS report which demonstrate widespread contamination with a wide range of gram-negative waterborne pathogens and biofilm throughout the water system during the first 6 months of occupation and again during the 'water incident' in 2018 when more extensive testing was carried out.

6.14. Dr Walker's view is that 'the microbiological data provide a pictorial and temporal evidential data-base that the entire domestic hot and cold-water system was

[51] Bundle 2, Document 1, page 7.
[52] Bundle 2, Document 2, page 8.
[53] Bundle 2, Document 20, page 52.
[54] Bundle 2, Document 26, page 67.
[55] Couchoud C, Bertrand X, Bourgeon M, Piton G, Valot B, Hocquet D. Genome-based typing reveals rare events of patient contamination with Pseudomonas aeruginosa from other patients and sink traps in a medical intensive care unit. J Hosp Infect. 2023 Apr;134:63-70.
[56] Bundle 21, Volume 1, Document 5, page 180.
[57] Bundle 19, Document 21, page 174.

contaminated with a wide range of waterborne pathogens that would have presented a HAI risk to patients from the day of occupation'.

6.15. There is evidence from pre- and post-flush samples carried out in PICU as early as 2016[58], that *Cupriavidus sp* was detected in both indicating that both the outlet and the pipework further back in the system were contaminated.

6.16. Health Protection Scotland and NHS NSS reported[59] in December 2018 that following a decision to decant wards 2A/2B to allow for further investigation of the environment in these wards, 4B was being prepared for the transfer of adult BMT patients from the Beatson. As a precaution, water testing was undertaken and revealed the presence of *Cupriavidus pauculus* and other gram-negative bacteria in water outlets in the ward. This prompted the suggestion that there may be widespread contamination of the water system across QEUH/RHC and further testing was undertaken.

6.17. Test results provided confirmation of widespread contamination with positive samples in multiple areas including ward outlets as well as basement risers. The working hypothesis of the investigators was that a combination of regressional contamination and contamination at installation/commissioning was the cause.

6.18. Dr Peters reported on environmental sampling on ward 2A/2B in March 2018 done in response to two cases of *Cupriavidus pauculus* BSI. Showers and taps from the ward and from ward 4B, dismantled and cultured. A diverse population of environmental organisms including *Cupriavidus pauculus, Burkholderia sp, sphingomonas paucimobilis, Delftia acidovorans, Serratia fonticola, Brevundimonas sp, Rhodotorula mucilaginosa, Bordetella bronchisepticum and candida guillermondii* was found.

6.19. At the Water Technical Group on 20 April 2018[60] it was confirmed that every floor in QEUH/RHC had positive and negative results and attendees agreed that this indicated widespread water infection (sic).

6.20. Susanne Lee reviewed the system on-site following a request from the WTG. Her report[61] identified that the system was likely contaminated prior to handover, with a 12-month lag between the system being filled and occupation, allowing biofilm to develop. She reported that temperature fluctuations were a likely contributory factor as they had been inconsistent. The high number of little used outlets, particularly the en-suite bathrooms was also a risk factor. She made recommendations around improvements

[58] Bundle 21 Volume 1, Document 5, page 180.
[59] Bundle 18, volume 1, Document 11, page 819.
[60] Bundle 10, Document 3, page 14.
[61] Bundle 8, Document 32 page 134.

to the safety management of the water system, the correct use of point of use filters and the reduction of the number of outlets in patient rooms.

6.21. In July 2019, Intertek[62] reported on an expansion tank from QEUH which showed areas of corrosion and contaminated and found widespread biofilm on the holding plate, the expansion bladder and debris from the expansion vessel, together with multiple bacterial strains found on culture.

6.22. Whilst individual outlets undoubtedly played a role in the contamination of water, in part due to the use of plastic flow straighteners which have been shown to become contaminated with biofilm[63] and have also been the source of outbreaks of infection elsewhere[64], there is evidence of poor management of the water system as a whole[65] and contamination of water tanks with debris and bypassing of the ultrafiltration units allowing micro-organisms and debris to enter the water system.[66]

6.23. In May 2018, black grime was seen coming out of the drains in wash hand basins[67] in ward 2A/2B suggesting that biofilm was not only prevalent in the water supply but also in the waste-water system. This was observed again in September 2018[68].

6.24. This widespread contamination with biofilm formation at QEUH/RHC has not been addressed by the HAD Report. Instead it concentrates on the part played by biofilm in the spread of multi-resistant organisms. It is well recognised that biofilm can protect bacteria from chemical (biocide) destruction, and extreme environments such as heat. When they occur on a medical device such as the lumen of central venous cannulas, they can enable bacteria to resist destruction by antimicrobials. Mature biofilms allow dispersal of bacteria from the biofilm[69]. This can be the mechanism of infection in central line associated blood stream infections (CLABSI) or the release of bacteria into water systems.

6.25. The issue of multi-resistant organisms, save for those which are inherently resistant, has only appeared once in IMT minutes, in November 2018 and related to a multi-resistant pseudomonas isolate which was found in at least two sinks and caused a BSI in one patient and was confirmed by typing[70].

[62] Bundle 18, Volume 1, Document 4, page 91.
[63] Bundle 18, Volume 2, Document 115, page 1016.
[64] JT Walker et al Investigation of healthcare acquired infections associated with *Pseudomonas aeruginosa* biofilms in taps in neonatal units in Northern Ireland. JHI 86 (2014) 16-23.
[65] Bundle 21, Volume 1, Document 5, page 180.
[66] Bundle 6, Document 29, page 122.
[67] Bundle 1, Document 22, page 91.
[68] Bundle 1, Document 35, page 149.
[69] Zhao A, Sun J and Liu Y (2023) Understanding bacterial biofilms: From definition to treatment strategies. Front. Cell. Infect. Microbiol. 13:1137947.
[70] Bundle 1, Document 54, page 241.

6.26. At 5.4.3 the authors of the HAD Report remark on the time period between September 2018 and April 2019 when there were no cases of *Stenotrophomonas maltophilia* seen in the Schiehallion cohort. In October 2018 wards 2A/2B were decanted to Wards 6A and 4B in the adult hospital and interventions in the water system were implemented on 2A/2B including removing and replacing all of the wash hand basin and associated pipework and taps. In addition, dosing of the water supply with Chlorine dioxide commenced. The episodes of BSI due to *Stenotrophomonas maltophilia* in April 2019 were on ward 6A QEUH. This could be seen as confirmation that the Stenotrophomonas BSI were not related to intrinsic patient factors such as GI colonisation and translocation as the patient cohort did not change and the only factor which did change was the location of the ward.

6.27. The QEUH/RHC water contamination incident appears to be unique in the extent of contamination and the range of micro-organisms implicated in infections. I have not been able to find a directly comparable incident in the published literature. There are many incidents related to *Legionella* contamination in the literature[71], some with contaminated cooling towers and whole cold-water systems suggesting widespread contamination but none with the diversity of organisms examined.

6.28. Water testing has been variable in range, volume and organisms tested across the QEUH/RHC campus[72]. Although some isolates have been stored and tested by WGS it is not possible to be confident that these are either contemporaneous with infections caused by environmental organisms or comprehensive enough to rule out, as the authors seem to do, an environmental link based on the limited WGS testing undertaken[73],[74],[75].

6.29. If comprehensive water testing looking for organisms relevant to the infections seen, had been undertaken earlier in the course of the investigations into all of the unusual infections seen at QEUH/RHC, it may have been possible to relate the water contamination to the infections more definitively. However, because of the lack of this comprehensive testing it is impossible to rule out a link as the authors appear to wish to do.

6.30. I would also argue that by the time WGS results would be available in an outbreak investigation, the IPC team would usually have implemented the necessary control

[71] CA Hart et al. *Legionella* in hospitals: a review. Journal of Hospital Infection (1991) 18 (Supplement A), 481-489.
[72] Bundle 21, Volume 1, Document 1, page 3.
[73] Bundle 8, Document 44, page 223.
[74] Bundle 8, Document 45, page 230.
[75] Bundle 8, document 46, page 239.

measures. Most NHS hospitals do not have direct, immediate access to WGS and in-house water testing, meaning that an investigation team would have to wait for external contractors to test the water, produce results and then return any significant isolates so that they could be paired with clinical isolates and sent away to a reference facility for further typing and WGS as necessary. This would take weeks rather than days to achieve which would be too long to wait to implement control measures. There are of course, more extensive outbreaks which are assisted by WGS in identifying epidemiological links between cases and may in turn, identify additional control measures, however initial IPC measures would have to be implemented at pace to control the outbreak.

6.31. As noted at paragraph 5.4 in this report, it would be expected that moving to a brand-new facility from a much older facility there would be a reduction in infections with environmental organisms[76]. From Sid Mookerjee's work[77] it is clear that this was the case for the Schiehallion Unit patients but the numbers of environmental infections rapidly increased to a peak in 2017 and a decline from 2018 due to multiple interventions including, but not limited to, point of use filters on taps, bottled water for washing and teeth cleaning, enhanced cleaning including silver hydrogen peroxide vapour (HPV) decontamination, mobile HEPA filters in patient's rooms and decant away from ward 2A/2B.

6.32. This is also seen in Dr Kennedy's paper[78] which shows by a rolling case count that the number of cases of BSI reduced at the time of the move to RHC but then steadily increased from May 2016 onwards to peak in June 2018. The nuance of the polymicrobial episodes is not visible in this graph (p 109) but is clearly seen on the preceding page.

6.33. HPS also showed this in their SPC analysis[79] discussed at 5.4.

6.34. As part of the works on ward 2A/2B, the ventilation system was significantly upgraded. In their proposal for changes required, Innovated Design Solutions noted that '*the original design philosophy was not intended for use by patients with immune response impairment/deficiency'* and that the existing ventilation was likely to increase the risks associated with ingress of infectious aerosols and would require a new dedicated air handling unit plant and installation of new distribution, as modification of the existing system was not thought to be feasible to create the desired outcome.

[76] Bundle 18, Volume 2, Document 130, page 1566.
[77] Bundle 21, Volume 1, Document 1, page 3.
[78] Bundle 6, Document 28, page 104.
[79] Bundle 7, Document 5, page 194.

6.35. It was only after the move back to the refurbished ward 2A/2B which had the environmental issues resolved and now met the SHTM guidance[80] [81], and the correct level of maintenance was being undertaken, that the rates of infection decreased to a similar level to comparator units elsewhere in the UK.

6.36. Comparing infection rates at RHC with those at Yorkhill gives false reassurance that there is no problem, as accepting a baseline without comparing it to a number of other units caring for a similar cohort of patients is misleading.

7. Chapter 6

7.1. Airborne pathogens such as aspergillus were not included in the CNR as they were not included in the Terms of Reference. One case where the child had a gram-negative BSI and aspergillus was included but not specifically commented on with reference to the aspergillus. Details of each case were, again, specifically excluded from the report by the Terms of Reference.

7.2. In my earlier joint report[82] with Ms Dempster, aspergillus was mentioned briefly but was not included in detail as we had no laboratory results for aspergillus infections from NHS GGC as the data set we worked from was specifically blood culture results.

7.3. In chapter 6 the authors again give a general review of ventilation requirements. They appear to pit the SHTM against NICE guidance and the JACIE requirements. Clearly the NICE and JACIE guidelines have to be suitable for existing buildings as well as new, hence the comparative lack of detailed standards and mandating of standards of ventilation but SHTM 03-01[83] is for new builds and should be followed without derogation wherever possible but particularly for vulnerable patients such as the Schiehallion cohort. As can be seen, in the case of QEUH/RHC the derogation away from the SHTM standards (reduction of air changes to 2.5ach/hr, not providing positive pressure and not providing sufficiently filtered air (H12) as described in Appendix 1, table A1) appears to have contributed to the increase in infection rates secondary to the built environment with three probable cases of aspergillus infection seen on ward 2A between July 2016 and March 2017.

[80] Bundle 15, Document 4, page 253.
[81] Bundle 1, Document 9, page 618.
[82] Bundle 21, Document 4, page 74-76.
[83] Bundle 16, Document 5, page 342.

8. Chapter 7

Analysis of data

8.1. When comparing cohorts of patients, it is important to ensure that they match in profile, for example the same diseases are seen in both groups, the hospital environments are similar and that the comparison is like for like. As discussed at 6.35, using Yorkhill as a comparison to RHC, gave false reassurance that infection rates were acceptable whereas using peer comparators from other areas of the UK provides a more accurate view of achievable infection rates.

8.2. The choice of splitting the data by consultant sector appears to be an arbitrary designation in that on review of the data provided, both the North sector and BMT admit patients to many wards in the Beatson and also to other hospitals including QEUH providing no standardisation to the patient cohort thus exposing them to the same hospital environments. There are also patients on the South list admitted to the Beatson. This means that a true comparison in the context of examining environmentally relevant infections cannot be made due to conflicts in the data.

8.3. The paediatric data set provided[84] contains data only for paediatric haemato-oncology patients. From this data 26 cases of *Burkholderia cepacia* blood stream infections can be identified from 16 patients. 24 of the cases were patients at Yorkhill and two at RHC. There are no blood culture results for patients from other specialties at Yorkhill. The data shows that on average 2 cases were seen every year, but cases were not distributed evenly across all years and this trend did not continue when the Schiehallion Unit moved to RHC, with just two infections seen in six and a half years. This could suggest that there was a factor influencing the rate of infections at Yorkhill which was not present at RHC and was not patient-mediated. In table 11A of the HAD Report, the cases are grouped as possible or probable clusters. This does not bear scrutiny in all cases, for example, by examining CHI numbers it can be seen that all of the episodes in 2005 and January 2006 were the same patient but they are assigned as a probable cluster. Patient level data is important to include in such an analysis to avoid assigning clusters where they do not exist.

8.4. The three cases of *Elizabethkingia meningoseptica* in 2014 were three episodes in two patients. The two episodes in the same patient were less than 28 days apart

[84] Final Combined GGC Paeds Onc CHI only 01.01.05 – 31.12.22.xlsx – This document contains datasets relied upon by the HAD Expert group, spanning January 2005 to December 2022, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.

suggesting a relapsing infection. The two patients were cared for by different consultants.

8.5. There were a further two cases at Yorkhill which were identified as *Chryseomonas meningosepticum*. This organism was reclassified as *E. meningoseptica* in 2005 and the cases should be recognised under the correct heading. When a change in name is made, diagnostic laboratories can on occasion, take time to change their diagnostic and identification protocols to include the new names.

8.6. There were three cases of *E. meningoseptica* at QEUH in a relatively short period of time between September 2016 and February 2017 which likely represents a cluster as no cases have been seen in the Schiehallion cohort at any other time in the data.

8.7. There were three episodes of blood stream infection with *Pantoea agglomerans* in two patients at Yorkhill. The two episodes in the same patient occurred 35 days apart and would be classed as recurrent infection. There is nothing to suggest a link between the two patients and their infections although they were cared for by the same consultant.

8.8. It is unclear from the HAD Report how the adult bed day data has been derived. One would expect that a highly specialised service such as BMT would have fewer bed days than non-BMT haemato-oncology, however, that is not the case here and the South sector appears to have fewer bed days than BMT and the North. It is also not clear whether this data is specific to a patient group or to the service or to individual consultants. Yet the patients involved in the data were admitted to 98 different wards, in at least 10 hospitals, across all sectors when their blood cultures were taken and there is no confirmation that any data cleansing or validation of the link between consultant and patient has been done. In addition, the authors appear to compare patients who have admissions to multiple sites and wards to patients using a single site. This makes direct comparison between patient cohorts and hospital sites impossible with any confidence that a like-for-like comparison is being made.

8.9. It is particularly important to ensure that there is a like-for-like comparison when one is investigating a link to the environment. Trying to compare so many wards and hospital sites in aggregated data with any accuracy is not feasible. The patient groups are said to all be haemato-oncology but there are patients within the list[85] who have benign conditions such as sickle cell anaemia, disorders of iron metabolism, etc which will skew the data.

[85] IP Haem Onc selected consultants from 2013 by sector.xlsx - This document contains datasets relied upon by the HAD Expert Group, covering the period from 2013 to 2023, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it includes patient-identifiable data, it has not been included in a Hearing Bundle.

8.10. Therefore, in my opinion and for the reasons set out above, comparing North, South and BMT sector data based on consultant affiliation rather than defined geographical location is a flawed approach to comparing infection rates.

8.11. The BMT patients returned to the Beatson in July 2015 after a month in QEUH and did not return to 4B until remedial works had been completed in June 2018. Other haematology patients were housed in 4C in a bed base of 10 beds.

Relevant infections

8.12. PAGs were held on three occasions for infection concerns on 4B and 4C.

- In August 2018 a PAG was held following 4 cases of Vancomycin resistant enterococci (VRE) on 4C. No IMT was required.

- In September 2020 four cases of VRE were seen in BMT patients on 4B. A second PAG was held but no IMT was required.

- In October 2020 two cases of *Stenotrophomonas maltophilia* bacteraemia were seen in haematology patients on 4C. No IMT was required.

8.13. I am not aware of any further concerns raised about infection rates in these patients whilst on 4C and 4B.

8.14. To date there has not been a detailed analysis of infection rates in QEUH 4B and 4C based on the ward patients and activity and comparing them with infection rates in comparable units that I am aware of.

8.15. Following remedial work 4B has a higher level of ventilation protection than many wards in QEUH (although still falls short of full compliance with SHTM 03-01 (2013)) with 5.8-8.9 air changes per hour (ach), HEPA filtration (rooms only, not corridor), no chilled beams and 6.6-13.9 Pa of positive pressure to the corridor. By comparison, 4C has the same ventilation as standard wards in the hospital with 2.8-3.3 ach, no HEPA filtration (mobile HEPA units in use), chilled beams and neutral to +1 Pa positive pressure[86] [87]. The ventilation on ward 4C does not meet the standards in SHTM 03-01 and therefore represents a risk of infection for neutropenic and immunocompromised patients.

[86] Bundle 21, Volume 1, Document 8, page 611.
[87] Bundle 21, Volume 1, Document 7, page 468.

8.16. There has been extensive reporting of infection rates in the Schiehallion unit at the RHC. All of the reports[88] [89] [90] [91] [92] show that the rates of bacteraemia with gram negative environmental organisms increased following the move of the Schiehallion Unit to RHC, peaking in 2017-18. Sid Mookerjee showed that the rates of infection decreased to rates similar to comparator units in the rest of the UK following the extensive environmental remedial works undertaken on 2A/2B and other remedial strategies such as system-wide chlorine dioxide dosing, which strongly indicates an environmental link between infections and the water and ventilation systems.

8.17. The final report of the Oversight Board noted that environmental data was not well organised with inconsistent labelling of samples and their locations together with the format in which they were recorded. The report also comments on the inconsistent recording of reference laboratory results and that there was no database recording al typing results for cases. This makes retrospective reviews difficult and potentially incomplete due to missing data.

8.18. The impression gained from the Executive Summary and Chapter 7 is that the authors of the HAD Report consider that if it could be shown that the incidence of bacteraemia caused by 'environmentally relevant' microorganisms in a particular hospital or patient population is in a pattern largely the same as the incidence of bacteraemia caused by microorganisms that are 'not environmentally relevant' then that would indicate that the environment did not play a significant role in harbouring and transmitting pathogens. This theory does not take into account the complete history of infections at QEUH/RHC.

Water

8.19. The water incident occurred in 2017-2018 and it is notable that this coincided with the peaks in incidence of environmental BSI in adult haem-onc patients in QEUH as shown on Figs 5 and 6. These peaks are evident even when the 'adjusted' bed day data is used as the denominator.

8.20. The remedial interventions undertaken by the Health Board including the implementation of point of use filters, chlorine dioxide dosing of the water system, remedial work on 4B and 4C have impacted on the rate of infections and were

[88] Bundle 21, Volume 1, Document 1, page 3.
[89] Bundle 7, Document 2, page 32.
[90] Bundle 7, Document 7, page 250.
[91] Bundle 6, Document 36, page 795.
[92] Bundle 6, Document 38, page 975.

implemented in response to the peaks in paediatric infection rates in 2017 and 2018 which were also seen in the adult data.

8.21. The authors have analysed the data using clustering of cases as a method of counting which is confusing and given the errors made in assessing the presence of clusters described earlier, due to not considering multiple infections/relapses in the same patient, are unlikely to reflect the true picture.

8.22. Chaput[93] reported the data on pseudomonas contamination in QEUH/RHC and showed that the percentage of out of specification samples was 1.8% for the period 2015-2020. This would be reassuring if a larger number of samples had been taken. From early 2016 until late 2018, in the vast majority of months less than 50 samples were taken for the entire new build site. This level of testing is not reassuring and is below what would be expected for the size of the QEUH/RHC building.

8.23. The authors comment on the more intensive water testing undertaken from November 2018 which commenced after the implementation of continuous Chlorine dioxide dosing which would inevitably have an impact on the bacterial counts.

8.24. The Coppry study[94] examined 10 intensive care units over a five-month period and found variable initial levels of tap water positivity varying from no contamination to 76% contaminated with Pseudomonas aeruginosa. All taps on the unit were tested on a weekly basis throughout the study. This study cannot be compared directly with the rate of contamination in QEUH/RHC as the level of water testing is not comparable either in volume or in frequency – to ascertain the contamination rate of 1.8% Chaput looked at 8 years of data with very small levels of testing for several years including when the infection rates in the haemato-oncology patients was at its height. The lack of data from water testing at QEUH/RHC does not allow a conclusion to be drawn on the extent of water contamination in the same manner as in this study.

8.25. Making a comparison with the Halstead study[95] has similar problems. In the Halstead study all outlets (881) in four augmented care units were tested on three occasions over a period of 16 weeks. This gave a picture of the level of contamination of water over the course of the study. At QEUH/RHC from 2016 to 2018, for most months less than 50 samples were taken for the whole site. For the Schiehallion unit where there were three outlets per room plus other outlets on the ward, this would not allow all

[93] Bundle 19, Document 18, page 107.
[94] M Coppry et al. Exogenous acquisition of *Pseudomonas aeruginosa* in intensive care units: a prospective multi-centre study (DYNAPYO study). JHI 104 (2020) 40-45.
[95] FD Halstead. *Pseudomonas aeruginosa* infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals. JHI (2021) 162-168.

outlets to be tested once. It is not possible to use this comparison to conclude that there was no widespread contamination at QEUH/RHC as the data is not comparable.

8.26. It is well known that the taps fitted to the wash hand basins in QEUH/RHC were manufactured by Horne and had plastic flow straighteners[96]. These have been shown to be a high risk for biofilm formation[97] and therefore present a risk of HCAI for neutropenic and immunosuppressed patients. These taps are also fitted with thermostatic mixer taps which deliver water at around 41°C, creating a favourable environment for *Pseudomonas aeruginosa* to grow. This high risk combined with very low levels of water testing also brings into question whether the conclusion that the water system did not have widespread contamination can be made with any confidence.

Susceptibility to infection

8.27. In general, in any population, susceptibility to infectious diseases change with age. The general approach is that the extremes of age, neonatal and very elderly have the highest risk of infection, this drops during childhood and then increases gradually from early-twenties onwards[98].

8.28. Hospitalised patients, due to underlying illness and treatment, are more susceptible to infection. Those who have diseases of the immune system or who are treated with immunosuppressive drugs are at greatest risk.

8.29. In any scientific analysis of infection rates, it is important to compare like with like. As discussed earlier, the comparison of mixed sets of patients does not stand up to scrutiny. The data used in this analysis, split by consultant sector results in a patient mix where not all of the patients have the same vulnerability to infection. The BMT patients will be more vulnerable than other haemato-oncology patients due to their profound neutropenia during transplant and ongoing immunosuppressive drugs. The patients with benign haematological conditions will be less vulnerable than the haemato-oncology patients depending on treatment.

8.30. This type of comparison is helpful when a problem is suspected and assurance is needed as to the correct baseline to be comparing with. If there is confidence that the ‘usual’ rate of infection is within acceptable levels compared to peer units, then

[96] Bundle 21, Document 75, page 188.
[97] JT Walker et al Investigation of healthcare acquired infections associated with *Pseudomonas aeruginosa* biofilms in taps in neonatal units in Northern Ireland. JHI 86 (2014) 16-23.
[98] Glynn, J.R., Moss, P.A.H. Systematic analysis of infectious disease outcomes by age shows lowest severity in school-age children. Sci Data 7, 329 (2020).

prospective monitoring of rates of infection is likely to detect ongoing infection issues in a more timely manner.

8.31. Children with haematological or solid tumours are more susceptible to infection than adults with the same diseases.

8.32. Overall paediatric haemato-oncology patients are more vulnerable to infection than children with solid tumours. In general, children with AML have a higher risk of infection than those with ALL[99].

8.33. It is also known that infection risk persists into adulthood for child survivors of cancer[100].

Blood stream infections

8.34. The statement at 7.2.1 of the HAD Report that '*growth of pathogens in blood samples, i.e., bacteraemia, is not always a true indicator of a bloodstream infection (BSI), as contamination of the blood culture can occur when collecting or processing samples. Therefore, retrospective analyses of blood cultures are not confirmatory of a BSI, and may overestimate true numbers of infections, which is most often true for microorganisms commonly found on human skin'* is largely true, however, cultures which grow skin colonising organisms can also reflect significant infection in a relevant clinical scenario. Features in the clinical history (e.g. extensive immunosuppressive therapy, prolonged fever, embolic events) may aid clinical differentiation between infection and contamination[101]. Dismissing organisms as contaminants without the clinical context is inaccurate at best.

8.35. Bloodstream infections may result from a more localised infection, for example and urinary tract infection may result in bacteraemia and blood stream infection known as urosepsis. Alternatively, a primary BSI may result in localised infection, for example in a prosthetic joint may become infected due to seeding of bacteria from the blood.

8.36. Bacteraemia can be transient and not result in a symptomatic BSI; for example, where there is a source of infection such as an abscess or infected joint, bacteria can enter the blood in low numbers and be cleared quickly by the body's immune system. In these circumstances the blood culture may not be positive. In subacute endocarditis, several blood cultures are recommended to be taken as the bacteria causing the infection are intermittently released into the blood stream. It is usually recommended

[99] F Carlesse, et al. Infections in children and adolescents with Acute Leukaemia, EJC Paediatric Oncology, Volume 3, 2024.
[100] Chehab et al. A population-based study of the Long-term risk of infections associated with hospitalisation in childhood cancer survivors. Journal of clinical oncology. 2022 Vol 41, issue 2.
[101] RM Martinez and DM Wolk. Bloodstream Infections. Microbiology Spectrum, Vol 4, No 4, August 2016.

where this diagnosis is suspected that cultures are taken when the patient's temperature is rising to have the best chance of isolating the causative organism.

8.37. The taking of blood cultures is usually subject to protocols which include the patients displaying signs of infection such as fever (or very low temperature), increased heart rate, low blood pressure, raised serum lactate, or specific infection related clinical signs, etc. This is in order to maximise the yield from the test and ensure that there is a valid clinical reason for taking the test.

8.38. Taking more than one set of blood cultures from the same patient, ideally from a different site such as both arms, or sequentially from a single site such as a central line, can increase both the sensitivity yield from 65-91% of cases to 80-99%[102] and aid in the differentiation between a skin contaminant and a true pathogen (when taken from different sites). In addition, a skin contaminant will likely only grow in one bottle.

8.39. Skin organisms tend to be gram positive with those grouped as coagulase negative staphylococci being the commonest contaminant of blood cultures. The analysis around gram negative environmental organisms should not be affected by these contaminants.

8.40. The lists of environmental and non-environmental micro-organisms broadly match lists used in other reports. *Proteus spp* are considered normal human gut flora[103] and I would argue that they should not be included in this list. A notable absentee from the list is environmental mycobacteria.

8.41. Several environmental organisms have not been considered before in earlier reports including *Cellulosimicrobium cellulans* and *Psychrobacter phenylpyruvicus* which have been seen in adult and not paediatric blood cultures, and *Leclercia adecarboxylata, Ochrobactrum anthropi* and *Scopulariopsis brevicaulis* (a fungal pathogen) which have only been seen in paediatric, not adult, blood cultures from Yorkhill in one patient each and prior to the date of the data set used in the HPS report[104].

8.42. *Cellulosimicrobium cellulans* is a gram-positive bacterium and would therefore have been excluded from other data sets.

8.43. In table 16, the authors compare a 10-year period of BSI at Yorkhill with a 6-year period at RHC and make a finding based on the number of clusters which is erroneous as the time scales are not equivalent.

[102] ML Towns and LB Reller. Diagnostic methods current best practices and guidelines for isolation of bacteria and fungi in infective endocarditis. Inf Dis Clin North Am. 2002 Jun;16(2):363-76.
[103] AL Hamilton et al. Proteus spp as putative gastrointestinal pathogens. Clinical Microbiology Reviews 2018 vol 31 Issue 3 pages 1-19.
[104] Bundle 7, Document 6, page 214.

8.44. The examination of potential clusters of infection is useful but has limitations as described at 8.2.2.

8.45. Although the same patient cohort is followed throughout, there has not been any comparison of infection levels at Yorkhill with other comparable sites in the UK. This lack of assurance questions the validity of its use as a baseline. As discussed earlier, the expectation of moving to a brand-new facility should be that infection rates, particularly in relation to environmental organisms, will decrease.

8.46. In figure 18 it is clearly shown that immediately after the move from Yorkhill to the RHC, environmental BSIs reduced until early 2016. Thereafter rates began to rise again with the mean rate increasing above the baseline with no months returning to zero.

8.47. The authors comment that the rate of all bacteraemias reduced following the peak of early 2018 and conclude that the same interventions that had an impact on the environmental BSI also impacted on the non-environmental infections. In May 2017, two years of retrospective data were analysed, showing an increase in line infections from 3.25 per 1000 line days to 6.33 per 1000 line days. This resulted in the establishment of a Quality Improvement central line associated blood stream infection (CLABSI) working group. The full impact of this working group was not seen until 2019 and the rate of CLABSI fell. This contributed to the fall in BSI seen in figure 17 of the HAD Report.

8.48. Figure 18 demonstrates the reduction of environmental BSI happened concurrently with the implementation of Chlorine dioxide dosing of the water system, the decant of the Schiehallion unit to ward 6A/4B and implementation of mobile HEPA filtration together with the impact of the CLABSI reduction actions.

8.49. The move back to 2A/2B, following a full refurbishment, installation of an upgraded ventilation system including full HEPA filtration and improvements to the water system and outlets, in March 2022 shows the environmental infection rates fall even further. This strongly suggests that rectifying the environmental issues such as contaminated water supply and inadequate, non-compliant ventilation have decreased the risk of environmental infection in this highly vulnerable cohort of patients.

8.50. As stated at paragraph 6.26, I have been unable to identify any publications based on similar situations to that which has arisen at QEUH/RHC. This suggests that the events, their persistence and the widespread nature of the contamination are extremely unusual.

8.51. The lack of comprehensive water testing contemporaneous to the height of the infection episodes cannot exclude an environmental source for the infections with

unusual organisms which were seen in the Schiehallion cohort of patients and to some extent the adult haemato-oncology patients.

8.52. Whole genome sequencing is a useful tool if used in the manner described in many published works on outbreak investigation or prospective monitoring where it has been used. However, that has not been the case here. The water testing was of a very low level, there were issues with recording of results, and identification of stored clinical isolates, and therefore as mentioned at paragraph 5.7 the results reported by Prof Leanord and Dr Brown[105] and those reported by Prof Evans[106] [107] [108] cannot be used to exclude a link.

9. Other papers by the authors

9.1. I have been provided with two further documents [109] [110]. The '*Initial bullet point thoughts on executive summary for report'* from Prof Hawkey is repeated in the executive summary of the final HAD Report. The document '*Glasgow Hospitals Case Note Review D3 - notes and commentary on environmental bacteria'* is largely repeated in Chapters 3 and 4 regarding the Case Note Review.

9.2. I note that the comparison of infection rates with peer units which Prof Hawkey identifies as important in his final paragraph of Chapter 4 (page 40) and *Glasgow Hospitals Case Note Review D3 - notes and commentary on environmental bacteria'* has been completed by Sid Mookerjee[111] and confirms the high rates of infection in the Schiehallion cohort of patients.

[105] Bundle 6, Document 40, page 1195.
[106] Bundle 8, Document 44, page 223.
[107] Bundle 8, Document 45, page 230.
[108] Bundle 8, Document 46, page 239.
[109] Bundle 44, Volume 1, Document 40, page 667.
[110] Bundle 44, Volume 1, Document 41, page 700.
[111] Bundle 21, Volume 1, Document 1, page 3.

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Understanding bacterial biofilms: From definition to treatment strategies

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Bacterial biofilms are complex microbial communities encased in extracellular polymeric substances. Their formation is a multi-step process. Biofilms are a significant problem in treating bacterial infections and are one of the main reasons for the persistence of infections. They can exhibit increased resistance to classical antibiotics and cause disease through device-related and non-device (tissue) -associated infections, posing a severe threat to global health issues. Therefore, early detection and search for new and alternative treatments are essential for treating and suppressing biofilm-associated infections. In this paper, we systematically reviewed the formation of bacterial biofilms, associated infections, detection methods, and potential treatment strategies, aiming to provide researchers with the latest progress in the detection and treatment of bacterial biofilms.

KEYWORDS

bacterial biofilms, infection, antibiotic resistance, biofilm detection, treatment

1 Introduction

Bacterial biofilms have become an essential contributor to global health problems due to antibiotic resistance, the host's immune defense system, and other external pressures. Biofilms are commonly found on the surface of hospital instruments and body tissue, in industry, food processing units, and natural environments (Schulze et al., 2021). Almost all bacteria can form biofilms.

Bacterial biofilms are usually defined as fixed microbial communities encased in extracellular polymeric substances (EPS). It is characterized by changes in the irreversible adhesion of microbial cells to surfaces or substrates or each other, embedded in EPS, and exhibiting specific phenotypes in terms of gene transcription and growth rates. A bacterial biofilm is composed of a single microorganism or a mixture of bacteria, fungi, archaea, protozoa, and yeasts. It has a channel structure that controls the release of gases, nutrients, and antimicrobials.

Free-floating bacterial cells can also aggregate to form biofilms, which exhibit similar characteristics to medical device-related biofilms (Hall-Stoodley et al., 2012). With the improvement of medical technology, the widespread use of medical devices, and the

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pursuit of high quality of life for patients, medical device-associated biofilms pose a severe threat to the life and health of patients. Microorganisms can adhere to almost all medical devices and cause medical device-associated biofilm infections. Device-associated infections usually occur during treatment, where some microorganisms originate from the host. When these microorganisms attach and colonize the surface of a medical device, they can form a biological container. The pathogenesis of medical device-associated infections is related to microorganisms in complex communities that adhere to and grow on device surfaces. Medical device-related biofilms can consist of single or multiple species, depending primarily on the type of device and the time it is left in the patient's body. In most cases, device-associated infections are associated with biofilm formation on device surfaces. When floating bacteria come into contact with the surface of a medical device, they secrete polymers that create a three-dimensional matrix, which eventually sticks to the surface of the device, forming a biofilm structure. When the biofilm on the surface of implanted medical devices reaches a critical level, it can induce an inflammatory response in the host and may even cause implant failure. The most common microorganisms for medical device-associated infections are *Staphylococcus aureus* and *Staphylococcus epidermidis*. Multi-resistant gram-negative bacteria (e.g., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) can also cause medical device-associated infections in complex hospital settings (Niveditha et al., 2012). In addition, microorganisms can adhere to various tissue surfaces in the body (e.g., skin, connective tissue, intestinal mucosa, vascular endothelium, oral cavity, airway, bone tissue, and vagina), which in turn can cause non-device (tissue) -associated biofilm infections and lead to various diseases (Wi and Patel, 2018). When microorganisms in the oral cavity attach to enamel, dentin, and mucosal epithelial tissues, they can form a dental plaque, which is influenced by the nature of the attachment surface, the intraoral environment, and the state of oral health.

When stimulated by the harsh environment, the exopolysaccharides, fibrins and lipoproteins secreted by bacteria adhere to the surface of inert objects and form microbial substances. EPS is conducive to bacterial adhesion and promotes the formation of a biofilm matrix composed of extracellular polysaccharides, exogenous DNA, proteins, and lipids (Schilcher and Horswill, 2020; Chiba et al., 2022). The EPS matrix reduces the effect of antibiotics by neutralizing antimicrobial agents or limiting diffusion using extracellular polysaccharides. EPS facilitates intercellular communication, protects cells from chemical damage, provides oxygen diffusion, releases extracellular enzymes for nutrition and, in turn, stimulates the spread of bacteria within the biofilm (Costa et al., 2018; Galdiero et al., 2019). Bacterial biofilms are highly structured, functional, specific and coordinated. Various substances in biofilms coordinate to complete several life activities, such as bacterial biofilms' morphological diversity, adhesion and protective barrier function. Taking *P. aeruginosa* as an example, Table 1 shows the main components and basic functions of biofilms.

Bacterial biofilms are communities of microorganisms derived from single or multiple bacterial strains. Compared with single-species biofilms, multi-species biofilms showed the following new characteristics through interspecies interactions: increased biofilm mass, increased community cell count, enhanced metabolic activity of community members, increased antimicrobial tolerance, and changes in spatial organization and structure (Sadiq et al., 2021). Multispecies biofilms exhibit new characteristics different from their floating state, resulting from competition or cooperation. The interactions among bacteria of multiple species are synergy, mutual benefit, cooperation, utilization, antagonism, and competition (Liu et al., 2016). Among them, synergistic interactions play an important role in regulating bacterial microbial activity and constructing complex spatial structures of multispecies biofilms. The cooperation and competition between bacterial cells promote the formation of multispecies biofilms. Multispecies biofilms are found in many natural environments, such as the oral cavity, implantable medical devices, and mammalian intestines. The physical interactions, co-adhesion, and metabolic cooperation among bacterial cells promote the formation of multispecies biofilms in natural environments. Until now, research has focused on single-species biofilms. But now microbiologists are paying more attention to multispecies biofilms and cell interactions between communities. The early stage of multispecies biofilm formation is the adhesion and aggregation of microbial cells. Different biofilm-forming abilities and strategies of various bacteria and microorganisms lead to the formation of multiple types of biofilm (Yao et al., 2022). Multispecies biofilms are associated with developing various diseases, such as cystic fibrosis, diabetic foot ulcers, chronic wounds, and otitis media (Chan et al., 2017; Loera-Muro et al., 2021). The development and outcome of multispecies biofilm-associated diseases are related to the physiological organization and distribution of biofilms.

TABLE 1 Main components and basic functions of *P. aeruginosa* biofilm.

Components	Percentage (%)	Functions	Authors
Exopolysaccharides	1-2	Maintaining the structure and stability of biofilm matrix	Rather et al., 2021
Proteins (including enzymes)	<1-2	Maintaining the stability of biofilm matrix and surface colonization; Maintaining the structural integrity of biofilm	Fong and Yildiz, 2015
Extracellular DNA	<1-2	Promoting biofilm formation; Protecting the integrity of bacterial biofilms; Maintaining structural stability; Protecting the host immune system	Okshevsky and Meyer (2015)
Water	Up to 97	Keeping the biofilm hydrated to prevent it from drying out	Flemming and Wingender (2010)

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Due to species diversity, the various stages of bacterial biofilm formation vary but are generally close. The formation of bacterial biofilms is a multi-step process, as shown in Figure 1, including molecular attachment to the surface of an object, bacterial adhesion and secretion of extracellular polymeric substances, maturation of the biofilm through the formation of colonies, and bacterial cell escape and dispersion and formation of a new biofilm structure (Kranjec et al., 2021). The process of bacterial biofilm formation is detailed below.

The attachment of bacterial microorganisms to a surface is the first stage of bacterial biofilm formation. The bacterial cells are transported to the object's surface by convection, Brownian motion, or sedimentation (Palmer et al., 2007). Chemotaxis is prevalent in bacterial microorganisms and refers to the ability of planktonic bacterial cells in fluids to move along a distribution of material concentrations towards chemically induced substances (i.e., sugars and amino acids) or nutrient sources. It promotes interaction between bacterial cells and the surface of an object, which in turn stimulates the colonization and growth of planktonic bacteria on the surface. When planktonic bacteria reach the surface of an object, the sum of the attractive or repulsive forces between the cell surface and the object's surface determines the interaction between the two characters. When the repulsive force is greater than the attractive force, bacteria cannot adhere to the surface; conversely, when the attractive force is greater than the repulsive force, bacteria can stick to the surface (Carniello et al., 2018). Planktonic bacteria adhere to the surface of objects by non-specific physical forces such as van der Waals forces, electrostatic forces, and hydrophobic interactions (Carniello et al., 2018), and planktonic microbial cells are transformed into stable cells. Still, the process is reversible, and the adhesion is weak. Reversible bacterial cells can maintain a two-dimensional structure Brownian motion and can separate from the surface of an object by their mobility and shear effects. The substrate required for bacterial biofilm growth refers to all substrates that come into contact with planktonic bacteria. Physico-chemical properties such as the substrate surface's roughness, hydrophobicity, film modulation, and surface charge can influence the efficiency of planktonic bacterial colonization and biofilm formation.

Hydrophobicity plays a vital role in the attachment of bacterial microorganisms to surfaces. A hydrophobic surface is more conducive to bacterial microbial colonization than a hydrophilic material, probably due to the hydrophobic effect, which reduces the repulsion between the bacterial cell surface and the substrate. Yu et al. (2016) suggest that *Streptococcus mutants*' attachment can influence the hydrophobicity and roughness of the substrate. Bacterial cell surface hydrophobicity is influenced by multiple factors such as bacterial species, growth rate, and culture medium. Hydrophobicity affects the attachment of bacterial microorganisms to surfaces, and when the surface of a bacteria or object is more hydrophobic, the stronger the adhesion between the two. The structural and physicochemical properties of bacterial cells and object surfaces determine whether bacteria attach to hydrophilic or hydrophobic surfaces. Another physical factor affecting bacterial microorganisms' attachment surface is the surface charge. Many amino, carboxyl, and phosphate groups in most bacterial cells give them a negative surface charge. A positively charged surface facilitates the attachment of planktonic bacteria, while a negative charge hinders the attachment of bacteria (Tuson and Weibel, 2013). The surface charge of bacterial cells is influenced by bacterial species, age, environmental pH, ionic strength, and culture medium. The regulatory film is an integral part of the bacterial adherent surface. Almost all planktonic bacteria come into contact with the regulatory film as they transit from the medium to the surface. It is formed as a nutrient adsorbed onto the surface of an object, resulting in a change in the physicochemical properties of the material surface, which in turn affects the attachment of bacterial cells.

The second phase is irreversible adhesion, where microbial cell surface components can recognize bacterial adhesion molecules and consolidate bacterial interconnections (Nourbakhsh and Namvar, 2016). This phase is accomplished through bacterial cell surface hydrophobicity, hydrogen bonding, covalent bonding, ionic bonding, and dipole-dipole interactions. As bacterial cell surface

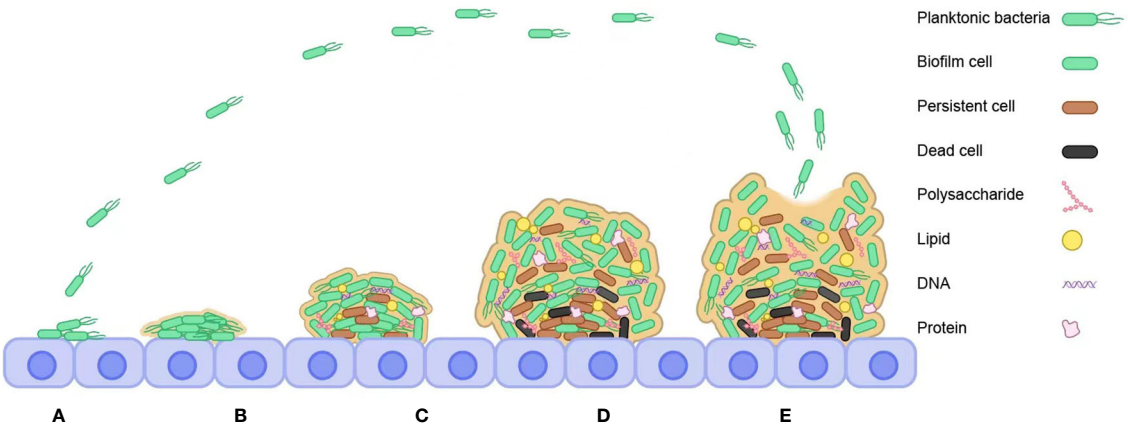

FIGURE 1
Steps to bacterial biofilm formation. **(A)** Reversible attachment. **(B)** Irreversible attachment. **(C)** Bacterial cells synthesize and secrete EPS. **(D)** Maturation. **(E)** Dispersal.

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adhesion structures, flagella and fimbriae can form bacterial biofilms and contribute to the physical contact of planktonic bacteria with the substrate (Carniello et al., 2018). Flagella can swim in liquid substances and swarm on the surface of wet solid substances. Through these two forms of movement, various species of bacterioplankton direct cell adhesion to surfaces and transfer to favorable environments. Flagella initiate the adhesion of planktonic bacterial cells to surfaces, primarily because of the ability of flagella to overcome the resistance that prevents cell-surface interactions. Fimbriae also contribute to the early adhesion of bacterial cells to surfaces and each other. *P. aeruginosa* generates bacterial cell surface motility through fimbriae in a manner known as twitching motility. As an intercellular signaling mechanism, the Quorum sensing (QS) system also significantly facilitates the formation of bacterial biofilms in single cells (Abraham, 2016). Bacterial cells use means through QS to synthesize and release first messengers, such as chemical signals, to enable communication between bacterial flora. Both Gram-positive and Gram-negative bacilli affect the formation of bacterial biofilms. Gram-positive bacilli utilize oligopeptides, while Gram-negative bacilli utilize acyl homoserine lactones (Muhammad et al., 2020).

Bacterial cell synthesis and secretion of EPS is the third stage of bacterial biofilm formation. EPS substrate is hydrophobic and ion bridging, which can promote bacterial condensation and biofilm adhesion. EPS can affect surface adhesion, bacterial biofilm formation, biofilm internal structure, mutual recognition between cells, signal transduction system, nutrient acquisition, cell maintenance, and genetic information exchange, playing a crucial role in various aspects (Costa et al., 2018). The second messenger, c-di-GMP, can stimulate bacterial adhesion from a reversible to irreversible state, mainly due to its ability to produce the EPS matrix and form bacterial cell surface structures. As an essential component of the EPS matrix, extracellular polysaccharides are necessary for most bacteria to form biofilms and promote their development. EPS is also rich in proteins (i.e., enzymes)and protein structures (i.e., fimbriae). At the same time, eDNA and lipids are also components of the EPS matrix. The former can connect to bacterial cells, while the latter can affect the attachment of *Thiobacillus ferrooxidans* (Flemming et al., 2016).

The maturation of bacterial biofilms is the fourth stage of biofilm development. Bacterial biofilms mature as bacteria replicate and multiply in the EPS matrix, forming small microbial colonies and generating three-dimensional structures. As the EPS matrix accumulates and bacterial colonies form, this leads to altered gene expression, and the induction products of these genes can be used for EPS matrix generation. When the matrix is formed, water channels can be generated, which act by a mechanism similar to the body's circulatory system to deliver available nutrients to the cell community while rejecting extraneous products.

The process of dispersal of bacterial biofilms is the final stage of biofilm development. After a while, the mature bacterial biofilm may suffer damage, and the biofilm structure evolves. Still, the released bacterial microorganisms can infect other parts of the organism and form new biofilm structures. The mechanisms of dispersal are different due to the specificity of the bacteria. Still, all broadly involve the following three standard processes: detachment of bacterial cells from small colonies, transfer of bacterial cells to other substrates, and attachment of bacterial cells to new substrates (Shen et al., 2018). The process of detachment of bacterial biofilms can be either active or passive behavior. Active behavior refers to seeding dispersion, where bacterial cells in the biofilm undergo their detachment to adapt to environmental changes when the bacterial biofilm is subjected to matrix-degrading enzymes, antimicrobials, and nutrient deprivation. Passive behavior refers to shedding and erosion dispersion mediated by external forces (e.g., shear). Shedding dispersion refers to the abrupt shedding of a large proportion of the bacterial biofilm, and erosion dispersion refers to releasing a portion of bacterial cells in a bacterial biofilm. Low expression levels of c-di-GMP can inhibit the formation of bacterial biofilms and promote biofilm separation (Kaplan, 2010). Thus, inhibition of the c-di-GMP signaling pathway can effectively disperse bacterial biofilms. Changes in environmental factors such as temperature, pH, oxygen, and nutrient content can also affect the dispersion process of bacterial biofilms. For example, low-oxygen environments can facilitate bacterial biofilm dispersion by accelerating the rate of c-di-GMP degradation. Increased glucose levels can reduce the amount of c-di-GMP in the organism and promote flagellar synthesis, which slows down the progress of separation (Lee and Yoon, 2017).

The process of bacterial biofilm formation is influenced by temperature and blood pH changes, nutrient content, quorum sensing, Brownian motion, and surface properties. At the same time, different strains and signal transduction also affect the formation of bacterial biofilm. The structure of mature biofilm is a matrix layer, regulatory layer, connective layer and bacterial biofilm layer from inside to outside.

Microorganisms can form biofilms on the surface of the device, causing the development of infectious diseases in the organism. Medical implants have brought hope to the medical revolution and provided new opportunities for human life and health progress. Still, they also increase the risk of infections in the body tissues. Bacterial adhesion is the first step in forming biofilms for device-related infections and is divided into two stages: initial non-specific reversible adhesion and specific irreversible adhesion. *S. aureus* and *S. epidermidis* are the main strains (Pietrocola et al., 2022). These microorganisms can come from the patient's skin, the healthcare worker's skin, or the environment. *S. epidermidis* adheres to each other and medical devices by using adhesins. *S. aureus* relies on adhesin ligands (fibronectin, fibrinogen, and collagen) for its adhesion (Darouiche, 2001). The prerequisite for device-related biofilm formation is the coating of the medical device with plasma proteins (Arciola et al., 2018). The type and amount of plasma proteins adhered to the device surface are mainly determined by the surface's physicochemical properties and the plasma proteins' characteristics. When *S. aureus* sticks to the surface of a medical device, it can interact through adhesins. Adhesins specifically recognize plasma proteins coated on the surface of the device. *S. aureus* can then proliferate and produce EPS composed of extracellular polysaccharides, proteins, and eDNA. The final stage is when the *S. aureus* community disperses and spreads the infection (Pietrocola et al., 2022). Inhibition of EPS production, enzyme-promoted EPS degradation, and surfactants all

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contribute to the bacterial dispersion process (Solano et al., 2014). The characteristic change of biofilm dispersion in *S. aureus* is the formation of phenol-soluble modulins (PSM) and extracellular enzymes. PSM plays an essential role in the diffusion stage of biofilm. It disrupts the non-covalent binding forces that strengthen the biofilm matrix, helping to form channels for transporting nutrients to deeper biofilm layers (Peschel and Otto, 2013).

Biofilms are a persistent cause of infection in implanted medical devices. The formation of device-related biofilms is closely related to the interactions between microbial cells. The common microorganisms that can form biofilms on the surface of equipment include *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Enterococcus faecalis*. When a medical device is contaminated with these microorganisms, the formation of biofilms depends on several factors. First, the microorganisms must adhere to the exposed surface of the implant long enough to reach the irreversible attachment stage. The adhesion speed of microbial cells on the surface of the device mainly depends on the content and types of bacterial cells in the liquid exposed by the equipment, the liquid's flow speed, and the physical and chemical properties of the surface. At the same time, the composition of the fluid can change the properties of the device's surface and affect the rate of cell attachment. When microbial cells irreversibly adhere to the surface of a medical device and produce EPS to form a biofilm, the rate of growth and development is influenced by the flow rate, nutrient content, antimicrobial concentration, ambient temperature, and pH (Donlan, 2001). In the absence of antimicrobial agents, when a medical device is implanted in a host, the surface of the device is immediately surrounded by multiple fluids (e.g., blood, saliva, urine, and other fluids). Mucopolysaccharides, glycoproteins, and metal ions then appear within minutes. These substances can penetrate and adhere to the surface of the device. The bacterial cells can use their surface-specific adhesion molecules to recognize the device surface receptors and thus undergo the adhesion process. Once the microorganism adheres to the device surface, the gene expression level is immediately affected. During the growth and reproduction phase, bacterial cells can synthesize EPS, form microbial communities, and then form biofilms (Huang et al., 2022).

Microorganisms colonize almost all central venous catheters implanted in patients. The most common microorganisms that form catheter biofilms are *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *K. pneumoniae* (Elliott et al., 1997). These microorganisms can move outward from the skin or inward from the port along the outer surface of the central venous catheter. Colonization of the catheter surface by microorganisms can occur within 24 hours. Biofilms can commonly form on the surface of central venous catheters, but the location and extent of bacterial biofilm formation are influenced by the time of catheter implantation. When the catheter is implanted <10 days, more biofilms can form on the external surface of the catheter. When implanted >30 days, biofilms are more likely to form in the catheter lumen (Raad et al., 1993). Common contaminating microorganisms on urinary catheters are *S. epidermidis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. When the catheter is implanted in the body, these microorganisms tend to form biofilms on the internal and external surfaces. The longer the catheter is left in place, the greater the ability of these microorganisms to form biofilms and the greater the likelihood of causing urinary tract infections.

Microorganisms can form biofilms on non-device (tissue) surfaces. There are several stages of dental plaque biofilm formation. First, the tooth's surface is covered with an organic "membrane" composed of immunoglobulins, carbohydrates, and glycoproteins. These substances can adhere to the surface of hydroxyapatite through electrostatic interactions. This interaction is generated between Ca2+, phosphate, and molecular groups with opposite charges in saliva. Among other things, carbohydrates comprise intracellularly stored polysaccharides and other intracellularly present polysaccharides. Water-insoluble glucans and fructans promote the attachment of bacterial cells to the tooth surface (Jakubovics et al., 2021). Bacterial cells can bind to surface organic membranes, leading to interactions between adhesion factors and fimbriae, capsules, and complementary receptors. Subsequently, the bacterial cells adhering to the tooth surface can produce various exopolymers to synthesize biofilm EPS (Mirzaei et al., 2020). Maturation and dispersion of dental plaque biofilm is the final stage. During the initial process of dental plaque biofilm formation, the source of nutrition for microbial cells is through the breakdown of salivary substrates (e.g., mucins and other glycoproteins) (Jakubovics, 2015). *P. aeruginosa* is the leading cause of death in patients with cystic fibrosis combined with *P. aeruginosa* infection due to its high virulence factor, biofilm formation, and resistance to antimicrobials. High levels of c-di-GMP expression generally promote matrix production and biofilm formation. In contrast, low levels of c-di-GMP expression down-regulate matrix production and can lead to a bacterial planktonic lifestyle. Carbon starvation and nitric oxide signaling can affect phosphodiesterase activity in *P. aeruginosa* cells, reducing the intracellular expression of c-di-GMP in the cells (Bjarnsholt et al., 2010). *P. aeruginosa* can produce a small molecule that can induce the dispersion of mature biofilms (Davies and Marques, 2009).

Bacterial biofilms provide an excellent and stable homeostasis environment that prevents host immune cells and antibiotics from entering the bacterial biofilm community while protecting bacterial microorganisms from the effects of blood pH, osmotic pressure, and nutrient deficiency. Thus, by providing a physical barrier for bacteria and microorganisms, bacteria can communicate with each other and co-exist, even in harsh conditions. This communication mechanism, called the quorum sensing system, comprises extracellular chemical signals (known as autoinducers). The QS system can help bacteria and microorganisms sense population density and influence biofilm formation and maturation, antibiotic resistance, bacterial communities, and bacterial-host interactions (Whiteley et al., 2017; Paluch et al., 2020). At the same time, some signaling molecules in bacterial microorganisms, such as c-di-GMP, can affect bacterial behavior, including cell cycle, cell movement, pili synthesis, RNA regulation, stress response, and bacterial virulence (Loera-Muro et al., 2021). Bacterial biofilms have specific immune escape mechanisms, including inhibition of immune cell function, alteration of gene expression, obstruction of immune recognition, and mechanical protection (Campoccia et al., 2019; de Vor et al., 2020). When

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bacterial organisms accumulate on the surface and form biofilms, the clearance function of immune cells is impaired, a phenomenon known as impaired phagocytosis (Urwin et al., 2020).

To provide additional research on bacterial biofilms, we review bacterial biofilm-associated infections, describe current methods used to detect biofilms and effective strategies for treating bacterial biofilms, and give an outlook on the development and future of bacterial biofilms.

2 Biofilm-associated infection

Bacterial biofilms can cause serious infections, such as multidrug-resistant, broad-spectrum drug-resistant, and complete drug-resistant bacteria. Currently, more than 80% of bacterial infections are caused by the formation of bacterial biofilms (Fleming and Rumbaugh, 2017). Bacterial biofilms can cause disease in the body through device-related and non-device-related infections. The following will describe the biofilm infections in terms of both and the relationship of bacterial microorganisms to infectious diseases and adhesive surfaces.

2.1 Device-related infections

The use of medical devices improves the quality of life for patients. Still, suppose bacterial biofilms form on the surfaces of medical implants (e.g., dental devices, catheters, heart valves, ventricular shunts, joint prostheses). In that case, they may cause bloodstream and urinary tract infections, posing a severe threat to global health. Medical device-associated infections are closely linked to biofilm formation, and the bacterial biofilm on the surface of most devices is composed of a variety of bacteria. Bacterial organisms first attach to the surface of the medical device or the surrounding tissue of the breakage. Bacterial cells proliferate, develop, and form a bacterial biofilm, which is then encapsulated in the EPS. At the same time, the bacterial cells are released from the bacterial biofilm. They can be transmitted through the bloodstream leading to infection or recurrence of localized lesions elsewhere in the body.

Cardiac implants, including pacemakers, artificial heart valves, cardioverter-defibrillators, and cardiovascular implantable electronic devices, are associated with higher morbidity and mortality due to infections (Habib et al., 2013). Gastrointestinal devices are associated with a wide range of microorganisms. A study using scanning electron microscopy showed defects in percutaneous endoscopic gastrostomy cannulas (PEGs), which can provide suitable sites for the attachment of bacterial microorganisms and biofilm formation (Dautle et al., 2003). The study found that bacterial biofilms were present on the surfaces of all devices included in the examination and that PEGs are a risk factor for colonization of the gastrointestinal tract by drug-resistant bacterial microorganisms and the formation of bacterial biofilms. Orthopedic implant surgery is often a safe and efficient treatment modality that restores hip and knee function and enhances patient well-being. However, there are still postoperative complications, the most common of which is an artificial joint infection, on top of which the formation of a combined bacterial biofilm may lead to osteomyelitis.

Neurosurgical implants include cerebrospinal fluid shunts, extra-ventricular cerebrospinal fluid drains, and neurostimulators. The main microorganisms in cerebrospinal fluid shunts are *S. epidermidis* and *S. aureus* (Fux et al., 2006). The microorganisms capable of forming biofilms on extra-ventricular cerebrospinal fluid drains are mainly *S. aureus*, followed by *Propionibacterium acnes* (Strahm et al., 2018). In neurostimulator-associated infections, common pathogenic microorganisms include *S. aureus*, *P. aeruginosa*, and *P. acnes* (Conen et al., 2017). The incidence of infection with these devices is 3-15%, with a higher incidence of infection with extra-ventricular cerebrospinal fluid drains (Caldara et al., 2022). These infections can be fatal to patients and increase morbidity and mortality. Permanent or temporary urinary catheters, nephrostomy tubes, penile implants, and ureteral stents are the most commonly used devices in the genitourinary system. Bacterial biofilms can be found on the surfaces of all these devices. In most clinical situations, infections from implanted stents occur, with fever and urinary tract infections being the most common complications, and even bacteremia and death may occur (Kehinde et al., 2002). Endovascular devices commonly used in clinical practice include intravenous infusions, hemodialysis, haemodilution, and parenteral nutrition. The risk of infection is increased by irregular disinfection, environmental contamination, and incomplete biofilm removal from the surface of implanted devices. To reduce the risk of infection, we should improve disinfection protocols and procedures in the future and detect the propensity for contamination of all types of implanted devices. Breast implants can be used for breast reconstruction and cosmetic surgery, helping improve patients' quality of life. However, breast implants are susceptible to complications such as infection, hematoma, contracture of the envelope, and scar formation. Contracture can lead to the removal or revision of the implant, causing discomfort and swelling of the breast and affecting its appearance. The researchers detected bacteria in 85% of the breast implants that developed periosteal contracture. They examined them using scanning electron microscopy and found bacterial biofilms in over half of the implants (Pajkos et al., 2003). *P. acnes*, *Streptococcus* spp., *Lactobacillus* spp., *Bacillus* spp., and *Mycobacterium* spp. can survive in the environment around breast implants and form a bacterial biofilm.

Bacteria in the mouth are closely linked to diabetes, heart disease, lung inflammation, and systemic diseases. Prevention and treatment Kalamaraof oral diseases can help prevent these diseases. Oral implants can be used to restore the normal function of the mouth. When bacterial microorganisms colonize the surface of these materials, a solid bacterial biofilm usually forms, leading to inflammation around the implant, which may damage healthy gums. The microorganisms can degrade the composite resin, and the bacteria can invade the implant and tooth interface, leading to severe consequences. Clinicians can apply prophylactic antibiotic treatment to avoid this outcome. At the same time, proper treatment planning, proper implant placement, attention to changes in condition, medication history, and monitoring of

underlying health conditions (e.g., diabetes, hypertension, obesity, osteoporosis) are essential to prevent implant failure. Bacterial keratitis is a cornea infection characterized by forming a bacterial biofilm on the eye's surface. If patients do not receive timely and effective treatment, it will likely lead to vision loss or even loss of vision. Bacterial keratitis is associated with various risk factors, such as corneal trauma, contact lens wear, surgical treatment of the eye, and immunocompromised systemic disease (Ng et al., 2015). Corneal trauma is a significant risk factor for bacterial keratitis in developing countries. Contact lenses are the primary source of bacterial keratitis infection in more developed countries. It can alter the corneal epithelium and carry bacterial organisms to the eye's surface. Bacterial keratitis is associated with the formation of a variety of bacterial biofilms. The lens materials (e.g., water retention, hydrophobicity) and contact lenses' physical and chemical properties can influence the colonization of bacterial biofilms. We should follow up with a vigorous search for novel materials to prevent implantable device-related biofilm infections. The most common bacterial organisms on the surface of medical devices are described in detail in Supplementary Table 1. In conclusion, medical device implant-related biofilm infections increase morbidity and mortality in patients, especially hospitalized patients, and pose a severe threat to their quality of life. We should pay more attention to the composition of different bacterial microorganisms on the surface of specific medical devices, continue to study biofilms *in vivo*, and systematically describe the interrelationship between bacteria on device surfaces and the surrounding environment of implants.

2.2 Non-device-related infections

Non-device-related infections also have a significant impact on health problems. Dental plaque (Rabe et al., 2022), urinary tract infections (Singh et al., 2022), cystic fibrosis (Vandeplassche et al., 2020), otitis media (Bair et al., 2020), infective endocarditis (Han and Poma, 2022), tonsillitis (Klagisa et al., 2022), periodontitis (Prado et al., 2022), necrotizing fasciitis (Grier et al., 2021), osteomyelitis (Huang et al., 2022), infective kidney stones (Fayez Hassan et al., 2021), chronic inflammatory diseases (Wu et al., 2015), bacterial vaginitis (Arroyo-Moreno et al., 2022), and bladder infections (Mirzaei et al., 2020) are all examples of non-device-related bacterial biofilm infections.

The human oral environment, with its favorable temperature and humidity and rich in micro-nutrients, can provide adequate conditions for bacterial growth, survival, and the formation and maturation of dental plaque biofilms. The most common microbial cell grouping in the oral cavity is the bacterial cell, followed by various fungi, viruses, and protozoa. Oral microorganisms co-exist with each other, maintain mutually beneficial relationships with their hosts, and generally do not cause disease. If this community changes, the symbiotic and mutually beneficial balance will be disrupted, leading to various conditions, such as dental caries. Bacterial microorganisms in the mouth can enter the circulation, affect the heart's function, and bind fatty plaque in the coronary arteries. Dental biofilms can cause periodontitis and gingivitis and even lead to tooth decay. Chronic bacterial infections cause almost all periodontal diseases. Periodontal infections increase the incidence of inflammation, promote the formation of plaque, and lead to edema in the coronary arteries. During the maturation of bacterial biofilms, anaerobes are the main colonizing bacteria in the human mouth. Several studies have shown that dental biofilms can cause not only the development of periodontitis but also various systemic diseases such as diabetes mellitus, infective endocarditis, and rheumatoid arthritis (Larsen and Fiehn, 2017; Marsh and Zaura, 2017). The main bacteria that play a role include *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Aggregatibacter actinomycetemcomitans*.

Lactobacillus strains can be associated with health problems by producing lactic acid to maintain a low pH environment in the vagina, thereby protecting the female genitourinary tract from bacterial microorganisms not of its origin. *Lactobacillus* spp. can play a role in maintaining the homeostasis of the vaginal environment while preventing bacterial microbes from colonizing and infecting it. Women are more prone to urinary tract infections than men because of the proximity of the female urethra to the anus, vagina, and rectum. Disturbances in the body's vaginal bacterial microbiota also increase women's risk of urinary tract infections. Estrogen plays a role in maintaining a low pH environment in the vagina, and post-menopausal women are more likely to develop urinary tract infections than younger women due to a lack of estrogen. The most common pathogenic bacteria for urinary tract infections in adult women is *E. coli*. Uropathogenic *E. coli* (UPEC) strains may evade the body's immune response by stimulating a pro-inflammatory reaction or obscuring the immunogenic bacterial component. Bacterial biofilms may play a key role in maintaining the sustainability of UPEC strains in the bladder and vagina. The second most common pathogen of urinary tract infections is *Proteus* spp. When they first adhere to surfaces, *Proteus mirabilis* can proliferate, develop and form biofilms, enhancing the antimicrobial properties of bacterial microbes and protecting the bacteria from the body's immune function. Bacterial biofilms are an essential factor in the persistence and existence of bacterial infections, and their diversity can influence the viability and survival of bacterial microorganisms (Delcaru et al., 2016). In addition, bacterial biofilms play an important role in renal pathology and can affect renal stones and the dialysis system. Pathogenic bacteria invade renal tissue and can cause chronic pyelonephritis and bacterial prostatitis. Most of the *E. coli* strains isolated from bacterial prostatitis exhibit the potential to form bacterial biofilms.

Bacterial vaginitis is the most common vaginal infection worldwide and severely impacts the quality of life of women who suffer from it. It is characterized by increased vaginal discharge, usually accompanied by a specific irritating odor. Various bacterial biofilms are present on the surface of the vaginal epithelium. *Gardnerella vaginalis* is associated with bacterial vaginitis infections and has been shown in several studies to produce bacterial biofilms on vaginal tissue and enhance the expression of various virulence factors (Castro et al., 2015; Gaspar et al., 2021).

Cystic fibrosis responds poorly to medications and is one of the chronic inflammatory diseases. Several studies have shown that

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most people with cystic fibrosis are susceptible to *P. aeruginosa* infections (Harrison, 2007; Filkins and O'Toole, 2015). *P. aeruginosa* infections in combination with cystic fibrosis are challenging to cure, mainly due to the formation of bacterial biofilms in the lungs of patients with cystic fibrosis. *P. aeruginosa* can colonize, develop and form bacterial biofilms in the lungs of people with cystic fibrosis due to the excessive release of mucus in the airways, providing an environment with low oxygen levels. Chronic *P. aeruginosa* infection can lead to complications such as epithelial tissue damage, mucus obstruction of the airways, respiratory dysfunction, and even accelerated death.

Infective endocarditis is a refractory disease that severely threatens human life and health. Although medical technology has been refined, the mortality rate of patients with co-infected endocarditis during hospitalization is still higher than 20% (Beynon et al., 2006). The ability to generate bacterial biofilms is critical in the virulence of bacterial microbes associated with infective endocarditis. Studies (Elgharably et al., 2016; Polewczyk et al., 2017) have confirmed that many bacterial microorganisms can form infectious neoplasms on the surface of the heart, which can lead to the development of infectious endocarditis. These neoplasms are essentially large bacterial biofilms. Using immunofluorescence staining techniques and electron microscopy, Bosio et al. (2012) found *Mycobacterium fortuitum* biofilms on infected artificial biological heart valves. Bacterial microorganisms shed from biofilm structures and enter the blood circulation, and the body will develop bacteremia and sepsis manifestations. Of these, bacteremia is controlled by the collective immune defense system and antimicrobials. Although bacteremia can be effectively prevented or even eliminated with aggressive treatment, the deeper biofilm and neoplasm structures can provide a "hideout" for the ever-present bacterial biofilm cells. When bacterial cells in biofilm structures are repeatedly shed, it is easy to develop septic embolism in the distal limb (Mirzaei et al., 2020).

Otitis media is a complex inflammatory disease, and its complications are a major cause of hearing loss. Approximately 75% of infants under three years suffer from middle ear-associated infections. Bacteria capable of forming biofilm in the middle ear are the leading cause of chronic bacterial infections. Using molecular methods, researchers have demonstrated that the DNA of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* can be detected in up to 80% of infants with otitis media (Otsuka et al., 2013).

The formation of bacterial biofilms can lead to severe chronic inflammatory and autoimmune diseases in the body. L-type pathogens and chronic biofilm infections can cause the development of inflammatory diseases. The vitamin D receptor is an alkaline substance that regulates the activity of immune cells and is the primary defense against bacterial microbial infections. Specific L-type and biofilm-forming pathogens can produce a substance that adheres to and kills the vitamin D receptor. Thus, as chronic bacterial biofilms and L-type pathogens accumulate in the body, most bacterial microorganisms can produce substances that inactivate the vitamin D receptor and severely compromise the body's immune defenses. Supplementary Table 2 describes non-device-related biofilm infections.

2.3 Bacterial biofilm cause tissue related and device associated infections

S. aureus and *S. epidermidis* can form biofilms on the surfaces of central venous catheters, heart valves, suture devices, and prostheses, in turn, can cause nosocomial infections, endocarditis, mucus cysts, and otitis media (Qu et al., 2010; Arciola et al., 2012). *P. aeruginosa* forms biofilms on the surface of contact lenses, central venous catheter, middle ear, and prosthesis, which can cause nosocomial infection, cystic fibrosis, and otitis media (Wiley et al., 2012; Huse et al., 2013). Bacterial biofilms formed by *S. aureus*, *E. coli* and *Streptococcus agalactiae* can cause mastitis (Loera-Muro et al., 2021). *E. coli* can form biofilms on medical devices such as catheters (Zhang et al., 2019), ultrasonic instruments (Khatoon et al., 2018), and contact lenses (El-Ganiny et al., 2017). The relationship between bacterial microbes adhering to surfaces to form bacterial biofilms and thus causing disease is detailed in Supplementary Table 3. The formation of bacterial biofilms will increase mortality in hospitalized patients (Batoni et al., 2016; Evans and Bolz, 2019). Multiple biofilm infections may exhibit different antibiotic sensitivities. Although the specific details of bacterial interactions in numerous microorganisms are not yet precise, there are metabolic links and spatial organization among bacteria, which may result in quorum sensing and resistance gene transfer to produce more antibiotic-resistant biofilms (Røder et al., 2020; Sadiq et al., 2021).

The three-dimensional structure of bacterial biofilm can act as a natural barrier to antibiotics and reduce the sensitivity of biofilm to antibiotics. Bacterial biofilm resistance to antibiotics is known to be 10–1000 times higher than planktonic bacteria (Ning et al., 2014), which may be related to the different antibacterial mechanisms between them. Biofilm communities produce antibacterial target mutations, efflux pumps, higher transverse transfer frequency, reduced cell permeability, and drug-neutralizing proteins (Kumar et al., 2013; Lata et al., 2015; Sharma et al., 2015; Sharma et al., 2016). In addition, there is a particular type of bacterial cell phenotype in biofilms – persistent cells, which can survive under the action of a powerful immune defense system and powerful antibiotics. These cells have metabolic inertia, slow growth and replication, and can regulate virus-antitoxic systems and enhance antioxidant and DNA repair systems. They show an inability to respond to antibiotics (Rather et al., 2021). Anoxia, nutrient deficiencies, antibiotic modification enzymes, and oxidative stress can also lead to antibiotic resistance in bacterial biofilms (Hall and Mah, 2017). Due to the phenotypic diversity of bacterial biofilms, the content of antimicrobials entering the biofilms will be reduced, and the microenvironment within the biofilms will be changed, causing the biofilms' resistance mechanism to antibiotics (Sharma et al., 2019), making it difficult to eradicate. In addition, genetic mutations can also lead to antimicrobial resistance in bacterial biofilms. As a result, the effect of antibiotics commonly used in clinical practice on bacterial biofilms is insignificant.

In recent years, due to the increasing incidence of iatrogenic bacterial biofilm-associated infections, long-term infections are difficult to cure, which will pose a new challenge to preventing and controlling infectious diseases caused by biofilms. In addition,

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bacterial biofilms can avoid the scavenging action of conventional antibiotics and the killing effect of the body's immune system and become a possible source of infection. Currently, methods for detecting, inhibiting and treating biofilm-associated infections are inadequate, and managing bacterial biofilm-associated infections remains a significant challenge. In addition, due to the specific environment within the biofilm, we cannot treat biofilm-associated infections with traditional antibiotics, and researchers must propose new antimicrobial or anti-biofilm-associated treatment strategies.

3 Methods for detecting bacterial biofilms

3.1 Nuclear medical imaging technology

Nuclear medicine imaging remains the standard method for detecting infectious diseases (Salmanoglu et al., 2018). As radionuclides, technetium-99m, indium-111, and iodine-125 have been shown to be useful for radio-labelling compounds. Still, some drawbacks exist, such as low expression of target receptors on the bacteria studied, non-specific adsorption, and complex radiochemical synthesis (Eggleston and Panizzi, 2014). A recent study found that as a metabolic product of bacteria, the maltodextrin transport system can also radially label compounds and trace them to detect bacterial biofilm infection (Auletta et al., 2019). Nuclear medicine imaging technology also has certain disadvantages, such as the need for specialized equipment and instruments, operator training, and patient exposure to radiation (Cruz et al., 2021).To further improve the sensitivity of nuclear medicine imaging, relevant researchers have developed an MH18F nuclear imaging agent, which can participate in the metabolism of bacterial carbohydrates and be internalized by maltodextrin transporter specific to bacteria (Auletta et al., 2019), contributing to the early detection of bacterial biofilms.

3.2 Ultrasonic technology

Ultrasonic technology can monitor dirt on instrument surfaces in real-time and has been shown to monitor the formation and growth of some bacterial microbial colonies (Kujundzic et al., 2007). Combined with other methods, it can enhance the detection strategy of bacterial biofilms (Vaidya et al., 2014). Ultrasound echo enhancers and microbubble contrast agents have opened up new avenues in diagnostic ultrasound medicine (Calliada et al., 1998). Ultrasonic medical imaging technology has matured with the application of contrast media, which contributes to the accuracy of medical diagnosis. The application of targeted ultrasonic contrast media is conducive to determining the difference between healthy and infected tissues (Unnikrishnan and Klibanov, 2012). The acoustic impedance of bacterial biofilms is similar to that of human tissue, making detection and *in vivo* targeting of bacterial biofilm substrates difficult. The combination of ultrasound and targeted ultrasound contrast agents can aid in the early detection and identification of bacterial biofilms and can help to improve therapeutic efficacy. Ligand-targeted ultrasound contrast agents can be a non-invasive imaging method for detecting early and late-stage bacterial biofilms. These agents can target, image, and detect the formation of the *S. aureus* biofilm matrix *in vitro* (Anastasiadis et al., 2014).

3.3 Crystal violet staining method

Crystal violet (CV) is the most commonly used staining method for the quantitative determination of microtiters of biofilms grown *in vitro* in polystyrene pore plates (Hassan et al., 2011). After CV staining, the structure of the biofilm can be observed directly by scanning electron microscopy. However, this staining method also has certain limitations, as it can reduce the number of bacterial biofilms after multiple washing (Hou et al., 2022), and the incubation time is longer. It is unsuitable for the rapid detection of biofilms. Castro et al. (2022) found that CV staining was highly effective for single-species biofilm detection. Still, in bacterial vaginosis, there might be bias in evaluating the formation of multiple bacterial biofilms.

3.4 Other detection methods

Bioluminescence analysis, tissue culture plate method, and percentage transfer method are also used to detect bacterial biofilms. Certain imaging techniques, such as infrared spectroscopy, reflection spectroscopy, optical fluorescence imaging, confocal laser scanning microscopy, target fluorescence imaging, and fluorescence *in situ* hybridization of peptide nucleic acid, can also be used to detect the formation of biofilms and provide spatial information on the distribution of strains and biofilms (Roy et al., 2018; Cruz et al., 2021). However, these imaging techniques depend on clear samples and are unsuitable for *in situ* bacterial biofilm detection. Raman and surface-enhanced spectroscopy offer high sensitivity and are non-invasive molecular detection techniques (Xu Y. et al., 2020). Laser capture micro-cutting technology has a high resolution, which can help researchers quickly separate or sample the required cells from solid tissue by laser beam, which is helpful for the detection of living biofilms. Ultra-wide-spectrum imaging is a labeling-free detection method showing bacterial biofilms in natural and wound environments.

One of the components of bacterial biofilm is protein, which is related to the types of pathogens and the stages of biofilm development and virulence. Several different proteins are listed below for their role in forming and maintaining biofilm structures. *S. epidermidis* expresses a variety of cell wall-anchored surface proteins that promote the formation of biofilms on the surface of medical devices, aid in binding to the EPS, and are a significant determinant of the virulence of *S. epidermidis*. For example, SdrG/Fbe protein can promote adhesion to the surface of conditioned biomaterials. SdrF protein enhances the adhesion between bacteria and fixed collagen. Embp protein can promote bacterial adherence to fixed fibronectin. Sesl protein may promote adhesion between bacteria and non-living surfaces. Sbp protein can

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promote the formation of amyloid protein and maintain biofilm integrity (Foster, 2020). McCourt et al. (2014) found that surface-bound fibronectin FnBPA and FnBPB could mediate the formation of methicillin-resistant *S. aureus* (MRSA) biofilm, increase bacterial aggregation, promote initial microbial adhesion on the surface, and facilitate biofilm accumulation. Manfiolli et al. (2018) showed that MAP kinases (MpkA, MpkC, and SakA) and phosphatases could regulate the *Aspergillus fumigatus* cell wall composition and affect cell adhesion and EPS production, as well as have essential effects on signaling pathways during biofilm formation. Liang et al. (2011) identified a novel cell surface protein, BapA1, from *Streptococcus parasanguinis* FW213 and found that it can affect biofilm formation. The BapA1 protein contains multiple putative fimbriae isopeptide junction structural domains that promote the aggregation of bacterial fimbriae in Gram-positive bacteria and is a novel streptococcal adhesin. When the BapA1 protein is deficient, it can inhibit the auto-aggregation of bacterial cells. Ye et al. (2018) found that outer membrane protein W (OmpW) contributed to the survival of *Cronobacter sakazakii* cells in a planktonic mode under the stress of NaCl and that the ability of cells to survive and form biofilms increased with increasing OmpW concentration.

Quantitative proteomics technology based on iTRAQ is helpful for the detection of bacterial biofilms and the search for promising targets for biofilm elimination (Sauer, 2003). The meta-proteomic analysis is an important technique for showing the interactions and functional roles of individual members of bacterial microbial communities. The areas of proteomics include protein function, expression level, post-translational modification, localization, stability, and adequate genome sequencing. Proteomic patterns are responses to the physiological state of cells and can elucidate bacterial biofilm phenotypes (Khemiri et al., 2016). Through the proteomic analysis of *Burkholderia pseudomallei* in the floating state and biofilm state, Khan et al. (2019) found that the change of proteome contributed to the survival of the biofilm by increasing the abundance of pressure proteins and reducing the presence of metabolic proteins. Ali Mohammed et al. (2021) used proteomic analysis to determine the proteome of *Fusobacterium nucleatum* and *P. gingivalis* expressed in the planktonic or biofilm state. The results showed that *P. gingivalis* produces fewer proteins due to the presence of *F. nucleatum* in the mouth. Resolution and dynamic range limit the initial development of proteomics techniques based on electrophoresis. The addition of transcriptomic analysis has led to the rapid development of proteomics because of its ability to detect solid structures capable of detecting functional cells. Charlebois et al. (2016) confirmed that by RNA transcriptional sequencing technology, 25.7% of genes were different between *Clostridium perfringens* biofilms and planktonic cells. About 12.9% of genes in biofilm cells were down-regulated, and about 12.8% were up-regulated. After leptospira forms a mature biofilm, some fundamental biological processes, such as DNA replication and cell division, are down-regulated. Transcriptome-based sequencing techniques can focus on transcriptional changes associated with leptospirosis biofilm formation and maturation (Iraola et al., 2016). *Fusobacterium nucleatum* stain ATCC 25586 at the planktonic cell and biofilm stages by RNA sequencing. It was confirmed that 110 genes of the *F. nucleatum* biofilm state differed from those of planktonic cells. The 85 down-regulated genes in the biofilm state are mainly related to cell proliferation, division, and oxidative stress. The 25 up-regulated genes are primarily associated with amino acid and carbohydrate metabolism (Zhao et al., 2022).

Metabolites are generated in the presence of metabolic enzymes and are the end products of gene expression processes. Many of the life activities in microbial cells (e.g., energy release, cellular signal release, and intercellular communication) are regulated by metabolites. Metabolites can reflect the microenvironment in which a bacterial cell is located and are also closely related to the nutritional status of the cell, the effects of drugs, contaminants, and other external factors. Several metabolites are listed below for their functions in biofilms formed by bacterial pathogens. Glucose, as the primary carbon source and metabolite, can upregulate the expression of extracellular polysaccharide-related gene pslA, which is conducive to promoting the formation of *P. aeruginosa* biofilms and changing metabolic pathways. Glucose could lead to decreased expression of 18 metabolites (including inositol, glutamine, 4-acetyl butyrate, myristic acid, and β-alanine) and increased expression of 7 metabolites (including fructose, 3-hydroxy propionic acid, and glucose-6-phosphate) in *P. aeruginosa* biofilms (She et al., 2019). Guanosine 59-diphosphate 39-diphosphate is also a metabolite that regulates the expression of a large number of genes and plays a vital role in the formation of biofilms of *E. coli*, *E. faecalis*, and *S. mutans* (de la Fuente-Núñez et al., 2014). Short-chain fatty acids, including butyrate, are also strongly associated with the pathogenesis of the periodontal disease. In the initial stage of biofilm formation, butyrate can promote early *Actinomyces oris*-dependent colonization and stimulate biofilm formation (Barbour et al., 2022). *Bacillus subtilis* can produce a variety of specific metabolites. Most of these metabolites (e.g., bacteriolysin, subtilisin A, surfactin, spore-killing factors) are associated with antimicrobial properties (Kalamara and Stanley-Wall, 2021). Schoenborn et al. (2021) investigated the role of nine specific metabolites in biofilm formation. They found that most of them (surfactin A, ComX, subtilin A, spore delaying protein, spore killing factor) could promote biofilm formation.

Metabolomics analysis techniques can reveal the processes of bacterial cell metabolism and are the study of metabolites (e.g., carbohydrates, amino acids, and lipids), intermediate metabolites, and other signaling molecules. The technique emphasizes discrete altered metabolic pathways and highlights biological small molecule metabolites. The Metabolite group is a complete assemblage of all metabolites in the tissues, organs, and compartments of biological cells that are extracted from cells or expressed as bodily fluids (Zhang and Powers, 2012). Metabolomics techniques focus on the broad or total analysis of cellular metabolites through high-throughput detection methods rather than localized and targeted analysis of specific numbers of individual metabolites (Fiehn, 2002). Many metabolic pathways between planktonic bacterial cells and biofilms were changed. Through instrumental analysis, bioinformatics, stoichiometry, and cell biology, metabolomics analysis can show the status of the overall metabolites, simplify the process of metabolite detection, and cover almost all metabolic changes of major pathways. Metabolomics analysis provides a systematic method for characterizing complex bacterial

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communities, showing the behavior of bacterial cells in biofilms, and contributing to the understanding of biofilms. In addition, biofilms' strain type and antimicrobial resistance are phenotypic by their differences from the metabolome. Shen et al. (2020) showed that carnosol could inhibit the formation of *S. aureus* biofilm by metabonomics analysis technique.

The fiber optic biosensor is capable of monitoring the growth quality of bacterial biofilms, quantifying analytes, and displaying biofilm properties. It works by monitoring the environment around the sensing element through changes in the refractive index. The sensor offers the following advantages: lightweight construction, compact size, biocompatibility, low fabrication costs, and real-time monitoring. The spherical resonance sensor is highly sensitive and can detect changes in the surface of bacterial cells, such as planktonic cells attached to the surface of an object, and has great potential for early detection of the presence of bacterial biofilms (Rakhimbekova et al., 2022). SiNW-FET, as a new nanosensor, can be combined with microfluidic technology to realize real-time, rapid, and fully automated detection of bacterial biofilms. It can reveal the biological and metabolic processes occurring in bacterial biofilms and has the advantages of high sensitivity, low consumption, non-invasive and traceless (Yeor-Davidi et al., 2020). Dai et al. (2022) reported a PH-responsive branched polymer [poly(MBA-AEPZ)-AEPZ-NA] capable of reducing the dose of antibiotics and overcoming antimicrobial resistance. It can emit intense green light rays in the local bacterial biofilm microenvironment (pH 5.5) to detect biofilm formation in real time. AmyGreen, a water-soluble amino ketone fuel, is a stain that enables the visualization of the amyloid component of the extracellular polymeric substances of bacterial biofilms. It can detect pathological amyloid proteins *in vitro* as a potent fluorescent dye. The application of the AmyGreen stain effectively reduced the risk of false positives when measuring the amyloidogenic fibrils of biofilms. In combination with other stains can be used for confocal fluorescence microscopy (Moshynets et al., 2020). Pandit et al. (2021) have developed a simple sensor of raw, non-functional graphene that is simple to manufacture and can be powerful without the need for precise species identification. It can distinguish and detect different bacterial types according to different growth dynamics, adhesion density, adhesion pattern, and colony formation between bacterial cells, which is helpful for the early detection of bacterial microbial colonization and biofilm formation. A new diagnostic kit is the product of a combination of two reagents, one that relies on a substance that promotes hydrogen peroxide to produce oxygen through catalase and the other a mobile biosensor. The kit can detect *P. aeruginosa* infection in sputum with high sensitivity and specificity within 8 minutes (Clemente et al., 2020). Bacterial biofilms attached to the mucous membranes of the mouth are difficult to visualize with the naked eye. Quantitative light-induced fluorescence (QLF) can detect bacterial infections in the oral cavity and the formation of dental biofilms. Park et al. (2022) demonstrated using QLF to detect and remove pathological biofilms from oral mucosa in elderly patients during hospitalization. The test tube method and Congo red agar technique can help detect biofilm formation by obtaining isolates of bacterial biofilm formation from contact lenses, the conjunctiva of contact lens wearers, and decorative contact lens cases (Raksha et al., 2020). Kouijzer et al. (2021) demonstrated that microvesicles in vancomycin-modified bacteria could detect the formation of *S. aureus* biofilms and could potentially treat *S. aureus* biofilm-associated infections.

Artificial intelligence technologies have also been found to be beneficial for detecting bacterial biofilms due to their powerful computational and learning capabilities. For example, biosensors based on electrochemical impedance spectroscopy can be used to detect *E. coli* biofilms (Xu et al., 2022). Convolutional neural network (CNN) can be used to detect the presence of biofilms and the formation of multiple biofilms through deep learning, with a CNN accuracy of up to 90%. Oh et al. (2022) have developed a material capable of precisely guiding the diagnosis and removal of bacterial biofilms. The material is a magnetic field-directed assembly of nanomaterials into surface topography adaptive robotic superstructures (STARS). It can adapt to complex bacterial surface topography and use automatic motion patterns to target the complex three-dimensional structure of human teeth to detect dental biofilm content with high accuracy while the effect of removing formed biofilm.

Currently, non-invasive techniques used in clinical practice have yet to provide the best method for detecting biofilms. Low practicability, low resolution and low cost-effectiveness limit the development of biofilm detection tools. Therefore, there is an urgent need to develop more accurate and practical detection techniques and diagnostic tools.

4 Strategies against bacterial biofilm removal

Bacterial biofilm inhibition is mainly achieved by physical, chemical or biological methods. Supplementary Figure 1 shows the different therapeutic strategies that inhibit bacterial biofilm formation. Physical methods include ionizing and ultraviolet radiation, damaging instruments and affecting material quality (Galié et al., 2018). Ultrasonic treatment is also one of the physical methods. Its mechanism of action is mainly through chemical and mechanical energy, including pressure, vibration, shear stress, shock waves and agitation. Stable pressure and cavitation can produce multidirectional acoustic microjets, which can damage proximal bacterial microorganisms and their biofilms. Relevant studies have confirmed the effect of plasma technology on therapeutic biofilms (Govaert et al., 2019; Patange et al., 2019). The influence of plasma on bacterial biofilms is mediated by biological activators, such as charged particles, ions, electrons, electric fields, and ultraviolet rays (Lu et al., 2016). Cold plasma can kill bacterial microorganisms and destroy the biofilm matrix. Still, the specific mechanism remains unclear, which may be related to ROS/RNS penetrating bacterial cells, oxidizing and nitrosating lipids and proteins, and then lipid peroxidation, inhibiting enzyme function, and changing DNA structure (Van Impe et al., 2018). Patange et al. (2021) found that atmospheric plasma and acoustic ultrasonic treatment technology can destroy the integrity of bacterial biofilm

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structure and effectively inactivate *E.coli* and *Listeria innocua* biofilm. They also found that atmospheric plasma was more effective than aeroacoustic ultrasound in treating both early and mature biofilms. When applied in combination, the two technologies have the potential to enhance the inactivation effect. The main mechanisms of atmospheric plasma damage to bacterial biofilms include cell membrane damage, structural changes, and biological and genetic changes. Future studies are needed to understand further the distribution of active particles in atmospheric plasma and acoustic ultrasonic techniques and the detailed mechanism of inactivating bacterial biofilms.

Microneedles (MNs) are an effective and minimally invasive method for the treatment of bacterial biofilms. MNs can not only destroy the tight physical barrier of EPS and directly penetrate the antibacterial agent into the biofilm, but also provide a large specific surface area to promote the diffusion of antibiotics in the biofilm. Yi et al. (2021) combined chitosan and zinc nitrate with MNs structure to produce CS-Zn[II] MNs. This substance has good cytocompatibility, rich acicular design and a large specific surface area, and is conducive to eradicating *S. aureus* and *E.coli* biofilms.

Chemical methods are unstable, cannot play a role in mild conditions, and the price of chemistry is relatively high, prone to producing toxic byproducts. Therefore, it is not usually the preferred method for eradicating bacterial biofilms.

Biological methods have higher inhibition efficiency, which is a relatively new method against biofilm, including the application of bacteriophage, bacteriocin and enzyme treatment of bacterial biofilm. In recent years, emerging anti-biofilm preparations have been developed to limit the adhesion of bacteria on the surface to eliminate the biofilm grown or replace cells from the established biofilm (Batoni et al., 2016). The EPS matrix in biofilms plays a crucial role in evaluating the drug resistance mechanism of biofilms. The ideal anti-biofilm preparation is characterized by its unique structure, antibacterial activity, restriction of EPS accumulation, facilitation of EPS penetration into cells, interference with communication mechanisms between cells, and synergistic action with other antibacterial agents (Wiens et al., 2014).

4.1 Antimicrobial peptide

Novel antimicrobial peptides (AMPs), as a kind of cation, can down-regulate biofilm formation genes, prevent bacteria from adhering to the cell-matrix surface, down-regulate QS system signals, and produce a wide range of antibacterial activities against bacterial microorganisms (Yazici et al., 2018). When used alone or in combination with antibiotics, AMPs can effectively inhibit the formation of biofilms or even destroy mature biofilms (Mulani et al., 2019) and can be used to treat biofilm-associated infections. AMPs bind rapidly to cell membranes, reducing bacterial load while circumventing the development of resistance, and it has strong antibacterial, fungal, and viral properties (Pontes et al., 2022). One of the mechanisms of action of AMPs against bacterial biofilms is the down-regulation of gene expression and inhibition of the biological behavior of bacterial cells (e.g., synthesis of DNA, RNA, proteins, and cell walls) (Graf and Wilson, 2019). At the same time, AMPs can interact with signaling molecules, which can control the maturation process of bacterial biofilm and cooperate with antibiotics to resist bacterial microorganisms.

Heinonen et al. (2021) proposed that AMP TAT-RasGAP 317-326 could effectively inhibit the formation and development of biofilms of *P. aeruginosa* and *A. baumannii*, and had little anti-biofilm activity against *S. aureus*. In the human oral environment, AMPs are widely present in the oral mucosa and salivary glands of the epidermal cells and neutrophils (Dale and Fredericks, 2005). AMPs are small in shape and have a positively charged amphiphilic structure, which can enter bacterial microbial cells through transmembrane pores and cause their cracking. AMPs are a potentially effective treatment for bacterial biofilm-associated oral infections due to their extensive antibacterial activity, low drug resistance, and ubiquity in the oral cavity. LL-37 is one of the most widely studied AMPs, which can modulate the immune response, regulate the inflammatory response, accelerate angiogenesis, promote wound healing, and help in oral defense against bacterial biofilms (Wuersching et al., 2021b). Levels of LL-37 are associated with various chronic inflammatory diseases, such as periodontal disease, gingivitis, systemic lupus erythematosus, and psoriasis (Pahar et al., 2020). Lactoferrin peptides are functional AMPs hydrolyzed by pepsin and are an effective anti-biofilm preparation. Wuersching et al. (2021a) demonstrated that LL-37 and human lactoferrin could interfere with the planktonic growth of anaerobic bacteria and the formation of bacterial biofilms, thus reducing the incidence of dental caries and periodontitis. Lactoferrin chimeras have a wide range of antibacterial activities. Ruangcharoen et al. (2017) proved that, compared with minocycline hydrochloride and chlorhexidine digluconate, lactoferrin chimeric could better inhibit the formation of oral multispecies biofilms *in vitro*, and it could reduce the activity of various bacterial cells in the biofilms. Antimicrobials depend on active cells, whereas AMPs are less specific for targeting molecules and can even target metabolically dormant cells in most regions of the mature bacterial biofilm.

At present, treating cystic fibrosis combined with *P. aeruginosa* infection remains difficult. Metal AMP piscidin 1 and piscidin 3 showed activity against *P. aeruginosa* biofilm infection, mainly due to their ability to tolerate an acidic environment and high ionic strength and to cut eDNA in the presence of Cu^2+. Metal AMP Gaduscidin-1 (Gad-1) is a broad-spectrum AMP that binds Cu^2+ efficiently in acidic and neutral environments. Holo-Gad-1 can eradicate mature bacterial biofilms and prevent the formation of new biofilms, mainly because eDNA is required for adhesion during the formation of nascent biofilms (Whitchurch et al., 2002). Portelinha and Angeles-Boza (2021) demonstrated that Gad-1 has multiple forms of action and can effectively remove *P. aeruginosa* biofilms in acidic, neutral, and high-salinity environments. When GaD-1 is combined with several commonly used antibiotics (e.g., Kanamycin and ciprofloxacin) to treat bacterial and microbial infections, it can play a synergistic role and improve the survival outcome of patients. This paper lists the acting bacteria and mechanisms of several peptides with antibiofilm activity in Supplementary Table 4.

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Most AMPs can be combined with antibiotics to enhance the role of antimicrobials in preventing bacterial biofilm formation and killing mature biofilms. Antimicrobial peptides are expected to improve the antibiofilm effect and reduce the dosage of antibacterial agents. However, there are limitations to the application of AMPs. Because AMPs are trapped by anionic biofilms, are easily broken down by enzymes in the biofilms, and may even cause acute hemolytic and toxic reactions.

4.2 Nanoparticles

Nanomaterials can reduce the adhesion of bacterial biofilms, promote the delivery of antimicrobial agents, improve the permeability of antibiotics, maintain the stability of antibiotics, and directly produce resistance to biofilms through specific mechanisms without antibiotic treatment. Due to their unique physical and chemical properties, namely biological response, surface charge and small-scale effect, nanomaterials can acquire a variety of antibacterial modes and perform multi-potency interactions with bacterial cells. Nanomaterials are effective carriers of antibacterial agents and inhibit the growth of biofilms through thermal damage, oxidative stress, and physical damage, which is conducive to the treatment of bacterial biofilm infection (Makabenta et al., 2021), and are not prone to drug resistance (Pelgrift and Friedman, 2013). Because of their special structure, nanomaterials can increase antibacterial activity in the following four ways. The surface charge of nanomaterials can enhance the interaction with bacterial microbial cells and lipid molecules, thus extending the exposure time of bacterial cells to antibiotics. Some nanomaterials reduce the generation of bacterial drug resistance through the self-cracking mechanism. Nanomaterials can enhance antimicrobials' solubility, prolong antimicrobials' life cycle, maintain the effective release of antimicrobials at the target site, and deliver multiple antimicrobials to the same target site to achieve combined therapy (e.g., photothermal and photodynamic therapy). Some nanovesicles inhibit the pre-degradation or release of drugs and deliver antimicrobials to designated targets *via* membrane fusion mode (He et al., 2022). Supplementary Figure 2 shows the mechanism of action of nanomaterials for inhibiting bacterial biofilm formation.

It is known that most organic nanoparticles can improve the dispersion performance of bacterial biofilms and have good biocompatibility, which is an important research direction in the field of antibacterial biofilms. Nanomaterials provide a new idea for the treatment of bacterial biofilms in the future, which can effectively improve the therapeutic efficiency of antibiotics and reduce the drug resistance caused by biofilms. The therapeutic effect of nanomaterials in the treatment of bacterial biofilms is mainly affected by their unique physical and chemical properties and the characteristics of biofilms. The combined application of organic nanomaterials with different functions can improve the efficacy of anti-biofilm. New composites composed of organic nanomaterials and other materials with antibacterial biofilm activity can significantly enhance the ability to resist biofilms and are currently the most widely used antibacterial substances.

Nanoparticle-based therapeutic regimens are expected to be effective for removing bacterial biofilms because of their advantages: functional versatility, selectivity, traceability, high loading efficacy, and controlled drug release (Datta et al., 2018; Khan et al., 2018). Unlike conventional antimicrobials, the activated nanoparticles can be designed to work in the presence of only a trigger, which reduces the side effects of being off-target (Schwartz-Duval et al., 2020). Therefore, the active development of nanomaterials for treating bacterial biofilm-associated infections has important research implications (Gao et al., 2020M; Yu et al., 2020). Ostadhossein et al. (2021) proposed a "particle-in-particle" treatment scheme for the first time. In the absence of antibiotics, they can deliver small therapeutic nanoparticles through simple chemical methods, which can target the characteristic pH of EPS, promote the killing of bacteria mediated by bacterial biofilm pH, and then use nanoparticles to kill caries pathogen S. Under the low pH value, the metabolic state of dental biofilm can produce pathogenicity. Targeting this factor can stabilize the original ecological balance and inhibit harmful pathogenic microorganisms. Particle-in-particle therapeutic approaches have been shown to provide excellent drug delivery, especially during oncology treatment (Xu F. et al., 2020; Zhang et al., 2020). Carbon dots (CDots), a new class of carbon-based nanoparticles, have the advantages of a simple fabrication process, adjustable luminescence function, and wealthy off-energy groups (Nguyen et al., 2020), which can provide unique conditions for antibacterial biofilms. One study confirmed that CDots could reduce the amount of caries pathogen S while killing bacterial biofilm EPS. *In vivo*, nanoparticles can effectively kill *S. mutans* and balance the oral environment (Ostadhossein et al., 2021).

Nanomaterials are effective in preventing the formation of bacterial biofilms on the surface of implantable medical devices. Metal nanoparticles are the key types of nanoparticles that have intrinsic anti-biofilm activity. Some metallic nanomaterials interact with the EPS of bacterial biofilms through surface charge interactions, and they can release soluble ions targeting bacterial microorganisms or EPS (Hiebner et al., 2020). Silver is one of the metal nanoparticles with high sterilization capacity. Silver nanoparticles (Ag-NPs) effectively prevented biofilm formation of *S. aureus* and oral mixed flora (*Streptococcus oralis*, *P. gingivalis*, and *Actinomyces naeslundii*) biofilm formation. Ag-NPs with maximum antibacterial activity after three repeated irradiations (Pérez-Tanoira et al., 2022). Habimana et al. (2018) developed a substance (GNP+PK) that treated gold nanoparticles (GNP) with the enzyme protease K (PK). They treated the bacterial biofilm on the surface of medical devices for 24 hours with enzyme protease K, gold particle, and GNP+PK, respectively. The total biomass of *Pseudomonas fluorescens* biofilm decreased by 40%, 77%, and 74%, while the thickness was reduced by 52%, 72%, and 78%, respectively. This study confirmed that GNP+PK particles could kill cells in *P. fluorescens* biofilm, mechanically separate cells in suspension, and damage the structure of bacterial biofilm, showing better anti-biofilm efficacy than PK or GNP. Slomberg et al. (2013) combined NO and silica nanoparticles to examine the effects of these materials on *S. aureus* and *P. aeruginosa* biofilms. In this process, the bacterial biofilm was first placed in a biological

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reaction incubator for 48 hours and then treated the biofilm with nanoparticles for 24 hours. They found that the morphology and volume of nanoparticles both affected the effectiveness of bacterial biofilm elimination. Chitosan (CS) carries a positive charge on its surface and can adsorb to the surface of bacterial microorganisms. It binds to the surface of bacterial cells carrying a negative charge using electrostatic interaction, and the permeability of bacterial cell membrane changes, leading to apoptosis (Rashki et al., 2021). At the same time, chitosan can penetrate the bacterial cell membrane and interact with nucleic acids to interfere with the DNA/RNA synthesis process, triggering intracellular reactions that lead to cell death. Pan et al. (2022) combined CS with artificial nano enzymes. They found that the conjugate is an efficient, economical, and environmentally friendly antibiofilm preparation, which may eventually become an effective alternative to eradicate bacterial biofilms.

Nanosponges can prevent the formation of biofilms or eliminate biofilms those already formed and exhibit broad-spectrum antimicrobial membrane activity against pathogenic single and double-species biofilms, with no toxicity to mammals. Nabawy et al. (2021) reported the ability of biodegradable polymer-stabilized oil-in-water nanosponges to inhibit methicillin-resistant *S. aureus* and *P. aeruginosa* biofilms. Raj et al. (2022) synthesized chitosan-gum arabic-coated liposomes-alizarin nanocarriers. They found that this material can eliminate the biofilm formed by *S. aureus* and *Candia albicans*, which helps improve drug penetration and release in biofilm cells. However, low stability and water solubility limit the broad application of nanomaterials. At present, a new method has been proposed for the treatment of biofilm-associated infections, namely "nanoscale bacterial debridement", which can separate bacteria from biofilm in a specific way, effectively kill bacteria, and reduce the biomass of biofilm (Li et al., 2021). However, as a new type of nanomaterial, "nanoscale bacterial debridement" has received little relevant research. Biofilm removal by separating bacteria and biofilm has broad research prospects and needs further investigation. Most nanomaterials have been shown to be effective solutions for preventing bacterial biofilm formation (Baelo et al., 2015; Cheeseman et al., 2020). Since biofilms exhibit a high antibacterial bacterial microbial community embedded in EPS, not all nanoparticles will be able to destroy bacterial biofilms. Although organic nanomaterials play an excellent role in inhibiting the formation of bacterial biofilms, they are less effective against stationary and persistent bacteria (Li et al., 2021). The cytotoxicity and abnormal distribution of complex tissues are common problems in nanomaterials application. Therefore, more in-depth research is needed to focus on the overlooked aspects of bacterial biofilm processing.

4.3 Catabolite control protein A inhibitor

Catabolite control protein A (CcpA) is the primary regulator of the trans-activation of carbon catabolite repression. It can induce and stimulate the upregulation of cidA and icaA gene expression. The cidA and icaA genes can play a role in synthesizing polysaccharide intercellular adhesins. They can be involved in encoding cave proteins that are involved in lysing bacterial cells and releasing eDNA (Sadykov et al., 2019). At the same time, CcpA can inhibit small noncoding RNA RsaI transcription and then affect bacterial biofilm formation (Bronesky et al., 2019; Bulock et al., 2022). CcpA of *S. aureus* (SaCcpA) can also affect bacterial biofilm formation, antimicrobial resistance development, and virulence factor expression. Catabolite control protein A inhibitor can inhibit the DNA-binding ability of SaCcpA, which is expected to eliminate the virulence factor of *S. aureus* strains. Silver, as an inhibitor, can target SaCcpA, eliminate alpha-hemolysin expression in bacterial cells, and reduce bacterial virulence (Liao et al., 2017). However, silver ions are toxic, and silver-containing preparations (e.g., silver sulfadiazine) are generally used only as topical treatments. Huang et al. (2020) identified a specific small molecule inhibitor that disrupts the SaCcpA-DNA complex product and reduces the expression of α-hemolysin encoded by the hla gene. The Sak gene is a staphylokinase that can convert plasminogen into plasmin (Tam and Torres, 2019). It can inhibit the formation and development of biofilms and separate mature biofilms by activating plasminogen and splitting fibrin from the host. After treatment with the Sak gene, *C. albicans* and *S. aureus* biofilms integrity and biomass decreased (Liu et al., 2019). Zheng et al. (2022) proposed that CcpA can bind to the promoter region of the Sak gene and inhibit Sak gene expression, thus regulating the formation and development of bacterial biofilms. This method has low cost and minor side effects and is expected to be a drug for treating *S. aureus*-related biofilm infections.

4.4 Bacteriophage

Bacteriophage is a virus that can infect and kill bacteria. Compared to common antibiotics, bacteriophage has the advantages of functional specificity, more robust tolerance, higher safety, narrower scope of action, and cost-effectiveness (Principi et al., 2019), and it cannot infect human or animal cells. Bacteriophages can penetrate the structure of bacterial biofilm and eradicate or prevent bacterial biofilm (Łusiak-Szelachowska et al., 2020), which is expected to be an alternative therapy for antibiotics. Supplementary Figure 3 shows the multiple mechanisms by which bacteriophages counteract bacterial biofilm formation. Haemolysinase is a phage-derived enzyme that can hydrolyze the peptidoglycan of the cell wall, which in turn can disrupt the structure of bacterial biofilms (Gray et al., 2018; Sharma et al., 2018). Bacteriophage infection of host cells mainly involves the following steps: (i) adhesion to the bacterial cell surface using phage receptor binding proteins; (ii) the phage genome enters the cytoplasm; (iii) phage for protein assembly; (iv) release of progeny phage (Tian et al., 2021).

Novel phage Φ 15 produces a polysaccharide depolymerase that hydrolyzes the EPS of a single species of single-species *Pseudomonas putida* and inhibits biofilm formation (Cornelissen et al., 2011). Rai et al. (2020) used CV staining to determine the biofilm content. They found that phage PD1 and PE2 could effectively prevent the formation of *S. aureus* biofilms, while PD1, PE1, and P3 could disperse mature biofilms. Phage Φ29 and phage T4 could inhibit the formation of *S. aureus* biofilms (Sybesma et al.,

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2016). Phages from the order Caudovirales, Myoviridae family, could reduce the proliferation of *P. aeruginosa* in the floating state. At the same time, this phage could also reduce the metabolic activity of endotracheal cannula-associated biofilm and destroy the already-formed *P. aeruginosa* biofilms (Oliveira et al., 2020). The phage mixture (LPSTLL, LPST94, LPST153) has a wide host range and lytic activity, which can play a role in reducing the biofilm of *Salmonella* spp. (Islam et al., 2019).

Combining bacteriophage and antibiotics can improve the therapeutic effect of bacterial biofilms and achieve reduced phage resistance without increasing the toxicity of antimicrobials. For example, the combination of bacteriophage T4 and tobramycin can significantly reduce *E. coli* tolerance to tobramycin (Hemmati et al., 2021). Combining specific phage and amoxicillin can improve the synergistic effect on *K. pneumoniae* B5055 biofilm (Bedi et al., 2009). Bacteriophages can be used alone or in combination with various bacteriophages in treating bacterial biofilms. Phage mixtures are effective in preventing the formation of bacterial biofilms and removing mature biofilms because they produce fewer types of phage resistance and significantly increase the host spectrum compared to phage alone. However, bacteriophage therapy also has limitations: for example, bacteriophages increase antibiotic resistance, bacteriophage DNA replication and protein synthesis need to interfere with bacteria metabolism, and bacteriophages are related to the growth conditions of bacterial microorganisms (Tagliaferri et al., 2019; Łusiak-Szelachowska et al., 2020). Since bacteriophages are viruses and pathogenic factors, there may be potential hazards. Therefore, when combining phages and antibiotics for treating bacterial biofilms, we should fully consider the possible adverse effects to prevent contraindications to the pairing.

4.5 Quorum sensing inhibitors

The communication mechanism between bacteria and microorganisms is called the quorum sensing system, which can control the expression of various virulence genes at different stages of bacterial biofilm formation and development (Dijkshoorn et al., 2007). Inhibition of this system can impair the formation of bacterial biofilm. As a small signal molecule, an autoinducer can mediate the communication between bacteria and microorganisms in the QS system to coordinate the development of bacterial cells. Significant changes in intracellular gene expression levels may occur when bacterial population densities reach concentration thresholds set by autoinducer agents while under the influence of the external environment (Hemmati et al., 2020). Gene expression level can induce or inhibit various virulence factors in bacterial cells and affect biofilm formation. Autoinducer peptides (AIP), Autoinducer-2 (AI-2), and N-acyl-homoserine lactones (AHLs) are three of the most studied QS signaling molecules (Muras et al., 2022). AIP is synthesized by Gram-positive bacteria. It cannot penetrate bacterial cells but binds to specific transmembrane receptors in cell membranes or cells to stimulate signal transduction pathways and promote the transcription of target genes. AHLs are usually produced by Gram-negative bacteria and can spread into bacterial cells to bind to specific receptor proteins that activate corresponding transcription factors and regulate gene expression (Duplantier et al., 2021). Blocking the production of AHLs can reduce the biological activity of AHL synthase and decrease the concentration of AHLs, which in turn interferes with the QS system. The AI-2 molecule is an autoinducer that mediates the signaling process between Gram-negative and Gram-positive bacteria, which is influenced by osmotic pressure and pH. The signaling molecule will be broken down when exposed to low osmotic pressure. AI-2 signaling molecules can affect bacterial biofilm formation, antibiotic resistance, virulence factor secretion, and cell motility Li and Zhao (2020).

Quorum sensing inhibitors (QSI) are produced by prokaryotes or eukaryotes. It can block the quorum sensing system, which may reduce efflux pump expression and destroy bacterial biofilm formation (Hemmati et al., 2020). QSI has been shown to interfere with AI-2 and the Competence Stimulation Peptide system to prevent the formation of oral bacterial biofilms (Muras et al., 2020). Non-steroidal anti-inflammatory drugs (e.g., Meloxicam and aspirin) can be used as potential QS inhibitors, which can affect the QS signaling molecules of *P. aeruginosa* and the formation and maturation of its biofilm (Almeida et al., 2018). Some commonly used antimicrobials (e.g., erythromycin, azithromycin, piperacillin-tazobactam, streptomycin, and ciprofloxacin) have high levels of QSI activity (Skindersoe et al., 2008). Natural products, antibiotics and compounds can affect the function of QSI. Bacteria may be resistant to a single QSI preparation, reducing its effective biological activity. Bacteria may be resistant to a single QSI preparation, reducing its effective biological activity. Therefore, it is recommended to combine the application of QSI and antibiotics to inhibit resistance to QSI without increasing the toxicity of antibiotics (Wang et al., 2016), which can effectively improve the therapeutic effect. When resveratrol was combined with aminoglycoside antibiotics (gentamicin, streptomycin, amikacin) to treat bacterial microorganisms, it significantly reduced the formation of bacterial biofilms compared with various substances alone (Zhou et al., 2018). Therefore, QSI can target the quorum sensing system in bacteria and may be a potential therapeutic solution for bacterial biofilms.

4.6 Enzymes involved in biofilm degradation

Enzymes are involved in ESP generation, intercellular communication, maturation and dispersion of biofilms, with high specificity. It can remove specific components from biofilms, helping to inhibit the extracellular matrix and quorum-sensing system of bacterial microorganisms (Ivanova et al., 2015). Enzymes capable of hydrolyzing ESP components have anti-biofilm activity, including oxidase (Nguyen and Burrows, 2014), polysaccharide degrading enzyme (Saggu et al., 2019), and proteolytic enzyme (Wille and Coenye, 2020). Therefore, enzymes are preferred among biological methods for inhibiting biofilms (Tan et al., 2020). Table 2 shows several enzymes and their mechanisms of action.

Soluble enzymes are unstable under different biofilm growth conditions and are not reusable. Enzyme immobilization can achieve enzyme stability, improve utilization, and reduce activity

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loss (Perwez et al., 2017). The immobilization includes adsorption, crosslinking, embedding, encapsulation and covalent coupling. Perwez et al. (2021) proposed m-combi-Cross linked Enzyme Aggregate (m-combi-CLEA), a novel biofilm inhibition method that can inhibit the biofilms of *E. coli* and *S. aureus*. The bacterial biofilms treated with enzyme mixtures in the form of CLEA showed inhibition rates could reach 75-78%, promising an alternative to antibiotics. The magnetic effect of CLEA helps achieve enzyme recirculation.

4.7 Aptamers

Aptamers are single-stranded oligonucleotide molecules or peptides produced *in vitro*. Because of its specific three-dimensional structure can select target molecules (e.g., cells, proteins, antimicrobial agents, small molecules, and metal ions) by specific linkage and high affinity. These properties make the aptamers highly active against bacterial biofilms and antimicrobials. Their antibacterial effects may be generated through the depolarization of cell walls of bacterial microorganisms. Aptamers may be an effective alternative to inhibit the development of biofilms (Shatila et al., 2020). Supplementary Figure 4 briefly shows the primary mechanism of action of aptamers in inhibiting bacterial biofilm formation. Aptamers are suitable alternative substances in the treatment of biofilms due to their flexible diagnostic and therapeutic properties. Mao et al. (2018) developed an aptamer-targeted graphene oxide (GO) therapeutic strategy against bacterial biofilms, defining the coupling as aptamer-GO. They found it can inhibit 93.5 ± 3.4% of *Salmonella typhimurium* biofilms, showing superior antibacterial biofilm properties and effectively becoming a long-term therapeutic option for treating bacterial biofilms. As a special targeting agent, aptamers are expected to improve the effective concentration of antibiotics and reduce the possibility of missing targets, which can be used to treat bacterial microbial infections. The synergistic action of aptamers and antibiotics may affect more bacterial microbial cells (Ning et al., 2015).

Shatila et al. (2020) demonstrated the inhibitory effect of DNA aptamer of *Salmonella* spp. invasion protein A (Apt177) on bacterial biofilms. They found that Apt177 could alter the three-dimensional structure of biofilms and was effective in reducing bacterial biofilm formation when applied alone or in combination with ampicillin. Enteropathogenic *E. coli* (EPEC) is a biological agent that causes diarrhea by forming a bacterial biofilm. It was found that the nucleic acid aptamer SELEX 10 Colony 5 could reduce the motion diameter of EPEC K1.1 and showed the highest biofilm inhibition effect. This aptamer can reduce the mRNA expression level of bacterial biofilm formation genes (e.g., curli gene, interaction, and motility), hinder EPEC K1.1's motility, and prevent bacterial biofilm formation (Oroh et al., 2020). Sengupta et al. (2020) reported the effectiveness of aptamer-DNA template silver nanocluster (Ag-NC) for inhibiting *P. aeruginosa* biofilm formation, and Ag-NC as a sensor is expected to be a new way to detect planktonic cells and biofilm formation. Ning et al. (2022) found that simultaneous delivery of penicillin-binding protein 2a-specific DNA aptamer and berberine *via* graphene oxide effectively inhibited the formation of MRSA biofilms, and this complex could potentially be an effective strategy for the treatment of chronic infections caused by MRSA biofilms. *P. gingivalis* can cause the occurrence of periodontitis. DNA-aptamer-nanographene oxide (NGO) achieves real-time, *in situ* detection and removal of *P. gingivalis* biofilm. The DNA-Aptamer-NGO complex serves as an antimicrobial photodynamic therapy and is a promising method that may inhibit the formation of bacterial biofilms (Pourhajibagher et al., 2022). Therefore, aptamers combined with other reagents can improve targeting specificity and inhibit the formation and development of bacterial biofilms, which is considered an ideal measure for antibacterial biofilms.

4.8 Peptide nucleic acid

Peptide nucleic acids (PNA) is a synthetic analogue of nucleic acid, composed of nucleic acid and peptide, similar in structure to DNA or RNA, and identical in physical and chemical properties to

TABLE 2 Enzymes with antibacterial biofilm activity.

Enzyme	Mechanism of action	Bacteria	Authors
Trypsin	Hydrolysis of the peptide bond on the carboxyl side of protein arginine and lysine residues	Inhibition of biofilm formation in *P. aeruginosa* and *Erysipelas rubbery* C208	(Gilan and Sivan, 2013; Banar et al., 2016)
Cellulase	Hydrolyses the β-1, 4 glycosidic bonds of hemicellulose and cellulose	Decomposition of EPS in *P. aeruginosa* biofilms	(Ibrahim et al., 2021)
α-amylase	Hydrolysis of α-1,4 glycosidic bonds in glycogen and starch		(Fleming et al., 2017)
Pectinex ultra clear	Contains polygalacturonase, pectin ester, hemicellulase and cellulase activity		(Perwez et al., 2021)
Disintegrin B produced by Aggregatibacter actinomycetem-comitans	Hydrolysis of glycosidic bonds in polysaccharides	Decomposing the mature *Staphylococcus* spp. biofilms	(Donelli et al., 2007)
Alginate lyase	Resistant alginate substances in *P. aeruginosa* biofilm substrates	*P. aeruginosa*	(Franklin et al., 2011)

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protein. PNA has a high affinity and specific binding ability, showing great potential in removing drug-resistant bacteria (Lee et al., 2019), and can hinder the formation of bacterial biofilms. Combined with conventional antibiotics, it can improve the antibacterial efficacy of antibiotics and anti-biofilm activity. There are two main reasons why pre-PNA has yet to be widely used. One is that PNA is hydrophobic and not easily dissolved in an aqueous solution. Second, due to the lack of effective carriers to transport PNA to biofilms, bacterial biofilms have low permeability to PNA (Wojciechowska et al., 2020). Meanwhile, components of bacterial cells, such as lipopolysaccharide and peptidoglycan, are also major barriers that restrict PNA entry into biofilms (Kurupati et al., 2007). Various methods have been proposed to increase the penetration of PNAs into biofilm cells: chemical changes in the structure of PNAs can enhance the hydrophilicity of PNA. Some cell-penetrating peptides (e.g., chloroquine, photochemical internalization, cationic lipids) can covalently bind to PNAs. This process helps PNAs form complementary base pairs with bacterial DNA. Filamenting temperature-sensitive mutant Z (FtsZ) remains silent in numerous bacterial cells and is one of the essential structures involved in the bacterial division process, making ftsZ a potential target for developing new antimicrobial agents. Narenji et al. (2020) showed that antisense PNAs targeting the efaA gene could reduce the formation of *Enterococcus* spp. biofilms, that anti-ftsZ materials could inhibit bacterial cell growth by interfering with *E. coli* division, and that PNAs were shown to inhibit the function of ftsZ. AcpP-PNA14-5'L is a PNA peptide based on targeting the acpP gene with a 5' membrane penetrating peptide and junction. It exhibits efficient antibacterial activity against *H. influenzae* in both planktonic and biofilm states and is not susceptible to drug resistance (Otsuka et al., 2017). Castillo et al. (2018) found that PNA can directly block the transcription of mRNAs encoding acyl carrier proteins. Certain antibiotics (such as polymyxin B) can interfere with cell wall formation and, when used in combination with PNA, are effective in preventing the formation of biofilms from Enterohemorrhagic *E. coli* 0157: H7. The combination of PNA with conventional antimicrobials has the potential to be an effective therapeutic option for the treatment of Gram-negative bacteria. In conclusion, PNA is expected to be an effective way to increase anti-biofilm activity.

4.9 Vaccine

Currently, vaccines formulated using biofilm-derived antigens are an effective way to prevent infectious diseases, which can improve the protective efficacy of existing vaccines (Loera-Muro et al., 2021). The outer membrane vesicles (OMV) derived from *Bordetella pertussis* can effectively prevent the colonization of bacterial cells in the lung of mice. Carriquiriborde et al. (2021) used OMV from the planktonic state (OMVplank) and biofilm state (OMVbiof) to create a vaccine. The OMVbiof vaccine was more affinity and immunogenic than the OMVplank vaccine in antibody induction. In addition, the *B. pertussis* biofilm-derived vaccine was found to be more protective and immunoreactive against bacterial strains with defective pertactin antigen expression than the OMVplank vaccine. Zurita et al. (2019) found that the OMVs vaccine induced respiratory CD4 tissue-resident memory cells with long-lasting protection against *B. pertussis*. It caused a durable immune response, making it an excellent alternative to third-generation pertussis vaccines. The treatment of pathogens with biofilm-derived vaccines is still in the research stage and may help treat biofilm infections. More advanced technologies are needed to identify bacterial biofilm-derived antigens in the future.

5 Conclusion and prospect

Clinically, most chronic infections are associated with biofilms of bacterial microorganisms, which are resistant to antibiotics and can grow and mature even under poor survival conditions. Biofilm-associated infection is a significant clinical problem. Biofilms can form on the surface of devices and non-devices, increasing patient morbidity and mortality and seriously threatening human life and health. In addition, the increased spread of multidrug-resistant bacteria has made biofilm infections a significant threat to hospitalized patients. Although many studies have been done to elucidate devices and non-devices surface biofilm formation, they are mainly limited to a few surfaces and specific bacterial microorganisms. Therefore, it is critical to focus on bacterial biofilm infections and work to raise awareness of the different microbial populations present on device and non-device surfaces to develop strategies for detecting and treating bacterial biofilms. Early detection of bacterial biofilms can improve treatment effectiveness and reduce medical costs. There are many strategies to resist biofilms, but relatively few to clinical treatment. In the future, we should also focus on more efficient, durable, and environmentally friendly methods and further study the safety and effectiveness of each strategy.

Author contributions

Conceptualization- YL; collecting data- AZ and JS; writing and editing- AZ; supervision- YL. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1137947/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Different therapeutic strategies for inhibiting bacterial biofilm formation.

SUPPLEMENTARY FIGURE 2
Mechanism of action of nanomaterials against biofilms.

SUPPLEMENTARY FIGURE 3
Mechanism of action of bacteriophages with anti-biofilm activity.

SUPPLEMENTARY FIGURE 4
Mechanism of action of aptamers with anti-biofilm activity.

SUPPLEMENTARY TABLE 1
Most common bacterial organisms on the surface of medical devices.

SUPPLEMENTARY TABLE 2
Non-device-related biofilm infections.

SUPPLEMENTARY TABLE 3
Microbial and bacterial biofilm-associated infections and their adhesion surfaces.

SUPPLEMENTARY TABLE 4
Mechanism of action of antimicrobial peptide.

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REVIEW

Proteus spp. as Putative Gastrointestinal Pathogens

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SUMMARY *Proteus* species, members of the *Enterobacteriaceae* family, are usually considered commensals in the gut and are most commonly recognized clinically as a cause of urinary tract infections. However, the recent identification of *Proteus* spp. as potential pathogens in Crohn's disease recurrence after intestinal resection serves as a stimulus to examine their potential role as gut pathogens. *Proteus* species possess many virulence factors potentially relevant to gastrointestinal pathogenicity, including motility; adherence; the production of urease, hemolysins, and IgA proteases; and the ability to acquire antibiotic resistance. Gastrointestinal conditions that have been linked to *Proteus* include gastroenteritis (spontaneous and foodborne), nosocomial infections, appendicitis, colonization of devices such as nasogastric tubes, and Crohn's disease. The association of *Proteus* species with Crohn's disease was particularly strong. *Proteus* species are low-abundance commensals of the human gut that harbor significant pathogenic potential; further investigation is needed.

KEYWORDS bacteriology, Crohn's disease, *Enterobacteriaceae*, gastrointestinal disease, infections, inflammatory bowel disease, *Proteus*

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INTRODUCTION

Proteus species are members of the *Enterobacteriaceae* family of bacteria. Most commonly, they are recognized clinically as a cause of urinary tract infections. Although *Proteus* spp. are typically considered commensals in the gastrointestinal (GI) tract, their abundance as a proportion of the microbial community is very low (<0.05%) (1). As a result, their detection in disease states using 16S profiling, and possibly metagenomics, may have rendered *Proteus* spp. undetectable due to bioinformatic abundance thresholds.

The recent identification of *Proteus* spp. as potential pathogens in Crohn's disease recurrence after intestinal resection (2, 3) serves as a stimulus to examine their potential role as gut pathogens. This review aims to provide an overview of the genus *Proteus* in terms of its known virulence factors as well as to collate the evidence surrounding the role of *Proteus* spp. in the pathophysiology of gastrointestinal diseases.

CHARACTERISTICS OF THE *PROTEUS* GENUS

Proteus spp. are Gram-negative bacteria belonging the *Enterobacteriaceae* family and are common commensals of the gastrointestinal microbiota (4). The first isolates were reported and characterized by Hauser in the late 19th century (5). The genus is currently comprised of *Proteus mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, *P. terrae*, and *P. cibarius*, along with the unnamed genomospecies 4, 5, and 6 (6–10). In humans, all current members of the genus, except for *P. cibarius* and *P. terrae*, have been isolated from clinical specimens (4, 5, 7, 9, 11). Typically, the human gut is colonized by various combinations of *P. vulgaris*, *P. mirabilis*, and *P. penneri*, but they comprise less than 0.05% of the gut microbiota of healthy subjects (Fig. 1) (1, 12). While *Proteus* spp. are widely recognized as pathobionts and the gut is the reservoir of these bacteria, the research focus on this genus has been on their role in urinary tract infections rather than intestinal manifestations (13–15).

Many recent studies of the gut "microbiome" in health and disease have revealed that there are gross alterations in the relative proportions of key bacterial taxa associated with active disease, which is generically referred to as "dysbiosis." One of the hallmarks of dysbiosis in the inflammatory bowel diseases (IBDs) is the population expansion of the phylum *Proteobacteria*, specifically the *Enterobacteriaceae* (16). Other genera within the *Enterobacteriaceae* family, such as *Escherichia*, *Shigella*, *Salmonella*, and *Klebsiella*, have received due attention in this regard, while *Proteus* has not been comprehensively investigated.

Pathogenic Features

Proteus species are short (1.5- to 2-μm) straight rods that demonstrate dimorphism as "swimming" and "swarming" forms, as do some other members of the *Enterobacteriaceae* family (17). Swimmer cells predominate in liquid environments as single cells with 4 to 10 peritrichous flagella (Fig. 2, bottom right) (5, 14). The swarming behavior of *Proteus* species results in a characteristic bull's-eye pattern on a plate culture, as a result of a cyclic process of swarming and consolidation phases (18). When *Proteus* cells are placed in a viscous environment or on a solid surface, they undergo differentiation to filamentous, multinucleated, highly flagellated swarmer cells (Fig. 2, top and bottom left). Following this differentiation, a consolidation phase occurs, where the cells revert to a shorter morphotype, and metabolic preparation occurs prior to the next swarming cycle (18).

Proteus mirabilis undergoes swarming differentiation at much higher concentrations of agar (1.5 to 2%) than other swarming bacteria (19). When *Proteus* spp. swarm, there is a dramatic increase in the production of secreted proteins, including virulence factors such as the protease ZapA (17, 20, 21). *In vivo*, swarmer cells have been demonstrated in mouse models of ascending urinary tract infection only infrequently, with the occasional swarmer cell being isolated from the kidneys and bladder stones of infected mice (22, 23). The swarming phenotype can occur under both aerobic and anaerobic conditions (24) and can be induced by the concentration of amino acids, in particular

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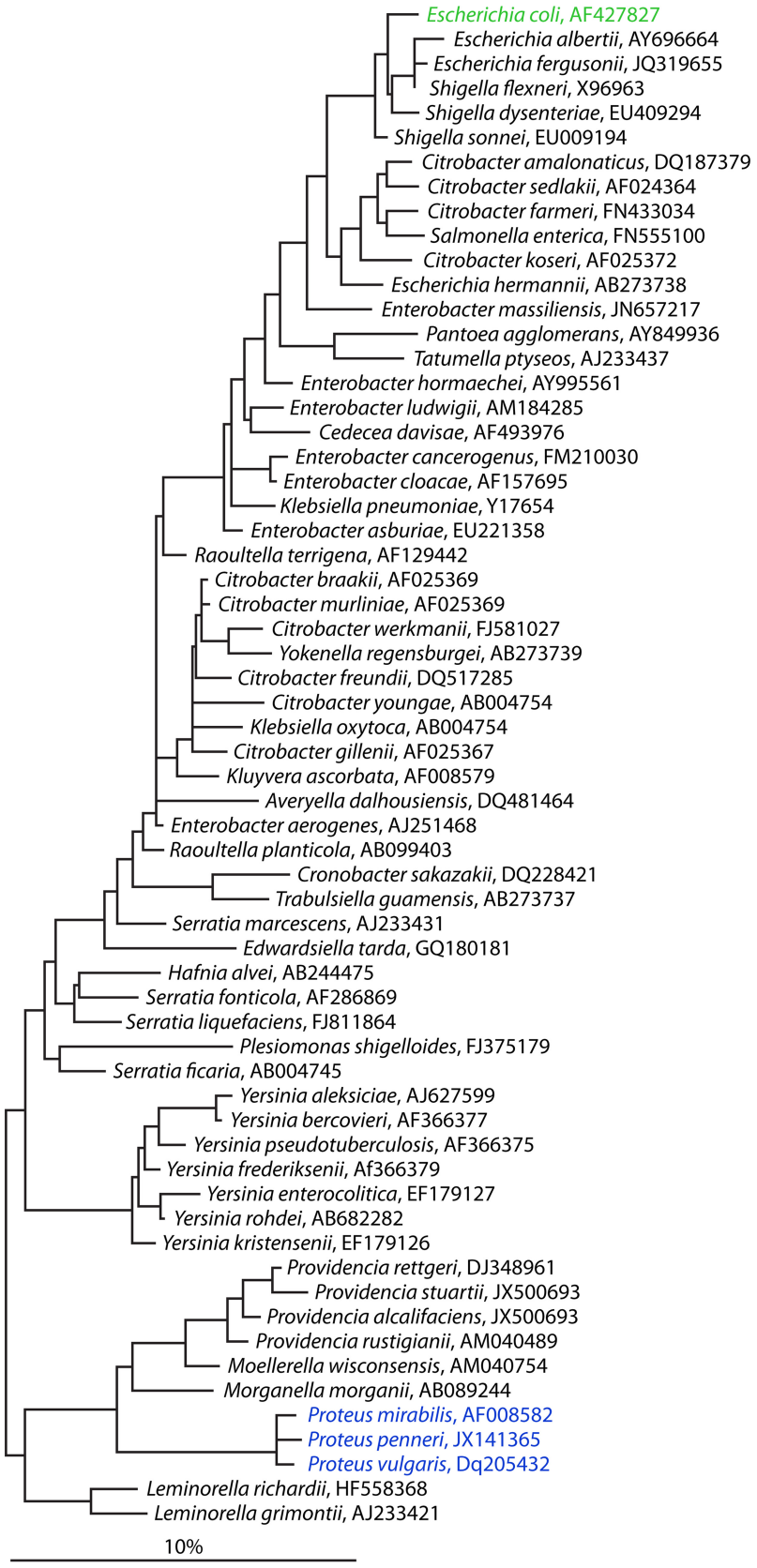

FIG 1 Phylogenetic tree showing the species from the *Enterobacteriaceae* family that colonize the human gastrointestinal tract. GenBank accession numbers of the16S rRNA gene sequences are provided for each species, and the family names are indicated. *E. coli* is highlighted in green, and the *Proteus* genus is shown in blue. (Reproduced from reference 133.)

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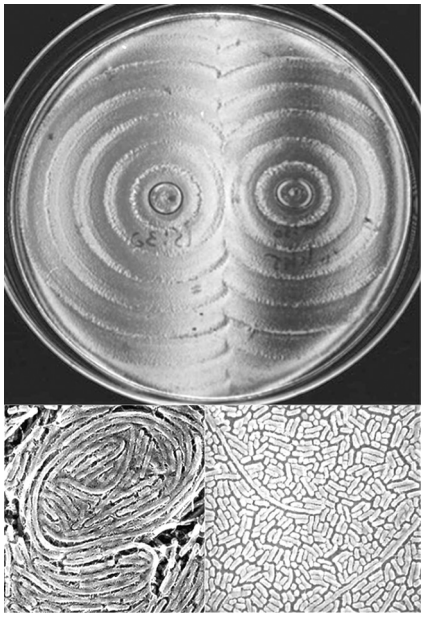

FIG 2 (Top) Strain of *P. mirabilis* inoculated twice, 1 h apart, demonstrating the macroscopic characteristic bull's-eye pattern produced by periodic swarming. (Reproduced with permission from reference 134.) (Bottom left) Interacting *P. mirabilis* swarmer cells; (bottom right) combination of swimmer and swarmer cells within a biofilm. (Both panels reproduced from reference 135 with permission from Elsevier.)

glutamine (25, 26). It has also been demonstrated that a more acidic pH, as might be expected to occur in the proximal small bowel and cecum, dramatically increased swarming behavior (27–29). Swarming has been shown to be an important factor in intracellular invasion and persistence, with 15- to 20-fold more swarmer cells than swimmer cells being capable of the intracellular invasion of uroepithelial cells (30). There is some evidence that swarming *Proteus* strains are more invasive in urinary tract mouse models than are swarm-defective mutant strains (22). Furthermore, a number of metabolites present in the intestinal tract have been shown to promote swarming, including choline, glutamine, and the most abundant polyamine in the gut, putrescine (25, 31–33). While we cannot yet conclude definitively that swarming behavior occurs in the gut *in vivo*, the combination of a viscous surface (such as the gut mucosa), the high availability of glutamine and polyamines such as putrescine (26, 31, 33), and electron acceptors for anaerobic respiration such as choline (32) makes it likely that the gut environment may be permissive for swarming.

Adhesion and mucosal attachment. Adhesion to epithelial surfaces is essential for the pathogenesis of *Proteus* infections in both the urinary and gastrointestinal tracts. Sequencing of *Proteus mirabilis* strain HI4320 revealed 17 fimbrial gene sets (operons), more than any other bacterial genome currently characterized (34–36). Six of the fimbrial types that *Proteus mirabilis* can produce have been characterized (Table 1), including mannose-resistant *Proteus*-like fimbriae (MR/P fimbriae), mannose-resistant *Klebsiella*-like fimbriae (MR/K fimbriae), nonagglutinating fimbriae (NAF) (also known as uroepithelial cell adhesin [UCA]), ambient-temperature fimbriae (ATF), *P. mirabilis* P-like pili (PMP), and *P. mirabilis* fimbriae (PMF) (5, 36). These fimbriae and adhesins play a major role in the formation of bacterial biofilms, a common complication of both urinary and gastrointestinal instrumentation (37). It is likely that the MR/P and NAF/UCA fimbrial types are most important in gastrointestinal pathogenesis, due to their role in epithelial adhesion (38–41).

A comparison of the 17 individual chaperone-usher fimbrial operons across the 7 sequenced *P. mirabilis* strains as well as 58 clinical isolates showed 99% conservation in 13 of 17 fimbrial operons, demonstrating that these genes are highly conserved across

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TABLE 1 Fimbriae and pili expressed by *Proteus* species[a]

Fimbrial type	Structure	Characteristic(s)	Contribution(s) to gastrointestinal pathogenicity	Reference(s)
MR/P	7-nm–8-nm, "thick" channeled fimbriae	Important for epithelial cell adhesion	Expression may allow mucosal cell adhesion in the gastrointestinal tract and contribute to intestinal persistence	38, 39, 69, 136
		MR/P expression undergoes phase variation, allowing for a molecular "switch" that turns on/off the expression of the *mrp* operon depending on the environment and oxygen availability, e.g., on in bladder colonization and off in kidney colonization	The repeating structures of flagellin proteins contribute to immunogenicity in the gut	
		MR/P fimbriae are potent immunogens and potential vaccine candidates		
		MR/P$^+$ *Proteus* strains are more genotoxic and cytotoxic to bladder/kidney-derived epithelial cells than MR/P$^-$ mutants	MR/P$^+$ *Proteus* strains are also likely more cytotoxic to intestinal epithelial cells	
MR/K	4-nm–5-nm, "thin" nonchanneled fimbriae	Expression is more common in *P. penneri* strains than in *P. mirabilis* strains	*P. penneri* has not been linked to gastrointestinal pathogenicity	42, 137
NAF/UCA	4-nm, "thin" nonchanneled fimbriae	Recognizes glycolipids, including asialo-GM1, asialo-GM2, lactosyl ceramide, and galectin-3		40, 41, 138
		Important for epithelial cell adhesion and colonization		
		Not present in *P. vulgaris*		
ATF		Highly expressed at 23°C, moderately expressed at 37°C	The structural subunit AtfA has significant homology with the major subunit of type 1 fimbriae from other enteric pathogens, such as *S. enterica* serovar Typhi	37, 40, 139–141
		Not considered to contribute to UTI or biofilm formation; may be more important for enteric colonization and environmental survival		
		Believed to be important for survival and persistence outside a mammalian host, at room temp		
PMP		Identified in uropathogenic *P. mirabilis* strains isolated from dogs		142
		Not present in *P. vulgaris*		
		PmpA fimbrial subunits show high homology to P-fimbriae from uropathogenic *Escherichia coli*		
		Cell surface assembly has not been confirmed		
PMF		Important in biofilms and responsible for the formation of higher-volume biofilms		37

[a]UTI, urinary tract infection.

strains isolated from various clinical sites (36). Of these, it is likely that at least two, and up to six, of the characterized fimbriae can be assembled on the cell surface at any one time (36, 42).

The regulation of motility and the expression of adhesion factors are tightly coupled; of the 17 fimbrial operons, at least 10 gene clusters possess a homolog of the *mrpJ* gene, a repressor of motility (43). MrpJ downregulates the flagellar master regulator *flhDC* via binding to the promoter sequence (43). When these cells revert back to the swimming morphology, the expression of MR/P and NAF fimbriae returns (37, 44).

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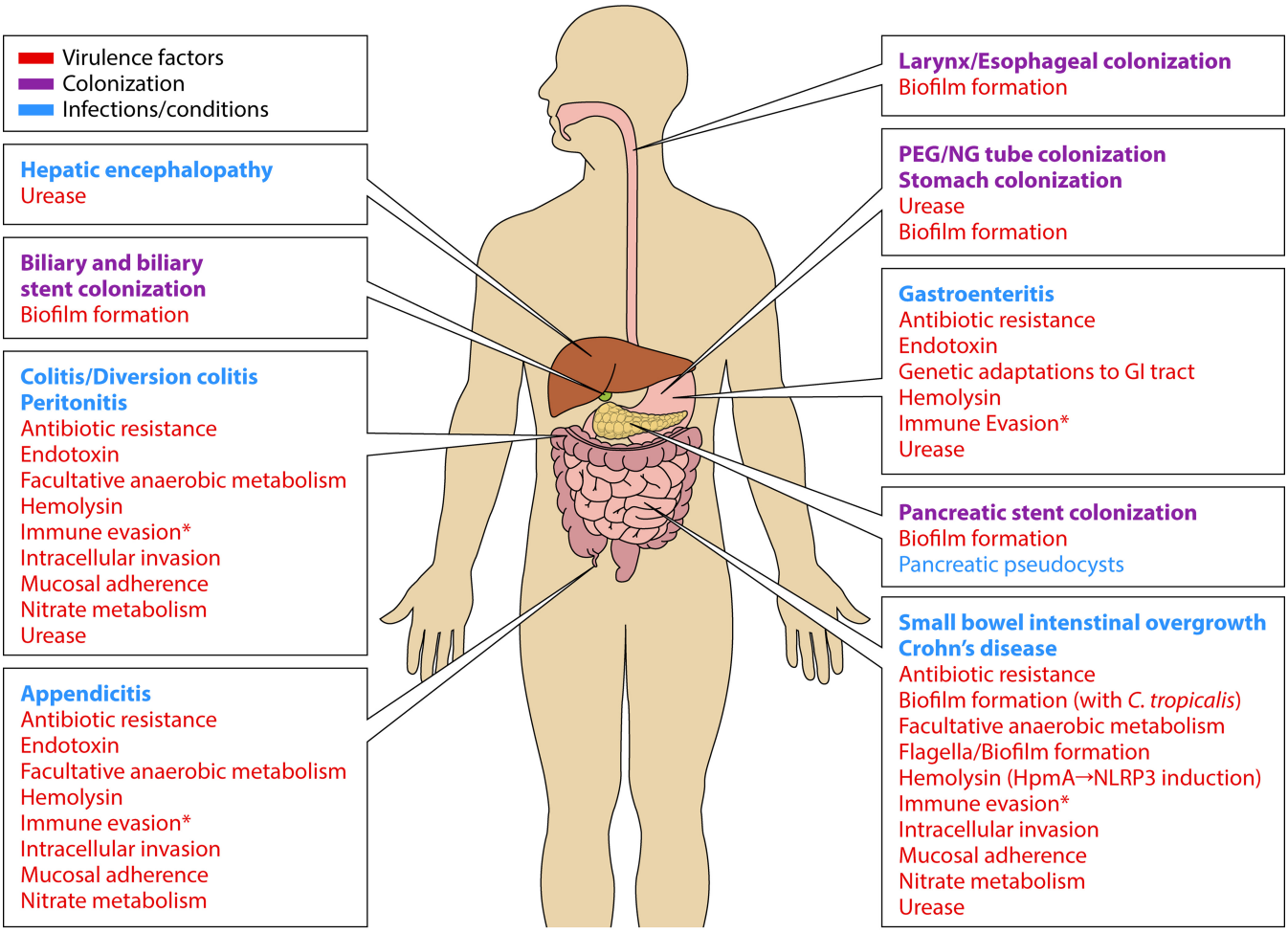

FIG 3 Potentially important *Proteus*-related virulence factors in relation to anatomical disease location and disease. *, immune evasion includes the production of the ZapA metalloprotease, O-antigens, and flagellin variation.

Furthermore, the induction of MR/P fimbriae appears coupled to the availability of oxygen (45). MR/P fimbriae are phase variant and can be "switched" on or off by a site-specific DNA recombinase (MrpI) that inverts a promoter region flanked by inverted repeats. This "invertible element" switches on or off the MR/P fimbrial operon, depending on the environmental conditions. There appears to be a growth advantage to MR/P fimbrial expression under low-oxygen conditions, such as those that would be present in the intestinal tract, likely contributing to the adhesiveness and persistence of *Proteus* species in the gut (45). The adaptation of *Proteus* species to mucosal surfaces by way of both fimbrial expression (for adherence) and swarming motility could increase the invasiveness, persistence, and pathogenicity of these species in the gut (Table 1 and Fig. 3).

Urease. The urease enzyme is a microbiological adaptation to metabolize urea, the most abundant nitrogenous waste product of human metabolism (46). Urease generates ammonia and carbonic acid as end products, with this ammonia providing a rich source of nitrogen for microbial metabolism in the gut (47). Urease confers a survival advantage to *Proteus* by providing nitrate for nonfermentative anaerobic respiration. This in turn promotes the population expansion of the *Enterobacteriaceae* (including *Proteus*) (48, 49). Additionally, as with *Helicobacter pylori*, the presence of this enzyme likely confers a survival advantage through increasing the local pH of the environment, allowing urease-positive organisms to survive in more-acidic environments such as the

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TABLE 2 Cell culture lines capable of intracellular uptake of *Proteus mirabilis*

Cell line	Cell type	Reference(s)
CaCo-2[a]/CaCo-2BBe[a]	Colorectal adenocarcinoma/C2BBe1 (brush border-expressing cells) Models for apical microvilli and tight junctions	60, 62, 132, 143, 144
HT29[a]	Enterocytes, undifferentiated or differentiated (polarized, in the absence of glucose)	60, 62, 132, 143, 145
HT29-18N2[a]	Mucus-secreting differentiated cells	60, 132
HT29-FU[a]	Mucus-secreting differentiated cells	
HT29-MTX[a]	Mucus-secreting differentiated cells	
INT407[a]	Embryonic intestinal epithelial cells (possibly HeLa-contaminated line)	61
HCT-8[a]	Ileocecal colorectal adenocarcinoma	
T24	Transitional cell bladder carcinoma	
HeLa	Cervical carcinoma	146
Vero	African green monkey kidney cells	
L-929	Mouse fibroblasts	
Human blood lymphocytes		

[a]Gastrointestinal cell line.

upper digestive tract. *Proteus* spp. have a wide pH range at which they are able to grow, from pH 5 to 10, with an optimal pH of 7 to 8 (9). Urease activity has been confirmed for *P. mirabilis*, *P. vulgaris*, and *P. penneri*, and production is regulated by both the environmental concentration of the substrate (urea) and increased chromosomal transcription in swarming cells (50–52).

Colonization of the upper gastrointestinal tract by urease-producing *Proteus* species may cause occasional false-positive results for the [^{13}C]urea breath test (UBT), especially if the patient has already been treated with proton pump inhibitors and antibiotics for *H. pylori* eradication (53). Given that urease is a known contributor to the pathogenesis of *H. pylori*-mediated damage in the upper gastrointestinal tract, it may also contribute to the pathogenicity of *Proteus* spp. in the gut in the setting of environmental perturbations, such as changes in pH.

Hemolysins. The *Proteus* genus produces two distinct cytotoxic hemolysins, HpmA and HlyA (51, 54, 55). *P. mirabilis* and most *P. vulgaris* strains produce only HpmA, most *P. penneri* strains produce HlyA, and a few isolated *P. vulgaris* strains produce both HpmA and HlyA (54, 56, 57). HpmA has been shown to lyse erythrocytes, bladder epithelial cells, B-cell lymphoma cells, and monocytes, while HlyA can lyse erythrocytes, fibroblasts, and neutrophils (55, 56). HpmA is a cell-associated hemolysin, encoded on the *hpm* locus along with HpmB (an activator and chaperone of HpmA). The expression of these hemolysin proteins is tightly coupled to the swimming-swarming cycle, with swarming cells being 18-fold more cytotoxic than swimmer cells (30). HpmA has also been shown to lyse erythrocytes under anaerobic conditions and at multiple temperatures (58). The contribution of hemolysins to gastrointestinal pathogenesis may occur through the lysis of innate immune cells, the induction of the NOD-like receptor protein 3 (NLRP3) inflammasome, and downstream interleukin-1β (IL-1β) release (59).

Intracellular invasion and persistence. Intracellular invasion by *Proteus mirabilis* has been assessed mainly by using cellular invasion assays in cell lines ranging from uroepithelial cells to colonic cell types.

In a cell-based urinary tract model, swarming cells were 15-fold (0.18% intracellular entry) more invasive to uroepithelial cells than swimmer cells (0.012% intracellular entry) (30). After the invasion of uroepithelial cells, swarmer cells start to divide, develop septums, and differentiate back to an average of 50 to 300 swimmer cells within the cytoplasm (30). *P. mirabilis* has been identified as being intracellularly invasive in a number of cell lines (summarized in Table 2). There are differences in the intracellular invasion and uptake pathways depending on the cell type, with intracel-

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TABLE 3 Substrates degraded by *Proteus mirabilis* ZapA metalloprotease

Substrate	Locations	Reference(s)
Structural cell components		
Actin, β-tubulin, fibronectin, collagen, laminin	All cells	21
Innate immune components		
Immunoglobulins (IgA1, IgA2, IgG)		21, 147
Complement proteins (C1q, C3)		21
Defensins (human beta defensin 1)	Epithelial surfaces, small bowel, and large bowel	21, 148, 149
Cathelicidin peptide LL-37	Epithelial surfaces, wounds	21, 150

lular vacuoles containing *P. mirabilis* having a single membrane within urothelial cells and a double membrane within intestinal cells (60, 61). These mechanisms may contribute to effective intracellular colonization (cytoplasmic colonies), evasion of the host immune system, and resistance to antibiotics (60).

Translocation of Gram-negative *Enterobacteriaceae* (including *P. mirabilis*) has been demonstrated *in vitro*, in terms of both uptake and intracellular persistence. By using Caco-2 and HT-29 cells (enterocyte cell lines), it was demonstrated that *P. mirabilis* not only is invasive but also can survive intracellularly for ≥20 h without affecting the viability of enterocytes (62). The origin of the *P. mirabilis* isolate influences the invasiveness of *Proteus* species in laboratory studies, with fecal isolates showing higher invasion efficiencies (61).

Proteus mirabilis is a common cause of pathogenic infection of bladders augmented with bowel segments (enterocystoplasty). A study investigated the colonization of colonic cell lines by *P. mirabilis* as a model for stone formation in enterocystoplasty. The HT29-18N2 cell line (a mucus-secreting intestinal goblet cell subclone of HT-29 [63]) underwent widespread cellular destruction, with extensive intracellular bacterial colonies. Furthermore, upon confocal microscopy, there was colocalization of *P. mirabilis* and both human colonic mucin MUC2 and human gastric mucin (MUC5AC) (63). There is experimental evidence that *Proteus* spp. can invade at least the outer mucous layer of the gut and the inner layer to the epithelial surface if there are certain immune defects. These defects include the absence of the Lypd8 protein or defects in innate immunity (the T-bet$^{-/-}$ × Rag2$^{-/-}$ colitis [TRUC] mouse model) (64–66). *Proteus mirabilis* has many invasive characteristics; however, they remain to be directly characterized in the context of gastrointestinal disease.

Immune evasion. In addition to intracellular invasion, *P. mirabilis* also secretes an extracellular metalloprotease (ZapA) that has been shown to degrade a wide range of substrates (Table 3). This enzyme has a key role in the evasion of innate immune destruction by *Proteus* species by the proteolytic digestion of secretory IgA, IgG, and other cellular components in the urinary and gastrointestinal tracts (20, 21). Kerr et al. showed ZapA cleavage products (IgA and IgG) in the urine of patients with *P. mirabilis*, reflecting protease activity *in vivo* (67). The expression of this protein has been shown to be correlated tightly with cellular differentiation from swimmer to swarming cells by *P. mirabilis*, with increased transcription of the ZapA locus (20). ZapA hydrolyzes human β-defensin 1, a constitutively expressed innate immune antimicrobial peptide that is expressed in the colonic epithelium, as well as secretory IgA (20, 21). The expression of ZapA in the gut may provide a survival advantage to *Proteus* spp. by perturbing host mucosal immune responses, increasing the likelihood of persistent colonization. The genomic rearrangements encoding the flagellin proteins of *Proteus mirabilis* and the antigenic variability of *Proteus* species lipopolysaccharide (LPS) (both reviewed below) also contribute to evasion of the host immune system (Fig. 3).

Endotoxin and flagellins. As a Gram-negative pathogen, *Proteus* species possess intrinsic characteristics similar to those of other *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, including the production of flagellin

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and the proinflammatory cell wall component LPS (68, 69). A constituent of LPS, endotoxin (lipid A), is highly immunostimulatory (70). Endotoxin is sensed by the innate immune system (specifically Toll-like receptor 4) and activates the downstream signaling of NF-κB (71). This, in turn, triggers a proinflammatory cascade (mediated by tumor necrosis factor alpha [TNF-α]) that can lead to acute sepsis (72, 73).

In addition to lipid A, *Proteus* species LPS includes O-antigens (polysaccharides that are repeating oligosaccharide units, each made up of 2 to 8 sugar residues) that are highly structurally diverse. Approximately 80 O-antigen serogroups have been reported, derived from a total of 60 O-antigen gene clusters (11, 73). Antibodies to O-antigens are not uncommon in human sera, for example, 25% of blood donors have anti-*P. mirabilis* antibodies to the O36 serogroup, which is just one of the many serogroups (74). The expression levels of virulence factors, such as urease, proteases (ZapA), and hemolysin, can vary significantly between the O-antigen serogroups, with negatively charged O-polysaccharide serogroups having higher ureolytic, proteolytic, and swarming activities (75). This heterogeneity of surface structures occurs across all *Proteus* species.

In addition, bacterial flagellins, the repeating protein subunits from which flagella are built, are highly immunogenic due to their three-dimensional structure (76). Bacterial flagellin is sensed by Toll-like receptor 5, which activates a number of downstream inflammatory pathways, including MyD88 (72, 76, 77). Recombination of the flagellin genes *flaA* and *flaB*, leading to hybrid flagellin proteins with significant antigenic variation, also contributes to innate immune evasion by *Proteus* spp. (78, 79) These features contribute to the overall pathogenicity of *Proteus* spp. via stimulation of the host innate immune system by bacterial products (Fig. 3).

Conjugation, plasmid acquisition, and antibiotic resistance. *Proteus* species are inherently antibiotic resistant. Resistance to polymyxins is mediated via covalent modifications of lipid A (80). The substitution of L-arabinoso-4-amine for either the Kdo residue or the ester-linked lipid A phosphate moiety increases the overall charge of the usually negative LPS to zero, reducing the binding of cationic polymyxins (69, 80, 81). They also possess intrinsic resistance to colistin, tigecycline, and tetracycline (82). Sequencing of *P. mirabilis* strain HI4320 demonstrated the presence of genes for a conjugal transfer pilus, which allows the horizontal genetic transfer of plasmids encoding antibiotic resistance (35).

While most species of *Proteus* remain sensitive to a range of antibiotics, increasing rates of acquired antibiotic resistance in the *Enterobacteriaceae* are a growing problem (83). In 2007 to 2008, observations of *P. mirabilis* strains acquiring *Salmonella* genomic island 1 (SGI1) were reported by groups in China and Palestine (84). SGI1 is a mobile genomic element first identified in *Salmonella* Typhimurium that integrates into the recipient chromosome and carries multiple genes encoding resistance to streptomycin, trimethoprim, tetracycline, sulfonamides, chloramphenicol, fluoroquinolones, and a broad spectrum of β-lactam antibiotics (84). A further SGI1-positive *P. mirabilis* strain (NKU) has also been identified in Europe (84). Recently, an SGI1-positive *P. mirabilis* strain acquired a plasmid containing the New Delhi metallo-β-lactamase 1 gene, leading to the identification of extensively drug-resistant (XDR) *P. mirabilis* strain PM58, which is resistant to all antibiotics used for *Enterobacteriaceae* except aztreonam (82). A recently sequenced *P. mirabilis* isolate (NO-051/03) from a patient with a soft tissue infection in Europe had acquired the resistance genes for trimethoprim, β-lactams, phenicols, sulfonamides, and aminoglycosides (85).

In gastrointestinal disease, the antibiotic sensitivity profile of *Proteus* species is relevant to pathogenicity under conditions that may be exacerbated by antibiotic perturbation of the gut microbiome. Given the potential for a "bloom" of *Enterobacteriaceae* (including *Proteus* species) both in the presence of an inflammatory process (86) and with a perturbation of the enteric environment via surgery (87), the use of antibiotics should be considered a possible potentiating factor.

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Vaccine Candidates

A full review of the treatment of *Proteus* infection is outside the scope of this review.

Purified MR/P fimbrial proteins have been tested for their antigenic potential as vaccine candidates (88, 89). There has been success by using the intranasal delivery of MrpH, the fimbrial tip adhesin of *P. mirabilis* (88). A fusion protein comprised of MrpH and mannose-binding protein delivered intranasally provided 75% protection from *P. mirabilis* ascending urinary tract infection in a mouse model (88). A fusion protein comprised of MrpH from *P. mirabilis* and FimH from uropathogenic *E. coli* delivered intranasally with monophosphoryl lipid A (as an adjuvant) induced robust IgG and IgA responses in mice (89). A clinical study of an inactivated bacterial cell suspension of four bacterial species, including a strain of *P. vulgaris*, was trialed in 159 patients with a history of recurrent urinary tract infections, compared with 160 patients maintained on prophylactic sulfamethoxazole-trimethoprim (90). The group of patients who received the vaccine had a mean number of 0.36 urinary tract infections in 3 months, versus 1.60 for those receiving sulfamethoxazole-trimethoprim ($P < 0.0001$) (90).

PROTEUS SPECIES AS GASTROINTESTINAL PATHOGENS

Colonization by *Proteus* Species

Proteus species are known human digestive tract commensal organisms with abundances varying according to location (Fig. 3). Colonization occurs early. Infants from Sweden and Pakistan were assessed for *Enterobacteriaceae* based on mode of delivery (vaginal versus cesarean) and breastfeeding behavior (91). Cesarean births in Pakistan were associated with *Proteus* species colonization within 3 days, with 11 of 21 cesarean-delivered and 1 of 9 vaginally delivered infants being positive for *Proteus* spp. ($P = 0.049$) (91).

Zilberstein et al. cultured mucosal samples from the upper ($n = 20$) and lower ($n = 24$) digestive tracts of healthy controls. *Proteus* species were present in 8% of gastric samples, 46% of duodenal and jejunal samples, 19% of ileal samples, 13% of cecal samples, and 38% of samples from the transverse colon (12).

Müller compared the recovery of *Proteus* species from the stool specimens of 1,422 healthy subjects. *P. mirabilis* was identified in 2.7% of healthy subjects, which is a probable underestimate as the epithelial preference of *P. mirabilis* means that it is likely undersampled in stool specimens (92). *Proteus penneri* and *P. vulgaris* were isolated from 0.9% and 4.2%, respectively, of the same population (92). A smaller culture-based study of 60 patients with gastrointestinal symptoms who tested negative for parasites demonstrated a colonization rate of 33% (20/60 patients) for *P. vulgaris* (93).

Proteus species, especially *P. mirabilis*, are often antibiotic resistant, conferring a survival advantage when colonizing the gastrointestinal tract. In a study of multiple-drug-resistant Gram-negative bacteria in the rectum of long-term-care patients, 52 drug-resistant strains were identified, 15 of which were *P. mirabilis* strains (94). A total of 87% of patients colonized with *P. mirabilis* were also cocolonized with at least one other resistant Gram-negative bacterium (range, 1 to 4 species; median, 2 species). Of the 15 patients with a resistant *P. mirabilis* strain, only 1 patient spontaneously cleared the organism, compared to 30 to 75% clearance for other bacterial species ($P = 0.007$ for clearance of other species versus *P. mirabilis*, as determined by a log rank test), demonstrating the ability of *P. mirabilis* to cause more-persistent colonization than other Gram-negative species (94). Of the 15 *P. mirabilis* strains recovered, 13 were genetically distinct, demonstrating the heterogeneity of *P. mirabilis* populations between patients within the same health care facility (94).

Proteus spp. in Gastroenteritis

Proteus species have been associated with infectious gastroenteritis. In a comparison of 1,271 patients with diarrhea, *P. mirabilis* was more prevalent in patients with diarrhea (10.8% in affected cases versus 2.7% in healthy subjects; $P < 0.001$) (92). However, that study did not take into account the possible administration of antibiotics to affected patients or the potential bystander or overgrowth effect (92). *P. mirabilis* has also been

associated with foodborne gastroenteritis in an outbreak in Beijing, China, associated with the consumption of stewed pork (95). Shi et al. investigated genetic adaptation to the digestive tract of *P. mirabilis* species isolated from the vomit and feces of patients, obtained at the time of a foodborne outbreak (96). When three clinical isolates were compared to four local and reference strains of *P. mirabilis*, obtained from food, a healthy subject, and two patients with urinary tract infections (including reference strains HI4320 and BB2000), there was evidence of strain-level genetic adaptation to the digestive tract (96). All seven isolates harbored drug resistance genes, but only the three isolates (one from vomit, one from stool, and one from infected food) contained digestive tract toxicity genes, including one with a complete type 4 secretion system (T4SS) not identified previously in *P. mirabilis*. Active horizontal gene acquisition has been demonstrated for *Proteus mirabilis*, including a protein from *Yersinia enterocolitica*, a known GI pathogen and member of the *Enterobacteriaceae* family (96). In summary, *Proteus* species can be linked to diarrheal states, but their primary pathogenic role has not been confirmed.

Proteus spp. in the Upper Gastrointestinal Tract

Colonization of the upper gastrointestinal tract, including the esophagus and stomach, by *Proteus* species in infants and older adults has been reported, often associated with instrumentation of the oropharynx (97–101). In 13 infants with feeding tubes without gastrointestinal symptoms, *Proteus* was isolated from the throat in 8% of patients, from the gastric juice in 15% of patients, and from the duodenal fluid in 8% of patients (98). In elderly patients with nasogastric feeding tubes (NGTs), *Proteus* species were isolated from the oropharynx in 24% of patients and from gastric fluids in 26% of patients (100). In another study, colonization of the oropharynx with *Proteus* species was present in 13% of patients with percutaneous endoscopic gastrostomy (PEG) and 21% of patients with NGTs, and colonization of the stomach was present in 4% and 23% of the same patients, respectively (99).

Association with Hepatobiliary Disease

Early culture-based surveys of patients undergoing biliary surgery showed the occasional isolation of *Proteus* species from the biliary tract (13% of bile samples) (102). *P. mirabilis* has also been recovered from bile obtained during endoscopic retrograde cholangiopancreatography (ERCP) in 6% of cultured samples (103).

In a metagenomic analysis using multitag pyrosequencing, the rectosigmoid mucosal community of healthy individuals was compared with that from patients with cirrhosis. Patients with cirrhosis had an elevated proportion of *Proteus* species compared with controls; the relative abundance in healthy controls was 0.0%, versus 0.1% in patients with cirrhosis ($P < 0.00001$) (104). Urease-producing microbes, such as *Proteus* spp., in the gut are known to contribute to the pathogenesis of hepatic encephalopathy through the breakdown of urea to ammonia and carbonic acid (104, 105).

In a series of patients undergoing liver resection, *Proteus vulgaris* bacteremia was identified in two patients, and polymicrobial infections were identified in eight patients (106). In the hepatobiliary tract, *Proteus* spp. are an uncommon cause of infection and are usually related to surgical interventions, such as ERCP or abdominal surgery. Implantable devices, such as stents, are also at risk of colonization and biofilm formation (107).

Pancreatic Disease

There are isolated reports of *Proteus* species infections of the pancreas, including a patient with a large infected pancreatic pseudocyst compressing the common bile duct. The cystic contents were polymicrobial, including *Proteus vulgaris*, *Morganella morganii*, *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa* (108).

Proteus species were found in biofilms that form on biliary and pancreatic stents placed via ERCP in 14 of 100 stents (107).

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Intestinal Disease

There are a number of reported links between *Proteus* spp. and various intestinal conditions, including small bowel intestinal overgrowth, Crohn's disease, and ulcerative colitis.

A small study of the downstream effects of small bowel ulceration caused by nonsteroidal anti-inflammatory drugs in rats identified a mixed population of *E. coli* and *P. mirabilis*. When treated with metronidazole, rats were protected from ulcer development (109). *Proteus* species were recovered from jejunal fluid in 11% of patients with small intestinal bacterial overgrowth syndrome (SIBOS), which often follows the expansion of facultative anaerobic bacterial communities (110). Viable bacterial translocation of *Proteus mirabilis* across the intact intestinal barrier has been demonstrated from the cecal and colonic mucosa of a monoassociated mouse model, with bacteria being additionally isolated from the mesenteric lymph nodes and the liver (111).

Crohn's disease. Recent research has implicated *Proteus* spp. in inflammatory bowel diseases, with evidence being derived both from patient-based microbiome surveys and mechanistic research. Ambrose et al. used culture-based techniques to compare the recovery of pathogenic gut bacteria from the ileal serosa and mesenteric lymph nodes in 45 Crohn's disease patients and 43 patients having surgery for other indications (112). Overall, Crohn's disease cases were more likely to have pathogenic bacteria recovered from the small bowel serosa (12/45 [27%] patients versus 6/41 [15%] controls). Of the 12 patients with positive serosal cultures, 4/12 patients were positive for *Proteus* spp. (33%). Additionally, involved and uninvolved mesenteric lymph nodes were assessed: 15/45 patients (33%) with involved nodes had a positive culture, and of these samples, 1/15 (7%) grew *Proteus* spp. Eleven of 45 samples of uninvolved nodes harbored bacteria, with 1/11 (9%) being positive for *Proteus* species (112). Although these data are not significant due to small numbers, they demonstrate the recovery of *Proteus* species from patients with Crohn's disease, while the recovery of *Proteus* species from lymph nodes establishes bacterial translocation, in line with the other members of the *Enterobacteriaceae* family (113).

Two microbiome studies have linked an overabundance of *Enterobacteriaceae* and *Proteus* spp. to Crohn's disease. In a pediatric study, *Proteus* species comprised 3/18 (16.7%) Gram-negative bacterial strains recovered from 12 Crohn's disease patients, compared to the total absence of *Proteus* species recovered from patients with ulcerative colitis, indeterminate colitis, and lymphonodular hyperplasia and from controls (114). A microarray-based study comparing patients with active Crohn's disease with matched healthy controls identified an overrepresentation of *Proteus* species overall, and *Proteus vulgaris* in particular, and other members of the *Proteobacteria* phylum in patients with active ileal disease requiring surgery (115).

A number of studies have addressed the mechanisms by which *Proteus* and other *Enterobacteriaceae* may contribute to the development of inflammatory bowel diseases. The TRUC mouse model of ulcerative colitis has been used to demonstrate that *Proteus mirabilis* and *Klebsiella pneumoniae* can elicit colitis and that this propensity for the development of colitis can be transmitted to wild-type mice via microbiome transfer (65).

Crohn's disease has been associated with changes in nitrogen metabolism. Ammonia produced from the breakdown of urea by bacterial urease provides a source of nitrogen for respiration and amino acid synthesis by pathogenic facultative anaerobes from the *Proteobacteria* phylum (49).

Interactions between the *Enterobacteriaceae*, which include *Proteus* species, and fungi (*Candida tropicalis*) have recently been implicated in the dysbiosis that characterizes Crohn's disease (116). When Crohn's disease patients were compared with first-degree relatives of Crohn's disease patients and healthy controls, bacterial dysbiosis was identified in affected patients and unaffected first-degree relatives. Crohn's disease patients had an increased presence of *Candida tropicalis*. Complex interactions between *Enterobacteriaceae* species (*Serratia marcescens* and *E. coli*) and *Candida*

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tropicalis were confirmed in laboratory studies, showing that flagellated and/or fimbriated bacteria combined with fungal hyphae to form a robust biofilm, with these three species combined forming the thickest biofilm ($P < 0.0001$). It was postulated that biofilms enriched for immunomodulatory microbial components (lipopolysaccharides and oligomannans, etc.) may perpetuate inflammation in dysbiotic patients through the induction of proinflammatory cytokine responses and of apoptosis. *Proteus* spp. were also strongly positively correlated with the abundance of *Candida* in patients with familial Crohn's disease ($r = 0.709$; $P < 0.005$), raising the possibility that *Proteus* spp. are capable of the same interactions, although this was not demonstrated, possibly due to their low abundance (116).

Seo et al. demonstrated that in the presence of intestinal injury and colonization with *P. mirabilis*, a marked proinflammatory IL-1β response occurs, via the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome (59). This occurs only in the presence of the *P. mirabilis* hemolysin HpmA, which appears to induce host macrophage induction of NLRP3. Preexisting injury or inflammation was necessary for the induction of NLRP3 activity and IL-1β by *P. mirabilis* (dextran sodium sulfate [DSS]-induced colitis), as inflammatory monocytes were required, suggesting that *P. mirabilis* may act to perpetuate and accelerate preexisting inflammation rather than induce it. *Proteus mirabilis* was as efficient at inducing IL-1β as pathogenic *Salmonella* spp., but the presence of the HpmA hemolysin was essential for the induction of IL-1β. IL-1β has been shown to be associated with disease activity in IBD patients (117, 118); however, in that study, most other anaerobic or facultative anaerobic commensals induced TNF-α expression but not IL-1β (59). When hemolysin expression levels were compared across *P. mirabilis* strains from multiple clinical sources (pyelonephritis, catheter-associated, and fecal isolates), fecal isolates had the highest hemolytic activity and had significantly higher hemolytic titers than those of the catheter-associated strain ($P = 0.001$) but not those of the pyelonephritis strain ($P = 0.065$) (119).

Proteus species have been associated with the postoperative recurrence of Crohn's disease by two independent groups (2, 3). Metagenomic surveys of patients at the time of surgery and 6 and 18 months postoperatively demonstrated that patients were more likely to have disease recurrence in the presence of detectable *Proteus* genera ($P = 0.008$) and the absence of detectable *Faecalibacterium* ($P < 0.001$) (3). The combination of detectable *Proteus* species and absent *Faecalibacterium* (<0.1%) in postoperative ileal biopsy specimens was associated with an increased risk of recurrence, with an odds ratio (OR) of 14 (95% confidence interval [CI], 1.7 to 110; $P = 0.013$). Smoking, an independent risk factor for postoperative disease recurrence, was also associated with an increased presence of *Proteus* spp. ($P = 0.0130$) (3). In another study by Mondot et al. of 20 Crohn's disease patients undergoing ileocolonic resection, the presence of a *Proteus mirabilis* operational taxonomic unit (OTU) was predictive of recurrence at 6 months postoperatively (2).

A recent review of consecutive Crohn's disease patients with intra-abdominal abscesses as a result of active disease demonstrated infection with *Proteus* spp. in 4.8% of cases, which was associated with high rates of quinolone resistance (120). Whether the presence of *Proteus* spp. in association with postoperative recurrence is a primary pathogenic event or secondary to disease recurrence remains to be elucidated. However, the association in both studies was established prospectively and longitudinally, with predictive association, making a pathogenic role more likely.

Other large intestinal diseases. Kanareykina et al. obtained samples (mouth, stomach, small intestine, and feces) from 65 patients with ulcerative colitis; performed culture-based enumeration; and identified *Proteus mirabilis*, *Proteus vulgaris*, or the closely related species *Morganella morganii* or *Providencia rettgeri* (Fig. 1) in nearly all cases (121). In 40/65 patients, these species were recovered from more than one anatomic site. A *Proteus* species protein "vaccine" was then administered and resulted in clinical improvement in moderate to severe cases of ulcerative colitis as well as a decrease in bacterial counts. However, no details of the vaccine composition or any

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objective disease activity metrics were described, and the study overall was of low quality (121).

A recent study of children with and without appendicitis showed increases in the relative abundances of 12 genera, of which *Proteus* species were the only representatives of the *Enterobacteriaceae* family (0.015% versus 0%; $P = 0.028$) (122).

Proteus bacteria have been implicated in the perpetuation of colonic inflammation in diversion colitis (123). Inflammatory conditions of the bowel increase the local concentrations of inducible nitric oxide synthase, leading to high levels of nitrate that cannot be metabolized, except by the microbiota (124). This favors the expansion of bacteria that are able to metabolize nitrate under anaerobic conditions, leading to a survival advantage and population expansion of *Proteus* spp. and other nitrate-reducing *Enterobacteriaceae* (48, 123).

Nosocomial Infections and *Proteus* Species Complicating Gastrointestinal Disease

Proteus species, especially *P. mirabilis* and *P. vulgaris*, are common causes of nosocomial opportunistic infections. Many patients with preexisting gastrointestinal diseases are liable to secondary *Proteus* infections, often in the context of polymicrobial infections. *Proteus* species can also cause peritonitis following perforations of the gastrointestinal tract; in one report of 383 patients with peritonitis, *Proteus* species were identified in 87 (23%) patients (125).

Proteus species can colonize medical devices placed in the gastrointestinal tract, including ventriculoperitoneal shunts (126), nasogastric tubes (99, 100), biliary and pancreatic stents (107), and tracheoesophageal voice prostheses (127). *Proteus* bacteria have been shown to be contaminants of gastroscopes and colonoscopes after insufficient disinfection (128). Infections can also be acquired in the hospital setting due to environmental contamination, with *P. vulgaris* persisting on dry, hard surfaces for up to 2 days (129). There are reports of hospital-based and community epidemics of infection with person-to-person spread, with most patients acquiring gastrointestinal carriage prior to infection (13, 130).

CONCLUSIONS

Proteus species are hardy, adaptable, and potentially pathogenic residents of the human gastrointestinal tract and have been underappreciated as a cause of gastrointestinal disease. Host-microbe and microbe-microbe interactions by *Proteus* spp., and the pathogenicity of this genus that may result from population expansion in response to environmental changes, are emerging as important aspects of disease associated with this genus. The possible contribution of *Proteus* spp. to intestinal diseases and infections has been somewhat neglected. Research into the virulence of *Proteus* spp. in the urinary tract using the bacteriology of ileal conduits (131) and intestinal segments (60, 132) for bladder augmentation suggests that *Proteus* spp. should be examined more closely for their potential as gastrointestinal pathogens.

There is increasing evidence that *Proteus* species may play a role in inflammatory bowel disease through the direct action of the bacteria, compounded by host immune evasion and perturbation. As Gram-negative organisms, *Proteus* species are intrinsically proinflammatory as a result of the production of lipopolysaccharide (LPS) and immunostimulatory flagellin proteins. There may be an association between *Proteus* species and inflammatory bowel disease, especially Crohn's disease, mainly through population expansion and immune activation. Their low population abundance does not preclude a potential large pathogenic effect.

Genetic characterization of enteric isolates compared to urinary tract isolates will be important for determining the effect of virulence factors on gastrointestinal pathogenesis.

Research on the gut microbiome as an ecosystem is informing our understanding of *Proteus* species, yet there are still unanswered questions. These include obtaining confirmation that *Proteus* species can swarm within the human gut and addressing the

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effect of individual environmental changes (e.g., surgery, pH, or oxygen concentrations) on the mucosa-associated *Proteus* population.

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M.A.K. and A.L.H. devised the concept. A.L.H. acquired data, screened papers, interpreted the data, and wrote the manuscript. M.A.K., S.C.N., and M.M. provided critical revision of the manuscript for important intellectual content.

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Characteristics and Outcomes of Polymicrobial Bloodstream Infections in the Emergency Department: A Matched Case–control Study

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Abstract

Objectives: Polymicrobial bloodstream infection (BSI) is a critical condition and has been increasingly reported; however, the authors were unable to find an emergency department (ED) patient-based study in the literature.

Methods: A retrospective matched case–control study with a ratio of 1:3 among patients with polymicrobial BSIs in an ED was conducted. The case group was patients aged > 16 years with polymicrobial BSIs. Patients matched for age and sex with monomicrobial BSIs were sampled as the control group. Demographic information, underlying conditions, microbiologic data, and outcomes were collected for further analysis.

Results: From January 2005 to December 2007, a total of 112 episodes of polymicrobial BSIs among 109 patients were included. Two pathogens were isolated among 87 (77.7%) episodes and three were found among 25 (22.3%) episodes. A history of hospitalization within 90 days was an independent risk factor for polymicrobial BSIs ($p = 0.003$). Intraabdominal infection ($p < 0.001$) and respiratory tract infection ($p = 0.017$) were more likely to be associated with polymicrobial BSIs. Gram-negative and Gram-positive bacteria were documented in 95.5 and 46.4% episodes of polymicrobial BSIs, respectively. Inappropriate antimicrobial treatment was observed in 53.6% of polymicrobial BSIs, but only accounted for 23.8% of monomicrobial BSIs ($p < 0.001$). The overall 30-day mortality rate of the polymicrobial group was significantly higher than those with monomicrobial BSIs (30.3 and 11.6%, respectively; $p < 0.001$).

Conclusions: Patients with polymicrobial BSIs had a high mortality rate. Acknowledgment of the clinical and microbiologic characteristics and recognition of patients at risk for polymicrobial BSIs are critical in EDs.

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Keywords: bacteremia, bloodstream infection, polymicrobial infection, emergency department, risk factors

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Blood cultures are frequently used to evaluate patients with fever in emergency departments (EDs). More than 3.1 million blood cultures, associated with 2.8% of all ED visits, are ordered annually in the United States.[1] Although almost half of all blood cultures ordered in the ED may not be necessary, patients with positive blood cultures more often than not have a serious infection and need admission for further treatment.[1,2]

Polymicrobial bloodstream infection (BSI), defined as the presence of at least two different microorganisms found from the blood cultures, has been reported increasingly, with rates ranging from 6% to 32% of all BSI episodes.[3–7] The mortality rate of hospitalized patients with polymicrobial BSIs ranged from 21% to 63%, approximately twice the rate of those with monomicrobial infections.[3,4,8,9] Most patients with

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polymicrobial BSIs have underlying medical conditions including malignancy, neutropenia, gastrointestinal disease, genitourinary disease, recent surgical procedures, or the presence of central venous catheters.[3–7,10,11] Nevertheless, the majority of investigations of polymicrobial BSIs are limited to unselected populations or neutropenic patients with underlying malignancy.

Despite the critical nature of polymicrobial BSIs in EDs, to our knowledge, no emergency patient-based study for polymicrobial BSIs could be found in the literature. Therefore, we performed a retrospective matched case–control study among patients with polymicrobial BSIs with the following objectives: 1) to explore clinical characteristics and underlying diseases, 2) to identify isolated microorganisms, 3) to define the sources of infections, 4) to evaluate empirical antimicrobial treatment, and 5) to compare their survival rates.

METHODS

Study Design

We conducted a retrospective matched case–control study to compare polymicrobial with monomicrobial BSIs in patients arriving at an ED. This study was approved by the institutional review board of E-Da Hospital in Taiwan.

Study Setting and Population

This study was carried out at E-Da Hospital, a 1,000-bed university-affiliated hospital in southern Taiwan with approximately 60,000 annual ED visits. The records of all positive blood cultures collected at an ED between January 2005 and December 2007 from patients aged > 16 years were reviewed.

Study Protocol

The medical records of included patients were each reviewed by two authors. If any discrepancy was found, the medical records were inspected again by these two authors together. Demographic information of age and sex, underlying illness, clinical condition, microbiology, source of bacteremia, and outcome were collected from the medical records. Clinical data were analyzed by case episodes. All patients with polymicrobial BSIs were enrolled into the case group, whereas those with monomicrobial BSIs were enrolled in the control group. For the purpose of comparison, we performed matching by a blinded observer unaware of the clinical outcomes. Patients with polymicrobial BSIs were matched by sex and age (±3 years) with a ratio of 1:3 to those with monomicrobial BSIs during the same study period.

Episodes were considered distinct if separated by at least 7 days and if the reason for visiting the ED was different. Monomicrobial and polymicrobial BSIs were defined as one species and two or more species that were isolated from blood cultures, respectively. Shock was defined as systolic pressure less than 90 mm Hg or requiring inotropic agents to maintain blood pressure during the ED stay. The sources of BSIs were determined clinically on the basis of the presence of an active infection site coincident with BSIs or isolation of the organism from other clinical specimens prior to or on the same date as the onset of BSIs. If a polymicrobial BSI was presumed to be due to two different sources, the episode was excluded from our study. If the source of a BSI could not be attributed to any known source, it was classified as a primary BSI.

According to the blood culture collection guidelines of E-Da Hospital, two sets of blood cultures collected 30 minutes apart with proper site preparation and aseptic technique were ordered routinely by physicians if infection was suspected. If patients received antibiotics within 24 hours, BACTEC resin-containing blood culture bottles were used (Becton Dickinson Diagnostic Instrument System, Sparks, MD). All blood culture samples were processed by the BACTEC 9240 system (Becton Dickinson). Susceptibilities to antimicrobial agents were determined according to the Clinical and Laboratory Standards Institute criteria of the year.[12–14] If the blood cultures yielded skin flora, including coagulase-negative staphylococci, micrococci species, diphtheroids, *Bacillus* species, or *Propionibacterium acnes* in only one culture, these microorganisms were regarded as contaminated,[15] and these episodes were excluded from our study.

Antimicrobial therapy was considered inappropriate if the regimen included antimicrobial agents that were not effective against all of the pathogens isolated from the blood cultures via in vitro susceptibility testing or the lack of antibiotic therapy during the ED stay. We classified the inappropriate antibiotics therapy into four groups: 1) use of improper kinds of antibiotics (such as enterococci, which should be treated with penicillin but were treated with a cephalosporin); 2) use in resistant microorganisms (such as extended-spectrum β-lactamase-producing *Enterobacteriaceae* and methicillin-resistant *Staphylococcus*); 3) combination therapy needed but not received (such as two or three isolates in polymicrobial BSIs, which cannot be treated by only one antibiotic); and 4) no antibiotics use during the ED stay.

Outcome Measures

We used the overall 30-day mortality for outcome analysis. The causes of mortality were determined by the medical records. If patients were discharged within 30 days after admission and were not followed up at our hospital, telephone contact was made to collect the required information. Patients who were lost to follow-up were excluded from our study.

Data Analysis

We used the SPSS software package (version 14.0, SPSS Inc., Chicago, IL) to analyze the results. Categorical variables were analyzed using the chi-square test or Fisher exact tests, as appropriate. Continuous variables were analyzed using the Student's t-test. Univariate odds ratios (ORs) were computed by the Mantel-Haenszel test. To identify the risk factors for polymicrobial BSIs, underlying conditions that could contribute to polymicrobial BSIs and were associated with a level of significance of less than 0.20 in univariate analyses were included in a logistic regression model for multivariate analysis (conditional backward stepwise model). The OR, 95% confidence interval (CI), and p-value

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were calculated for each factor. The Hosmer-Lemeshow goodness-of-fit test was used to assess the fitness of the model. Survival data were analyzed by Kaplan-Meier plots. Associations between variables and survival were compared using the log-rank tests. All p-values were two-tailed and a p-value < 0.05 was considered statistically significant.

RESULTS

Characteristics of Enrolled Episodes

During the study period, a total of 4,290 positive blood cultures were documented among 162,760 ED visits (Figure 1). A total of 112 episodes of polymicrobial BSIs among 109 patients were included in our study. Three patients had two episodes of polymicrobial BSIs. Two pathogens were isolated among 87 (77.7%) episodes, and three pathogens were found among 25 (22.3%) episodes. Sixty patients were male and 52 were female, and their median age was 65 years (mean ± SD = 64.0 ± 15.7; range = 18–89 years).

Underlying Conditions

After matching, no significant differences by age or sex were noted between case and control groups. The prevalence of underlying conditions is shown in Table 1. The presence of malignancy (p = 0.018) and a history of hospitalization within 90 days (p = 0.003) were associated with polymicrobial BSIs. However, multivariate regression analysis showed that only patients with a history of hospitalization within 90 days were predisposed to polymicrobial BSIs (OR = 1.92; 95% CI = 1.25 to 2.97; p = 0.003).

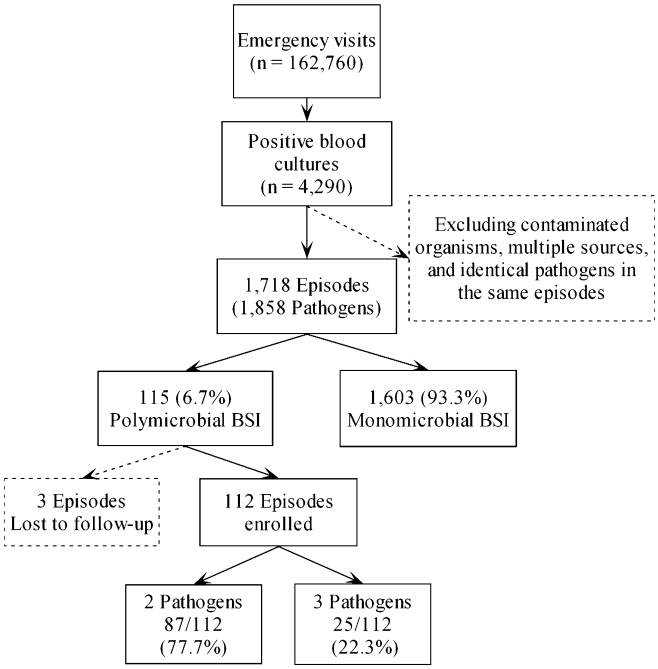

Figure 1. Patient enrollment, number of bacteremic episodes, and number of isolates in polymicrobial BSIs. BSI = bloodstream infection.

Table 1
Demographic Characteristics, Underlying Conditions, and Clinical Manifestations of 112 Polymicrobial and 336 Monomicrobial BSI Episodes

Characteristics	No. (%) of Episodes: Polymicrobial (*n* = 112)	Monomicrobial (*n* = 336)	OR (95% CI)
Age (year, mean ± sd)	64.0 ± 15.7	64.4 ± 15.5	—
Sex, male	60 (53.6)	180 (53.6)	1.0 (0.65–1.54)
Clinical condition			
Diabetes mellitus	35 (31.3)	135 (40.2)	0.68 (0.43–1.07)
End-stage renal disease	3 (2.7)	18 (5.4)	0.49 (0.14–1.68)
Malignancy	36 (32.1)	71 (21.1)	1.77 (1.10–2.84)
Liver cirrhosis	18 (16.1)	47 (14.0)	1.18 (0.65–2.13)
HIV infection	0	3 (0.9)	—
COPD	7 (6.3)	13 (3.9)	1.66 (0.64–4.26)
Nosocomial infection	8 (7.1)	22 (6.5%)	0.91 (0.39–2.11)
Hospitalized within 90 days	56 (50.0)	115 (34.2)	1.92 (1.25–2.97)
Catheter/tube			
Port-A Cath	15 (13.4)	33 (9.8)	1.42 (0.74–2.73)
Bile drainage tube	0	2 (0.6)	—
Urinary catheter	3 (2.7)	7 (2.1)	1.29 (0.33–5.09)
Clinical manifestation			
Anemia*	35 (31.3)	89 (26.5)	1.26 (0.79–2.01)
Neutropenia†	5 (4.5)	6 (1.8)	2.57 (0.77–8.59)
Thromocytopenia‡	36 (32.1)	78 (23.2)	1.57 (0.98–2.51)
Shock	18 (16.1)	33 (9.8)	1.76 (0.95–3.27)
Inappropriate antimicrobial therapy	60 (53.6)	80 (23.8)	3.69 (2.36–5.78)
30-day mortality	34 (30.4)	39 (11.6)	3.32 (1.97–5.60)

BSI = bloodstream infection; COPD = chronic obstructive pulmonary disease; HIV = human immunodeficiency virus.
*Hemoglobin < 10 g/dL.
†Absolute neutrophil count < 0.5×10^9/L.
‡Platelet count < 100×10^9/L.

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Table 2
Sources of 112 Polymicrobial and 336 Monomicrobial BSIs

Source of BSI	No. (%) of Episodes: Polymicrobial (n = 112)	No. (%) of Episodes: Monomicrobial (n = 336)	OR (95% CI)	p-value
Urinary tract infection	19 (17.0)	131 (39.0)	0.32 (0.19–0.55)	<0.001
Lower respiratory tract infection	20 (17.9)	32 (9.5)	2.07 (0.02–1.23)	0.017
Catheter-related infection	3 (2.7)	8 (2.4)	1.23 (0.29–4.33)	1
Intraabdominal infection	38 (33.9)	64 (19.0)	2.18 (1.36–3.52)	0.001
Skin and soft tissue infection	19 (17.0)	36 (10.7)	1.70 (0.93–3.11)	0.081
Bone/joint infection	0	5 (1.5)	—	0.338
Intravascular infection	1 (0.9)	8 (2.4)	0.37 (0.05–2.99)	0.461
Primary bacteremia	12 (10.7)	48 (14.3)	0.72 (0.37–1.41)	0.337
Others*	0	4 (1.2)	—	—

BSI = bloodstream infection.
*Including central nervous system infection and ear, nose, and throat infection.

Sites of Infections

The sources of infections could be identified in 89.3% of polymicrobial BSIs and in 85.7% of monomicrobial BSIs. The most common source of polymicrobial BSIs was intraabdominal infection (33.9%). Comparing with monomicrobial BSIs, sources of polymicrobial BSIs were significantly more likely attributed to intraabdominal infection (p = 0.001) and lower respiratory tract infection (p = 0.017). On the contrary, monomicrobial episodes were significantly more likely to arise from the urinary tract infection (p < 0.001; Table 2).

Pathogens of Bacteremia

The isolates of polymicrobial and monomicrobial BSIs are presented in Table 3. Only one episode of *Candida albicans* was found in the group of polymicrobial BSIs. Gram-positive bacteria were found in 95.5% of polymicrobial BSIs, and Gram-negative bacteria were identified in 46.4%. The most frequent isolates of Gram-negative and Gram-positive bacteria in polymicrobial BSIs were *Escherichia* species (57.1%) and streptococci (26.8%), respectively.

Table 4 shows the microorganisms isolated from patients with polymicrobial BSIs according to their infection sites. Gram-negative bacteria could be recognized in the majority of urinary tract infections (73.8%), lower respiratory tract infections (86.7%), catheter-related BSIs (85.7%), and intraabdominal infections (78.4%). In contrast, Gram-negative bacteria could be identified in only half of skin and soft tissue infections. The most common polymicrobial combination in both lower respiratory tract infection and intraabdominal infection was *Escherichia coli* and *Klebsiella pneumoniae* coinfection (25 and 23.7%, respectively).

Antimicrobial Therapy

Inappropriate antimicrobial treatment was found in 53.6% of patients with polymicrobial BSIs and was significantly more frequent than in those with monomicrobial BSIs (23.8%; OR = 3.69; 95% CI = 2.36 to 5.78; p < 0.001; Table 1). Figure 2 shows the proportions of inappropriate antimicrobial therapy in polymicrobial BSIs by different sites of infections. The types of inappropriate antibiotics use are demonstrated in Figure 3. One-third of polymicrobial bacteremic patients should have received combination therapy of antibiotics but did not.

Survival Analysis

The overall 30-day mortality rates of patients with polymicrobial and monomicrobial BSIs were 30.3 and 11.6%, respectively. Patients receiving inappropriate antibiotics had a higher mortality rate than those who were treated with appropriate antibiotics (p = 0.011). Among these mortality cases, 70 died directly due to infections, and three patients died of other etiologies (hepatocellular carcinoma rupture, spontaneous subarachnoid hemorrhage, and respiratory failure due to pulmonary metastasis in a hepatocellular carcinoma patient). In the survival analysis, patients with polymicrobial BSIs had a significantly higher overall mortality rate than those with monomicrobial BSIs (p < 0.001; Figure 4). Patients infected with two or three microorganisms had a significantly higher overall 30-day mortality rate than those with only one isolate (p < 0.001 and p = 0.001, respectively). However, there was no statistical difference between the group of two-pathogen and three-pathogen polymicrobial BSIs (p = 0.234).

DISCUSSION

Our study found that polymicrobial BSIs accounted for 6.7% of all BSI episodes in the ED, which is similar to the previous reports of general population studies.[4,16] We also identified that a history of hospitalization within 90 days was an independent risk factor for polymicrobial BSIs, as was bacteremia due to lower respiratory tract infection or intraabdominal infection. Polymicrobial BSIs are known to be associated with hospital-acquired infections in several studies.[8,9,17] Intraabdominal infection is also a well-known cause of polymicrobial bacteremia.[9] Lower respiratory tract infections have also been associated with multiple pathogens. Lauderdale et al.[18] reported that polymicrobial infection occurred in 4% to

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Table 3
Microorganisms Among 112 Polymicrobial and 336 Monomicrobial BSIs

Microorganism	No. (%) of Episodes: Polymicrobial (*n* = 112)	No. (%) of Episodes: Monomicrobial (*n* = 336)	OR (95% CI)	p-value
Gram-positive bacteria*	60 (53.6)	80 (23.8)		
Staphylococcus spp.	15 (13.4)	38 (11.3)	1.21 (0.64–2.30)	0.554
S. aureus	14 (12.5)	36 (10.7)	1.19 (0.62–2.30)	0.603
MRSA	9 (8.0)	15 (4.5)	1.87 (0.80–4.40)	0.146
Streptococci	30 (26.8)	29 (8.6)	3.87 (2.20–6.82)	<0.001
S. pneumoniae	1 (0.9)	2 (0.6)	1.51 (0.14–16.75)	1
Enterococcus spp.	14 (12.5)	7 (2.1)	6.71 (2.64–17.10)	<0.001
Other‡	0	4 (1.2)	—	—
Gram-negative bacteria†	189 (168.8)	255 (75.9)		
Escherichia spp.	64 (57.1)	136 (40.5)	1.96 (1.27–3.02)	0.002
E. coli	63 (56.3)	136 (40.5)	1.89 (1.23–2.91)	0.004
ESBL production	3 (2.7)	5 (1.5)	1.82 (0.43–7.75)	0.417
Klebsiella spp.	50 (44.6)	52 (15.5)	4.40 (2.74–7.09)	<0.001
K. pneumoniae	46 (41.1)	52 (15.5)	3.81 (2.36–6.14)	<0.001
ESBL production	3 (2.7)	0	—	—
Pseudomonas spp.	19 (17.0)	15 (4.5)	4.37 (2.14–8.94)	<0.001
Proteus spp.	11 (9.8)	7 (2.1)	5.12 (1.93–13.55)	0.001
Enterobacter spp.	10 (8.9)	5 (1.5)	6.49 (2.17–19.42)	0.001
Aeromonas spp.	4 (3.6)	6 (1.8)	2.04 (0.56–7.35)	0.277
Salmonella spp.	0	7 (2.1)	—	0.200
Acinetobacter spp.	4 (3.6)	8 (2.4)	1.52 (0.45–5.14)	0.505
Citrobacter spp.	6 (5.4)	4 (1.2)	4.70 (1.30–16.96)	0.019
Morganella morganii	5 (4.5)	2 (0.6)	7.80 (1.49–40.81)	0.012
Other‡	15 (13.4)	13 (3.9)	3.84 (1.77–8.35)	0.001
Fungi				
Candida albicans	1 (0.9)	1 (0.3)	3.02 (0.19–48.65)	0.438

BSI = bloodstream infection; ESBL = extended-spectrum β-lactamase; MRSA = methicillin-resistant *S. aureus*.
*The rate of Gram-positive bacteria in one episode of polymicrobial BSI was 46.4% (52/112), significantly higher than monomicrobial BSI (OR = 2.77; 95% CI = 1.77 to 4.34; p < 0.001).
†The rate of Gram-negative bacteria in one episode of polymicrobial BSI was 95.5% (107/112), significantly higher than monomicrobial BSI (OR = 6.80; 95% CI = 2.68 to 17.25; p < 0.001).
‡Including *Providencia alcalifaciens, Chryseobacterium meningosepticum, Serratia marcescens, Moraxella* spp., *Plesiomonas shigelloides, Weeksella virosa, Vibrio cholerae, Vibrio vulnificus, Flavobacterium* spp., *Shewanella putrefaciens* Bv. 1, *Stenotrophomonas maltophilia*, *Pasteurella multocida*, *Methylobacterium* spp., *Pantoea agglomerans*, *Lactobacillus* spp., *Leclercia adecarboxylata*, *Listeria monocytogenes*, *Stomatococcus mucilaginosus*, and *Roseomonas* spp.

39% of patients with community-acquired pneumonia. *Streptococcus pneumoniae* is found to be the most common pathogen mixed with other pathogens in western countries.[19–21] However, our study revealed that *K. pneumoniae* is the most common isolate in the polymicrobial bacteremic patients. This may be due to the high prevalence rate of *K. pneumoniae* infection in Taiwan.[22]

With regard to the microbiology of polymicrobial BSIs, we noted that nearly all episodes included at least one isolate of Gram-negative bacilli, but only around half of episodes involved Gram-positive bacteria. In a study of inpatient polymicrobial BSIs, 44% of the infections involved at least one Gram-negative bacterium and 43% involved Gram-positive bacteria.[4] In another large study of polymicrobial BSIs among malignancy patients, 76% of the infections involved at least one Gram-negative bacillus, and 50% involved Gram-positive organisms.[16] The culture rate of Gram-positive bacteria was similar to our study of emergency-based patients, but the yielding rate of Gram-negative bacteria has differed in the literature. Because these results are important to provide the information for empirical antimicrobial treatment in clinical practice, more studies involving patients with different underlying diseases and different populations are necessary.

Empirical treatment is given before results of blood cultures are available, and the prescription of empirical antibiotics may be dependent on a clinical basis alone. Our study showed that the empirical antibiotics treatment was inappropriate in 53.6% of patients with polymicrobial BSIs, which is much higher than those with monomicrobial BSIs. Regarding the causes of inappropriate use of antibiotics, we found that no combination therapy occurred in one-third of episodes. In contrast to monomicrobial BSIs, treating multiple pathogens with only one antibiotic could be inadequate, even when a broad-spectrum antibiotic was prescribed. For example, enterococci are intrinsically resistant to cephalosporins. If a broad-spectrum cephalosporin is used for polymicrobial infections including enterococci, the treatment will be inadequate. So acknowledging the common causative microorganisms in each site of infection is important to guide empirical antibiotics treatment.

Our study also found that almost a fourth of bacteremic patients received no antibiotics during their ED stay, neither for polymicrobial nor for monomicrobial

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BSIs. Indeed, there is no indicator available to precisely predict the presence of bacteremia.[23,24] Delayed or absent antibiotic treatment is most often due to inaccurate clinical evaluation of the risk factors for severe infection or bacteremia.[25] Adequate education and interdisciplinary coordination may improve clinical performance.

The mortality rate of patients with polymicrobial BSIs ranged from 14% to 43%, approximately two times the mortality rate of those with monomicrobial BSIs.[26] The higher mortality in polymicrobial BSIs was reported to be related to inappropriate antimicrobial treatment.[4,16,17,27,28] As a result, the empirical antimicrobial therapy that could cover the possible pathogens is

Table 4
Pathogens Isolated from 112 Polymicrobial BSIs

Pathogens	UTI (*n* = 19)	LRI (*n* = 20)	CRBSI (*n* = 3)	IAI (*n* = 38)	SSTI (*n* = 19)	Intravascular Infection (*n* = 1)	Primary (*n* = 12)
Gram-positive bacteria	11	6	1	19	20	0	3
Staphylococcus spp.	2	2	1	2	8	0	0
S. aureus	2	2	1	2	7	0	0
MRSA	2	1	1	1	4	0	0
Streptococci	4	2	0	11	10	0	2
S. pneumoniae	0	1	0	0	0	0	0
Enterococcus spp.	5	1	0	6	2	0	1
Gram-negative bacteria	31	39	6	69	21	0	20
Escherichia spp.	11	7	1	32	10	0	4
E. coli	11	7	1	31	10	0	4
ESBL production	1	0	0	1	1	0	1
Klebsiella spp.	5	11	0	23	2	1	5
K. pneumoniae	5	10	2	21	1	1	5
ESBL production	1	1	0	0	0	0	0
Pseudomonas spp.	4	10	0	2	0	0	4
Proteus spp.	3	2	0	2	4	0	0
Enterobacter spp.	1	1	3	1	0	1	3
Aeromonas spp.	0	0	0	2	0	0	2
Acinetobacter spp.	1	0	0	1	2	0	0
Citrobacter spp.	3	1	0	2	0	0	0
Morganella morganii	2	0	0	1	1	0	1
Other*	1	7	0	3	2	0	1
Fungi							
C. albicans	0	0	0	0	0	0	1

CRBSI = catheter-related bloodstream infection; ESBL = extended-spectrum β-lactamase; IAI = intraabdominal infection; LRI = lower respiratory tract infection; MRSA = methicillin-resistant *S. aureus*; SSTI = skin and soft tissue infection; UTI = urinary tract infection.
*Including *P. alcalifaciens, C. meningosepticum, S. marcescens, Moraxella osloensis, P. shigelloides, Flavobacterium* spp., *Roseomonas* spp., and *V. cholerae.*

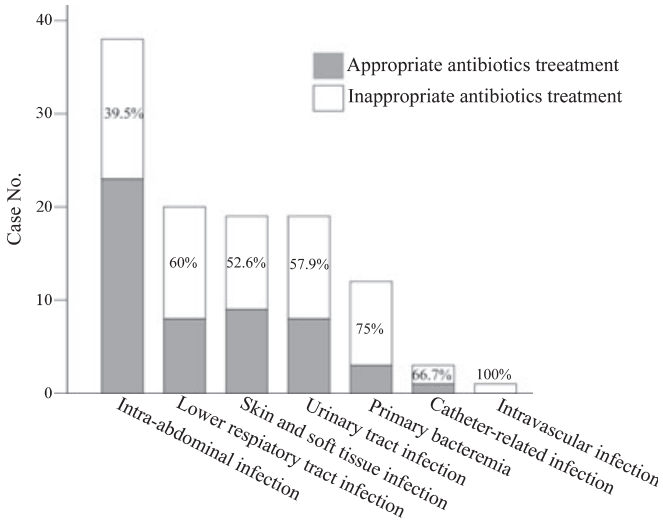

Figure 2. Proportions of inappropriate antimicrobial therapy in polymicrobial BSIs by different sites of infections. BSI = bloodstream infection.

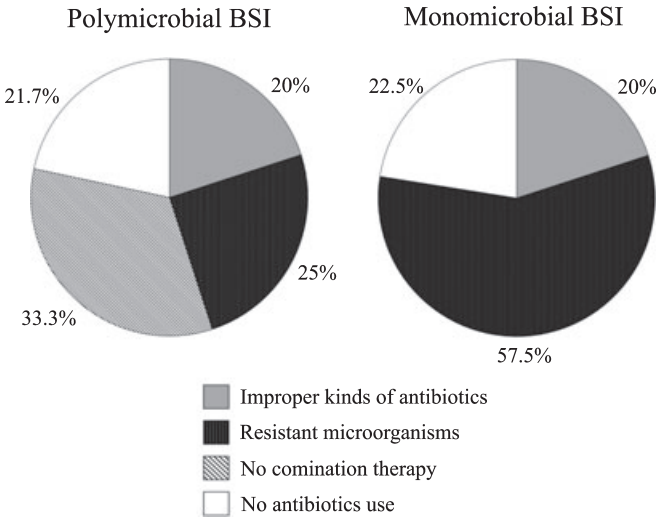

Figure 3. Types of inappropriate antimicrobial therapy in polymicrobial and monomicrobial BSIs. BSI = bloodstream infection.

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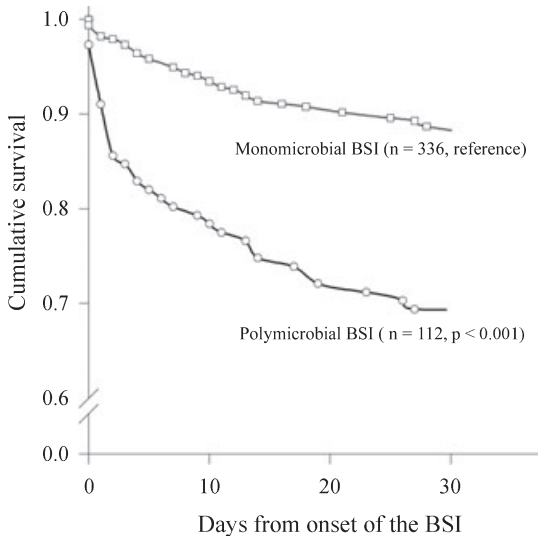

Figure 4. Kaplan-Meier survival curves of the 30-day cumulative survival rate for patients with polymicrobial and monomicrobial BSIs in the ED. BSI = bloodstream infection.

essential for treating patients at risk for polymicrobial BSIs. Effective and early antimicrobial therapy administered during an ED stay for BSI has been demonstrated to improve survival rates.[25,28,29] Although the organisms and the number of causative isolates will not be known at the time of blood cultures, recognizing patient-specific risk factors, the suspicious infection sources, and the most common pathogens, as well as the regional and institutional patterns of antibiotic resistance, will help physicians to provide adequate empirical antibiotics therapy.

LIMITATIONS

First, our study was conducted at a single hospital in southern Taiwan, limiting its generalizability. Second, although there is a standard practice regarding the use of blood cultures, we could not ascertain that all emergency physicians adhered to established guidelines. Therefore, omission of patients with polymicrobial BSIs could exist. Third, while there is an antibiotics use policy in our hospital, the prescription of empirical antibiotics may be dependent on the personal experiences of physicians. Fourth, the data used in our analysis were collected from medical records. There may be inconsistencies among the completeness of these data. Finally, because all of the univariate and multivariate analyses were carried out in an exploratory fashion, some risk factors may not have been detected or explored in our study. Further prospective studies with specific hypotheses are necessary to accurately determine these risk factors.

CONCLUSIONS

Our study provides important information about the clinical and microbiologic manifestations of ED patients with polymicrobial BSIs. Inasmuch as the mortality rate of patients with polymicrobial BSIs is significantly higher than those with monomicrobial infection, identifying patients at risk for polymicrobial BSIs, knowing the most common microorganisms in different infection sites, and being familiar with the epidemiology of regional and institutional patterns of antibiotic resistance are of paramount importance to provide adequate antimicrobial therapy in EDs.

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ARHAI Scotland 2023 Annual Report

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ARHAI Scotland

Antimicrobial Resistance and Healthcare Associated Infection

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Alternative formats

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About ARHAI Scotland

ARHAI Scotland overall vision is to:

Enable Scotland to adopt an evidence based and innovative approach to minimising the burden of infection and antimicrobial resistance (AMR).

Our mission is to:

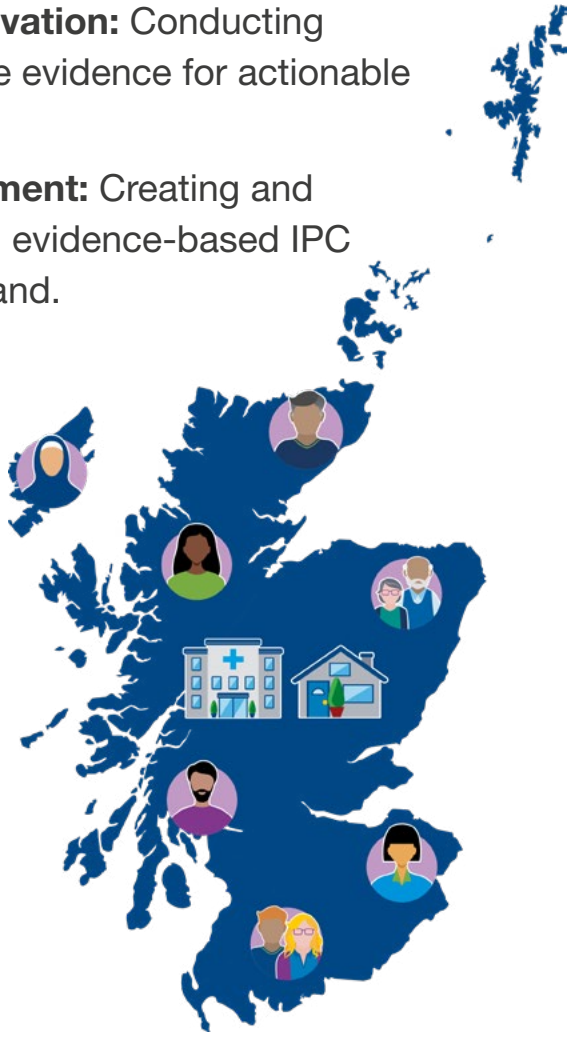
Enhance the health and wellbeing of the population by reducing infection and antimicrobial resistance within Scottish care settings.

We will achieve this by:

- establishing a robust evidence base for best practices
- developing mechanisms for monitoring key priority areas
- connecting with the broader health, social care, and public health systems
- collaborating with key delivery partners, including NHSScotland boards, care providers, and other national bodies as commissioned by the Scottish Government

The work of ARHAI Scotland is grounded in delivering a comprehensive range of functions in collaboration with stakeholders across health, care, and other sectors. ARHAI Scotland's functions include:

- **Surveillance and monitoring:** Assessing the impact of infections and antimicrobial resistance on health.
- **Clinical assurance:** Reducing risk in the healthcare built environment.
- **Programme coordination:** Overseeing national Infection Prevention and Control (IPC) and Antimicrobial Resistance (AMR) programmes.
- **Expert advice and horizon scanning:** Providing specialised IPC/AMR guidance and foresight.
- **Outbreak response:** Preparing for and responding to healthcare-associated infection (HCAI) outbreaks and incidents.
- **Workforce development:** Collaborating with NHS Education for Scotland to cultivate a confident, knowledgeable, and competent IPC workforce.
- **Professional practice:** Promoting high standards of professional conduct.
- **Research and innovation:** Conducting research to generate evidence for actionable interventions.
- **Guidance development:** Creating and maintaining national evidence-based IPC guidelines for Scotland.

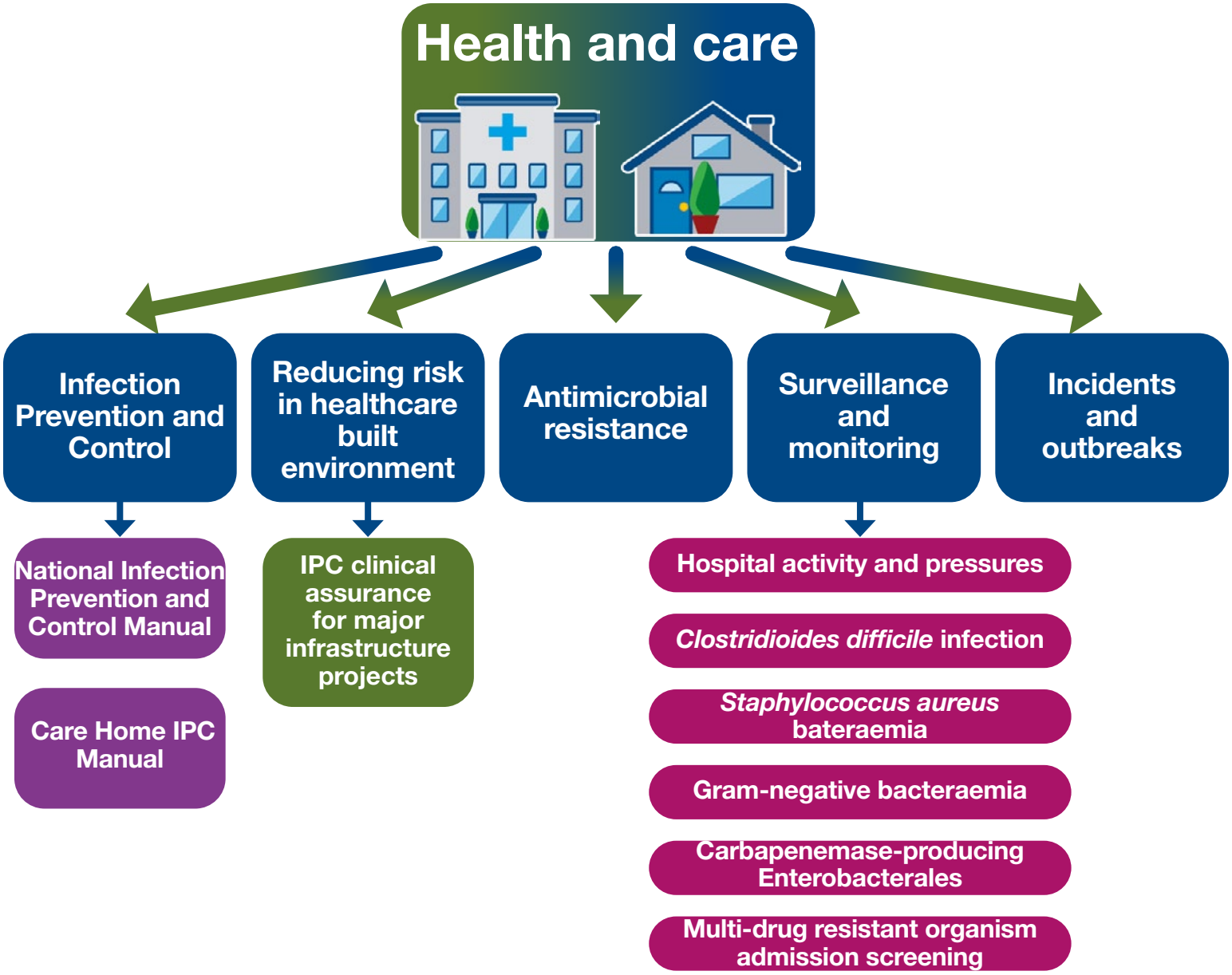


ARHAI Scotland Activities in 2023

Globally, healthcare associated infections (HCAI) and antimicrobial resistance (AMR) continue to pose a significant threat to patient safety in all settings where care is delivered.

This annual report showcases the key initiatives undertaken by ARHAI Scotland in 2023 to support Infection Prevention and Control (IPC), prevent HCAI, and contain AMR. Alongside a planned programme of work, ARHAI Scotland continued to provide responsive IPC expert advice throughout the year.

Click below to read more about ARHAI Scotland's work in 2023.

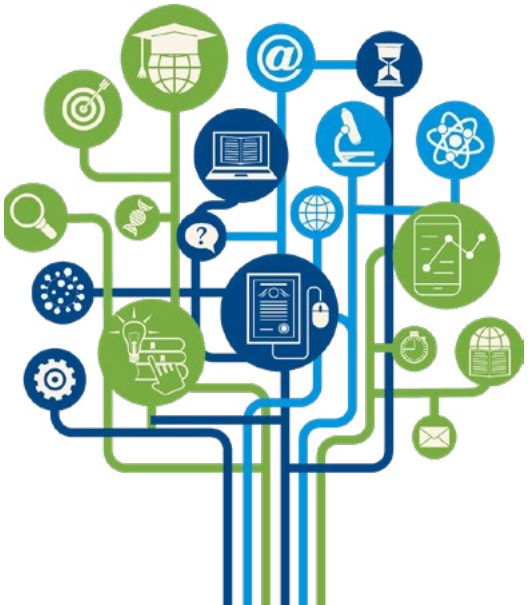


Infection Prevention and Control

National Infection Prevention and Control Manual (NIPCM)

The National Infection Prevention and Control Manual (NIPCM) first published in 2012 provides national evidence-based guidance for Infection Prevention and Control (IPC) which is considered best practice for all health and care settings in Scotland. The NIPCM has continued to expand year on year. The development of new content is informed by horizon scanning, national learning via incidents and outbreaks and stakeholder needs, leading the way as a single national resource for IPC guidance. The NIPCM aims to provide the most up to date evidence-based guidance, enhanced where necessary by expert opinion, to allow healthcare workers to make informed risk assessments in the application of IPC measures.

Following the removal of dedicated COVID-19 pandemic content as part of a phased process in 2022, 2023 has refocused efforts on ensuring the NIPCM is maintained and populated with new guidance which is evidence based, user friendly and meets accessibility standards. A series of annual activities are undertaken to support NIPCM functionality and the delivery of IPC guidance and associated tools. An annual review of the development methodology was also undertaken and 2023 saw the commencement of a new pilot methodology which aimed to increase the robustness and transparency of NIPCM content development where a considered judgement form (CJF) is applied to each pilot literature review. In addition to recommendations and good practice points, the CJF records the reliability of the evidence considered. Expert opinion is used to consider the benefits versus harms as well as any feasibility issues. Doing so supports the practical clinical operationalisation to inform the recommendations and good practice points and consistency of the conclusions within the evidence. The pilot methodology has been published and can be found **here**. The pilot will continue in 2024 when the final development process will be ratified via internal governance groups.



Following completion of three literature reviews in 2023, the NIPCM was strengthened to reflect the following points:

Hand hygiene: skin care. This update provided some additional background information on what skin integrity is and how to maintain it, and the signs and symptoms of contact dermatitis.

Surgical hand antisepsis. The review update clarified that where surgical hand rubbing is the preferred method for surgical hand antisepsis, hand hygiene should be performed using a non-antimicrobial liquid soap and water prior to entering the theatre or care area. The rationale for this is to physically remove contamination (which hand rub products are unable to do).

Indication and techniques for hand hygiene. The need to remove hand and wrist jewellery before performing hand hygiene was expanded to include embedded jewellery (dermal piercings).

It was recognised that after the COVID-19 pandemic, it was necessary to address the use of Personal Protective Equipment (PPE) in the delivery of patient care and make clear the risk assessment required in selecting individual items of PPE. **Appendix 15** underwent a significant change to stipulate the PPE required based on exposure to blood and body fluids and suspected infection. The Appendix 15 tables are intended to be more user friendly than previous versions.

Notable was the commencement of the transmission-based precautions (TBPs) definitions literature review update in 2023 with significant progress made in the analysis of the vast evidence base to inform re-classification of transmission routes. The need for this review was made apparent during the COVID-19 pandemic and ARHAI Scotland are leading the way in this work by challenging the historical droplet/aerosol dichotomy and reviewing the key factors which impact transmission in health and care settings. A dedicated working group will be convened in 2024 to finalise new recommendations and oversee implementable changes to Chapter 2 of the NIPCM. Alongside this work, a review of the literature pertaining to surgical face masks and respiratory protective equipment was commenced to ensure full alignment with published changes to TBPs.

ARHAI Scotland completed a **rapid review of the literature** to consider post cataract surgery endophthalmitis in July 2023 which was used to inform the development of a **cataract IPC pathway** in support of the Centre of Sustainable Delivery (CfSD). This group brought together key IPC and specialist ophthalmology stakeholders from across Scotland to develop a nationally approved evidence-based set of principles aimed at reducing variation and mitigating against any perceived IPC risks associated with increasing the throughput of cataract surgeries, whilst providing a means of monitoring through national data sets. This work was published in the Journal of Hospital Infection and will be included in the NIPCM in 2024.

Care Home IPC Manual

Within the NIPCM website, ARHAI Scotland have a dedicated section for all Care Home IPC guidance, supporting materials and resources. As part of the Care Home IPC section, staff can navigate through the Care Home Infection Prevention and Control Manual (CH IPCM) which is based on evidence and promotes best practice. The content of the CH IPCM is fully aligned to the evidence based NIPCM.

The content of the CH IPCM continues to evolve since publication of Chapter 1: Standard Infection Control Precautions (SICPs) and Chapter 2: Transmission Based Precautions (TBPs) in 2021. Within the CH IPCM, there is a National Cleaning Specification (NCS) section which provides a guide for care home staff to plan their cleaning services and to record local cleaning activities. The NCS supports care home staff to plan, train and monitor standards of cleaning services provided throughout their care home.

In addition to the core chapters, the Care Home section in the NIPCM website also contains multiple appendices and supporting materials which are constantly updated as the evidence base evolves. Throughout 2023, collaboration with Care Home stakeholders ensured that ARHAI Scotland Care Home IPC guidance and materials were relevant and context specific.

ARHAI Scotland continues to support the interim Scottish Health Protection Network Guidance Group from a community IPC perspective and in 2023 developed an IPC Chapter for inclusion in the 'Health protection in children and young people settings, including education' Guidance. Assistance was provided for an IPC review of the National Childsmile Executive Standards for Nursery and School Toothbrushing Programme content and to the Care Inspectorate publication entitled 'Space to Grow'. During 2023, the IPC Respiratory and Gastrointestinal support resources for Care Homes was also updated and refreshed.

Reducing risk in the healthcare built environment

During 2023, ARHAI Scotland continued to focus on reducing risk in the healthcare-built environment by providing access to infection prevention and control (IPC) guidance and supportive tools. ARHAI Scotland also supported NHS Boards through the design, construction and commissioning of new health care facilities.

ARHAI Scotland assesses potential IPC risks at all stages of health care construction and refurbishment as part of NHSScotland Assure's key stage assurance review (KSAR) process, and also supports local NHS boards and IPC teams with subject matter expertise during environmental incidents and outbreaks.

Expert IPC advice continued to be provided through developing guidance and educational materials to assist local IPC Teams in reducing risk in all healthcare buildings.

The water services literature review utilising the new National Infection Prevention and Control Manual (NIPCM) methodology gathered speed and involved extensive consultation with stakeholders. It is anticipated that the water services literature review and associated manual content/ recommendations will continue to progress with publication expected late summer 2024.The dental unit water lines literature review was initiated with publication also expected in 2024. A further notable review was the commencement of the ventilation literature review which will be for inclusion within Chapter 4 of the NIPCM. The question set for this literature review is being developed and is expected to significantly progress throughout 2024.

The focus for the year ahead is the continued development of Chapter 4 of the NIPCM content; tools specifically designed to reduce risk; and evidence to support the safe management of risks from the complex components within the built environment. This will include the review of outbreak and incident assessment to improve reporting of incidents relating to the built environment.

IPC clinical assurance for major infrastructure projects

The multidisciplinary team within the NHSScotland Assure Assurance service continues to review the design, construction and maintenance of major health care infrastructure developments across NHSScotland at key stages during the project lifecycle. ARHAI Scotland provide IPC support as subject matter experts through the KSAR processes, ensuring an overarching focus on IPC and infection risk during all stages of the building lifecycle.

Support has been given to board IPC teams which resulted in completion of a further 11 KSARs in 2023, enabling progression of design and construction of the healthcare built environment estate across NHSScotland.

During 2023, as part of the Assurance Service, ARHAI Scotland supported two NHS boards' project teams through the commissioning of National Treatment Centres which opened this year: NHS Fife (Victoria Hospital, Kirkcaldy) and NHS Highland (Raigmore Hospital, Inverness).

In addition to Assurance reviews, ARHAI Scotland have continued to support IPC project teams across respective boards regarding construction projects out with KSAR review specialist support or general built environment board enquiries.

During 2023, IPC support was formally included within the NHSScotland Design Assessment Process (NDAP). The NDAP process in addition to the review process is included within the Assurance process. IPC support is provided to NHS boards during the design of their facility with the aim of ensuring IPC requirements are met in advance of the Assurance Review being undertaken.

ARHAI Scotland are developing a suite of resources (Notes for Boards) to assist board IPC teams through health care construction projects and the KSAR process for delivery over 2024. The first resource is KSAR: Notes for Board Infection Prevention and Control Teams and advises IPC teams of what the KSAR process is and what can be expected throughout each of the stage reviews. Work has also commenced on Notes for Boards: Summary of guidance for the design of Neonatal units, level 2 and 3 care units, and Bone marrow transplant units and haemato-oncology units. These will be published in 2024. A Notes for Boards: Air sampling in operating theatres has also commenced and is expected to publish in 2024.

Antimicrobial resistance

Antimicrobial resistance (AMR) happens when microbes, such as bacteria, fungi, viruses and parasites change and no longer respond to medicines designed to kill them. This change is an adaption of the microbe's genetics and is driven by the exposure of the microbe to antimicrobials in humans, animals and the environment. The result of this AMR is that certain infections become increasingly difficult or impossible to treat. This is a global concern.

Causes of antimicrobial resistance

Antimicrobial resistance happens when bacteria change and become resistant to the antimicrobials used to treat the infections they cause.

Over-prescribing of antimicrobials

Patients not finishing their treatment

Over-use of antimicrobials in livestock and fish farming

Poor infection control in hospitals and clinics

Lack of hygiene and poor sanitation

Lack of new antimicrobials being developed

As **The King's Fund essay, 'What if antibiotics stopped working?'** highlighted, antimicrobials (including antibiotics) add 20 years on average to life expectancy across the world through their ability to prevent and treat infection. This ability is central to managing infectious disease, making surgery and childbirth safer, protecting people undergoing transplants and cancer treatments, and supporting animal health and food production.

AMR is a natural phenomenon therefore it cannot be fully prevented but human activity is accelerating the emergence and spread. Accordingly, the focus, as set out in the **UK Government 20-year vision for antimicrobial resistance**, is to effectively contain, control and mitigate AMR by 2040. This long-term vision is supported by five-year AMR national action plans (NAP). This ARHAI annual report is published at an important point with the transition from the 2019 to 2024 NAP **'Tackling antimicrobial resistance'** to the 2024–2029 NAP to **'Confronting antimicrobial resistance'**.

In 2023, ARHAI Scotland provided intelligence on the trends in antimicrobial use and resistance. ARHAI Scotland's work on monitoring antimicrobial use and resistance is aligned with the 'One Health' approach to tackling AMR in the UK AMR NAP. This 'One Health' approach works across sectors and recognises that many of the same bacteria infect humans and animals and may be found in the environment as they share the same ecosystem and calls for action across and between sectors.

Data and intelligence on the trends in antimicrobial use and resistance in humans and animals for 2023 will be published in the annual Scottish One Health Antimicrobial Use and Antimicrobial Resistance (SONAAR) report in November 2024. The publication on the **ARHAI Scotland website** will coincide with World Antibiotic Awareness Day and European Antibiotic Awareness Week.

ARHAI Scotland's 2023 achievements

- **Undertook near real time monitoring and reporting of use of antibiotics commonly used for respiratory infections to support antimicrobial stewardship activities coordinated by the Scottish Antimicrobial Prescribing Group aimed at optimising antibiotic use in the community.**

- **Using our AMR early warning system, undertook monitoring for instances of organisms displaying unexpected antimicrobial resistance (see NIPCM Appendix 13 Table 6). This work is intended to enable a timely scientific and public health response to potential emerging AMR issues, informs infection control practices and appropriate therapy, and is critical to contain the development and spread of AMR.**

- **Supported our stakeholders to access quarterly data on antimicrobial use and resistance through ARHAI Indicators dashboards in Discovery to inform clinical practice across NHSScotland.**

AMR is a serious and complex problem but there are actions which, if taken, will make a difference. In 2024, ARHAI Scotland will continue to play our part and will support the delivery of the UK AMR NAP implementation plan and measurement of impact within Scotland, building on our role in monitoring and reporting trends in antimicrobial use and resistance in human and animal health.

Key activities for ARHAI Scotland in support of the UK AMR NAP will include:

Reducing the burden of infections, AMR and exposure to antimicrobials through leadership, expert advice and guidance on infection prevention and control.

Developing and utilising data and intelligence to inform actions – this aims to enable decisions to be based on robust surveillance data and intelligence.

Identifying unwarranted variation and health inequalities – this aims to improve the information available to identify where the burden of antibiotic use and AMR is greatest. This will help to target future interventions where they will have the greatest impact.

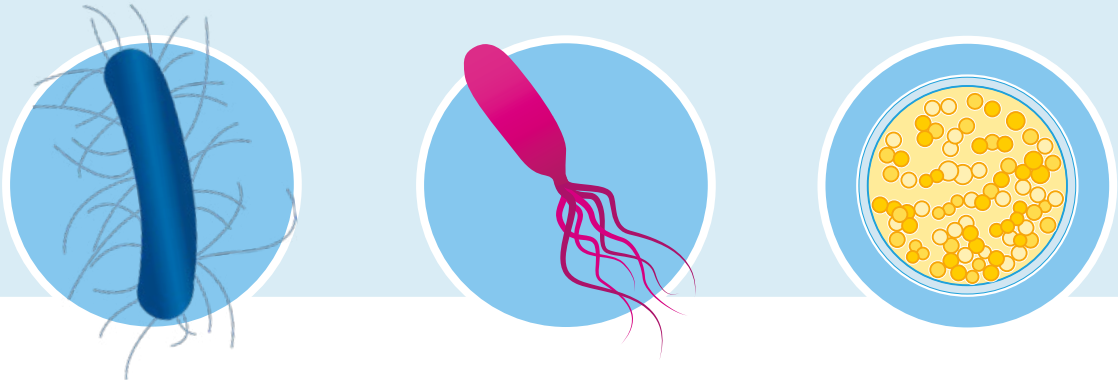
Surveillance and monitoring

Surveillance of healthcare associated infection in Scotland

ARHAI Scotland coordinate the national surveillance programme of healthcare associated infection (HCAI) monitoring the burden of key HCAI, supporting benchmarking and providing intelligence to inform the development of interventions.

In 2023, the surveillance of three key infection types continued:

- ***Clostridioides difficile*** **infection (CDI)**
- ***Escherichia coli*** **bacteraemia (ECB)**
- ***Staphylococcus aureus*** **bacteraemia (SAB)**



National surveillance of surgical site infection (SSI) remained paused during 2023 though some boards continued with local surveillance.

Trends in key HCAI rates **continued to be published quarterly** in 2023 and provided in **Discovery**, supporting local NHS boards with data for benchmarking and to inform quality improvement and reduction strategies.

Surveillance priorities for 2024

- Complete a review of the national HCAI surveillance programme ensuring a focus on reducing risk and harm.
- Complete review of paused SSI surveillance programme including a focus on smarter solutions for surveillance to reduce the burden of data collection.
- Support the implementation of the redeveloped surveillance system for healthcare associated infection in intensive care units (ICU).
- Continue to support the development of a competent and confident infection prevention and control (IPC) workforce by delivery of epidemiology and surveillance training in conjunction with NHS Education Scotland.

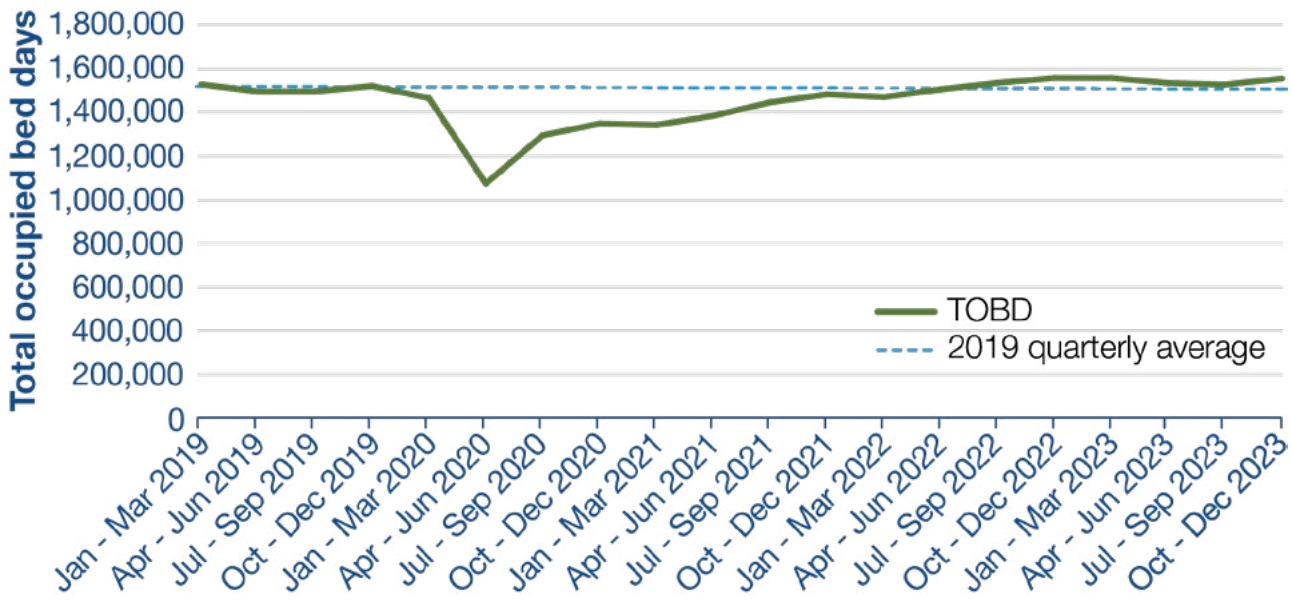
Hospital activity and pressures

Healthcare delivery, activity and the inpatient population is evolving and healthcare systems remain under pressure following the pandemic. Changes within the system can affect the risk of Healthcare Associated Infection (HCAI) in the hospital population and present a challenge to the interpretation of epidemiological data. Interpretation can be supported by consideration of other contextual measures describing healthcare. HCAI outcome data should be interpreted with caution and in the context of the wider healthcare system.

Hospital activity

6,217,510 occupied bed days in **2023**, compared to **6,112,174** in **2022**.

Total occupied bed days, by quarter*

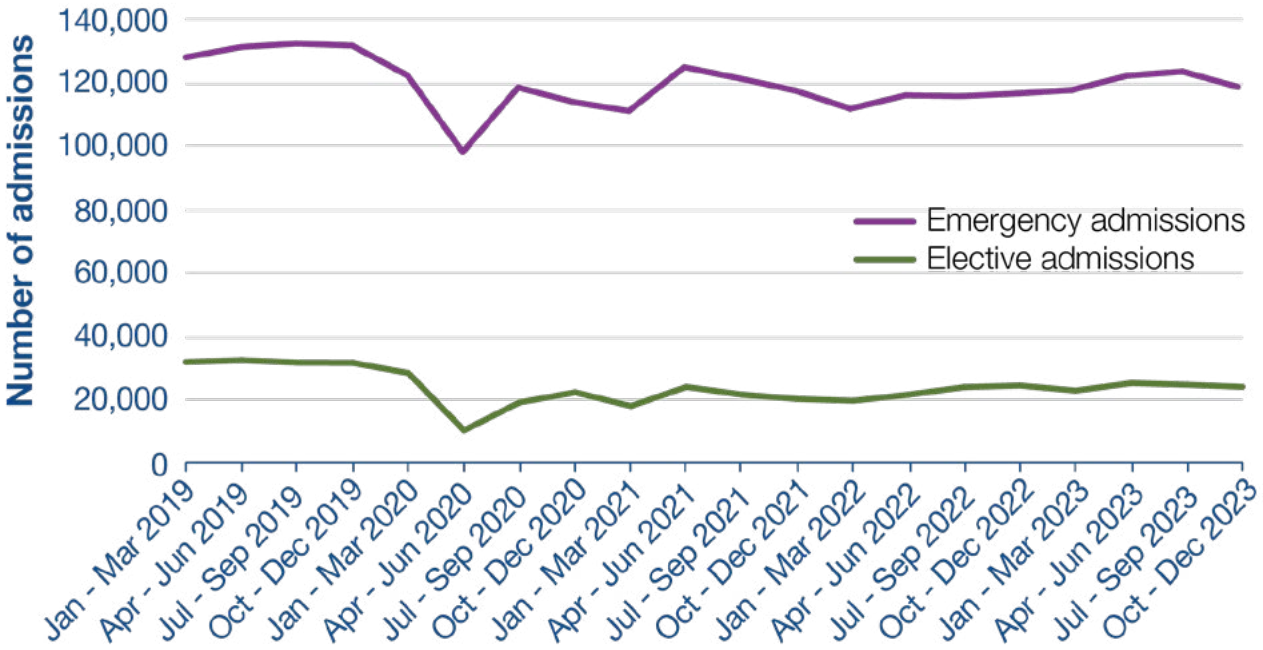


*The dashed line represents the quarterly average for the calendar year 2019 of 1,520,807 Total Occupied Bed Days (TOBD).

98,144 elective inpatient admissions in **2023** compared to **90,995** in **2022**.

482,997 emergency inpatient admissions in **2023** compared to **461,069** in **2022**.

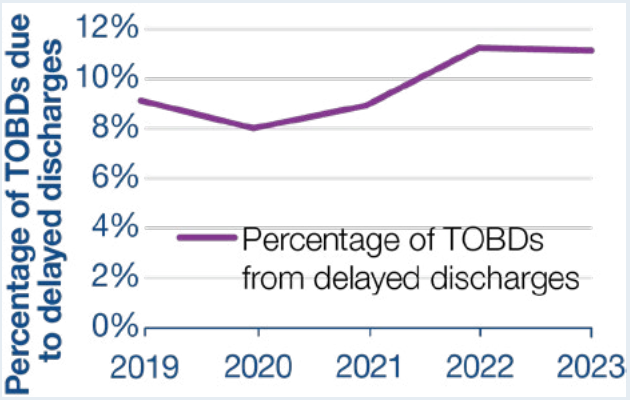
Number of elective and emergency admissions, by quarter



Hospital pressures

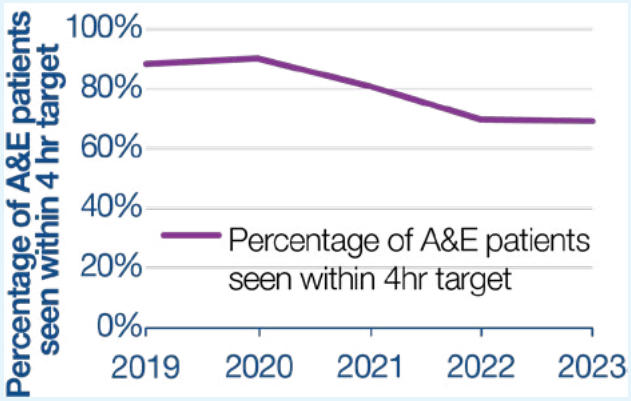
Delayed discharges

The percentage of total occupied bed days due to **delayed discharges** **decreased** by **0.9%** in **2023 (11.1%)** compared to **2022 (11.2%)**.



Accident and Emergency (A&E) attendances

69.6% of **Accident and Emergency (A&E) attendances** were seen within the **4-hour target** in **2023**, a **decrease** from **70.2%** in **2022**.



Clostridioides difficile infection

Epidemiological data

In **2023**, there were **1,209** cases of ***Clostridioides difficile* infection (CDI)** reported in **patients aged 15 years and older** in **Scotland**, **compared** to **1,053** cases in **2022**.

The **annual incidence rate** was **22.2** per 100,000 population.

There has been a **14.8% increase** in the **rate** between **2022** and **2023**.

The **rate in 2023** was **14.5% higher compared to 2019**.

No linear trend over the **past five years** was observed.

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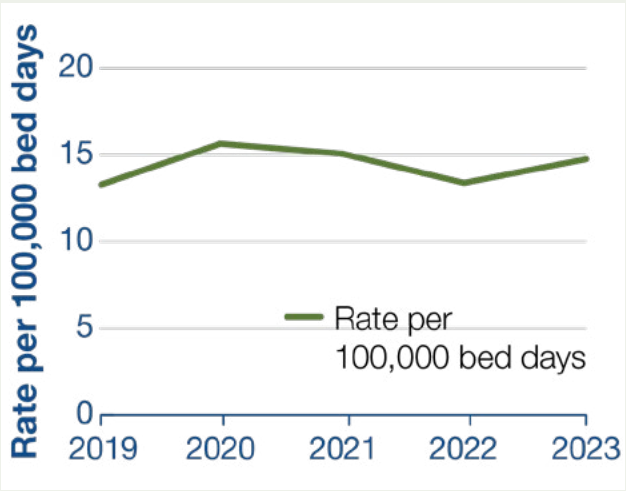
Healthcare associated CDI

There were **917 cases** in **patients aged 15 years and older** in **2023**.

The **annual incidence rate** was **14.7** per 100,000 bed days.

There has been a **10.2% increase** in the **rate** between **2022** and **2023**.



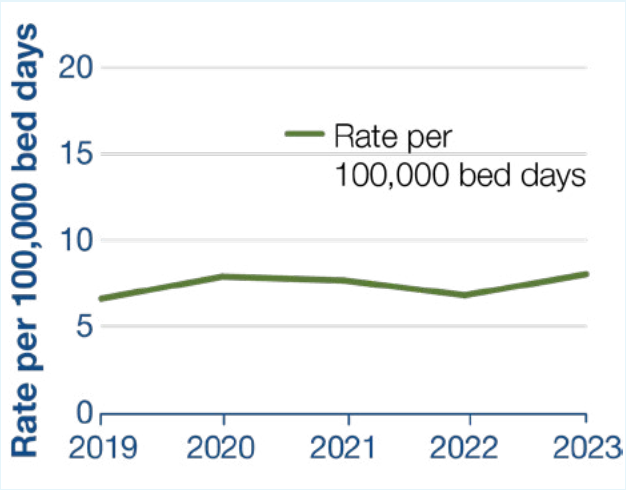
The **five year trend** has remained **stable**.

Hospital acquired CDI

Of the **917** healthcare associated CDI cases, **503** were **hospital acquired infections**.

The **annual incidence rate** was **8.1** per 100,000 bed days.

There has been a **17.5% increase** in the **rate** between **2022** and **2023**.



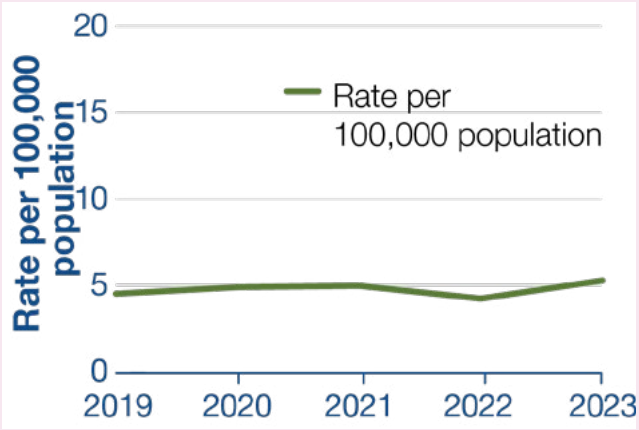
The **five year trend** has remained **stable**.

Community associated CDI

There were **292 cases** in **patients aged 15 years and older** in **2023**.

The **annual incidence rate** was **5.4** per 100,000 population.

There has been a **24.3% increase** in the **rate** between **2022** and **2023**.



The **five year trend** has remained **stable**.

Annual funnel plot analysis

NHS Highland and **NHS Lanarkshire** had **higher annual rates** of **healthcare associated CDI compared** with the **Scottish average rate**.

No NHS boards had **higher annual rates** of **community associated CDI compared** with the **Scottish average rate**.

Note: NHS board rates are **not adjusted** for **differences in the patient population**.

NHS boards are required to submit improvement plans in response to **higher than average quarterly rates** of **healthcare or community associated CDI**, as identified through the **exception reporting process**.

Three improvement plans were developed by **NHS boards** during **2023** in response to **higher than average quarterly rates** of **healthcare associated CDI**.

For further information please see the **quarterly epidemiological reports**.

All cause case fatality

In **2023**, the **30 day all cause case fatality rate** for **CDI** in **patients aged 15 years and older** was **10.8%**.

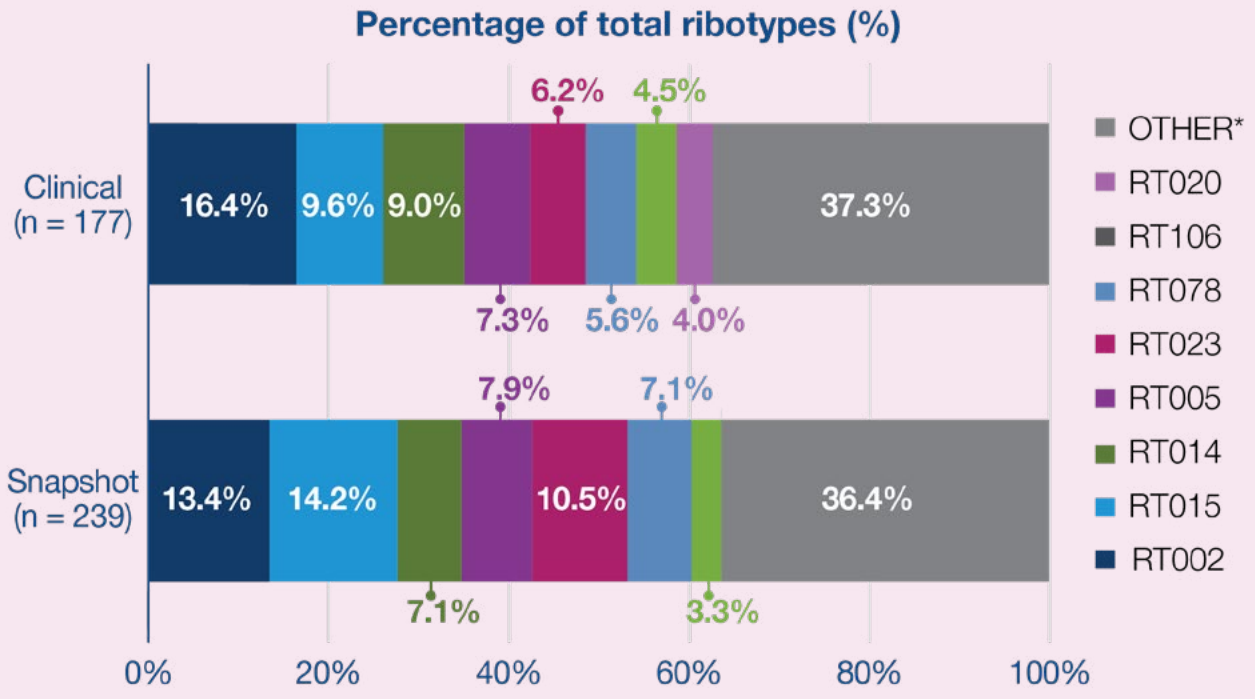
The **CDI case fatality five year trend** has remained **stable**.

Note: rates are **not adjusted** for **differences** in the **patient population over time**.

Molecular epidemiological data

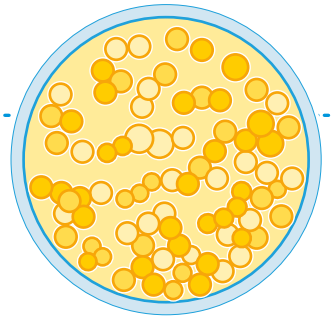
The **three most prevalent ribotypes** reported by **clinical surveillance** in **2023** were **RT002 (16.4%)**, **RT015 (9.6%)** and **RT014 (9.0%)**.

The **three most prevalent ribotypes** (RT) reported by the **snapshot surveillance scheme** in **2023** were **RT015 (14.2%)**, **RT002 (13.4%)** and **RT023 (10.5%)**.



*OTHER contains ribotypes, each with a prevalence of <3%.

Staphylococcus aureus bacteraemia



Epidemiological data

In **2023**, there were **1,712** cases of ***Staphylococcus aureus* bacteraemia (SAB)** reported in **Scotland**, compared to **1,590** cases in **2022**.

The **annual incidence rate** was **31.4** per 100,000 population.

There has been **7.7% increase** in the **rate** between **2022** and **2023**.

The **five year trend** has **increased** with a **3.4% average increase** each year.

96.7% (n = 1,656) of **all SAB cases** were **meticillin-sensitive *Staphylococcus aureus* (MSSA)**.

3.3% (n = 56) of **all SAB cases** were **meticillin-resistant *Staphylococcus aureus* (MRSA)**.

The **rate** of **MSSA bacteraemia** in **2023** was **30.4** per 100,000 population.

There has been a **7.5% increase** in the **rate** between **2022** and **2023**.

The **five year trend** has **increased** with a **3.3% average increase** each year.

The **rate** of **MRSA bacteraemia** in **2023** was **1.0** per 100,000 population.

The **rate** has **remained stable** between **2022** and **2023**.

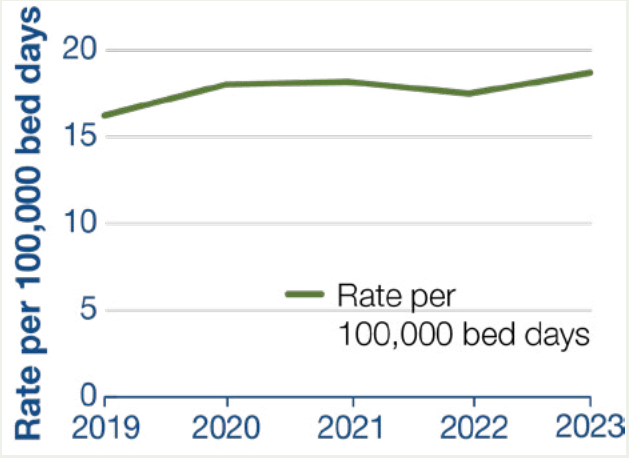
The **five year trend** has remained **stable**.

Healthcare associated SAB

There were **1,162** cases in **2023**.

The **annual incidence rate** was **18.7** per 100,000 bed days.

The **rate** has **remained stable** between **2022** and **2023**.



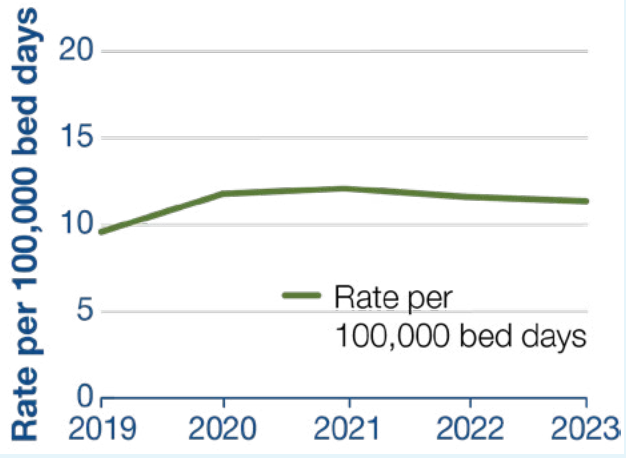
The **five year trend** has **increased** with a **2.6% average increase** each year.

Hospital acquired SAB

Of the **1,162** healthcare associated SAB cases in **2023**, **706** were **hospital acquired infections**.

The **annual incidence rate** was **11.4** per 100,000 bed days.

The **rate** has **remained stable** between **2022** and **2023**.



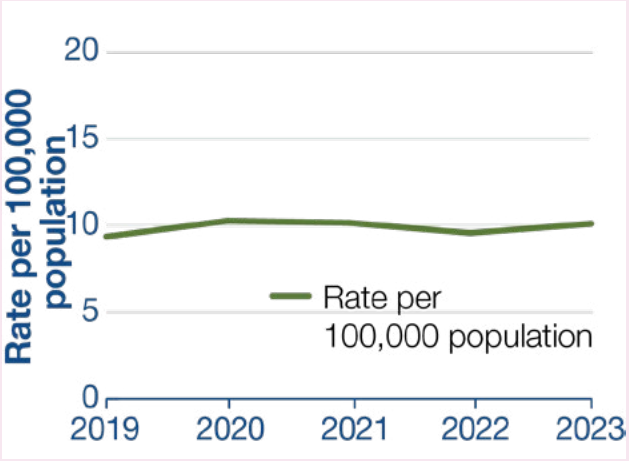
The **rate in 2023** was **18.5% higher compared to 2019**. **No linear trend** over the **past five years** was observed.

Community associated SAB

There were **550** cases in **2023**.

The **annual incidence rate** was **10.1** per 100,000 population.

The **rate** has **remained stable** between **2022** and **2023**.



The **five year trend** has remained **stable**.

Annual funnel plot analysis

NHS Tayside had a **higher annual rate** of **healthcare associated SAB**, compared with the **Scottish average rate**.

NHS Ayrshire & Arran and **NHS Shetland** had a **higher annual rate** of **community associated SAB compared** with the **Scottish average rate**.

Note: NHS board rates are **not adjusted** for **differences in the patient population**.

NHS boards are required to submit improvement plans in response to **higher than average quarterly rates** of **healthcare or community associated SAB**, as identified through the **exception reporting process**.

Two improvement plans were developed by **NHS boards** during **2023** in response to **higher than average quarterly rates** of **healthcare associated SAB**.

One improvement plan was developed by an **NHS board** during **2023** in response to **higher than average quarterly rates** of **community associated SAB**.

For further information please see the **quarterly epidemiological reports**.

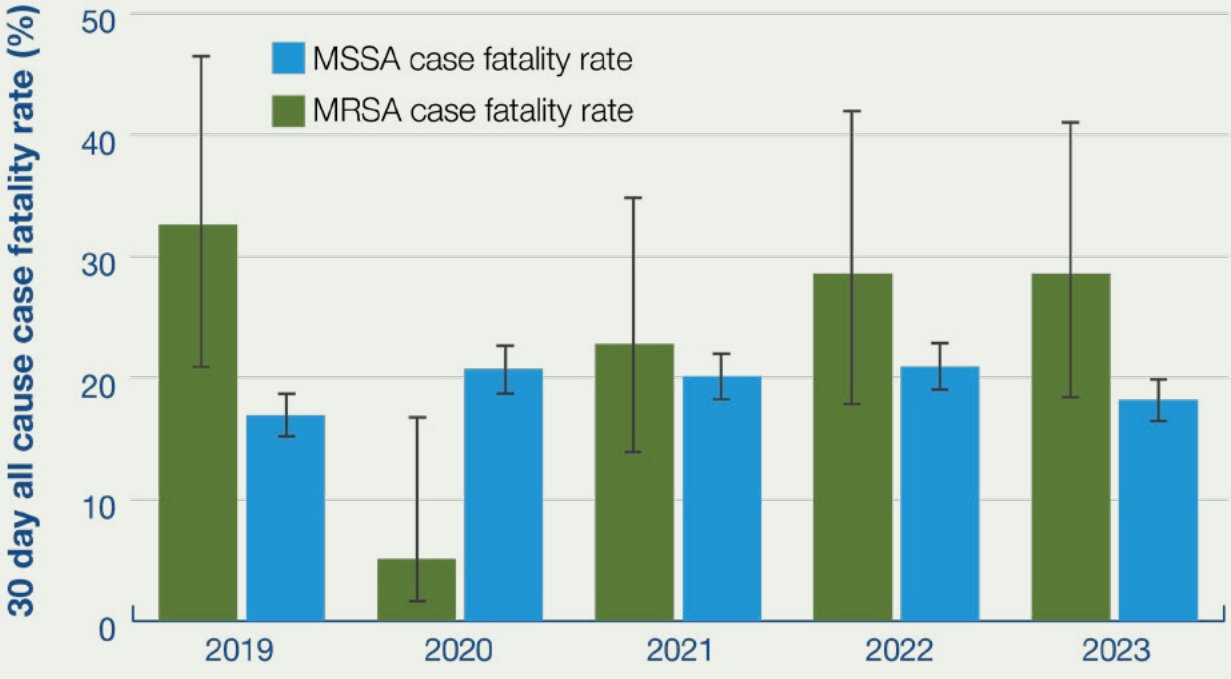
All cause case fatality

In **2023**, the **30 day all cause case fatality rates** for **SAB** were

28.6% for MRSA | **18.2% for MSSA**

The **MRSA** and **MSSA** **case fatality five year trends** have remained **stable**.

Note: rates are **not adjusted** for **differences** in the **patient population over time**.



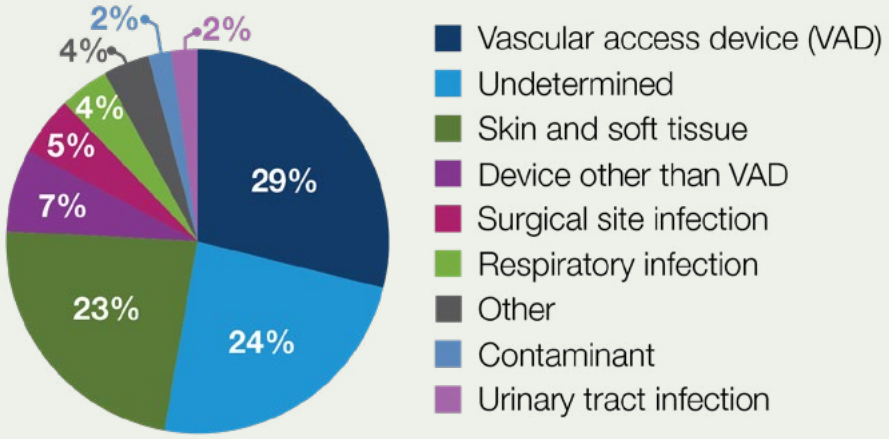
In **2023**, **entry points** of **healthcare associated SAB** were known for **75.7%** of cases.

The most common entry points (as determined by the clinical team) include:

29.4% Vascular access device (VAD)

22.9% Skin and soft tissue

Healthcare associated SAB entry points



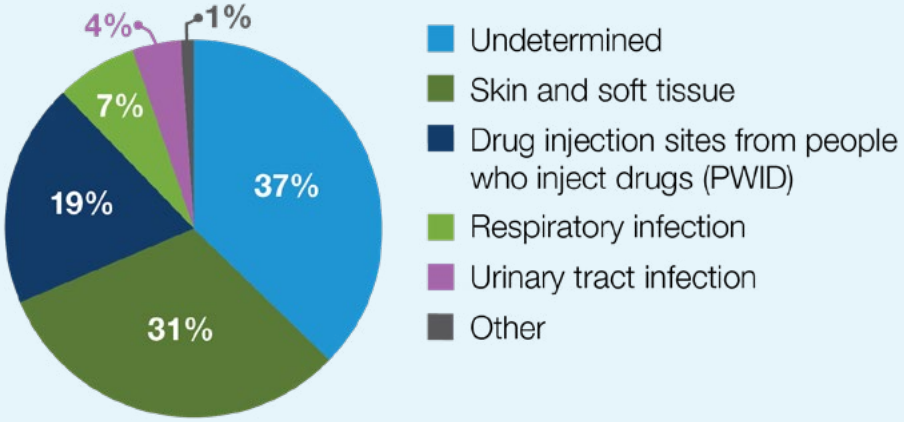
In **2023**, **entry points** of **community associated SAB** were known for **62.9%** of cases.

The most common entry points (as determined by the clinical team) include:

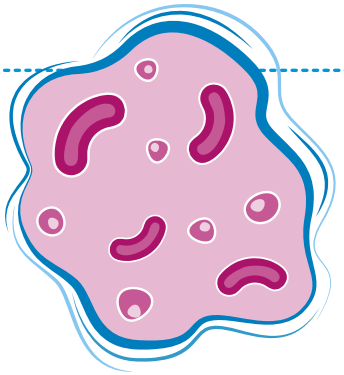
31.5% Skin and soft tissue

19.5% Drug injection sites from people who inject drugs (PWID)

Community associated SAB entry points



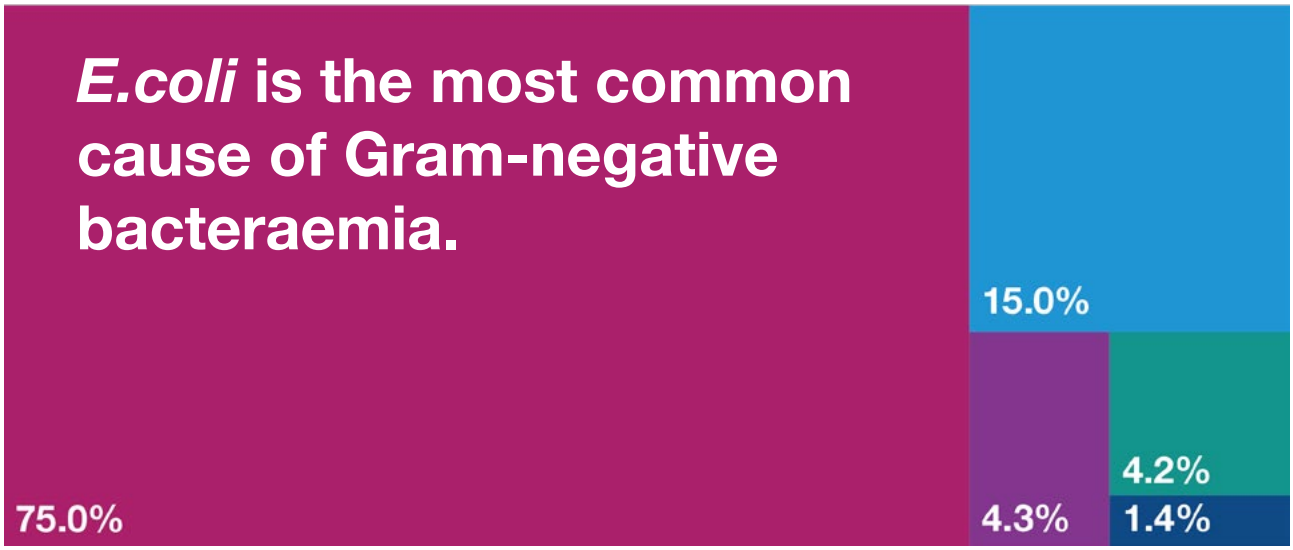
Gram-negative bacteraemia



Epidemiological data

In **2023**, there were **5,744 Gram-negative bacteraemia** in **Scotland** caused by **5 key Gram-negative pathogens**.

E.coli is the most common cause of Gram-negative bacteraemia.



- *Escherichia coli* (n=4,309)
- *Pseudomonas aeruginosa* (n=248)
- *Acinetobacter* species (n=82)
- *Klebsiella pneumoniae* (n=863)
- *Klebsiella oxytoca* (n=242)

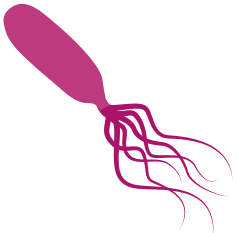
In **2023**, there were **4,309** cases of ***Escherichia coli* bacteraemia (ECB)** reported in **Scotland**, compared to **4,218** cases in **2022**.

The **annual incidence rate** was **79.1** per 100,000 population.

The **rate** has **remained stable** between **2022** and **2023**.

The **rate in 2023** was **9.3% lower compared to 2019**.

No linear trend over the **past five years** was observed.



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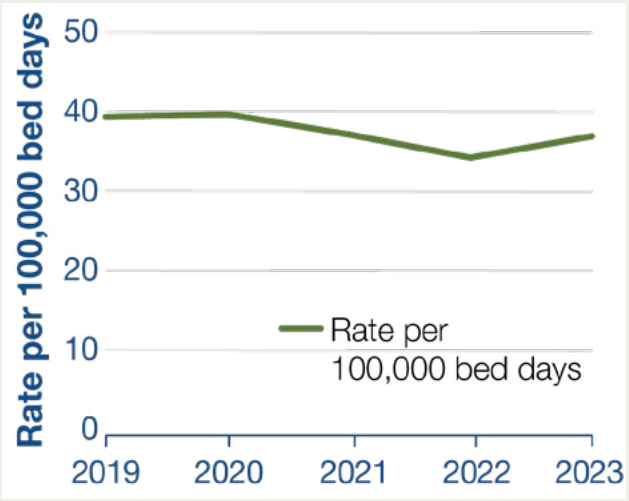
Healthcare associated ECB

There were **2,293** cases in **2023**.

The **annual incidence rate** was **36.9** per 100,000 bed days.

There has been a **7.9% increase** in the **rate** between **2022** and **2023**.



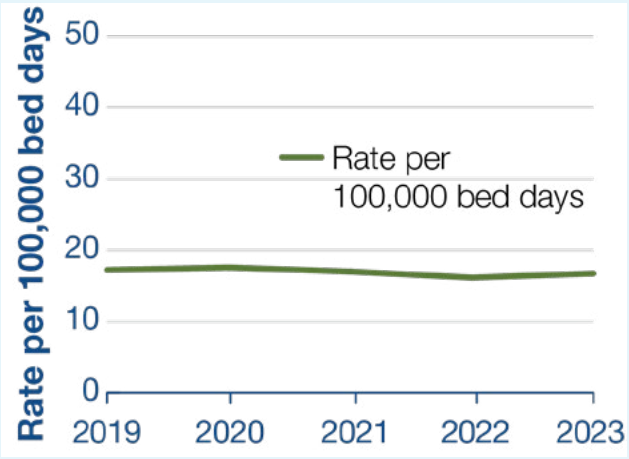
The **rate in 2023** was **6.2% lower compared to 2019**.
No linear trend over the **past five years** was observed.

Hospital acquired ECB

Of the **2,293 healthcare associated ECB cases**, **1,041** were **hospital acquired infections**.

The **annual incidence rate** was **16.7** per 100,000 bed days.

The **rate** has **remained stable** between **2022** and **2023**.



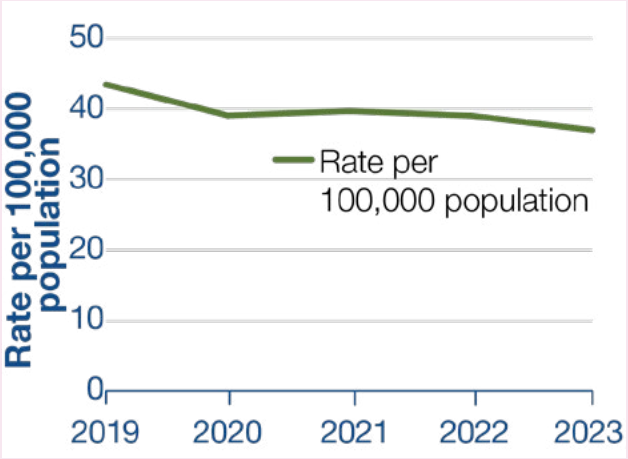
The **five year trend** has remained **stable**.

Community associated ECB

There were **2,016** cases in **2023**.

The **annual incidence rate** was **37.0** per 100,000 population.

The **rate** has **remained stable** between **2022** and **2023**.



The **five year trend** has **decreased** with a **3.2% average decrease** each year.

Annual funnel plot analysis

NHS Forth Valley and **NHS Tayside** had **higher annual rates** of **healthcare associated ECB compared** with the **Scottish average rate**.

NHS Ayrshire & Arran, **NHS Dumfries & Galloway** and **NHS Lanarkshire** all had **higher annual rates** of **community associated ECB compared** with the **Scottish average rate**.

Note: NHS board rates are **not adjusted** for **differences in the patient population**.

NHS boards are required to submit improvement plans in response to **higher than average quarterly rates** of **healthcare or community associated ECB**, as identified through the **exception reporting process**.

Three improvement plans were developed by **NHS boards** during **2023** in response to **higher than average quarterly rates** of **healthcare associated ECB**.

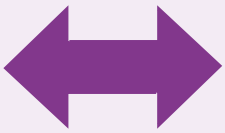
Six improvement plans were developed by **NHS boards** during **2023** in response to **higher than average quarterly rates** of **community associated ECB**.

For further information please see the **quarterly epidemiological reports**.

All cause case fatality

In **2023**, the **30 day all cause case fatality rate** for **ECB** was **12.8%**.

The **ECB case fatality five year trend** has remained **stable**.



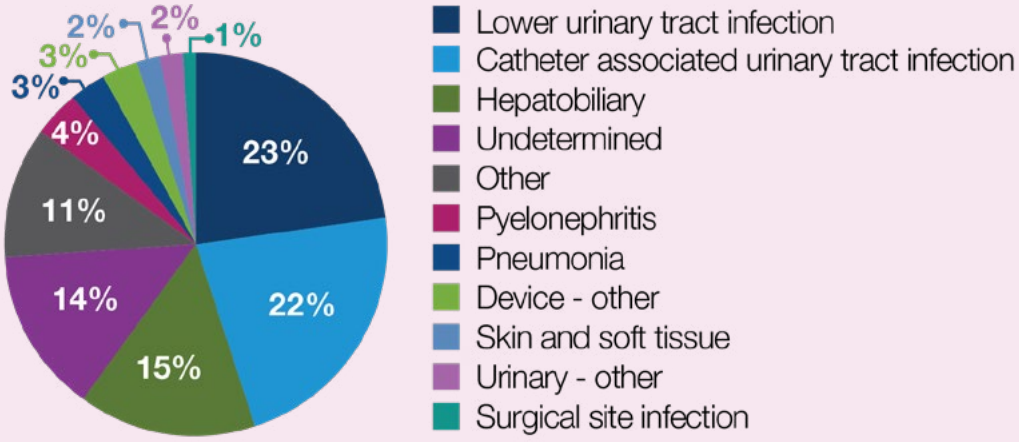
Note: rates are **not adjusted** for **differences** in the **patient population over time**.

In **2023**, the **primary infections** for **healthcare associated ECB** were known for **86.0%** of cases.

The most common primary infections (as determined by the clinical team) include:

23.2% Lower urinary tract infection
22.0% Catheter associated urinary tract infection
14.7% Hepatobiliary infection

Primary infections of healthcare associated ECB in 2023

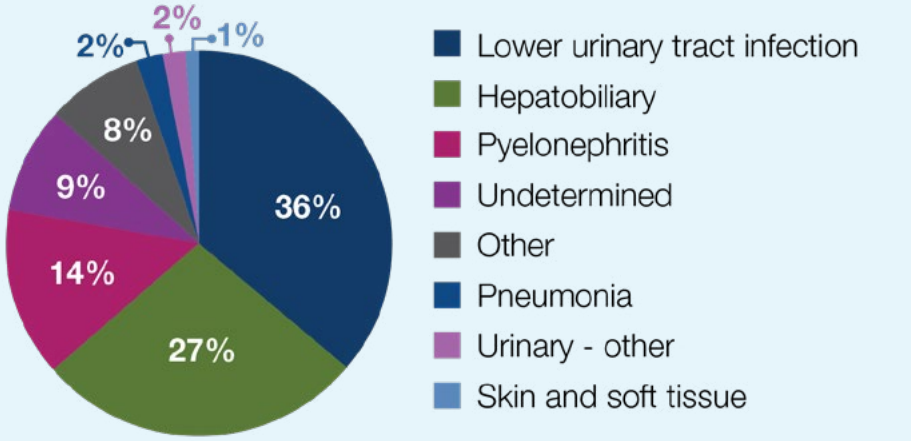


In **2023**, the **primary infections** for **community associated ECB** were known for **90.7%** of cases.

The most common primary infections (as determined by the clinical team) include:

35.7% Lower urinary tract infection
26.7% Hepatobiliary infection
14.5% Pyelonephritis

Primary infections of community associated ECB in 2023



Carbapenemase-producing Enterobacterales

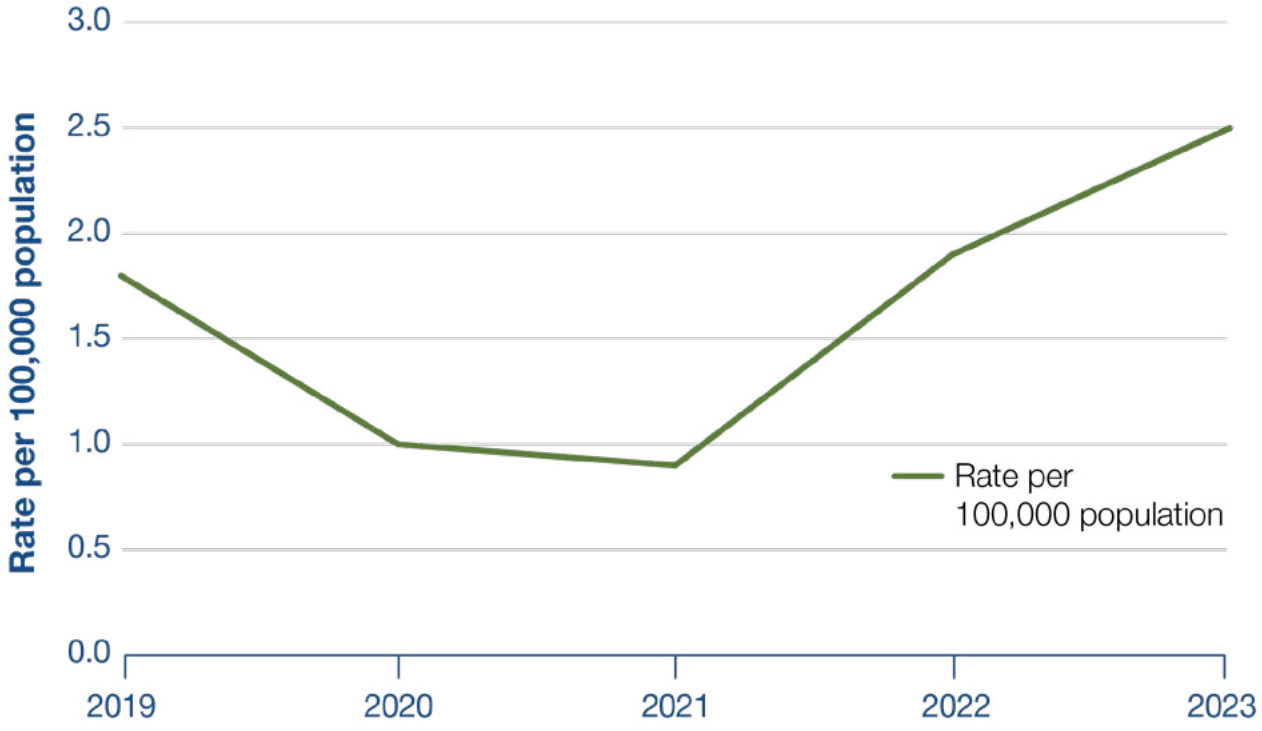
In **2023**, there were **135 cases** of **carbapenemase-producing Enterobacterales (CPE) reported** in **Scotland**, **compared** to **102** in **2022**.

The **annual incidence rate** of **CPE** was **2.5** per 100,000 population.

There has been a **32.4% increase** in the **rate** between **2022** and **2023**.

The **rate in 2023** was **39.6% higher compared to 2019.**

Rate of CPE per 100,000 population in Scotland, 2019 to 2023



Further detail on antimicrobial use and antimicrobial resistance, including Carbapenemase-producing organisms and CPE enzymes, will be published in the annual SONAAR report.

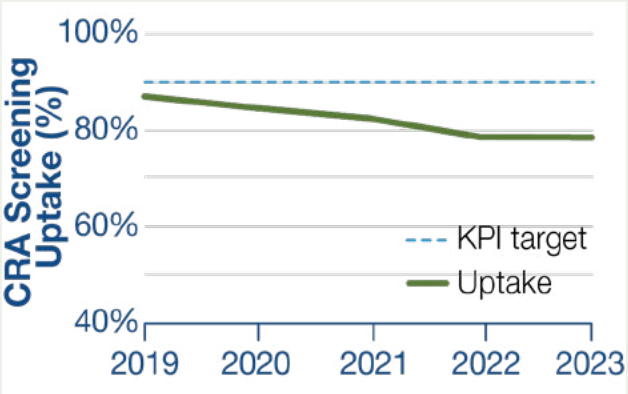
Multi-drug resistant organism admission screening

MRSA CRA screening

In **2023**, **78.5%** of patients audited underwent a **clinical risk assessment (CRA)** for **meticillin-resistant *Staphylococcus aureus* (MRSA)**.

This **remains below** the **90%** Key Performance Indicator and uptake has **remained stable** between **2022** and **2023**.

The **five year trend** in reported uptake has **decreased** with a **2.8% average decrease** each year.

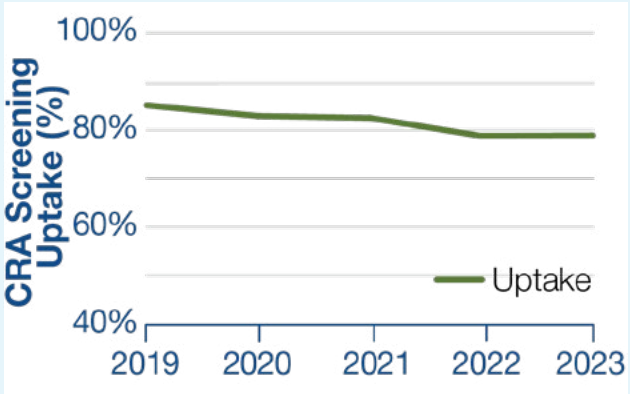


CPE CRA screening

In **2023**, **78.3%** of patients audited underwent a **clinical risk assessment (CRA)** for **carbapenemase-producing Enterobacterales (CPE)**.

Uptake has **remained stable** between **2022** and **2023**.

The **five year trend** in reported uptake has **decreased** with a **2.0% average decrease** each year.



These MRSA and CPE CRA uptake data continue to be monitored by ARHAI Scotland and feedback is provided to NHS boards on a quarterly basis.

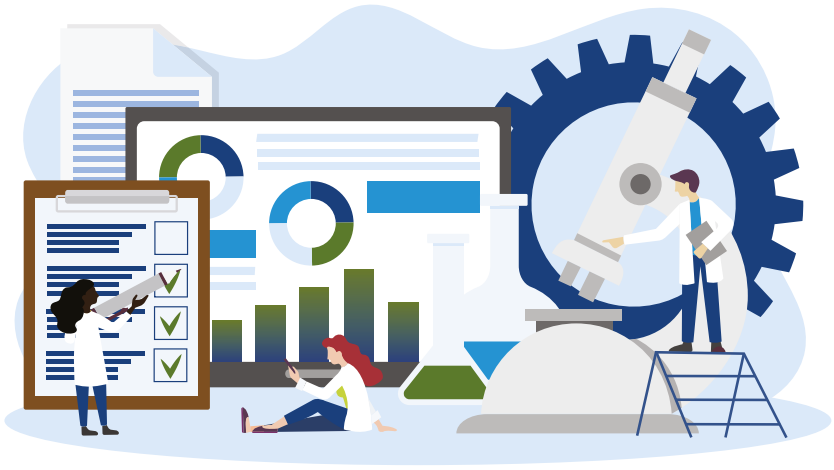
Education and training materials are available on TURAS to support NHS staff in carrying out the two-step admission screening process.

Incidents and outbreaks

ARHAI Scotland supports local Infection Prevention and Control Teams (IPCTs) and Health Protection Teams (HPTs) throughout Scotland to prevent, prepare for, and manage incidents and outbreaks, allowing the sharing of lessons learned throughout the region.

IPCTs and HPTs identify incidents, outbreaks and data exceedances, including decontamination incidents and near misses, in line with **Chapter 3 of the National Infection Prevention and Control Manual (NIPCM)**, which are assessed using the Healthcare Infection Incident Assessment Tool (HIIAT). In collaboration with key stakeholders, work is ongoing to revise the format of the HIIAT, with the aim to address potential variation that may occur with the assessment criteria, to provide a more structured approach in determining the risks associated with an incident or outbreak, and for consistency in the subsequent reporting across Scotland.

HIIAT assessed incidents, outbreaks and data exceedances are reported to ARHAI Scotland through the Outbreak Reporting Tool (ORT), which aims to facilitate the collation of epidemiological data and lessons learned which contribute to the development of national guidance and help inform local incident and outbreak management. The ORT continued to evolve in 2023 with the creation and introduction of the respiratory short form. Any incident/outbreak from key respiratory viruses (COVID-19, influenza and respiratory syncytial virus (RSV) only), where IPC measures align with the checklist and NIPCM and ARHAI support is not requested can be reported through the respiratory short form using a minimum dataset to ease the reporting burden for NHS boards.



In **2023**, there were **181** **healthcare infection incidents** and **outbreaks reported** to ARHAI Scotland (excluding incidents and outbreaks where COVID-19 or Norovirus is the sole pathogen), with HIIATs categorised as **Red**, **Amber** or **Green**.

Of these 181 reports, there were:

32 Red	21 Amber	128 Green

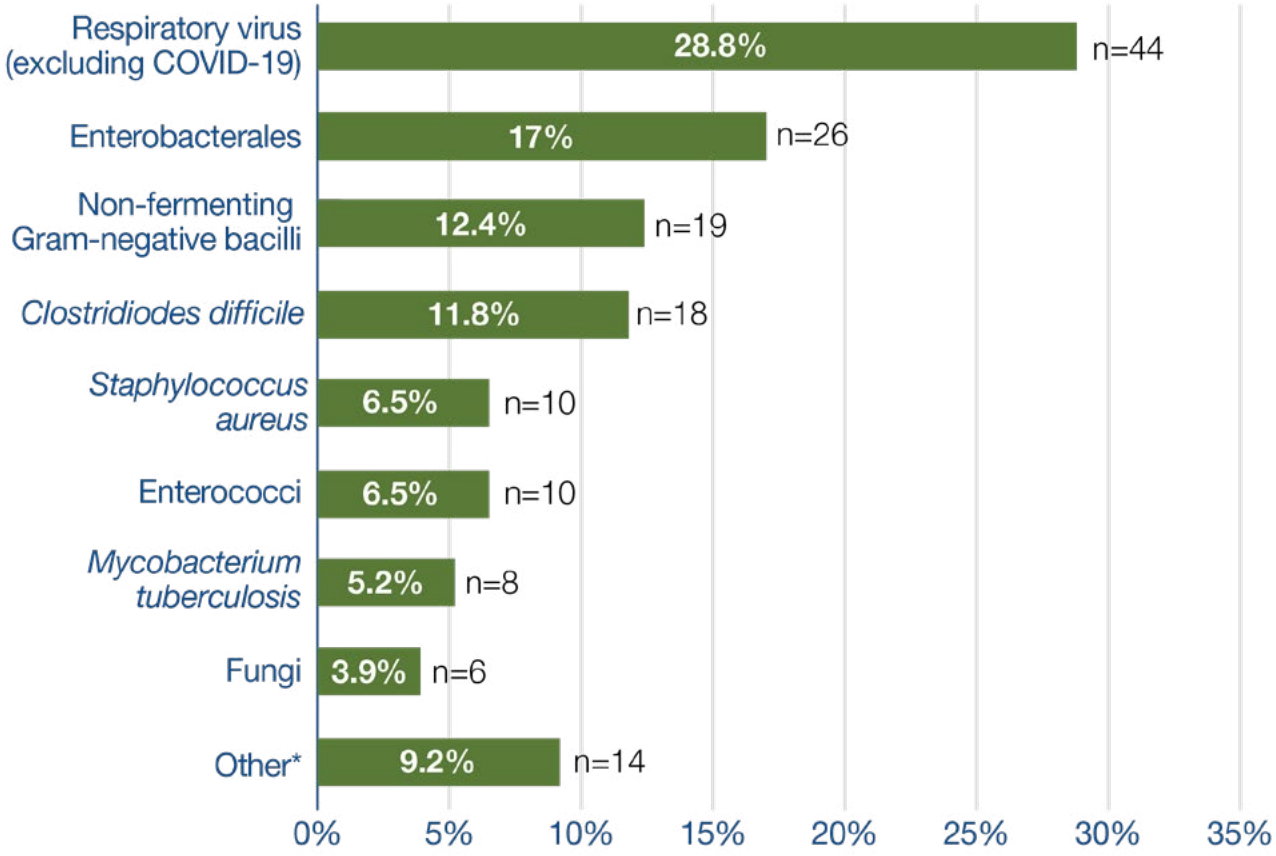
Of the **181** **incidents and outbreaks**, **153** were **reported with one or more patient cases**.

39 incidents involved a single patient case.

4 incidents stated two or more pathogens were involved.

3 incidents were from an unknown causative pathogen.

Percentage of key organism types as reported from incidents and outbreaks with one or more patient cases

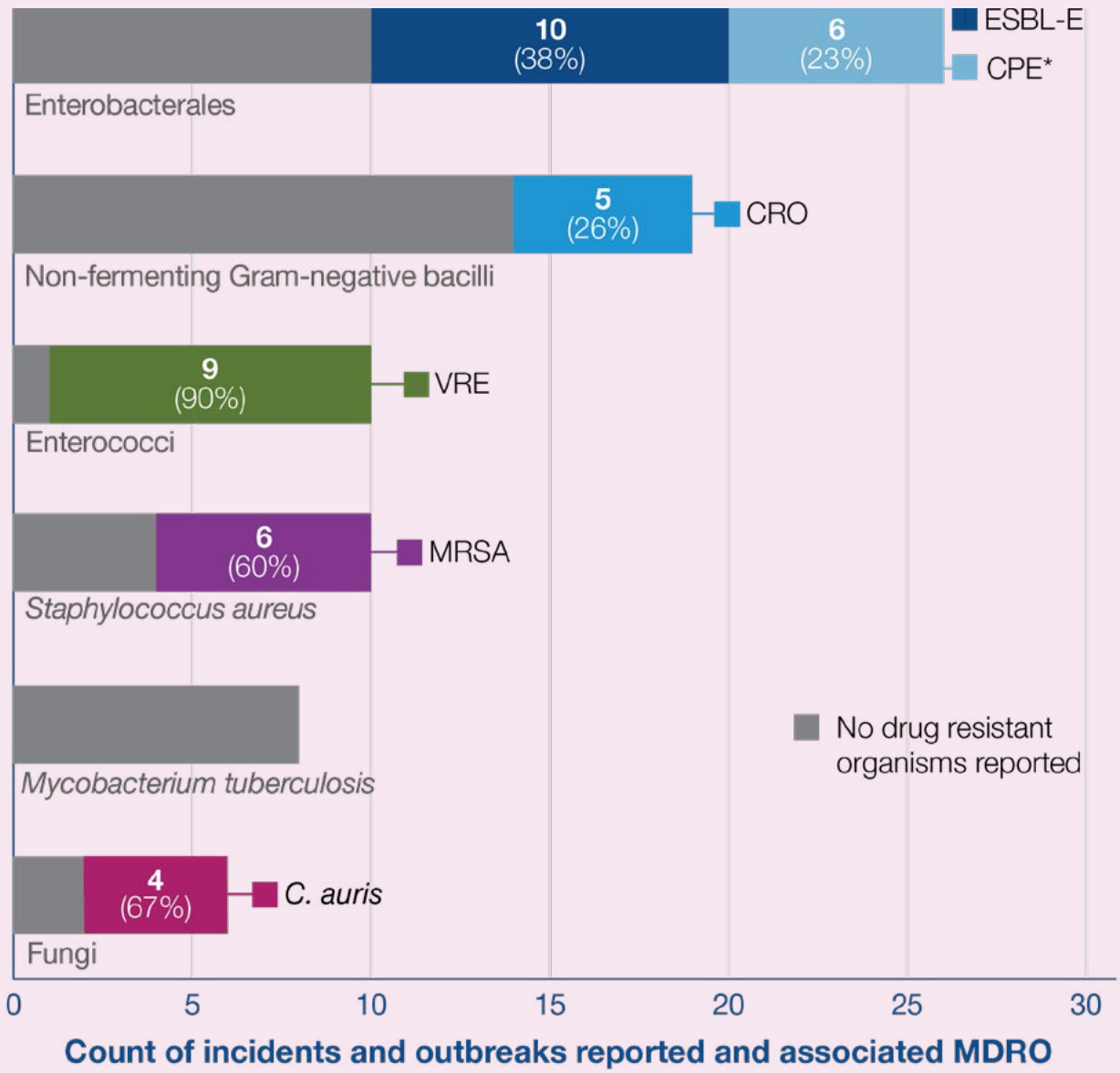


*Other includes: Gastroenteritis viruses (excluding norovirus), other Gram-negatives, other Gram-positives and scabies.

Note: some incidents included multiple organisms so total percentages may not add up to 100%.

Incidents and outbreaks with multi-drug resistant organisms

NHS boards also notify ARHAI Scotland if causative pathogens are multi-drug resistant organisms (MDRO).



*Organisms reported as CPE are not counted as ESBL-E separately.

- ESBL-E – Extended beta-lactamase producing Enterobacterales
- CPE – Carbapenemase-producing Enterobacterales
- CRO - Carbapenem-resistant organism
- VRE – Vancomycin-resistant Enterococcus
- MRSA – Meticillin-resistant *Staphylococcus aureus*
- *C. auris* – *Candida auris* (any susceptibility pattern)

Infection Prevention and Control workforce development

During 2023, NHS Education for Scotland (NES) and ARHAI continued to work collaboratively to embed education across key priority areas, to support improving people's health through a competent and skilled Infection Prevention and Control (IPC) workforce.

Winter preparedness

The winter preparedness campaign for 2023/24 launched 9 infographics containing key IPC messages. The report on our campaign that ended in February 2024 showed better engagement compared to the winter of 2022/23, with more people visiting our website and downloading our content. Our Winter Preparedness 2023/24 video on Vimeo was a hit, with 1,349 views, making it the most-watched video out of over 500 videos posted on our NES Vimeo account in the last 5 months.

Care home webinars

To support adoption and implementation of the Care Home Infection Prevention and Control Manual (CH IPCM), ARHAI Scotland delivered a series of themed national webinars entitled 'Clean Care Homes' to a target audience of care home staff across Scotland. The webinars were planned and jointly delivered with our partners in the Care Inspectorate and the Scottish Social Services Council and feedback was very positive.

Healthcare built environment

Project work was prioritised to develop and deliver learning and development opportunities for the specialist healthcare-built environment workforce. This included identifying priority areas for executive and non-executive NHSScotland board members and the delivery of two senior leadership development sessions.

IPC educational animations

An educational animation aimed at increasing staff awareness of potential risks associated with the improper use of clinical hand wash basins was produced and is hosted on **TURAS**. To support this work, **a safe use of clinical wash hand basin poster** was produced to be placed at all hand hygiene sinks.

A further suite of four educational animations which will identify IPC risks associated with the design, construction, commissioning and handover of healthcare water and above ground drainage systems also commenced. The preparatory work for the animations has begun and two of the four are complete with the remaining two to be undertaken in 2024. The development of the first animation is underway and will conclude in 2024 and sequentially all four will be available by the end of 2024.

Antimicrobial resistance

To continue to embed the importance of reducing the development and transmission of antimicrobial resistance (AMR), ARHAI Scotland provided sessions on post-graduation education to local universities. During Scottish Antimicrobial Prescribing Group network events, topics were presented and discussion groups led. Working in collaboration with NES, subgroups were facilitated on antimicrobial stewardship and AMR.

Surveillance and epidemiology

ARHAI Scotland and NES developed and delivered courses on **Epidemiology and Surveillance for IPC** and **Outbreak Simulation Training**. The learning outcomes for the epidemiology and surveillance course included foundational epidemiology for HCAI with a focus on epidemiological methods for outbreak investigations. The outbreak simulation training allowed participants to apply and enhance their knowledge, skills and confidence in outbreak management in a realistically simulated Incident Management Team environment. These courses will continue to be delivered during 2024.

ARHAI Scotland launched a Sharepoint site with training materials designed to support IPC staff with ongoing mandatory national surveillance programmes. In addition to links to key protocols and resources, ARHAI Scotland compiled additional video guides for users on the mandatory national surveillance programmes. These materials have been developed to support infection prevention and control teams to collect, submit and validate data according to surveillance definitions.

Education and training goals for 2024

- **Focus on educational content beyond social media campaigns to better reach our target audience.**
- **Introduce new educational tools and resources to support the launch of recommendations from the Transmission Based Precautions (TBPs) literature review.**
- **To continue support for adoption and implementation of national IPC guidance, a further series of Care Home themed webinars will be delivered**
- **Further development and creation of a suite of educational animations**
- **Continued education sessions feeding into the post-graduation programme**
- **Continue to deliver epidemiology and surveillance training to the IPC workforce.**
- **Expand the suite of training materials to support the provision of national surveillance data and outbreak reporting.**

List of abbreviations and acronyms

A&E	Accident and Emergency
AMR	Antimicrobial Resistance
ARHAI	Antimicrobial Resistance and Healthcare Associated Infections
C. *auris*	*Candida auris*
C. *difficile*	*Clostridioides difficile*
CDI	*Clostridioides difficile* Infection
CfSD	Centre of Sustainable Delivery
CHI	Community Health Index
CH IPCM	Care Home Infection Prevention and Control Manual
CJF	Considered Judgement Form
COVID-19	Coronavirus disease 2019 COVID-19
CPE	Carbapenemase-producing Enterobacterales
CRA	Clinical Risk Assessment
CRO	Carbapenem-resistant Organism
EBIS SMiRL	Enteric Bacterial Infections Service Scottish Microbiology Reference Laboratories
E. coli	*Escherichia coli*
ECB	*Escherichia coli* Bacteraemia
ECOSS	Electronic Communication of Surveillance in Scotland
ESBL-E	Extended beta-lactamase producing Enterobacterales
HAI	Hospital Acquired Infection
HCAI	Healthcare Associated Infection
HIIAT	Healthcare Infection Incident Assessment Tool
HIIORT	Healthcare Infection, Incident and Outbreak Reporting Template
HPT	Health Protection Team
ICU	Intensive Care Unit
IPC	Infection Prevention and Control
IPCT	Infection Prevention and Control Team
ISD	Information Services Division
KPI	Key Performance Indicator
KSAR	Key Stage Assurance Review
MDRO	Multi-Drug Resistant Organism

MLST	Multi-locus Sequence Typing
MRSA	Meticillin-resistant *Staphylococcus aureus*
MSSA	Meticillin-sensitive *Staphylococcus aureus*
NAP	National Action Plan
NCS	National Cleaning Specification
NDAP	NHSScotland Design Assessment Process
NES	NHS Education for Scotland
NHS	National Health Service
NIPCM	National Infection Prevention and Control Manual
NRS	National Records of Scotland
NSS	National Services Scotland
ORT	Outbreak Reporting Tool
PCR	Polymerase Chain Reaction
PHS	Public Health Scotland
PPE	Personal Protective Equipment
PWID	People Who Inject Drugs
RSV	Respiratory syncytial virus
RT	Ribotype
S. aureus	*Staphylococcus aureus*
SAB	*Staphylococcus aureus* Bacteraemia
SAMRS **SMiRL**	Scottish Antimicrobial Resistance Service Scottish Microbiology Reference Laboratories
SICPs	Standard Infection Control Precautions
SMR	Scottish Morbidity Record
SONAAR	Scottish One Health Antimicrobial Use and Antimicrobial Resistance
SSI	Surgical Site Infection
TBPs	Transmission Based Precautions
TOBD	Total Occupied Bed Days
UK	United Kingdom
VAD	Vascular Access Device
VRE	Vancomycin-resistant Enterococcus
WGS	Whole Genome Sequencing

Appendix 1 – Publication metadata

Publication title

ARHAI Scotland 2023 Annual Report

Description

This release provides information on activity within Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) Scotland for the period January to December 2023.

Theme

Healthcare associated infections in Scotland

Topic

Healthcare associated infections

Infection prevention and control

Format

Online resource (PDF)

Data source(s)

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures:

Total occupied bed days: **Public Health Scotland, ISD(S)1**.

Elective admissions data: **Public Health Scotland, Acute hospital activity and NHS beds information**.

Emergency admissions data: **Public Health Scotland, Acute hospital activity and NHS beds information**.

Delayed discharges data: **Scottish Health and Social Care Open Data platform, Delayed discharges in NHSScotland**.

Accident & Emergency (A&E) waiting times data: **Scottish Health and Social Care Open Data platform, Monthly A&E Activity and Waiting TimesScottish Health and Social Care Open Data platform, Monthly A&E Waiting Times**.

***Clostridioides difficile* infection**:

Case data: Electronic Communication of Surveillance in Scotland (ECOSS).

Data linkage source: general / acute inpatient and day case Scottish Morbidity Records (SMR01): Public Health Scotland.

Healthcare associated denominator: Public Health Scotland ISD(S)1 total occupied bed days.

Community associated denominator: National Records of Scotland (NRS) mid-year population estimates.

Case fatality data: NRS.

Molecular typing data: ECOSS, Enteric Bacterial Infections Service (EBIS SMiRL, Glasgow).

***Staphylococcus aureus* bacteraemia:**

Case data: ECOSS Enhanced Surveillance Web Tool.

Healthcare associated denominator: Public Health Scotland ISD(S)1 total occupied bed days.

Community associated denominator: NRS mid-year population estimates.

Case fatality data: NRS.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia):

Case data: ECOSS.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia):

Case data: ECOSS and ECOSS Enhanced Surveillance Web Tool.

Healthcare associated denominator: Public Health Scotland ISD(S)1 total occupied bed days.

Community associated denominator: NRS mid-year population estimates

Case fatality data: NRS.

Multi-drug resistant organism admission screening: National Multi-drug resistant organism (MDRO) Admission Screening Uptake Monitoring Tool.

Carbapenemase-producing Enterobacterales: ECOSS, Scottish Antimicrobial Resistance Service (SAMRS SMiRL, Glasgow).

Population denominator: NRS mid-year population estimates.

Incidents and Outbreaks: Healthcare infection incidents and outbreaks reported to National Services Scotland (NSS) through the Healthcare Infection, Incident and Outbreak Reporting Template (HIIORT) reporting process and the ARHAI Scotland Outbreak Reporting Tool (ORT).

Infection Prevention and Control Workforce Development: N/A

Date that data are acquired

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: 28 May 2024

***Clostridioides difficile* infection:** 19 March 2024

***Staphylococcus aureus* bacteraemia:** 19 March 2024

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia):
8 April 2024

Gram-negative bacteraemia (*Escherichia coli* bacteraemia):
19 March 2024

Multi-drug resistant organism admission screening: 27 March 2024

Carbapenemase-producing Enterobacterales: 9 April 2024

Incidents and Outbreaks: 11 June 2024

Infection Prevention and Control Workforce Development: N/A

Release date

15 October 2024

Frequency

Annual

Timeframe of data and timeliness

The latest iteration of data are to 31 December 2023, therefore ten months in arrears.

Continuity of data

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Please see each **data source** for any relevant information.

***Clostridioides difficile* infection:** Changes in the hospital population and activity during the pandemic period may have affected the epidemiology of *Clostridioides difficile* infection and comparison of results should be interpreted with caution.

In 2023, a small number of *C. difficile* isolates were typed using whole genome sequencing (WGS) derived from multi locus sequence type (MLST) due to an ongoing shortage of DNA polymerase, and ribotypes were inferred from the sequence type. Polymerase chain reaction (PCR) ribotyping was resumed in early 2023.Caution is advised in interpretation of the annual ribotype distributions due to the differences in typing methods.

***Staphylococcus aureus* bacteraemia:** Changes in the hospital population and activity during the pandemic period may have affected the epidemiology of *Staphylococcus aureus* infection and comparison of results should be interpreted with caution.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia):

Changes in the hospital population and activity during the pandemic period may have affected the epidemiology of Gram-negative bacteraemia and comparison of results should be interpreted with caution.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Changes in the hospital population and activity during the pandemic period may have affected the epidemiology of Gram-negative bacteraemia and comparison of results should be interpreted with caution.

Multi-drug resistant organism admission screening: Changes in the hospital population and activity during the pandemic period may have affected screening uptake; results of the clinical risk assessment (CRA) screening audits for meticillin-resistant *Staphyloccocus aureus* (MRSA) and carbapenemase-producing Enterobacterales (CPE) should be interpreted with caution.

Carbapenemase-producing Enterobacterales: Changes in the hospital population and activity during the pandemic period, including international travel restrictions, may have affected the epidemiology of CPE and comparison of results should be interpreted with caution.

Incidents and Outbreaks: Changes in the hospital population and activity during the pandemic period may have affected the epidemiology of healthcare incidents and outbreaks and comparison of results should be interpreted with caution.

Infection Prevention and Control Workforce Development: N/A

Revisions statement

These data are not subject to planned major revisions. However, our aim is to continually improve the interpretation of the data and therefore analysis methods are regularly reviewed and may be updated in the future.

Revisions relevant to this publication

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Please see each **data source** for any relevant information.

***Clostridioides difficile* infection:** Details provided in **quarterly publication**.

***Staphylococcus aureus* bacteraemia:** Details provided in **quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia): N/A

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **quarterly publication**.

Multi-drug resistant organism admission screening: N/A

Carbapenemase-producing Enterobacterales: Due to data quality updates the number of CPE cases in 2023 is reported as 102 cases, whereby this was previously reported as 103 cases in the ARHAI Scotland 2022 Annual Report.

Incidents and Outbreaks: N/A

Infection Prevention and Control Workforce Development: N/A

Concepts and definitions

Statistical significance:

Please note an increase or decrease stated in this report refers to a statistically significant change ($p < 0.05$), and where a trend is referred to as stable, or there has been no change, this means that there has been no significant increase or decrease.

Origin of infection:

Clostridioides difficile infection, *Staphylococcus aureus* bacteraemia and *Escherichia coli* bacteraemia: Cases are presented by origin of infection, where definitions are applied as per each surveillance protocol to classify cases as hospital acquired, healthcare associated, community associated or unknown. Please see the **quarterly publications**, corresponding **methods & caveats** document and individual surveillance protocols for **CDI** and **SAB/ECB** for full details.

Case fatality:

Clostridioides difficile infection, *Staphylococcus aureus* bacteraemia and *Escherichia coli* bacteraemia: The definition of 30 day all cause case fatality is any death occurring within 30 days of the first positive specimen date within each infection episode. Therefore, the data includes deaths where

Clostridioides difficile infection, *Staphylococcus aureus* bacteraemia or *Escherichia coli* bacteraemia may not have been either the underlying or contributory cause of death. All cause case fatality depends solely on the number of deaths identified and is not subject to bias that may be introduced as a result of inaccuracies in completion of the death certificate or coding of the cause of death. If more than one episode occurs in the 30 days prior to death, only the latest episode will be counted as resulting in a death. Some cases may not be able to be linked to NRS death data due to missing or invalid Community Health Index (CHI) numbers.

Year-to-year comparisons:

The current calendar year 2023 is compared to the previous calendar year 2022 using two sided z-tests for proportions and rate ratio tests (using Poisson counts) for incidence rates. A resulting p-value of less than 0.05 was deemed statistically significant to determine an increase or decrease relative to the previous year.

Five-year trends:

Incidence rates and incidence proportions over the past five years are modelled using Poisson regression and negative binomial regression respectively. This is performed to determine the presence of a significant upwards or downwards linear trend in the changing incidence rate/ proportion, and the corresponding rate of change of the best-fit gradient over the past five years from 2019 to 2023.

Where a year-on-year increase or decrease has been reported, this represents a significant linear trend over the past five years.

Where no five year increases or decreases have been detected, these are reported as stable.

Where a change over the past five years has been detected, but the trend is not linear, a direct year-to-year comparison between the incidence rate in 2023 and the incidence rate in 2019 has been made.

Funnel plots:

Funnel plot analyses are used to determine if any NHS board's rate is significantly higher than the NHSScotland average rate. The incidence rates for each NHS board are plotted against NHS board size/hospital activity,

with 95% confidence intervals calculated from the NHSScotland average. Any NHS board above the 95% confidence interval are deemed to be exceptions. Funnel plots are generated on a quarterly basis in the **quarterly epidemiological reports**; in this report the funnel plots incorporate the full year's data for 2023, and therefore as a result, some NHS boards may be above the 95% confidence interval upper limit in the annual funnel plot who may not have exceeded the quarterly funnel plots limits.

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Total occupied bed days: Total number of occupied bed days for years 2019 - 2023, as per **Public Health Scotland ISD(S)1**.

Elective admissions data: Details are provided within **Public Health Scotland Acute hospital activity and NHS beds information quarterly publication**.

Emergency admissions data: Details are provided within **Public Health Scotland Acute hospital activity and NHS beds information quarterly publication**.

Delayed discharges data: Details are provided on the **Scottish Health and Social Care Open Data platform**. These data include the number of hospital bed days occupied by people aged 18 and over who were clinically ready for discharge (excluding NHS Golden Jubilee). Data are presented as a percentage of TOBDs, whereby a comparable number of total hospital occupied bed days are used which excludes TOBDs from NHS Golden Jubilee, paediatric specialties and TOBDs from children's hospitals.

A&E waiting times data: Details are provided within **Scottish Health and Social Care Open Data platform, Monthly A&E Waiting Times.Scottish Health and Social Care Open Data platform**

***Clostridioides difficile* infection:** Details provided in **surveillance protocol and quarterly publication**.

***Staphylococcus aureus* bacteraemia:** Details provided in **surveillance protocol and quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia):
Gram-negative organisms including Enterobacterales and non-fermenters, cause serious infections including bacteraemia, pneumonia, meningitis, and surgical site infections (SSIs). Gram-negative bacteraemia is a public health and clinical concern because of:

1. The severity of infection, commonly occurring among vulnerable patients often at the extremes of life and/or with comorbidities.
2. The large number of cases of Gram-negative bacteraemias each year, and high prevalence of Gram-negative infections.
3. The association with receiving healthcare in community and healthcare settings.
4. Their ability to become resistant to multiple classes of antibiotics, limiting treatment options.

Further details provided in the **SONAAR Annual Report**.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **surveillance protocol and quarterly publication**.

Multi-drug resistant organism admission screening: Screening for MDRO on admission to hospital is a key intervention for early identification and management of patients who are colonised or infected, reducing the risk of introduction of MDRO into healthcare settings.

MRSA and CPE CRA screening policies are in place for all acute hospitals in Scotland. The two-stage screening process includes a CRA to identify patients at high risk of colonisation or infection with either MRSA or CPE, followed by microbiological testing as indicated by the CRA.

Uptake of the MRSA CRA has been a **level 3 HCAI Key Performance Indicator (KPI) since 2013**. The MRSA screening uptake monitoring tool was extended in 2018 to include assessment of CPE CRA uptake for the same patients included in the MRSA KPI audits.

Carbapenemase-producing Enterobacterales: CPE are bacteria that produce enzymes that inactivate carbapenems and other classes of antibiotics. Infections caused by CPE are difficult to treat and are associated with increased mortality.

A case of CPE is defined as one isolate per patient per enzyme and organism combination per year as confirmed by the Scottish Microbiology Reference Laboratory. Further details provided in the **SONAAR Annual Report**.

Incidents and Outbreaks: Healthcare infection incidents and outbreaks reported to NSS. Healthcare associated infection incidents and outbreaks are defined within **Chapter 3 of the National Infection Prevention and Control Manual**.

Individual NHS boards report the MDRO status of organisms. These are typically ascertained by local phenotypic susceptibility testing, reference laboratory phenotypic or genotypic testing or a combination. For more information see the **Reference laboratory referral guide**.

Further details provided in the **SONAAR Annual Report**.

Infection Prevention and Control Workforce Development: N/A

Relevance and key uses of the statistics

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: Details provided in the **SONAAR Annual Report**.

Hospital Activity and Pressures: Figures provided are used for management information for resource planning, surveillance and research in NHSScotland.

***Clostridioides difficile* infection:** Details provided in **quarterly publication**.

***Staphylococcus aureus* bacteraemia:** Details provided in **quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia): The outputs of the surveillance programme are intended to support the NHS boards in controlling and reducing the burden of Gram-negative bacteraemia.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **quarterly publication**.

Multi-drug resistant organism admission screening: These audit data measure uptake of the two-stage admission screening process of CRA followed by microbiological testing of those considered to be at risk of carrying MRSA or CPE based on CRA response, in NHSScotland as per policies. These audit data are collected to support the assurance element of quality improvement processes and gather intelligence to inform and target local interventions where CRA screening uptake is suboptimal. ARHAI Scotland provide enhanced feedback to NHS boards on a quarterly basis.

Carbapenemase-producing Enterobacterales: Details provided in the **SONAAR Annual Report**.

Incidents and Outbreaks: To identify risks or trends in the organisms, types of infection, procedures, patients, or clinical specialities associated with healthcare infection incidents and outbreaks. This informs the production of guidance, tools or policy and assists in preparing for, preventing, detecting, and managing healthcare infection incidents and outbreaks.

Infection Prevention and Control Workforce Development: N/A

Accuracy

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Details available as per each **data source**.

***Clostridioides difficile* infection:** Details provided in **quarterly publication**.

The **snapshot programme** aims to obtain a representative sample of isolates from CDI cases across all NHS boards in Scotland, however not all NHS boards have submitted the required number of isolates as specified by the Snapshot protocol therefore the data should be interpreted with caution.

***Staphylococcus aureus* bacteraemia:** Details provided in **quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia): Gram-negative bacteraemia data are the product of the ECOSS. Participating laboratories routinely report all identifications of organisms, infection or microbiological intoxication and where possible the antimicrobial resistance data unless they are known to be of no clinical or public health importance. The collected data are used for the identification of single cases of severe disease, outbreaks, antimicrobial resistance patterns and longer-term trends in the incidence of laboratory reported infections, enhanced surveillance, health protection, analytical and statistical use.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **quarterly publication**.

Multi-drug resistant organism admission screening: While audit data does not present the same robust scientific data as surveillance, it provides a nationally representative measure of CRA screening uptake and is valuable for identification of areas for targeted implementation of quality improvement measures. A minimum of 3,500 patients are audited annually for NHSScotland in order to measure uptake with sufficient precision, with each NHS board auditing a representative number of patients.

For each eligible patient admitted during the audit period, data are collected for the most recent opportunity for the CRA to have been undertaken. It is acknowledged that all patients are eligible for CPE CRA admission screening though not all areas are included in the screening audit. These areas will not be represented in these data. Patients are selected for audit based on the **Protocol for CRA MRSA Screening National Rollout in Scotland**, with the exception of NHS Golden Jubilee from which only CPE CRA screening uptake audits are submitted (as all patients are screened for MRSA). In order to maximise the representativeness of the national KPI measure whilst ensuring flexibility at a local level, NHS boards are asked that data collection throughout the year should be spread as much as possible across; hospital, wards, high/low impact specialties, medical/surgical specialties, elective and emergency admissions.

Carbapenemase-producing Enterobacterales: Details provided in the **SONAAR Annual Report**.

Incidents and Outbreaks: NSS are aware that the healthcare infection incident assessment tool (HIIAT) is subjective and there is variation in how NHSScotland boards assess and therefore report healthcare infection incidents. The extent of variation in assessment and unreported incidents has not been fully quantified.

Infection Prevention and Control Workforce Development: N/A

Completeness

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Details available as per each **data source**.

***Clostridioides difficile* infection:** Details provided in **quarterly publication**.

The **snapshot programme** aims to obtain a representative sample of isolates from CDI cases across all NHS boards in Scotland, however not all NHS boards have submitted the required number of isolates as specified by the Snapshot protocol therefore the data should be interpreted with caution.

***Staphylococcus aureus* bacteraemia:** Details provided in **quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia): All data for the reporting period have been included in the analysis.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **quarterly publication**.

Multi-drug resistant organism admission screening: A minimum of 3,500 patients are audited annually for NHSScotland in order to measure uptake with sufficient precision, with each NHS board auditing a representative number of patients. All NHS boards submitted at least their required minimum number of patients audited and all completed audits submitted data for the reporting period have been included in the analysis.

Carbapenemase-producing Enterobacterales: Details provided in the **SONAAR Annual Report**.

Incidents and Outbreaks: NSS are aware that the HIIAT is subjective and there may be some variation in how NHS boards assess and therefore report healthcare infection incidents. The extent of variation in assessment and unreported incidents has not been fully quantified.

Infection Prevention and Control Workforce Development: N/A

Comparability

Changes in the hospital population and activity during the pandemic period may have affected the comparison of results and therefore should be interpreted with caution.

Infection Prevention and Control Guidance: N/A

Reducing Risk in the Healthcare Built Environment: N/A

Antimicrobial Resistance: N/A

Hospital Activity and Pressures: Details available as per each **data source**.

***Clostridioides difficile* infection:** Details provided in **quarterly publication**.

***Staphylococcus aureus* bacteraemia:** Details provided in **quarterly publication**.

Gram-negative bacteraemia (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Acinetobacter species* bacteraemia): Details provided in **SONAAR Annual Report**.

Gram-negative bacteraemia (*Escherichia coli* bacteraemia): Details provided in **quarterly publication**.

Multi-drug resistant organism admission screening: There are no comparable data on CRA screening uptake.

Carbapenemase-producing Enterobacterales: Details provided in the **SONAAR Annual Report**

Incidents and Outbreaks: Reporting of all HCAI outbreaks is not mandatory elsewhere in the UK and comparable data are not published.

Infection Prevention and Control Workforce Development: N/A

Accessibility

It is the policy of NSS to make its website and products accessible according to published guidelines.

Coherence and clarity

Development of guidance: all National Infection Prevention and Control Manual (NIPCM) reviews and resources are produced using a **defined process** which ensures clarity and coherence.

Value type and unit of measurement

Total occupied bed days per year and quarter.

Count of emergency admissions per year and quarter. Count of elective admissions per year and quarter.

Percentage of total occupied bed days due to delayed discharges (%) = Number of bed days occupied by people aged 18 and over who were clinically ready for discharge (excluding NHS Golden Jubilee) / Total number of occupied bed days (excluding bed days from NHS Golden Jubilee and all paediatric specialties/children's hospitals) * 100.

Percentage of A&E attendances seen within 4-hour target (%) = Total number of patients seen within 4 hours in A&E / Total number of patients who attended A&E * 100.

Healthcare associated and hospital acquired cases and incidence rates (per 100,000 total occupied bed days) for *Clostridioides difficile* infection, *Escherichia coli* bacteraemia and *Staphylococcus aureus* bacteraemia.

Community associated cases and incidence rates (per 100,000 population) for Clostridioides difficile infection, *Escherichia coli* bacteraemia and *Staphylococcus aureus* bacteraemia.

30 day all cause case fatality rate for *Clostridioides difficile* infection, *Staphylococcus aureus* bacteraemia and *Escherichia coli* bacteraemia = count of cases with death occurring within 30 days of positive specimen date / total number of cases.

Percentage of *C. difficile* ribotypes (%) = count of each ribotype or ribotype group / total number of isolates submitted for typing.

Percentage of each SAB entry point (%) = count of healthcare associated or community associated SAB with a specific entry point / total number of healthcare associated or community associated *Staphylococcus aureus* bacteraemia.

Percentage of each ECB primary infection (%) = count of healthcare associated or community associated ECB with a specific source / total number of healthcare associated or community associated *Escherichia coli* bacteraemia.

Due to rounding percentages may not add up to 100%.

MRSA Clinical Risk Assessment (CRA) Uptake (%) = number of patients where CRA undertaken / all patients in audit sample *100.

Carbapenemase-producing Enterobacterales (CPE) CRA Uptake (%) = number of patients where CRA undertaken / all patients in audit sample *100.

Count of carbapenemase-producing Enterobacterales per year. Incidence rate of carbapenemase-producing Enterobacterales per 100,000 population per year.

Total number of reported incidents and outbreaks.

Number of reported incidents and outbreaks by HIIAT category.

Number of incident and outbreak reports where one or more patient cases where reported.

Number of incident and outbreak reports where two or more pathogens were reported.

Number of incident and outbreak reports where the causative pathogen was unknown.

Number and percentage of incident and outbreak reports from key organism types (%) = Number of incident and outbreak reports from a key organism type / Total number of incident and outbreak reports * 100. Note: some incidents included multiple organisms so total percentages may not add up to 100%.

Number and percentage of incident and outbreak reports from a key organism type where ARHAI has been notified of a multi-drug resistant organism (%) = Number of incident and outbreak reports from a key organism type where ARHAI has been notified of a multi-drug resistant organism / Number of incident and outbreak reports from corresponding key organism type * 100.

Disclosure

The PHS protocol on **Statistical Disclosure Protocol** is followed.

Official statistics accreditation

Not Assessed

UK Statistics Authority Assessment

Not Assessed

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Date of first publication

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Date form completed

15 October 2024

Appendix 2 – Early Access Details

Pre-release access

Under terms of the 'Pre-Release Access to Official Statistics (Scotland) Order 2008', NSS is obliged to publish information on those receiving Pre-Release Access ('Pre-Release Access' refers to statistics in their final form prior to publication). The standard maximum Pre-Release Access is five working days. Shown below are details of those receiving standard Pre-Release Access.

Standard pre-release access

Scottish Government Health Department, NHS board Chief Executives, NHS board Communication leads

Appendix 3 – NSS and Official Statistics

Our statistics comply with the **Code of Practice for Statistics** in terms of trustworthiness, high quality and public value. This also means that we keep data secure at all stages, through collection, processing, analysis and output production, and adhere to the **'five safes'**.

NHS National Services Scotland

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Alternative formats

This publication can be made available in large print, Braille (English only), audio tape and different languages. Please contact nss.equalitydiversity@nhs.scot for further information.

This report was designed by NHS National Services Scotland's Creative Services team.

ARHAI Scotland

Antimicrobial Resistance and Healthcare Associated Infection

A53210128

NSS3406

Genome-based typing reveals rare events of patient contamination with Pseudomonas aeruginosa from other patients and sink traps in a medical intensive care unit

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A53210128

Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genome-based typing reveals rare events of patient contamination with Pseudomonas aeruginosa from other patients and sink traps in a medical intensive care unit

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Article

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Abstract

Background

We used genome-based typing data with the aim of identifying the routes of acquisition of *Pseudomonas aeruginosa* (PA) by patients hospitalized in a medical intensive care unit (MICU) over a long period in a non-epidemic context.

Methods

This monocentric prospective study took place over 10 months in 2019 in a 15-bed MICU that applies standard precautions of hygiene. Lockable sink traps installed at all water points of use were bleach disinfected twice a week. We sampled all sink traps weekly to collect 404 PA environmental isolates and collected all PA isolates (n = 115) colonizing or infecting patients (n = 65). All isolates had their phenotypic resistance profile determined and their genome sequenced, from which we identified resistance determinants and assessed the population structure of the collection at the nucleotide level to identify events of PA transmission.

Results

All sink traps were positive for PA, each sink trap being colonized for several months by one or more clones. The combination of genomic and spatiotemporal data identified one potential event of PA transmission from a sink trap to a patient (1/65, 1.5%) and six events of patient cross-transmission, leading to the contamination of five patients (5/65, 7.7%). All transmitted isolates were fully susceptible to β-lactams and aminoglycosides.

Conclusions

Genome-based typing revealed the contamination of patients by PA originating from sink traps to be infrequent (1.5%) in a MICU with sink trap-bleaching measures, and that only 7.7% of the patients acquired PA originating from another patient.

Introduction

P. aeruginosa (PA) is an opportunistic Gram-negative bacillus that can thrive in a wide variety of niches. Hence, PA is widespread in soil and water and frequently found in the environment, including in the wastewater evacuation network of hospitals (1). PA is also one of the most frequent species responsible for nosocomial infection in Europe and the USA (2, 3). In intensive care units (ICUs), 10 to 15% of healthcare-associated infections are attributed to this pathogen (1). Such infections consist mostly of ventilator-associated pneumonia or bacteremia, associated with high mortality (4). A high number of such infections are nosocomial, especially among mechanically ventilated patients (5). The PA genome can also readily acquire genetic material and thus gain new antibiotic resistance (6).

The rate of colonization by PA is low (2.3%) among healthy humans, more frequent among patients admitted to ICUs (4.1 to 11.6%), and can reach higher rates (57.8%) during hospitalization (7–10). ICU patients can acquire PA from their environment and other patients, directly or via the hands of healthcare workers (8, 11). Hence, PA can contaminate respiratory equipment, endoscopes, and sections of the hospital water network, such as faucets, shower drains, and sink traps (also known as U-bends or P-traps) (12–16). The proportion of sink traps contaminated with PA varies from 15 to 50% in European ICUs and studies have reported that 7 to 50% of patients acquire PA from water points of use (17, 18). In addition, investigations of hospital outbreaks have identified the water supply system as the source of the PA outbreaks (19). This has led to infection control departments to recommend sink trap disinfection or sink removal or redesign in high-risk wards, such as ICUs and hematology units (20, 21).

The distribution of the sources of PA (endogenous, environmental, other patients) varies greatly between studies because of differing infection control procedures (contact precautions, sink trap disinfection). In addition, discrepancies in study conclusions may also result from differences in sampling protocols and bacterial typing methods. Hence, genome-based typing identifies

transmission routes of pathogens with a higher accuracy than older typing methods, which probably overestimate the number of transmission events (22, 23).

We aimed to identify the acquisition pathways of PA by patients in a medical ICU (MICU) in which patients positive for PA were managed according to the recommendations of the French Society of Infection Control and, additionally, in which sink traps were disinfected with bleach twice a week. We sampled patients and sink traps for 10 months to collect 519 PA isolates, for which the genomes were entirely sequenced and compared at the nucleotide level. Such analysis elucidated the precise network of PA transmission in this MICU and identified the routes of PA acquisition by the patients.

Methods

Study characteristics. This prospective monocentric study took place in a 15-bed MICU between January and November 2019 in a university hospital in France. This MICU has 11 individual rooms distributed within three subunits (A, B, and C) with one water point of use and a four-bed room with two water points of use (Fig. 1). The MICU admits ~ 700 patients/year and all 549 patients admitted during the time of the study were included. Each patient received care from ~ 10 members of the healthcare staff per day. Healthcare workers were dedicated to a subunit but could help in another subunit when needed. We conducted this study in the absence of an identified outbreak of PA.

Infection control procedures. All 13 water points of use were equipped with a lockable sink trap (Geberit, France) that was bleach-disinfected twice a week and at patient discharge. Briefly, locked sink traps were treated 15 min with 20 ml 2.6% liquid bleach and then rinsed with tap water. This unit systematically applies standard precautions of hygiene according to the recommendations of the French Society of Infection Control (24).

Bacteriological methods. Patients admitted to the MICU were screened for PA carriage upon admission and twice a week thereafter using nasal swabs, rectal swabs, and tracheal aspirates when intubated. In parallel, we collected all PA isolates retrieved from diagnostic samples. All sink traps were sampled every week for PA detection by collecting 50 ml sink-trap content with a suction catheter and a syringe. The sample was centrifuged 5 min at 5000 x *g* at room temperature and the supernatant discarded. Swabs and pellets were streaked on PA-selective cetrimide agar plates (Bio-Rad) and incubated 48 h at 35°C. All colony phenotypes were identified using a MALDI-TOF mass spectrometer (Maldi Biotyper, Bruker). We assessed the activity of 13 antipseudomonal agents (listed in Supplemental Table 1) from three classes (β-lactams, aminoglycosides, fluoroquinolones) by the agar diffusion method as recommended by the EUCAST (www.eucast.org). Morphologically different colonies of PA recovered from the same sink trap sample were retained for further analysis when they showed distinct resistance profiles. The resistance profile of isolates susceptible to all tested antibiotics was considered to be wildtype. Acquired genes encoding β-lactamases (including carbapenemases) were sought within the genomic data against the ResFinder database (25).

Data. Each isolate collected was associated with its date of isolation, its patient or sink trap of origin, and its antibiotic resistance profile. See Supplemental Material and Methods for details on bacteriological and sequence analysis. We sequenced the full genome of all PA isolates (Supplemental Table 1). Sequencing data are available in the NCBI BioProject PRJNA788732. We first identified the sequence type (ST) of each isolate by MultiLocus Sequence Typing (MLST) (26). Then, isolates for which the genome contained ≤ 30 different genes were clustered into groups with Core Genome MLST (cgMLST) using 3,867 core genes (27). We further measured the genetic relatedness of isolates within each group by the number of single nucleotide polymorphisms (SNPs) between genomes. See Supplemental Fig. 1 for the justification of the threshold.

Definitions. *(i)* Isolates from a given group were defined as clonal when their genomes clustered with a threshold of seven SNPs. (*ii*) Cross-contamination was defined by the identification of clonal isolates in two sampling points (patient or sink trap). (*iii*) In cases of cross-contamination between two sampling points ≥ 7 days apart, we defined the older one as the source. (*iv*) Infections were defined according to Sepsis-3.

Statistical analysis. The data were analyzed with R Studio (v 1.4) using the circlize and vegan libraries. Differences between the distributions of resistance profiles of the two PA reservoirs (patients and sink traps) were tested using Fisher's exact test at a threshold of 0.01.

Results

Contamination of sink traps and patients by **P. aeruginosa.** During the 10 months of the study, the 13 sink traps of the MICU were sampled 42 times each, for a total of 546 samples, of which 282 (51.6%) were positive for one or more isolates of PA. This led to the collection of 404 environmental isolates. All sink traps were positive for PA at some point of the study and could be contaminated with multiple STs (mean: 5, min: 2, max: 10), with clones persisting in each sink trap for long periods of time (mean: 242 days, max: 286 days). Among the 549 patients included, 65 (11.8%) were positive for PA. We collected 115 clinical isolates from these patients during their hospitalization. Three patients (3/65, 4.6%) were infected with PA.

Population structure of **P. aeruginosa**. The 519 isolates were distributed within 62 different STs, with five STs accounting for 54.9% of the entire collection. Hence, the high-risk clones ST253, ST308, ST298, and ST244 were represented by 90 (17.3%), 69 (13.3%), 39 (7.5%), and 32 (6.2%) isolates, respectively, and ST309 was represented by 55 isolates (10.6%) (Supplemental Table 1). Isolates from sink traps and patients were distributed within 27 and 48 different STs, respectively, with 13 STs in common. We then compared the clonal diversity of the population of isolates retrieved from the sink traps with that of the clinical isolates and found that the community of clinical PA was 4.1-times richer and 2.6-times more diverse than that retrieved from the sink traps (Supplemental Fig. 2).

Transmission routes of **P. aeruginosa**. The routes of transmission of PA within the MICU were accurately identified by comparing the genomes of all isolates with a pipeline that allowed variant calling. This method clustered the isolates into 36 groups (Supplemental Fig. 3). We combined these genomic data with spatiotemporal data to identify intra- and inter-reservoir transmission events (Table 1, Fig. 2).

Table 1
Details of the transmission of *P. aeruginosa* isolates involving patients in the medical intensive care unit at Besançon University Hospital (France) between January and November 2019.

Type of transmission	Isolate (ST, group)	Resistance phenotype	Reservoir 1	Room	Date (2019)	Direction of transmission[a]	Reservoir 2	Room	Date (2019)
Patient-to-patient									
	ST198, group34_1	FQs[b]	Patient43	A1	Apr. 14	Unknown	Patient49	A1	Apr. 18
	ST274, group30_1	Wild-type	Patient49[c]	C2	Apr. 12	Unknown	Patient32[c]	C2	Apr. 18
	ST1197, group25_1	Wild-type	Patient1	C1	Aug. 7	Unknown	Patient13	C2	Aug. 12
	ST1238, group27_1	Wild-type	Patient9	B5	Jul. 22	Unknown	Patient48	A1	Jul. 22
	ST3218, group15_1[d]	Wild-type	Patient32[c]	C2	Apr. 15	Unknown	Patient49[c]	C2	Apr. 18
	ST3218, group15_1[d]	Wild-type	Patient8	A3	Oct. 14	→	Patient39	C2	Oct. 22
Sink trap to patient									
	ST253, group0_6	Wild-type	Sink trap	B2	May 13	→	Patient14	A3	May 24
Patient to sink trap									
	ST27, group21_1	Wild-type	Patient36	B3	Feb. 23	→	Sink trap	B3	Mar. 4
	ST234, group33_1	Wild-type	Patient5	A5	May 2	→	Sink trap	A5	Jul. 29
	ST253, group0_3	Wild-type	Patient17	A1	Oct. 23	→	Sink trap	A1	Nov. 4
	ST308, group1_6	Wild-type	Patient54	A2	Jul. 8	→	Sink trap	A2	Jul. 15
	ST309, group2_3	Wild-type	Patient10	B2	Sep. 2	→	Sink trap	B2	Sep. 23
	ST671, group32_1	Wild-type	Patient19	A2	Mar. 30	→	Sink trap	A2	Apr. 8

[a] In cases of transmission between two sampling points ≥ 7 days apart, we defined the older one as the source.

[b] Isolated low-level resistance to fluoroquinolones.

[c] Patient32 and Patient49 shared both isolates ST274, group30_1 and ST3218, group15_1.

[d] ST3218, group15_1 was shared by Patient32 and Patient49 and transmitted from Patient8 to Patient39.

Most of the links occurred within a given sink trap, showing that such niches were contaminated with a signature flora that was stable over time. However, we identified 22 cross-transmission events between sink traps of different rooms, with 10 between sink traps of different subunits (Fig. 2A).

We identified six events of PA cross-transmission between patients, four involving one clonal isolate and one involving two (Table 1, Fig. 2B). These events involved five non-high-risk clones (ST198, ST274, ST1197, ST1238, ST3218) and nine patients. Isolate ST198 group34_1 was shared by two patients hospitalized in room A1 during the same week, but whose hospitalization

period did not overlap. Isolates ST1197 group25_1 and ST1238 group27_1 were transmitted between patients hospitalized during the same week in different rooms (C1 and C2 for ST1197, A1 and B5 for ST1238). Two patients from room C2 shared two isolates (ST274 group30_1 and ST3218 group15_1). The temporal proximity of the finding of these isolates prevented the identification of the source of contamination. In one case, we could document the direction of contamination of a patient of room C2 with the isolate ST3218 group15_1 from a patient hospitalized in room A3 (Table 1, Fig. 2B). Overall, four patients shared the isolate ST3218 group15_1: two patients were hospitalized in April 2019 in room C2 and two others six months later in rooms C2 or A3 (Table 1). We never retrieved this isolate from any sink trap (Supplemental Table 1).

In terms of environment-to-patient contamination, only one transmission of a PA isolate occurred from a sink trap to a patient. A high-risk ST253 clone, repeatedly found in the sink trap of rooms B2 and B3 from January to September 2019, was isolated from a patient hospitalized in May in room A3 (Table 1, Fig. 2B). In addition, we identified six transmission events of PA from patients to the sink traps of their rooms (Fig. 2B). The six STs involved (ST27, ST234, ST253, ST308, ST309, ST671) were transmitted in rooms A1, A2, A5, B2, and B3 (Table 1). Overall, among the 65 patients infected or colonized with PA, one patient (1.5%) acquired a PA isolate from a sink trap and five other patients (7.7%) were contaminated with a PA isolate from another patient.

Resistance profiles of **P. aeruginosa.** The proportion of isolates susceptible to all antibiotics tested was higher for the PA of human origin (74.8%; 86/115) than the PA found in the sink traps (48.0%, 194/404) (Fisher's exact test, $p = 2.8x10^{-7}$; Supplemental Table 1). The only clone that produced extended-spectrum β-lactamase (VEB-1) belonged to ST357 and was represented by eight isolates found in the sink trap of room B1. Isolates non-susceptible to carbapenems were more frequently found in sink traps (152/404, 37.6%) than in patients (19/115, 16.5%) (Fisher's exact test, $p = 1.6x10^{-5}$). The 13 isolates resistant to all antibiotics tested (Supplemental Table 1) were exclusively retrieved from sink traps and clustered within two clones belonging to ST111 (room C2) and ST357 (room B1).

Four of the five PA isolates transmitted between patients (ST274 group30_1, ST1197 group25_1, ST1238 group27_1, ST3218 group15_1) displayed wildtype resistance profiles. Of note, isolate ST3218 group15_1 was transmitted on two occasions involving four patients. The fifth isolate (ST198 group34_1) displayed an isolated low level of resistance to ciprofloxacin. Finally, the isolate ST253 group0_6 transmitted from a sink drain to a patient had a wildtype resistance profile to antibiotics (Table 1, Supplemental Table 1, Supplemental Fig. 3).

Discussion

We investigated the cross-transmission of PA between patients and sink traps over 10 months in the MICU of a university hospital in France in the absence of a recognized outbreak. Among the 65 patients infected or colonized with PA, one (1.5%) was contaminated with a clone originating from a sink trap and five (7.7%) from one originating from another patient.

The proportion of patients contaminated with a PA isolate previously found in a sink trap (1.5%) was lower than that previously reported (7–50%) (17, 18). Such a discrepancy could be due to the more accurate typing method used here relative to older typing techniques based on DNA restriction, such as pulsed-field gel electrophoresis (PFGE). Although PFGE can detect local outbreaks caused by PA, comparison at the nucleotide level is required to identify contamination routes of the pathogens (22, 23, 28). The implementation of infection control procedures, with improved hand hygiene, presumably accounted for the low transmission rate from the environment to patients. In addition, we identified five patients from among the 65 (7.7%) who acquired PA from another patient (Table 1, Fig. 2B). This is the first quantification of the rate of patient cross-contamination in a non-epidemic context. For each cross-contamination event, the genomes of the PA isolates retrieved from the two patients were completely identical (Supplemental Fig. 3). Three transmission events involved patients hospitalized in different rooms, indicating transmission by healthcare workers (Fig. 2B). Of note, the six cross-transmission events between patients were concentrated in the geographically close rooms A1 (two events) and C2 (four events), which frequently shared healthcare workers (Table 1, Fig. 1). Hence, the proximity of the beds and the sharing of sinks in the four-bed room C2 could enhance the risk of cross-contamination with PA. All clinical PA transmitted to the sink traps originated from patients occupying the room (Table 1), probably during their bathing.

Among the 65 patients positive for PA, 29 (44.6%) tested negative at admission. Only two (2/29; 6.9%) acquired a PA isolate from another patient. In other words, the vast majority of patients who became positive with PA during their hospitalization acquired an

isolate not previously found in the other patients or sink traps. Other studies have reported higher proportions (50.0-93.6%) of patients contaminated with an exogenous isolate in ICUs with no detectable PA outbreak and no bleach-disinfection of sink traps (22, 25). Although we cannot rule out contamination with PA isolates originating from unexplored sources (*e.g.* healthcare workers, invasive devices), our data show that endogenous sources (*i.e.* patient flora) predominate over exogenous sources in a non-epidemic context (22).

Overall, we can assume that the cleaning and disinfection procedures, together with infection control procedures, applied in this MICU limit the risk of PA transmission. Sinks were cleaned daily and sink traps were disinfected twice a week with 2.6% bleach. Disinfection procedures using bleach, acetic acid, electrochemically-activated solutions, or self-disinfecting sink drains fail to sterilize sink traps because bacterial biofilms in wastewater plumbing systems resist disinfectants and are not easy to access. However, such procedures likely limit the inoculum size, which, in turn, reduces the risk of contamination of the surrounding sink area and further transmission (29). Additionally, other mechanisms may limit the transmission of PA from sink traps to patients. Hence, sub-inhibitory concentrations of bleach can promote horizontal gene transfer (30), thus favoring the adaptation of pathogens to a harsh environment. Such adaptation, illustrated here by the stability and low diversity of PA populations in sink traps (Fig. 2A, Supplemental Fig. 2), could impair the ability of PA to colonize patients (31).

Our study focused on PA, but other pathogens (*Klebsiella pneumoniae*, *Acinetobacter baumannii, Elisabethkinga meningoseptica*) have already been retrieved from sink traps (32–34). One can assume that disinfection also limits the risk of outbreaks with these pathogens. However, the implementation of sink bleaching alone has not been shown to be systematically associated with the cessation of outbreaks and the implementation of a bundle of measures is recommended for infection control (35, 36).

Outbreaks of multidrug-resistant bacteria specifically linked to drains or sinks are overrepresented in the literature (35). We found all PA isolates transmitted from and to patients to be fully susceptible to β-lactams and aminoglycosides. Clinicians and microbiologists should not neglect the potential spread of strains with unspectacular resistance profiles (37).

This monocentric study was not designed to assess the effect of infection control procedures on the transmission of PA. Hence, the absence of a comparable ICU using different hygiene practices prevented assessment of the efficacy of sink trap disinfection in preventing the transmission of PA to patients. Instead, our design focused on breadth and depth sampling to identify the contamination routes of PA to patients. We isolated PA from both sink traps and patients over 10 months, collecting the largest dataset yet used for an epidemiological study (38). As we were aware of the complex flora of the sink traps (20), we sampled all sink traps weekly and analyzed all colonies with various colony phenotypes and resistance profiles in each sample to obtain a complete picture of the PA population in this environmental niche. The use of isolates collected over 10 months increased the chance of finding clonal isolates that overlapped among patients and sink traps. The genomes of all isolates were fully sequenced and compared at the nucleotide level. This contrasts with typing methods previously used in epidemiological studies (25, 39). We circumvented the absence of a consensus threshold for clonal isolate identification from genome-based data by performing a two-step analysis of the genomes. First, we clustered the isolates with cgMLST and grouped all isolates using a threshold (30 alleles of difference) higher than that found in the literature (15 alleles of difference) to avoid missing any clonal isolates (27). Second, we called variants between isolates within a group and performed a second clustering to access clonal strains with a threshold of seven SNPs of difference (Supplemental Fig. 3). This threshold was experimentally optimized (see Supplemental Fig. 2), consistent with previous studies (6–10 SNPs), and is fully compatible with the evolution rate of the bacterial pathogen (40).

Conclusions

Genome-based typing revealed the contamination of patients by PA isolates originating from sink traps to be rare (1.5%) in a MICU with sink trap bleaching measures and that 7.7% of the patients acquired PA from cross-contamination.

List Of Abbreviations

cgMLST, core genome multilocus sequence typing

ICU, intensive care unit

MALDI-TOF, matrix assisted laser desorption ionization - time of flight

MICU, medical intensive care unit

MLST, multilocus sequence typing

PA, *Pseudomonas aeruginosa*

SNP, single nucleotide polymorphism

Declarations

Ethics approval and consent to participate. This study is exempt from the need for ethical approval under local law as per advise received from the Human Protection Committee, East Area II, Besançon.

Consent for publication. Not applicable

Availability of data and materials. The datasets generated during the current study are available in the NCBI repository as the BioProject PRJNA788732.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. C.C., X.B., B.V., and D.H. designed the study; C.C., M.B., and B.V. performed the research; B.V. contributed analytical tools; C.C., G.P., B.V., and D.H. analyzed the data; and C.C., X.B., B.V., and D.H. wrote the paper.

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Figures

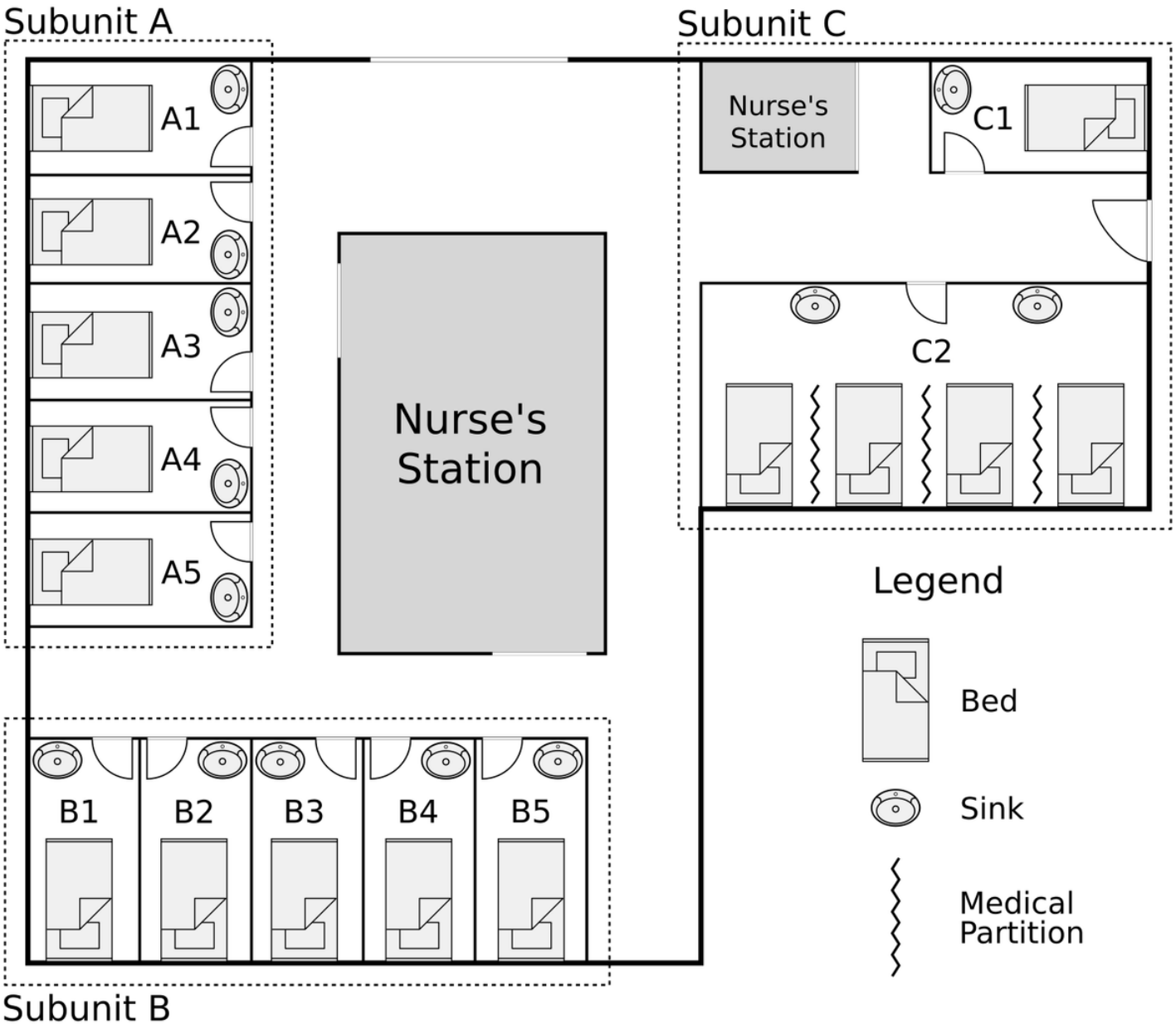

Figure 1

Layout of the medical intensive care unit at Besançon University Hospital (France). Subunits A and B are each composed of five rooms, with each room containing one bed and one water point of use. Subunit C is composed of two rooms, room C1 containing one bed and one water point of use and room C2 containing four beds and two water points of use.

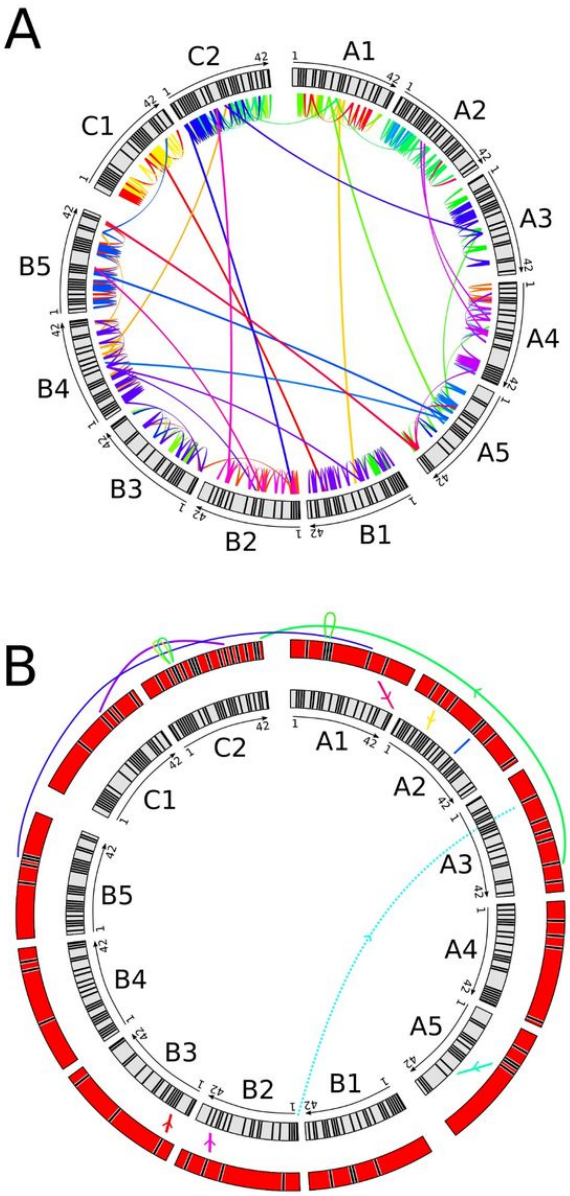

Figure 2

Representation of the transmission routes of *P. aeruginosa* between sink traps and patients in the MICU. Each sector represents a room with the chronology of PA isolation from week 1 to week 42 in a clockwise direction, with the black bars showing PA-positive samples in a given week. The gray circle represents the sink trap isolates and the red circle the human isolates. Each colored link connects two isolates for which the genomes clustered at a threshold of seven SNPs. Isolates from the same cgMLST cluster are connected with a link of the same color. The arrow in the link shows the orientation of the transmission, when determined. When a clone was repeatedly found in a sink trap, we only considered its first appearance to identify the potential links of transmission. (A) Transmission of PA within and between sink traps. (B) Transmission of PA from and to patients. The dotted link between the inner grey and outer red circles indicates the contamination of a patient in room A3 with a PA from the sink trap of room B2. The six links between the outer red and inner grey circles indicate sink traps contaminated with PA of human origin.

Supplementary Files

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Association of Secondary and Polymicrobial Nosocomial Bloodstream Infections with Higher Mortality

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The objective of this study was to characterize microbiological factors independently associated with higher mortality rates following nosocomial bloodstream infection. All patients admitted to the University of Iowa Hospitals and Clinics between 1 July 1989 and 30 June 1990 who developed a nosocomial bloodstream infection were included. The crude in-house mortality for the 364 patients with nosocomial bloodstream infections was 33 %. These deaths accounted for 25 % of all in-hospital deaths. Significant risk factors for death from bloodstream infection included diagnoses of cancers and diseases of the cardiovascular and respiratory systems ($p < 0.01$). Neither previous surgery nor neutropenia was associated with higher mortality rates. Whereas the crude mortality rates associated with gram-negative (33 %) and gram-positive (31 %) bloodstream infections were similar, that associated with fungemia was higher (54 %, $p < 0.02$). The mortality associated with secondary bloodstream infections (46 %) was higher than that associated with primary bloodstream infections (28 %, $p < 0.001$). Furthermore, polymicrobial infections had a worse prognosis than infections from which a single pathogen was isolated ($p < 0.05$). A multivariate, logistic regression model identified four variables that independently predicted mortality ($p = 0.025$): age (OR 1.01 per year; CI95 1.00–1.02); cancer (OR 2.35, CI95 1.26–4.37) or diseases of the cardiovascular or respiratory systems (OR 2.20, CI95 1.04–4.67); polymicrobial infection (OR 2.34; CI95 1.21–4.53); and secondary bloodstream infection (OR 2.46; CI95 1.50–4.02). The last variable was the strongest independent predictor. Our study demonstrates the importance of microbiological factors in the outcome of nosocomial bloodstream infections.

Bloodstream infections are associated with a crude mortality that approximates 35 %, ranging from 30 to 45 % in the largest series (1–7). In studies comparing the mortality rates associated with community-acquired versus hospital-acquired bacteremia, the latter was associated with the higher rates (5, 8–12). Surely, the underlying diseases contribute importantly to the crude mortality from bloodstream infection. The concept of attributable mortality has been applied to nosocomial bloodstream infections to define the mortality rate directly attributable to the infection and apart from the underlying diseases. The attributable mortality of bloodstream infection has averaged 27 % in six specifically designed studies, with point estimates ranging from 14 to 38 %, depending on the offending species (13).

Several studies have reported that the mortality of bacteremia also varies with the source of infection (1, 7, 14, 15); in most series, bacteremia complicating lower respiratory tract or intraabdominal infections has been shown to be more frequently associated with a fatal outcome than bacteremia secondary to other sites. Furthermore, polymicrobial bloodstream infections have been associated with higher mortality rates than monomicrobial infections (5, 7, 8, 16), even in series including only cancer patients (10, 17) or those examining specific organisms (18, 19).

Although most studies have suggested that distinct microbiological factors may influence the outcome of the infection, few have attempted to

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define the respective role of such factors after controlling for confounders, particularly underlying diseases or other demographic variables that may affect the prognosis. The results of our study demonstrate that microbiological factors independently affect the outcome of nosocomial bloodstream infection.

Patients and Methods

Setting. The University of Iowa Hospitals and Clinics (UIHC) is a 900-bed tertiary health care center serving Iowa and the border areas of surrounding states. Approximately 25,000 patients are admitted annually for a mean hospital stay of seven days. Prospective hospital-wide surveillance for nosocomial infections began in 1976 at UIHC. There is one infection control practitioner per 200 beds. Recent studies using standard methods have indicated the sensitivity and specificity of this hospital-wide reporting of nosocomial infections to be 81 and 97 %, respectively (20).

Methods. All patients admitted to the UIHC between 1 July 1989 and 30 June 1990 who developed a nosocomial bloodstream infection were included in the study. Patients with bloodstream infections were identified through the nosocomial infection surveillance program according to standard definitions (21). These definitions combine specific clinical and laboratory findings (21): positive blood cultures are always associated with significant signs of sepsis, thus blood culture contaminants were excluded. Additional chart reviews were performed for data completion when necessary.

The objective of the study was to characterize the microbiological factors predictive of in-hospital mortality. The demographic variables studied were age, sex, underlying disease and surgery (defined as admission to the operating room). The underlying disease was defined as the primary diagnosis on admission based on the International Classification of Diseases (ICD-9) classification. Five groups of diseases were categorized as follows: cancer patients; burn and trauma patients; diseases of the cardiovascular or respiratory system; diseases of the digestive or urogenital system; and other diseases. Neutropenia was defined as ≤ 500 granulocytes/mm^3.

Microbiological Characteristics and Definitions. The microbiological variables studied were the type of infection (primary vs. secondary bloodstream infection), the nature of the bloodstream infection (monomicrobial vs. polymicrobial) and the class of microorganism involved (gram-positive bacteria, gram-negative bacteria, fungi). Primary bloodstream infection refers to bacteremia for which there was no documented distal source (21). The term includes those episodes resulting from intravenous or arterial line infections. Secondary bloodstream infection defines an infection that develops subsequently to a documented infection with the same microorganism at another body site (21). Polymicrobial bloodstream infection refers to infections in which more than one microorganism was recovered from the blood culture within a 48 h period (2). Roberts (22) has recently proposed the use of this definition. Most of our patients with polymicrobial infection (44/48) had positive blood cultures on the same day. We defined subsequent episodes of bloodstream infection as those occurring more than 48 h after the initial positive blood culture.

Outcome Variable. In-hospital mortality was used as the measure of outcome. In the event that the same patient developed more than one episode of bloodstream infection, only the first episode was considered in the analysis, except when death occurred more than 30 days after bacteremia. In the latter situation, mortality of the subsequent episode was considered. We excluded from the analysis patients whose blood cultures were obtained post-mortem.

Statistical Analysis. Characteristics of patients who died but survived bloodstream infection were compared in a univariate analysis, using two-sample tests for binomial proportions. We used the Yates-corrected chi-square test. Odds ratios (OR) and 95 % confidence intervals (CI95) were based on the method proposed by Miettinen (23). The value for alpha (p) was set at ≤ 0.05 to be considered statistically significant. The model for the binary response variable (death) was built by using logistic regression with seven independent variables (age, sex, underlying disease, neutropenia, primary/secondary bloodstream infection, class of microorganism and uni/polymicrobial infection); two of the variables had either three (class of microorganism) or five (underlying disease) categories. All statistical analyses were carried out using the procedure Logist in the Statistical Analysis System (SAS, version 6).

Results

Type of Infection and Microbial Etiology. Three hundred sixty-four patients developed 412 episodes of bloodstream infections at UIHC in 1989–1990. In the same period, 20,274 patients were admitted. Thus, the rate of bloodstream infection was 1.8 per 100 patients admitted or 1.99 episodes per 1,000 patient-days of care. There were 283 episodes of primary bloodstream infection, accounting for two-thirds of the infections (69 %). Of these, 45 episodes were polymicrobial (16 %). Secondary bloodstream infections constituted a third (31 %) of the total number of bloodstream infections, and 10 % of them were polymicrobial.

Overall, gram-positive organisms were involved in 271 (66 %) and gram-negative organisms in 158 (38 %) of the 412 infectious episodes (Table 1). Fungi were involved in 9.7 % of all infections (40/412); candidemia alone accounted for two-thirds of those, or 6.5 % of all bloodstream infection episodes. Overall, coagulase-negative staphylococci constituted the leading nosocomial pathogens, responsible for 33 % of all bloodstream in-

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Table 1: Leading pathogens responsible for 412 episodes of nosocomial bloodstream infections at the University of Iowa Hospitals and Clinics, 1989–1990.

Primary bloodstream infections (n = 283 episodes, 324 isolates)	Percent	Secondary bloodstream infections (n = 129 episodes, 145 isolates),	Percent
Coagulase-negative staphylococci	35	Staphylococcus aureus	18
Staphylococcus aureus	12	Coagulase-negative staphylococci	16
Escherichia coli	8	*Serratia marcescens*	14
Enterobacter spp.	6	*Escherichia coli*	11
Klebsiella spp.	6	*Candida albicans* and *Candida* spp.	10
Enterococci	6	*Pseudomonas aeruginosa*	10
Streptococci (non enterococci)	5.5	*Klebsiella* spp.	6.5
Candida albicans and *Candida* spp.	4.3	Streptococci (non enterococci)	6
Pseudomonas aeruginosa	2.5	Enterococci	4.8
Serratia marcescens	2	*Enterobacter* spp.	3.8

fection episodes. *Staphylococcus aureus* was the second most common pathogen, causing 16 % of the infections, followed in order of importance by *Escherichia coli* (9.5 %), *Klebsiella* spp. (6.8 %), non-enterococcal streptococci (6.5 %), *Enterobacter* spp. (6 %), *Serratia marcescens* (6 %), enterococci (5.8 %) and *Pseudomonas aeruginosa* (5 %). The stratification highlights the relative importance of several microorganisms responsible for secondary bloodstream infections (Table 1). For example, the relative proportions of *Serratia marcescens* (14 %) and *Pseudomonas aeruginosa* (10 %) secondary bacteremia were seven and four times higher, respectively, than those of primary bacteremia (2 and 2.5 %, respectively).

The three leading sources of 114 episodes of secondary bloodstream infection were lower respiratory tract infections (24 %), surgical wounds (24 %) and urinary tract infections (19 %). Importantly, among urinary tract infections 19/22 (86 %) were related to the presence of a Foley catheter. Finally, among the 283 episodes of primary bloodstream infection, at least 49 (17 %) were related to a documented infection at the skin insertion site of an intravenous or arterial line.

Factors Associated with Mortality. Among the 364 patients who acquired a nosocomial bloodstream infection within the study period, 121 (33 %) died. Importantly, the overall hospital mortality at the UIHC was 2.38 per 100 admissions: 483 patients of the 20,274 admitted. Thus, the crude mortality of nosocomial bloodstream infection accounted for 25 % of the total hospital mortality.

The crude mortality associated with nosocomial bloodstream infection varied with age (Table 2). In this study the odds ratio for dying from the infection was 1.016 (CI95 1.01–1.02) per year of age with the corresponding parameter estimate: 0.01587 ± 0.005. The formula to translate the odds ratio to a definite age is $e^{0.01587 \times age \pm 1.96\,(0.005)}$, with the translation for a 10-year-old vs. a 70-year-old patient as follows: $e^{0.01587 \times 10 \pm 1.96\,(0.005)}$ and $e^{0.01587 \times 70 \pm 1.96\,(0.005)}$, i.e. 1.17 (CI95 1.16–1.18) and 3.03 (CI95 3.00–3.06), respectively. Sex did not affect the prognosis of bloodstream infection. A total of 108 patients had surgery before the episode of bloodstream infection, of whom 28 died (26 %). Surgery before the development of nosocomial bloodstream infection was not associated with an increased risk of death in this study (OR 0.62, CI95 0.37–1.01).

We stratified the patients according to the primary diagnosis to account for the effects of underlying disease in the analysis (Table 2). One-third (31 %) of our population had cancer, with an associated crude mortality in those who acquired a nosocomial bloodstream infection of 40 % (46/114). This rate was different from that of patients without cancer (75/250, 30 %) but did not reach the statistical threshold ($\chi^2 = 3.32$, $p = 0.07$). Importantly, the mortality rates were similar for leukemics and non-leukemics among cancer patients. Eighty-one of 114 cancer patients (71 %) had neutropenia before or at the time bloodstream infection occurred, compared to 13 patients with non-malignant conditions. The mortality rate among neutropenic cancer patients (38 %) was similar to that among non-neutropenic cancer patients (45 %, $p = 0.63$) as well as that among neutropenic (44 %) and non-neutropenic patients without cancer (29 %, $p = 0.45$). Considered separately, the estimated odds ratios for cancer patients and for patients suffering from diseases of the cardiovascular or respiratory system were significantly higher than those of

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Table 2: Factors associated with the mortality of nosocomial bloodstream infections as determined by univariate analysis.

	Total no. of patients (n = 364)	No. (%) of deaths (n = 121)	Odds ratio	CI95	p value
Age	364	121 (33)	1.016	1.01–1.02	< 0.001
Sex					
Male	195	65 (33)	1.00		
Female	169	56 (33)	0.99	0.64–1.53	0.97
Underlying disease					
Cancer	114	46 (40)	2.31	1.28–4.16	0.005
Leukemia	63	25 (40)			
Non-leukemia	51	21 (41)			
Trauma / burns	30	7 (23)	1.04	0.40–2.71	0.32
Diseases of the cardiovascular and pulmonary systems	67	29 (43)	2.61	1.34–5.06	0.005
Diseases of the gastrointestinal and urogenital systems	47	15 (32)	1.60	0.74–3.44	0.96
Other	106	24 (23)	1.00		
Surgery					
No previous surgery	256	93 (36)	1.00		
Previous surgery	108	28 (26)	0.62	0.37–1.01	0.07
Type of infection					
Primary bloodstream infection	250	69 (28)	1.00		
Secondary bloodstream infection	114	52 (46)	2.20	1.39–3.49	< 0.001
Class of microogranism					
Gram-positive bacteria	214	66 (31)	1.00		
Gram-negative bacteria	122	40 (33)	1.09	0.68–1.76	0.98
Fungi	28	15 (54)	2.59	1.17–5.74	0.019
Type of bacteremia					
Monomicrobial	316	99 (31)	1.00		
Polymicrobial	48	22 (46)	1.85	1.00–3.43	0.049

CI95 = 95 % confidence interval.

patients with other underlying conditions (OR = 2.31 and 2.61, respectively; Table 2).

We reviewed the microbiological variables associated with mortality after nosocomial bloodstream infections. Whereas mortality rates associated with gram-positive and gram-negative bacteremia were similar (31 % and 33 %, respectively), fungemia was associated with a significantly higher mortality (54 %, $\chi^2 = 4.70$, $p = 0.03$). The exposure odds ratio for fungemia was 2.59 (CI95 1.17–5.74, $p < 0.02$). The mortality associated with secondary bloodstream infections (46 %) was significantly higher than that associated with primary infections (28 %, $p < 0.001$). Among the former, bloodstream infections associated with the higher mortality rates were those complicating lower respiratory tract infections (67 %) and gastrointestinal tract infections (73 %). Secondary bacteremia complicating wound infections was associated with a lower mortality rate (15 %).

Thirty-one percent of the patients (99/316) with monomicrobial bloodstream infections died, whereas 46 % of the patients (22/48) with polymicrobial infections died. Thus, the mortality associated with polymicrobial bloodstream infection was significantly higher than that associated with monomicrobial infection (OR = 1.85, CI95 1.00–3.43, $p < 0.05$). Moreover, although not statistically significant, secondary polymicrobial bloodstream infections had a higher mortality (55 %) than primary polymicrobial infections (43 %).

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Microbiological Factors as Independent Predictors of Mortality. A model predicting mortality from nosocomial bloodstream infection was derived from the multiple logistic regression procedure. This analysis indicated that the best predictor of bloodstream infection mortality in the population studied was a model composed of four of the seven variables entered. Those variables were age ($p = 0.011$), underlying disease, and secondary ($p < 0.001$) and polymicrobial ($p = 0.013$) bloodstream infection. Among underlying diseases, cancer (OR = 2.35, CI95 1.26–4.37, $p = 0.007$) and diseases of the cardiovascular or respiratory system (OR = 2.20, CI95 1.04–4.67, $p = 0.039$) were the two conditions that independently affected the outcome of nosocomial bloodstream infection. The estimated odds ratios for the two microbiological factors that independently predicted mortality were 2.46 (CI95 1.50–4.02) for secondary bloodstream infection and 2.34 (CI95 1.21–4.53) for polymicrobial infection. The class of microorganism causing the infection did not appear as an independent predictor of fatality.

The application of the logistic equation derived from the model in the following example illustrates the importance of the microbiological factors for the outcome of nosocomial bloodstream infection. For example, with our model, the probability of death for a 60-year-old cancer patient with a single organism in a primary bloodstream infection would be 36 % (Pr(death) = log (p/1–p) = -2.1653 + 0.8988(0) + 0.8524(0) + 0.0126(60) + 0.8547(1)). In the event the same individual developed a polymicrobial secondary bloodstream infection, the probability of death would be 77 % (Pr(death) = log (p/1– p) = -2.1653 + 0.8988(1) + 0.8524(1) + 0.0126(60) + 0.8547(1)).

Discussion

In our study population the crude mortality associated with nosocomial bloodstream infection was 33 %, accounting for 25 % of the total hospital mortality. Our data were confirmed with data independently examined over a three-year period (1987–1990) at our institution, during which time nosocomial bloodstream infections accounted for 22 % of the total hospital mortality (D. Pittet et al., 31st ICAAC, Chicago, 1991, Abstract A800). In the present study the crude mortality of bloodstream infection varied with age, underlying disease and microbiological variables. Cancer and diseases of the cardiovascular or respiratory system were the only underlying disease conditions that significantly affected the outcome of nosocomial bloodstream infection. Importantly, both secondary and polymicrobial bloodstream infections proved to be independent predictors of mortality.

Several authors have recently reviewed the topic of polymicrobial bacteremia (6–9, 24, 25). Although comparison across these studies is difficult because of the diversity of methods and definitions used (22), the proportions of polymicrobial bacteremia varied from 6 to 22 %, ranging from 6 to 18 % in the largest series (2, 7, 9, 26). Higher rates have recently been reported in the geriatric population (27, 28) as well as in infants in neonatal intensive care units (25 %) (16). In the present study 58 episodes (14 %) of bloodstream infection were polymicrobial; among them, 45 (78 %) developed in the setting of a primary and 13 (22 %) in the setting of a secondary bloodstream infection.

Both early and more recent series suggested that polymicrobial bacteremia has a higher mortality than monomicrobial bacteremia (7, 10, 11, 26, 29). In the study by Weinstein and colleagues (8, 26), polymicrobial bacteremia was significantly more likely to be hospital-acquired than monomicrobial infection ($p < 0.005$) and was associated with higher mortality (63 % vs. 38 %, $p < 0.05$). Deaths directly related to bloodstream infection were two-fold higher in polymicrobial versus monomicrobial episodes. Polymicrobial bacteremia is a significant problem in patients with malignancies (10, 17, 19, 30, 31) and is associated with higher mortality rates (19, 31). In the study by Whimbey and colleagues (31), the overall mortality of single-organism bacteremia was comparable in non-neutropenic (37 %) and neutropenic (29 %) patients. In contrast, neutropenic patients with polymicrobial bloodstream infection had an extremely high mortality rate (80 %). In the present study mortality was similar in neutropenic and non-neutropenic cancer patients but was significantly higher in patients with polymicrobial (67 %) vs. monomicrobial (33 %, $\chi^2 = 4.64$, $p < 0.05$) bloodstream infections. Noteworthy, neutropenia was not an independent predictor of mortality in the logistic regression analysis, underscoring the role of microbiological factors in the outcome of bloodstream infections.

In a recent analysis of 1,972 positive blood culture episodes over a three-year period (1984–1987) at the Vancouver General Hospital (7), organisms

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associated with lower mortality (*Escherichia coli*, coagulase-negative staphylococci) in monomicrobial bacteremia were associated with higher mortality in polymicrobial bacteremia, similar to the crude mortality associated with virulent organisms. Overall, polymicrobial sepsis was always associated with higher mortality, even after stratification for the type of microorganism (7). In the present study patients with polymicrobial infections had a two-fold increase in the risk of dying from the infection, independent of their underlying disease or of the class of causal microorganism. Our results extend previous observations by using an appropriate statistical approach that controls for other confounders of mortality from bloodstream infection.

Secondary bacteremia was associated with a higher mortality rate, independently of the class of microorganism involved. Among the three leading sources of infections, infections of the lower respiratory tract and urinary tract were associated with mortality rates as high as 67 and 45 %, respectively, if complicated by secondary bloodstream infection. Krieger and colleagues (32) previously provided direct estimates of the risk for secondary bloodstream infections among patients with nosocomial urinary tract infections; importantly, patients with infections due to *Serratia marcescens* were more likely to develop secondary bacteremia compared to those with infections due to other organisms; the median interval between urinary tract infection and bacteremia was 24 days. Our study did not include a sufficiently large number of secondary infections to allow a valid statistical analysis of the role of each separate source of infection as potential independent predictors of mortality in cases of secondary bloodstream infection. Such an analysis would have required a minimum of 380 episodes of secondary infections.

Several studies have previously suggested that the mortality of secondary bloodstream infection might be higher than that associated with primary infections (7, 15, 26, 27, 33). As recently suggested (7), both the sources of infection and the class of microorganisms causing the infection need to be considered in analyzing the mortality from bacteremia. In the study by Roberts and colleagues (7), striking differences in mortality rates were apparent when the source of secondary bloodstream infection was considered. Pneumonia, gastrointestinal tract and multiple sources of infection were associated with the highest mortality rates, ranging from 43 to 55 %. This study, however, included both community- and hospital-acquired bloodstream infections and did not attempt to control for underlying conditions in the analysis. In the study by Gatell and colleagues (15), bacteremia arising from high-risk sources, defined in their study as intraabdominal, lower respiratory tract or primary bloodstream infection not related to intravenous lines, was shown to predict independently a poor outcome in the logistic procedure performed (OR 2.7). Our findings confirm this observation: patients who developed secondary versus primary bloodstream infections had odds ratio of death of 2.46 after correcting for other microbiological variables.

Secondary bloodstream infections, accounting for one-third of all bloodstream infections, are potentially preventable (12). The proportion of secondary bacteremia varied from 47 to 80 % in three recent studies focused on nosocomial bloodstream infections (15, 27, 33), urinary tract infection being the leading source. Infections related to the use of urinary catheters were not specified but certainly accounted for most of these infections, as reported in our series. Secondary bloodstream infections complicating catheter-related urinary tract infection constitute a target for prevention, and data presented here should prompt infection control practitioners to study ways to improve their management. In the present study the time between the documentation of primary-site infections and secondary bloodstream infection averaged 6.4 days, ranging from two to 42 days. Studies need to readdress the question of the appropriateness of the management of nosocomial infections, particularly those associated with high rates of secondary bloodstream infection. Our results suggest that optimal management of distal sources may constitute an effective way to prevent the development of secondary infections and would thus reduce the cost, morbidity and mortality of nosocomial bloodstream infections. As a result, total hospital mortality would be reduced.

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Infections in children and adolescents with Acute Leukemia

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ABSTRACT

The incidence and spectrum of infections in pediatric leukemia patients vary depending on the type of leukemia. AML, which involves intensive chemotherapeutic regimes, leads to profound and prolonged neutropenia, making children more susceptible to infection. The intensity of chemotherapy, the duration of neutropenia, and antimicrobial prophylaxis implementation all affect the risk of life-threatening infections. In AML studies have shown that during intensive treatment, children experience more than one episode of infection, with majority being bacteremias. Viridans group *Streptococci* comprises around 20% of all isolates. Infection related deaths range from 5.4% to 7.3% during chemotherapy being more frequent on intensive phase. Invasive fungal infections (IFI) were highly associated with mortality specially caused by *Aspergillus* spp. In ALL induction and consolidation phases posing a higher risk for infections due to severe neutropenia. Infection related mortality in ALL is 2–4%, and infections are the main cause of treatment related deaths. IFI are significant concern with risk factors including age and treatment intensity. High risk include older children, delayed response during early induction therapy and corticosteroid administration. In summary, while survival rates for childhood leukemia have improved, the risk of infections, particularly bacterial and fungal infections, remains a significant concern, especially during intensive treatment phases. Ongoing research is needed to better understand and manage these infections in pediatric leukemia patients

1. Introduction

Survival rates for childhood leukemias have been on a consistent upward in high-income countries (HICs), trend primarily due advances in cancer treatment, risk assessment, and support care [1,2]. Overall survival rates of approximately 83% to 94% have been achieved for pediatric acute lymphoblastic leukemia (ALL) [3] and 65% to 70% for pediatric acute myeloid leukemia (AML) [4]. It is important to mention that the chance of a cure is lower low-income countries (LICs) probably due to more advanced stage at diagnosis, differences in population biology, higher rates of relapse, abandonment of therapy, and higher rates of death from toxicity [5].

However, the intensification of combination chemotherapy for purposes of eliminating leukemic cell populations leads to substantial immunosuppression increasing the rates of infections complications.

Infections are an important cause of morbidity and mortality for children and adults with hematological malignancies especially acute leukemia. These patients are at particularly high risk of infection, likely related to the intensity of their therapy resulting in repeated episodes of prolonged and profound neutropenia. Infections not only lead to increased mortality but also extend hospital stays, hinder the administration of chemotherapy, impact the quality of life, and raise healthcare resource utilization. The majority of microbiological documented infections in children with hematological malignancies are bacterial, the most frequent being bacteremias. Limited data exists on documented systemic viral infections in children undergoing therapy for acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). Sung et al., in their analysis of 492 children with AML, reported that 7.5% to 8.8% of virus infections were associated with the treatment phase, with a higher prevalence during the consolidation phase. Similar findings were observed in other series. With advancements in diagnostic techniques, there has been an observed increase in viral infections, as demonstrated in a recent study by Zawitkowska, which reported an incidence of 18.4% in patients with ALL. [6–8]. Virus infections can be very serious and can be complicated by secondary bacterial and fungal pathogens.

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2. Incidence and spectrum of infections according with hematological malignancy

The occurrence of severe infectious complications in pediatric leukemia patients has been notably linked to the leukemia type, with AML displaying a higher incidence in comparison to ALL [9]. Nevertheless, as advances in treatment, including target therapies, with the aim of enhancing survival rates, it becomes imperative to reevaluated the risk of life-threatening infections. This reassessment should be predicated on several factors, including the intensity of chemotherapy, the duration of neutropenia, corticosteroids use and the implementation of antimicrobial prophylaxis.

a) Acute Myeloid Leukemia (AML):

Despite advances in therapy over the past decades, overall survival for children with acute myeloid leukemia has not exceeded 70%. In relation to the host and the disease, advances in cytogenetics and molecular profiling, as well as improvements in detection of minimal residual disease after induction therapy, have informed risk stratification. The treatment of childhood AML continues to be based primarily on intensive, conventional chemotherapy. However, recent trials focus on limiting treatment-related toxicity through the identification of low-risk subsets who can safely receive fewer cycles of chemotherapy, allocation of hematopoietic stem-cell transplant to only high-risk patients and optimization of infectious and cardioprotective supportive care.

In understanding the impact of different lines of treatment, during the last two decades, the intensification of antileukemic treatment has improved the long-term survival of both children and adults with AML. The cumulative doses of cytarabine were successively increased, and more effective anthracyclines such as idarubicin were given in the Acute Myeloid Leukemia-Berlin-Frankfurt-Muenster (AML-BFM) clinical trials. In this retrospective analysis the AML-BFM protocols between 1987 and 2012, the contribution of intensified first-line treatment, supportive care and salvage therapy were fundamental to this success. The BFM group used cranial radiotherapy until 2009 and maintenance therapy until the BFM 2012 protocol, obtaining results comparable to the groups that did not use these two components of therapy [10,11].

Therapy for AML targets the myeloid cell lineage, producing profound and prolonged neutropenia and it is therefore one of the most intensive chemotherapeutic regimens used in pediatric oncology. Consequently, many children experience one or more infectious complications during AML treatment [7,12].

The incidence and severity of infections are contingent upon the treatment protocol employed and the specific phase of treatment. Studies have been conducted with the objective of establishing the incidence and the risk of infection, subsequently informing more effective management strategies.

There was a significantly reduced infectious related mortality in AML-BFM 04 compared to historic data of the AML-BFM 93 e 98 studies, especially in the first 42 days (early deaths group) or after achieving clinical remission (CR).

On BFM-93, 295 episodes of infection were documented among 285 children receiving first induction therapy and 85/285 (29%) experienced a microbiologically documented infection, Viridans group *Streptococci* (VGS) comprised 56/252 (22%) of all isolates [13].

Lately in a multi-institutional clinical trial AML-BFM 2004, study by Bochennek et al., 405 patients experienced 1326 episodes of suspected or confirmed infection during intensive treatment, averaging 3.3 infectious complications per patient. These infections included fever of unknow origin (56.1%), clinically and microbiologically documented infections (17.5% and 32.4%, respectively). Among microbiologically documented infections 71.6% were bacteremia, with Viridans group *Streptococci* (VGS) responsible for over 30% of these cases. Invasive fungal infections occurred in just 3% of cases, and 1.5% of patients died due to infections, mainly from Gram negative pathogens or invasive aspergillosis [6].

The incidence of infectious complications was slightly higher compared to a previous study (AML-BFM 93). However, infection-related mortality was significantly lower (1.5% versus 5.4%) [6].

Reducing infectious-related mortality, particularly in non-responsive patients, enables the consideration of second-line therapy. This means that severely ill patients are now eligible for hematopoietic stem cell transplantation (HSCT), thereby enhancing the overall outcomes for these individuals [11].

Prospective trials of transplantation in children with AML suggest that overall, 60% to 70% of children with HLA-matched donors available who undergo allogeneic HSCT during their first remission experience long-term remissions with the caveat that outcome after allogeneic HSCT is dependent on risk-classification status [14].

The better supportive care contributed to reduce the early death rate and partly infectious-related deaths. These findings have important implications for the future, because intensification of anthracycline- and cytarabine-based chemotherapy will most likely not result in a major reduction of relapse or nonresponse. Although standard treatment of AML usually includes high-dose induction therapy, recent genomic studies have shown that applying regimens with reduced-intensity inductions can achieve long-term remissions with less treatment-related toxicity. Thus, the strategy of administering a minimally myelosuppressive regimen for the first induction remission and risk-adapted therapy for subsequent courses has, as its main objective, to avoid treatment-related early mortality [15].

On CCG 2961 protocol, 297/492 (60%) children experienced at least one microbiologically documented infection during induction (phase 1); 300/407 (74%) on phase 2 and 176/248 (71%) on phase 3. In induction, 191/297 (64%) of microbiologically documented infection were Gram positive organism, with 91/ 191 (18%) being Coagulase negative Staphylococci and 51/297 (17%) due to VGS. The rate of VGS bacteremia was higher in the fludarabine based regimen compared with idaDCTER (idarubicin, daunomycin, cytarabine, thioguanine, etoposide, and dexamethasone) [7]. On 2009, 872 children with AML on CCG 2891 protocol were evaluated. Of these 872 patients, 678 were randomized to intensive versus standard timing induction and 452 were allocated or randomized to chemotherapy, autologous SCT or allogeneic SCT in Phase 2. The results were similar with more infections on intensive phase of treatment and a significance increase in VGS infections associated with intensive timing induction [16]. It is noteworthy in both studies with large number of patients that the prevalence of infection varies depending on type of treatment with high risk of infections consisting in intensive time induction. (Table 1).

Gram negative infections were less common, the two most common Gram negative bacteria on both BFM-93 and CCG 2961 were *Escherichia coli* and *Pseudomonas aeruginosa*. In AML BFM 2004 *E. coli* and *Klebsiella pneumoniae* were more prevalent, while *Pseudomonas aeruginosa* ranked as the third most common agent. This suggest that maintaining anti-pseudomonal coverage remains a vital element of empirical antibacterial therapy in this population [6,12,16].

AML BFM 2004 Gram-negative pathogens were significantly more often isolated in consolidation and intensification as compared with induction [6].

It is crucial to highlight the observed disparity in the incidence of fungal infections between two studies using the AML-BFM 93 protocol and CCG 2961 protocol 3% vs 21% (Sung 2007 and Bochennek 2016). This difference could be attributed, at least partially, to the administration of antifungal prophylaxis in 47% of the chemotherapy cycles on Bochenneck study. This prophylaxis primarily involved the use of antifungal agents with activity against molds, such as amphotericin B, voriconazole, or posaconazole [6,17].

Polymicrobial invasive infections in pediatric AML are more related to infection-related mortality than that caused by a single pathogen [7].

Sung et al. in 2012 conducted a systematic assessment of the severity of infections in AML, aiming to identify organisms deemed life-

Table 1
Characteristics of infectious episodes according with protocol used in patients with AML:.

Author/year	Protocol	Number of patients/nº infection	Type of infection	Agents				Mortality	Associated risk of mortality
Lehrnbecher 2004 [12]	BFM-93	304/ 855	FUO:61.2% CDI: 6.7% MDI:32.1%	Induction GP:57/252 (23%) VGS: 12/ 252 (5%) GN: 11/ 252 (4%)	Consolidation GP: 58/252 (23%) VGS:13/252 (5%) GN: 14/252 (5%)	HAM GP: 50/ 252 (20%) VGS:18/ 252 (7%) GN:8/252 (3%)	HAE GP: 38/252 (15%) VGS: 13/252 (5%) GN:16/252 (6%)	20 (6.6%)	18 (90%) pneumonia: (4) Streptococci (5) GN (4) *Aspergillus* spp
Bochenneck 2016 [6]	BFM-2004	405/ 1326	FUO:56% CDI:17.5% MDI: 32.4%	**High Risk (n = 243)** **GP:** 175/243(72%)- VGS: 32.5% **GN:** 68/243 (28%) **Fungus:** *Aspergillus* spp:7	**Standart Risk (n = 87)** **GP:** 65/87 (74.7%)- VGS: 33/87 (37.9%) **GN:** 22/87 (25.3%) **Fungus:** *Candida* spp: 2 *Aspergillus* spp:3			6 (1,5%)	(1) *Salmonella* spp (1) *Pseudomonas aeruginosa* (1)*Stenotrophomonas maltophilia* (3) Aspergilosis
Sung 2007 [7]	CCG 2961	492	MDI Phase 1: 60% Phase 2: 74% Phase 3: 71%	**Phase 1: Intensive timing (n = 492)** **GP:** 191/492 (39%)- VGS: 51/492 (10%) **GN:** 87/492 (18%) *Klebsiella* spp: 15/492 (3%) *E coli:* 18/492 (4%) *Pseudomonas* spp: 21/492 (4%) **Fungus:** 88/ 492 (18%)	**Phase 2 (n = 407)** **GP:** 205/407 (50%)- VGS:109/407 (27%) **GN:** 106/407 (26%) *Klebsiella spp:* 44/407 (11%) *E coli:* 15/407 (4%) *Pseudomonas* spp: 29/ 407 (7%) **Fungus:** 86/407 (21%)	**Phase 3** (n = 248) **GP:** 111/248 (45%)- VGS:49/ 207 (20%) **GN:** 69/248 (28%) *Klebsiella* spp: *19/ 248 (*8%) *E coli:* 14/248 (6%) *Pseudomonas* spp: 11/248 (4%) **Fungus:** 34/248 (14%)		**11% IRM** 31% *Aspergillus* spp 26% *Candida* spp 15.5% VGS	Age > 16 years Non- white ethnicity Underweight status
Sung 2009 [16]	CCG 2961	872		**Phase 1: Intensive timing** (n = 343) vs **standart time** (n = 335) More infections bacterial (53/343 15.5% VGS); fungal 94/343 (27.4%) both yeasts (65/343-19.0% vs. 28/335-8.4%, P < .001) and molds (40/343-11.7% vs. 5/335-1.5%, P < .001) and viral (48/343-14.0% vs. 13/335-3.9%, P < .001)	**Phase 2** **Chemotherapy n = 168** **Allo SCT n = 147** Chemotherapy was associated with more bacterial infections (95/168- 56.5% vs. 59/147-40.1%, P = .005) and more VGS bacteremia (27/168-16.1% vs. 4/147-2.7%; P < .001) compared with allogeneic SCT.			**3.6% (33/ 872):** 19 (58%): Bacterial infection 11 (33%) molds	Infection deaths more common during intensive timing in induction (5.5% vs. 0.9%; P = .004)

AML: acute myeloid leukemia; **IRM:** infection related mortality; **CCG:** Children's Cancer Group; **VGS:** Viridans Streptococci Group; **GP:** Gram positive; **GN:** Gram negative; **HAM:** high-dose cytarabine 3 g/m^2 every 12 h for 3 days and mitoxantrone 10 mg/ m^2 on days 4 and 5; **HAE:** high-dose cytarabine and etoposide; **SCT:** seam cell transplantation

threatening or fatal. They explored whether the intensity of induction or the type of intensification therapy influenced the proportion of such infections. The study revealed a significant increase in life-threatening or fatal infections during intensive timing (60.3%) compared to standard timing (37.3%) in phase 1 induction ($p < 0.0001$). Infections caused by Gram-positive bacteria, Gram-negative bacteria, and fungi were more likely to be classified as life-threatening or fatal during intensive timing. Specifically, infections caused by VGS, Pseudomonas spp, and Escherichia coli had a higher likelihood of being life-threatening or fatal during intensive timing. Molds were considered life-threatening or fatal irrespective of whether they occurred during standard or intensive timing induction [18].

In an attempt to reduce infections rates and, consequently, decrease morbidity and mortality in these children, several authors have sought to demonstrate the effectiveness of antibiotic prophylaxis. However, further studies are necessary to estabilish the true benefits. Long-term monitoring is essencial to assess the emergence of multiresistant pathogens, *Clostridioides difficile* infection, invasive fungal infection and impact on children's [19,20].

Castagnola E, 2010, performed a retrospective, multi-center study on the epidemiology of bacteremias and invasive fungal infections (IFI) in 240 children with AML and showed that more than half of them developed at least one episode of severe infection. Bacteremias were significantly more frequent than IFI. The epidemiology of IFI alone was influenced by the status (first diagnosis or relapse) of AML, and cumulative risk of the IFI were significantly higher during treatment for relapsing AML [21].

Invasive fungal infections (IFI) were highly associated with mortality specially caused by *Aspergillus* spp [7,16]. The true incidence of IFI in AML children is probably underestimated once the diagnostic is difficult to be made the majority of studies were done in adult populations [22].

A recent publication evaluated a total of 298 episodes of febrile

neutropenia in 78 patients with AML revealing IFI rates of 3% for proven, 2.6% for probable and 9.4% for possible. Aspergillosis accounted for the majority of IFI cases. Importantly patients with relapsed AML had higher risk of developing IFI and in multivariate analysis it was the most substantial risk factor associated to mortality. This highlights the crucial role of fungal infections in AML and underscores the potential benefits of antifungal prophylaxis in mitigating their occurrence [23].

Infectious deaths are relatively common in this population, with an infection-related mortality rate between 5.4% and 7.3% during chemotherapy [12].

There are limited studies assessing the infection rate in children with AML in Latin America. A recent study conducted in Mexico evaluated the incidence of IFIs in children with ANL and demonstrated a high IFI incidence compared with literature (11.6% vs 8.2%) [24,25].

It is of utmost importance to understand the local epidemiology and further studies on IFIs in this population are necessary.

In Brazil, whose data are still under analysis, there is an open prospective study, by the Childhood Acute Myeloid Leukemia Study Group – LMA Protocol 2021, with the proposal of two induction cycles with low doses of citrarabine, with lower toxicity and expectation of complete remission without loss of dose intensity by reducing the incidence of severe infections at diagnosis.

b) **Acute Lymphoid Leukemia (ALL):**

Acute lymphoblastic leukemia (ALL) occurs ~5 times more frequently than acute myeloid leukemia (AML), accounting for 75% of all childhood leukemia. [26]

The treatment related mortality in currently published studies is 2-4% mainly due to infections. [3,27]

The risk for infections varies by phase of ALL therapy during induction, and consolidation phases, the duration and depth of neutropenia are more severe than in maintenance. During the first two phases, patients are usually hospitalized, and bacterial and fungal infections are more frequent, often originating from the patients' own flora or nosocomial settings. The incorporation of genomic and molecular data in pediatric ALL will allow for additional refinements in risk stratification to enable the tailoring of treatment intensity. These data will also dictate the incorporation of target therapies into frontline treatment, as immunotherapies, in the hope of improving survival while decreasing treatment-related toxicity.

Medical Research Council (MRC) Working Party on Leukemia in Children UK National Acute Lymphoblastic Leukemia (ALL) Trial, UKALL2003, the 5-year cumulative incidence of infection-related mortality was 2.4% and accounted for 30% of all deaths and 64% of treatment-related deaths. [26]

A retrospective multicenter study in Poland analyzed 1363 patients with ALL treated under the ALL IC-BFM 2002 and 2009 protocols. The incidence of microbiologically documented infections was 53% (726/1363), with 36.4% (497/1363) being bacterial infections, predominantly Gram-positive (57.5%, 298/497). Fungal infections comprised 406 episodes (probable 42; proven 28; possible 337), ranging from 1 to 7 per person. In terms of patients, fungal infections were observed in 20% (278/1363) of the children. Among the 726 patients with documented infections, twenty children (2.75%) died due to sepsis, and eight children (2.9%) died due to fungal infections. [8]

Other study performed by Christensen et al. analyzed 1652 children with ALL who were treated according to the NOPHO-ALL92 protocol between 1991 and 2001. Out of 1652 patients, 56/1652 (3.4%) died due to complications of therapy, being 68% (38/56) caused by infections. [28]

The largest cohort of pediatric ALL related sepsis deaths was documented in a 2016 study involving 3126 patients from MRC UKALL 2003 trial. Out of a total of 249 deaths, sepsis emerged as the leading cause of treatment related mortality (TRM), accounting for 75 deaths, or 2.4% of all trial patients. Sepsis was prominently observed in both the induction and post-induction phases, with 48% of infection related deaths occurring during induction, 9.3% during consolidation; 22.6% during delayed intensification and 20% during maintenance. Notably an identifiable pathogen was found in 75% of infection related mortality (IRM) with bacterial infections (68%) being the most common, followed by fungal (20%) and viral (12%) infections. The 5-year cumulative incidence of IRM on UKALL 2003 (2.4%) was comparable to other trials in which IRM has been reported (1.7–2.3%) [26]

It is noteworthy to mention that while infections can occur at any point during the treatment for ALL, the risk is higher during the induction chemotherapy, particularly before achievement remission. This elevated risk is thought to be associated with the use of central venous catheters, mucositis, neutropenia and immunodeficiency induced by chemotherapy specially use of corticosteroids and bone marrow disease.

In Latin America, a recent retrospective cohort study was conducted, involving 259 children and adolescents diagnosed with ALL and treated with the Brazilian Cooperative Group of Treatment of Lymphoblastic Leukemia in Childhood -GBTLI–LLA 2009 protocol. Blood stream infection (BSI) were documented in 19.3% (50/259) of the patients, with the isolated microorganisms comprising 39 Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli* more prevalent), 21 Gram positive bacteria and four fungi. Among the isolated microorganisms, there was resistance in 17/64 of cases (26.5%). [29]

To mitigate the incidence of bacterial infections in this population, a potential strategy involving antibacterial prophylaxis has been emerging. However, this approach encounters limitations due to concerns about antibiotic resistance. Data on this practice in children are scarce, with few observational studies and two published randomized trials in children with ALL. Wolf et al.'s study (2017) evaluated 344 newly diagnosed ALL patients, comparing infection-related outcomes with no prophylaxis, levofloxacin prophylaxis, or other prophylaxis during induction therapy in the XVI study. Levofloxacin prophylaxis reduced infections but also lowered exposure to cephalosporins, aminoglycosides, and vancomycin, thereby decreasing the odds of *C. difficile* infection by over 95%. No increase in antibiotic-resistant organism infections was observed. Similarly, in Brazil, Dufrayer et al. (20 patients) demonstrated that its use did not elevate cephalosporin enterobacteriacae colonization rates or lead to *C. difficile*-associated diarrhea [19,30]. More studies are necessary to do any recommendation

Invasive fungal diseases need to be highlighted as a significant cause of infection in this group.

The risk of IFD can be influenced by various factors, including age and treatment intensity. An extensive analysis was conducted involving 6136 patients enrolled in the AIEOP-BFM ALL 2009 Phase III clinical trial. Among the 419 reported serious adverse events related to IFI, the incidence of proven/probable IFI was 3.8%. Through logistic regression, it was determined that children aged 12 years or older and those with more than 10% bone marrow blasts on day 15 were at significantly higher risk for IFD (both $p < 0.0001$). [31]

It is well reported that higher treatment intensity increases IFD risk, but notably in this study insufficient treatment response during induction therapy emerged as an independent risk factor for IFD, and corticosteroid administration during the first 6 weeks of treatment, impacting neutrophils and macrophages, should be considered. [31]

All these factors emphasize the need for a reassessment of the infection risk in ALL, and, in certain cases, the consideration of prophylactic measures, including those against bacterial and fungal infections. [19,20,25]

Risk-based treatment assignment is a key therapeutic strategy utilized for children with ALL, and protocols are designed for specific patient populations that have varying degrees of risk of treatment

failure. This approach allows children who historically have a very good outcome to be treated with less intensive therapy and to be spared more toxic treatments, while allowing children with a historically lower probability of long-term survival to receive more intensive therapy that may increase their chance of cure. The use of bortezomib was based on compelling biologic rationale, strong preclinical data, and encouraging safety and efficacy in relapsed T-ALL/T-LL in COG AALL07P1. [32]

The advent of effective immunotherapy is now changing therapeutic paradigms, providing options for patients in whom cytotoxic chemotherapy has proven ineffective or intolerable. Immunotherapies can be exquisitely target-specific, conferring more limited toxicity to normal tissues than traditional chemotherapy. Blinatumomab is a bispecific monoclonal antibody with one site for CD3 (T cells) and the other site for CD19 (present on most B-ALL cells). Thus, blinatumomab promotes the binding of the patient's own cytotoxic T cells to B lymphoblast. After initial reinduction chemotherapy, the use of blinatumomab instead of intensive cytotoxic chemotherapy as pre-HSCT consolidation has been shown to be associated with superior outcomes. [33]

c) **Down Syndrome and Leukemia**

Children with Down syndrome (DS) constitute a distinct genetic population who has a greater risk of developing acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) compared to their non-Down syndrome counterparts. In addition to a higher risk of relapse, patients with DS have been shown to have an increased risk of treatment-related mortality with traditional induction and consolidation chemotherapy, such that they have here to fore often been excluded from clinical trials in ALL or have received adapted treatment elements with reduced intensity Approximately 2% to 3% of childhood ALL cases occur in children with Down syndrome. ALL in pediatric patients with Down syndrome is characterized by a lower incidence of both favorable (e.g., ETV6::RUNX1 and high hyperdiploidy with favorable trisomies) and unfavorable (e.g., BCR::ABL1, KMT2A rearrangements, low hypodiploidy, t(9;22)(q34;q11.2) or t(4;11)(q21;q23)) biology and a near absence of T-cell phenotype [34].

Patients with Down syndrome have an increased risk of developing toxicities from treatment, including infections, mucositis, and seizures. In some studies, outcomes of children with Down syndrome and ALL and AML [35].

Because of the well-established increase in toxicity experienced by patients with Down syndrome, some ALL protocols (such as those of the COG) have de-intensified risk-based treatment for patients with Down syndrome and ALL to minimize exposure to the morbid components of therapy. While this treatment reduction strategy reduces the frequency and severity of toxicities, its impact on antileukemic outcomes is not yet known [35].

3. Prophylaxis in patients with Acute Leukemia (AML and ALL)

3.1. Bacterial prophylaxis

Antibiotic prophylaxis consists in an important strategy in reducing bacterial infections and associated complications in pediatric patients with acute leukemia (AL) [20,36]. Quinolones, notably due to their efficacy in adults, have been widely used in this group. However, concerns exist regarding their true efficacy, toxicity, and the emergence of antibiotic-resistant strains. The recommendation for pediatric use is based on limited evidence, emphasizing the need for a careful risk-benefit assessment [20]. A comprehensive 2022 meta-analysis encompassing diverse risk classes, chemotherapy protocols, and leukemia phases suggests that quinolone prophylaxis effectively reduces bloodstream infections (BSIs) and may decrease febrile episodes in AL settings. However, conclusive evidence on mortality impact is lacking, warranting further research [37].

While quinolone prophylaxis may contribute to reduced hospitalization and healthcare costs, its role in preventing *Clostridium difficile* infections and antibiotic exposure remains inconclusive [30,36,38]. Discrepancies in study findings suggest a potentially nonsignificant role for quinolones in these aspects, underscoring the need for validation in larger cohorts. The prophylaxis's positive impact on febrile episodes may enhance patient quality of life by shortening hospital stays, potentially contributing to cost-effectiveness.

A noteworthy consideration is the potential impact of quinolones on gut microbiota. Margolis et al.'s study highlights resistome modifications, indicating that levofloxacin (LVX) may elevate the risk of colonization with resistant bacteria. However, recent evidence also emphasizes the detrimental role of antibiotic-mediated dysbiosis, emphasizing the need for a nuanced understanding of these dynamics in future research [39].

The ECIL-8 (European Conference on Infectious in Leukemia) group does not recommend routine antibacterial prophylaxis for paediatric patients with AL (recommendation 1; grade D, level of evidence III) [40].

3.2. Fungal prophylaxis

Systemic antifungal prophylaxis is a vital strategy in the reduction of invasive fungal disease (IFD). An array of pediatric studies, encompassing both retrospective and prospective analyses, has delved into the efficacy of various mold-active and mold-nonactive agents. In a pivotal multicenter randomized trial involving 517 AML patients, caspofungin exhibited a noteworthy reduction in the incidence of proven or probable IFD in comparison to fluconazole (3.1% vs. 7.2%; HR 0.37 [95% CI 0.15–0.94]; p = 0.03). and proven or probable invasive aspergillosis (0·5% vs 3·1%, HR 0·16 [95% CI 0·02–1·27]; p = 0·046) There were 23 proven or probable invasive fungal disease events (6 caspofungin vs 17 fluconazole) included 14 molds, 7 yeasts, and 2 fungi not further categorized. It is crucial to note that this study's interpretation is constrained by premature termination arising from an unplanned interim analysis, precluding observations on overall survival impact [41].

Paediatric guidelines strongly recommend primary antifungal prophylaxis in patients who are at high risk of developing invasive fungal diseases to reduce disease related morbidity and mortality (invasive fungal diseases with an incidence of ≥10% are usually considered of high risk) Patients receiving intensive chemotherapy for acute myeloid leukaemia, highrisk acute lymphoblastic leukaemia, and recurrent acute leukaemia, especially during prolonged courses of glucocorticosteroids or phases of long lasting and profound granulocytopenia are in this group A strong recommendation was made in this guideline to administer a mould active agent with an echinocandin or a mould active azole when systemic antifungal prophylaxis is warranted [40,42,43].

4. Conclusion

AML and ALL present varying infections risks. AML's intensive chemotherapy leads to prolonged neutropenia, making patients more susceptible to infections. Recent studies have highlighted the importance of antimicrobial prophylaxis and tailored treatments in managing AML related infections. Similary ALL patients, particularly during induction and consolidation phase, face a significant infection risk with mortality rate of 2–4%. IFI are a major concern influenced by factors like age, treatment intensity, responses to induction therapy and corticosteroids administration. Further research is necessary to understand the full scope of the problem and develop strategies for reducing infection-related morbidity and mortality in young leukemia patients. Despite progress in childhood leukemia treatment, addressing infection challenges remains a crucial area for ongoing study and improvement.

Looking ahead 5 to 10 years, advancements in leukemia research and treatment are expected to bring transformative changes to infection management. The new monoclonal and bispecific antibodies and CAR-T,

besides offering new perspectives in the overall survival and disease-free survival of patients, may also transform the epidemiology of infections in ALL by improving the toxicity of treatments. On the other hand development on the tools for early diagnose and target therapy will lead to a significant reduction in infection-related mortality rates.

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CRediT authorship contribution statement

Lopes de Sousa Ana Virginia: Writing – original draft, Writing – review & editing. **Carlesse Fabianne Altruda de Moraes Costa:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SCIENTIFIC DATA

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ANALYSIS

Systematic analysis of infectious disease outcomes by age shows lowest severity in school-age children

Judith R. Glynn[1]✉ & Paul A. H Moss[2]

The COVID-19 pandemic has ignited interest in age-specific manifestations of infection but surprisingly little is known about relative severity of infectious disease between the extremes of age. In a systematic analysis we identified 142 datasets with information on severity of disease by age for 32 different infectious diseases, 19 viral and 13 bacterial. For almost all infections, school-age children have the least severe disease, and severity starts to rise long before old age. Indeed, for many infections even young adults have more severe disease than children, and dengue was the only infection that was most severe in school-age children. Together with data on vaccine response in children and young adults, the findings suggest peak immune function is reached around 5–14 years of age. Relative immune senescence may begin much earlier than assumed, before accelerating in older age groups. This has major implications for understanding resilience to infection, optimal vaccine scheduling, and appropriate health protection policies across the life course.

Introduction

A striking feature of the COVID-19 pandemic is that disease and death are both much less common in children than in adults[1]. This unexplained phenomenon has renewed interest in age patterns of disease. Before COVID-19, most contemporary discussion focussed on vulnerabilities at the extremes of age, due to the immature immune system in infants, and immune deterioration or 'immunosenescence' in the elderly[2]. There has been little investigation of the comparative response to infection in between theses extremes: indeed, it is generally assumed that the response is broadly constant over this period and that immune function remains stable until around 60 years of age[2].

Infections which have been noted to increase in severity in young adults, such as Spanish influenza and tuberculosis, are typically discussed as outliers, and explanations sought. However the medical literature a century ago noted that the case fatality rate (deaths/cases) by age is J-shaped for a number of infections, with a 'honeymoon period' in school-age children and rising fatality throughout adulthood[3,4]. This pattern, which has never been systematically examined, appears to have been largely forgotten, and the explanation for it is unknown.

We present a systematic analysis of severity of infectious disease by age, concentrating on the age at which severity starts to rise after childhood. We investigated case fatality rates and other measures of severity for a wide range of viral and bacterial infections. We are interested in the effect of age on the outcome of infection, so do not include population mortality rates, as they would be influenced by different exposures to infection by age. For infections for which there are effective treatments or vaccines, older studies were sought, as the interest is in the natural host response. We have included all studies identified by our search that fit our inclusion criteria.

We find that for almost all infections, school-age children have the lowest severity, severity starts to rise well before old age, and for many infections, severity is already higher in young adults than in children.

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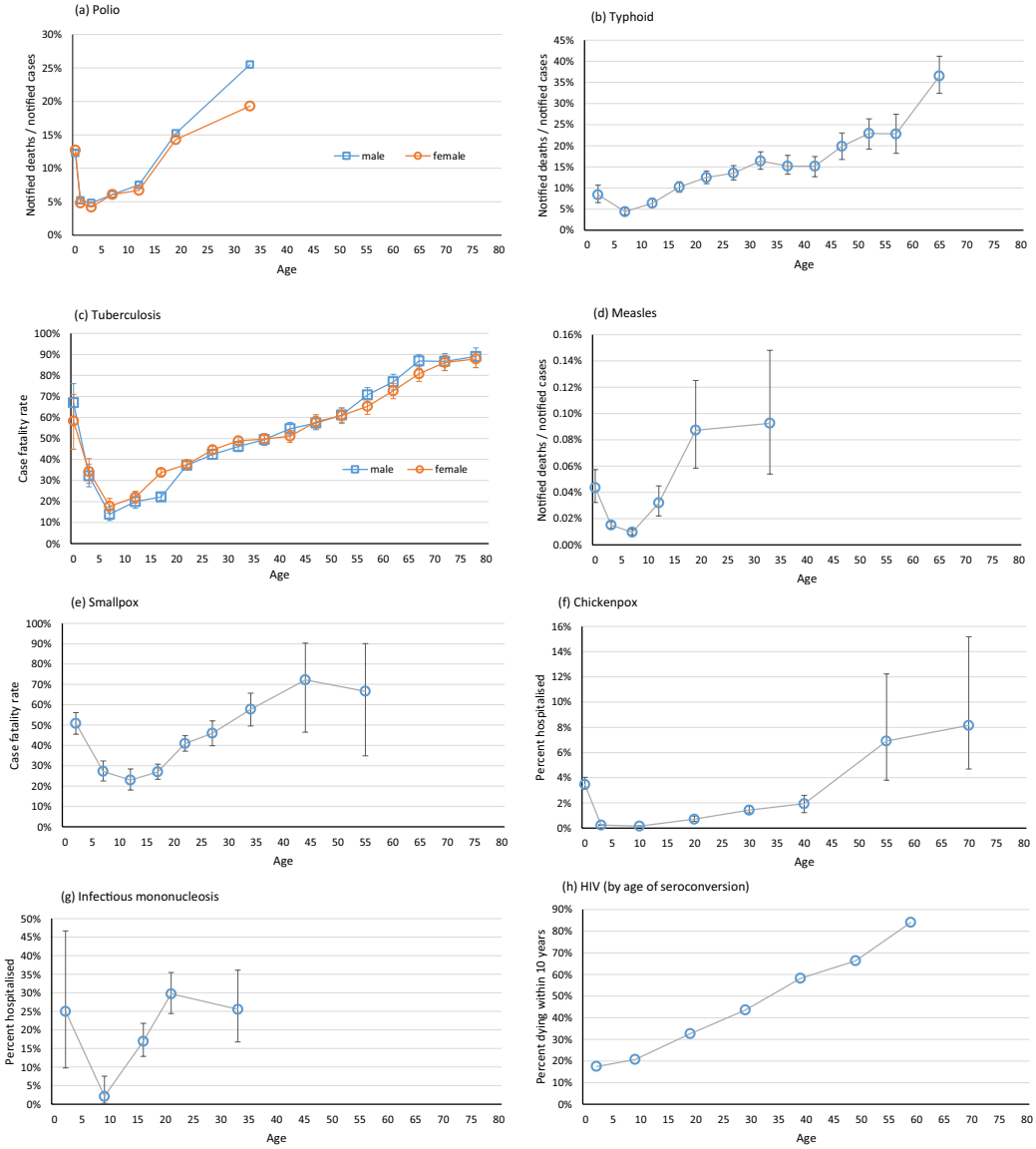

Fig. 1 Severity of infectious disease by age for polio, typhoid, tuberculosis, smallpox, chickenpox, measles, infectious mononucleosis and HIV. The 95% confidence intervals on the estimates are shown where they are known or could be calculated. (**a**) Polio in England and Wales 1947–50, notified deaths/notified cases (23,143 cases)[12]; (**b**) Typhoid in small towns in New York State, US, 1915–24, notified deaths/notified cases[13]; (**c**) Pulmonary TB in Denmark, 1925–34, percent of notified cases dying by 31st December 1934[21]; (**d**) Measles in England and Wales, 1970–88, notified deaths/notified cases[29]; (**e**) Smallpox in London Smallpox Hospital, UK, 1836–51, case fatality rate among unvaccinated patients[35]; (**f**) Chickenpox in France 1997–9, estimated hospitalisation rates based on national databases and surveillance[40]; (**g**) Infectious mononucleosis in Rochester, Minnesota, US, 1950–69, percent hospitalised[43]; (**h**) HIV in Europe, North America and Australia, 1983–1996, percent dying within 10 years, mother-to-child infections excluded (12,910 cases, survival estimated from graph)[44].

Results

Information on at least one measure of severity in at least one study was found for 32 infections. Severity is measured as case fatality rate, or the proportion of cases with severe disease (e.g. the proportion hospitalised), or as the proportion with symptomatic disease among those with infection or known exposure. For ease of comparison, a figure from the largest dataset for each infection is presented in Figs. 1–4. Details of all identified studies for each infection are given in the Supplementary Material[1,5–143], together with figures, including the number of cases in each age group, where known, and 95% confidence intervals where these could be calculated. Results are shown separately by sex when this was available and the sample size was sufficient.

For most infections clinical severity is relatively high in infancy, drops in early childhood and rises in adulthood and more steeply into old age. Figures 1–4, Supplementary Figures S1–32 and online-only Table 1 are arranged in order of the age at which the severity starts to rise.

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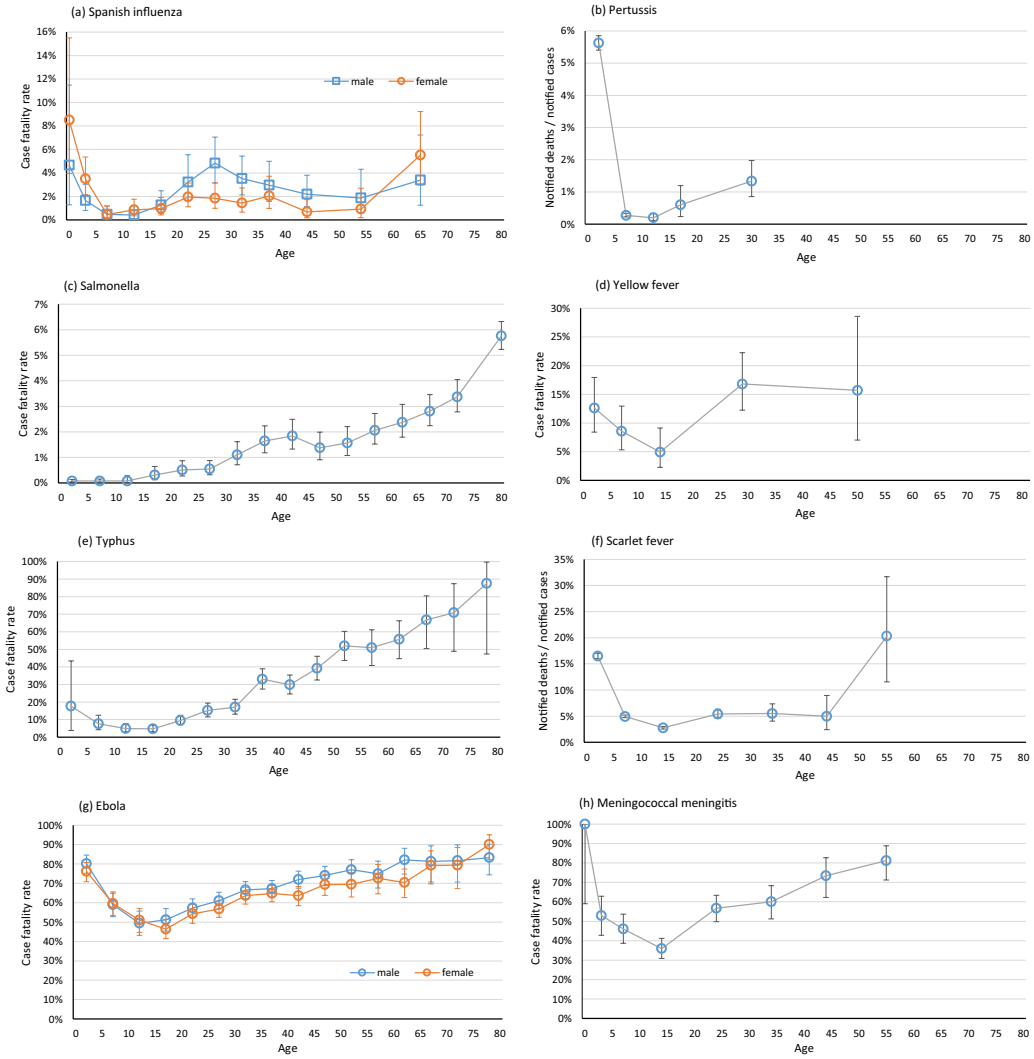

Fig. 2 Severity of infectious disease by age for influenza, pertussis, Salmonellosis, yellow fever, typhus, scarlet fever, Ebola, and Meningococcal meningitis. The 95% confidence intervals on the estimates are shown where they are known or could be calculated. (**a**) Influenza in Maryland, US, 1918–19, case fatality rate from household surveys[47]; (**b**) Pertussis in small towns in New York State, US, 1915–24, notified deaths/notified cases[13]; (**c**) Salmonellosis in Spain 1997–2006, case fatality rate among hospitalised patients (numbers estimated from graph and population data)[55]; (**d**) Yellow fever in New Orleans, US, 1878, case fatality rate[63]; (**e**) Typhus in London Fever Hospital 1848–57, case fatality rate[15]; (**f**) Scarlet fever in Pennsylvania, US, 1907–12, notified deaths/notified cases[9]; (**g**) Ebola in Guinea, Liberia and Sierra Leone, 2013–15. case fatality rate[66]; (**h**) Meningococcal meningitis in Cyprus, 1908–9, case fatality rate[70].

For many infections the rise in severity is apparent by age 20 years. For polio, typhoid, tuberculosis and measles (Fig. 1a–d, Supplementary Figures S1–4) some datasets suggest that the case fatality rate is already higher by age 10–14 years than in younger children[9,11–13,21,29]. A rise in severity by age 15–19 or 15–24 years is seen clearly in large datasets for case fatality rates for smallpox[35], HIV[44], Spanish influenza[47,48], pertussis[13], and Salmonella[55], for hospitalisation rates for chickenpox[40], and in smaller datasets for hospitalisation for infectious mononucleosis[43], and case fatality rate for Yellow fever[63] (Figs. 1d–h, 2a–d, Supplementary Figures S5–12).

A slightly later rise, from around 20 years, is seen for case fatality rates for typhus[15], scarlet fever[9], Ebola[66], meningococcal meningitis[70], and Japanese encephalitis[73] (Figs. 2e–h, 3a, Supplementary Figures S13–17). For cholera, case fatality rates were lowest around 10-24 years in a number of datasets (Supplementary Figure S18)[75,76], but mortality rates in the large 19th century waterborne outbreaks, in which all age groups would have been exposed, were lowest at 5-14 years[88,89]. For Lassa fever, case fatality rates in Nigeria[91,92] increased with age from 20–30 years but no particular pattern with age was seen in Sierra Leone (Supplementary Figure S19)[93,94].

For most of the remaining infections for which information was available, the severity rises with age from 30 years (seasonal influenza[48], brucellosis[96], plague[102], probably hepatitis B acute infection[99], Fig. 3d–f, Supplementary Figures S9c, S20–22), 40 years (Hepatitis A[111], SARS[114], COVID-19[117], MERS-CoV[124,125], St Louis encephalitis[126], Figs. 3g,h 4a–c, Supplementary Figures S23–27) or 50 years (Campylobacter[57], Western equine encephalitis[134], Fig. 4c,d Supplementary Figures S28, 29).

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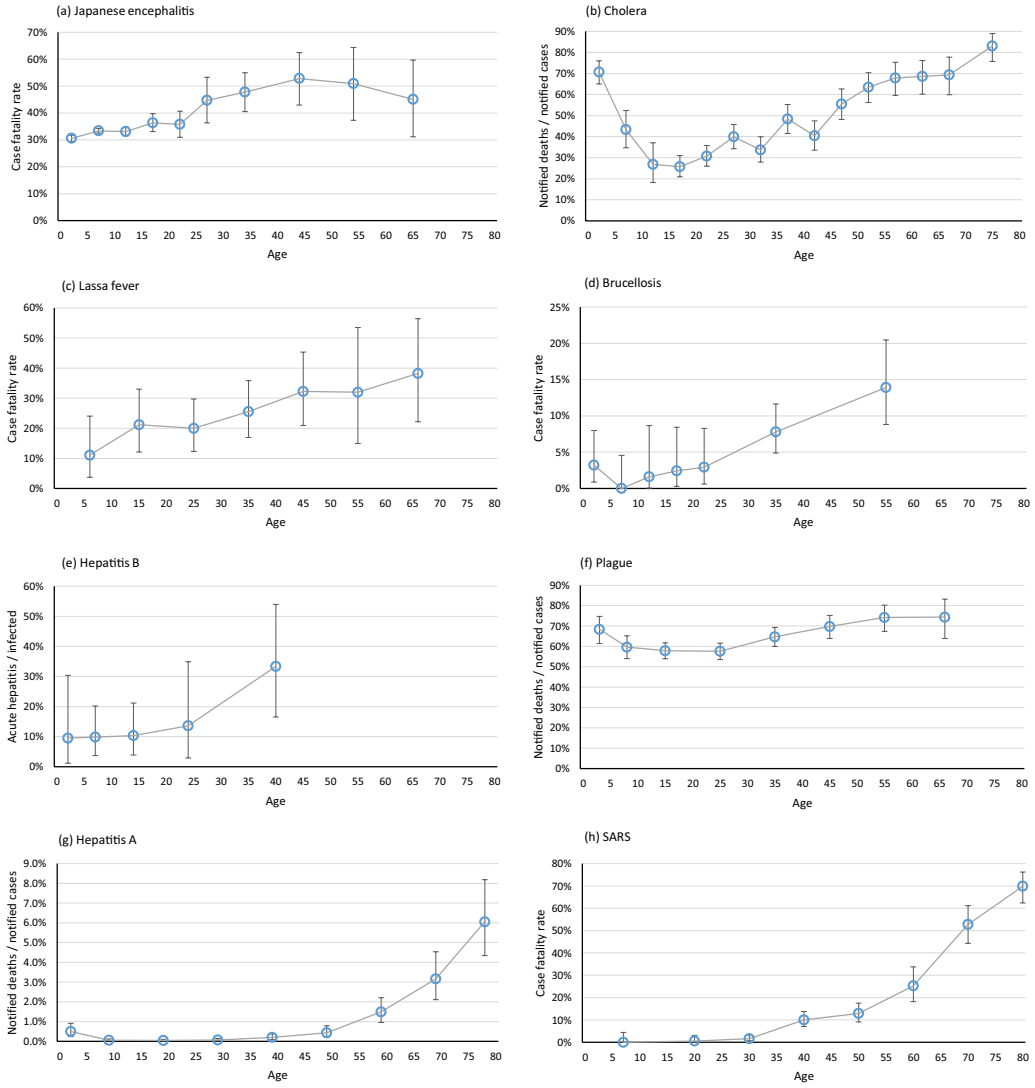

Fig. 3 Severity of infectious disease by age for Japanese encephalitis, cholera, Lassa fever, brucellosis, hepatitis B, plague, hepatitis A, and SARS The 95% confidence intervals on the estimates are shown where they are known or could be calculated. (**a**) Japanese encephalitis in Korea, 1955–66, notified deaths/notified cases[73]; (**b**) Cholera in Munich, Germany, 1873–4, notified deaths/notified cases[76]; (**c**) Lassa fever in Nigeria, 2018, case fatality rate in laboratory confirmed cases[91]; (**d**) Brucellosis in Malta, 1936, case fatality rate[96]; (**e**) Hepatitis B in Alaska, 1971–6, proportion of new infections with symptomatic hepatitis[99]; (**f**) Plague in 75 villages in Jalandhar District, India, 1897–8, notified deaths/notified cases[102]; (**g**) Hepatitis A in England and Wales, 1979–85, notified deaths/notified cases[111]; (**h**) SARS in Hong Kong, 2003, case fatality rate (numbers estimated from incidence and population data)[114].

Three infections show different patterns, with greater severity in children than in young adults. The case fatality rate from diphtheria drops steeply with age in childhood, and continues to fall during adolescence, so the lowest case fatality rate is in the 20 s and 30 s, before rising from age 40 years (Fig. 4f, Supplementary Figure S30)[9,135]. The proportion of people with Shiga-toxin producing *E coli* who develop the haemolytic uraemic syndrome follows a similar pattern, with a drop during childhood, and the lowest proportion in the 40 s and 50 s before rising from age 60 years (Fig. 4g, Supplementary Figure S31)[138,139]. For Dengue the proportion with more severe disease (measured as the proportion with haemorrhagic or any complications or the proportion hospitalised) is greatest in school-age children, dropping to a nadir in the 20–30 s before rising again (Fig. 4h, Supplementary Figure S32)[142].

Discussion

Our findings show that the severity of most infectious diseases is at its lowest in school-age children. Strikingly, the severity was higher among young adults than among school-age children for polio[12], typhoid[13–15], tuberculosis[21,22], measles[29], smallpox[34,35], chickenpox[40,41], infectious mononucleosis[43], HIV[44], influenza[47,48], pertussis[13], Salmonella[55], Yellow fever[62,63], typhus[15], scarlet fever[9,65], Ebola[144], meningococcal meningitis[70], Japanese

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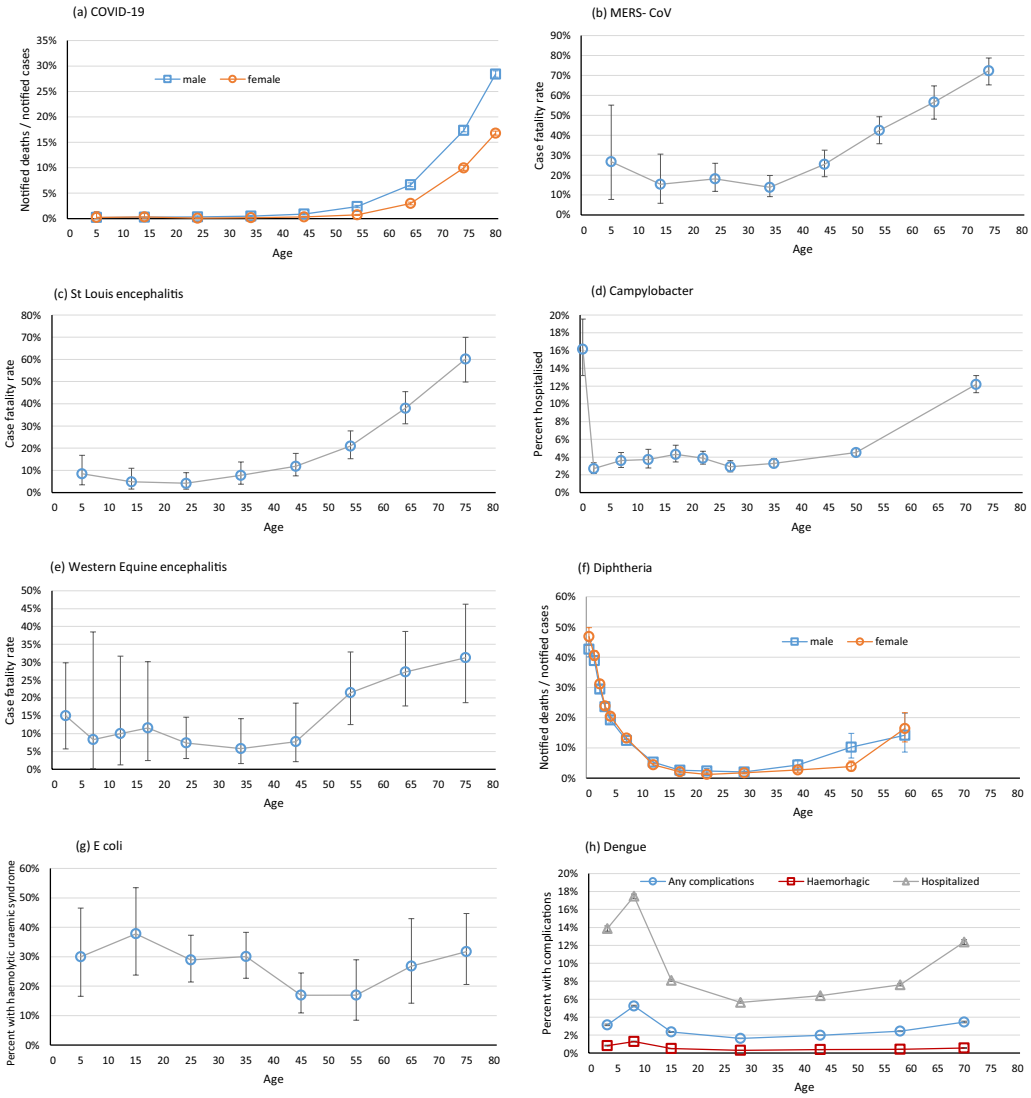

Fig. 4 Severity of infectious disease by age for COVID-19, MERS-CoV, St Louis encephalitis, Western equine encephalitis, diphtheria, Escherichia coli, and dengue. The 95% confidence intervals on the estimates are shown where they are known or could be calculated. (**a**) COVID-19 in Spain 2020, notified deaths/notified confirmed cases, as of 11 May 2020[117]; (**b**) MERS-CoV in Saudi Arabia June 2012-July 2014 and 2017–18, case fatality rate[124,125]; (**c**) S Louis encephalitis in St Louis, US, 1933, case fatality rate[126], (**d**) Campylobacter in Canada, 2001–4, percent hospitalised[57]; (**e**) Western equine encephalitis in Mannitoba, Canada, 1941, case fatality rate[134]; (**f**) Diphtheria in London, UK, 1894–1903, notified deaths/notified cases[135]; (**g**) *E coli* 0104/H4 Hamburg, Germany, 2011, proportion developing haemolytic uraemic syndrome[138]; (**h**) Dengue in Brazil 2000–2014, proportion haemorrhagic, with any complications, and hospitalised[142].

encephalitis[73] cholera[76], and perhaps Lassa fever[91]. Some infections show a slower rise of severity with age after childhood (brucellosis[96], plague[102], coronavirus infections[113,115,117,124,125], acute hepatitis B[99] and hepatitis A[111], St Louis encephalitis[126], Campylobacter[57], and Western equine encephalitis[134],) but for most this rise begins well before old age. For Diphtheria[9,135] and Shiga toxin-producing E coli[138,139] severity remains raised in childhood and adolescents compared to young or middle-age adults, and for Dengue[142] the greatest severity is in school-age children.

Since exposure may vary for children and adults we examined case fatality rates and other measures of severity, not mortality rates. Many reports relied on clinical diagnoses or were based on surveillance data or were limited to hospitalised patients, and it is possible that diagnoses, notifications and hospitalisations differ for children and adults. In order to measure outcomes that are unaffected by vaccination or effective treatments, some of the included studies are old. But even some older studies were carefully conducted. House-to-house surveys of smallpox[34] and influenza[47,48] ensured mild cases and all ages are included. Direct estimates of fatalities in well-characterized outbreaks of Yellow fever[62,63], meningococcal meningitis[70], and viral encephalitides[73,126] avoid any strain differences or notification differences by age. Long-term follow-up studies of patients with

tuberculosis[21,22] ensured all deaths are included. For each infection the different datasets tell a consistent story. The general pattern is remarkable, and unlikely to be explained by biases in the data.

A number of factors may explain these observations. The severity of infectious disease depends on the virulence of the infecting organism, the dose or route of infection, and the response of the host. Variation in strain virulence by age is unlikely, and many of the studies reported outbreaks of single strains (e.g. Spanish influenza, Ebola, smallpox, typhoid, cholera). Most infections have a single route or mode of infection, and for HIV the mode of infection does not explain the age pattern[44].

Adolescents and adults may be exposed to higher doses of infectious agents than are younger children, through caring responsibilities or because they eat more. Greater exposure often increases the risk of acquiring an infection, but the relationship with disease severity is less consistent. An association between infecting dose and severity of disease has been suggested for measles[145] and perhaps chickenpox[146,147], and for Salmonella food-poisoning but not typhoid[148–150]. Fatal cases of SARS and MERS had a slightly shorter incubation period, consistent with a higher infectious dose, but this was not found for influenza[151–153], and an association between severity and incubation period could be explained by host susceptibility. For Ebola, infecting dose (as estimated by degree of exposure to people with Ebola virus disease and their bodily fluids) is strongly associated with the risk of disease, but did not influence the case fatality rate[67,69]. Infectious dose cannot explain the continued rise in severity throughout adulthood.

The J-shaped pattern of severity, high in infancy, dropping in early childhood to a minimum around age 10 years, and rising in adolescence and through adulthood, with a steep increment in old age mirrors that seen for all-cause mortality across populations[154,155]. It is also seen for many of the more frequent causes of death including respiratory disease, diarrhoeal disease, and tuberculosis[154], consistent with a changing age-specific host response.

Co-morbidities tend to increase with age but are generally low in young adults. Interestingly, in a large study of trauma in the US, the case fatality rate, adjusted for severity of injury, only rose from age 55 years onwards[156], suggesting that the change in resilience to infection at younger ages is immunological, not dependent only on co-morbidities or physiological changes.

Further support comes from the immunological response to vaccine challenge by age. In clinical trials of human papilloma virus vaccine, the antibody levels to 9 different component vaccine sub-types measured 7 months post-vaccination decreased with each year increase in age from 9–26 years, with the steepest decline between ages 10 and 16 years[157]. A similar profile was seen in responses to Hepatitis B vaccine in China, with antibody titres in subjects vaccinated at ages 15–24 years half of those vaccinated at 5–14 years;[158] and in bacteriocidal antibody responses to Meningococcal group A conjugate vaccine in West Africa, with lower titres in those aged 18–29 years at vaccination than in those aged 2–10 or 11–17 years[159]. However this is not universal: responses by age to the Meningococcal B vaccine varied by vaccine strain[160].

Humoral responses to natural infection also indicate that immune function may be optimised in children and adolescents. Antibody titres against persistent herpesviruses such as cytomegalovirus and varicella zoster increase in response to recurrent episodes of viral reactivation so higher titres indicate less effective immune control, or re-infection. In the Gambia, where most people are infected with cytomegalovirus in infancy, cytomegalovirus-specific antibody levels show a 'J-shaped' pattern with age, with the lowest levels (suggesting optimal control) in adolescents, rising after age 20 years[161]. Similarly, Varicella zoster is ubiquitous where there is no vaccination, and studies in different settings suggest that antibody levels in seropositive individuals are at their lowest (suggesting optimal control) at 8–9 or 15–19 years[162,163].

The immune system changes in infancy and old age are well described[2]. After birth the weak innate immune system of neonates strengthens rapidly, and the adaptive immune system develops an expanded repertoire of memory B and T cells triggered by infections (and vaccinations), the microbiome, and allergens in food and the environment[2]. With ageing, there is a decline in naïve T-cell numbers and T-cell receptor diversity, cytokine production by CD4 and CD8 T-cells is impaired, the CD4 to CD8 ratio is inverted, and the cells of the innate immune response function less well[164]. It is likely that the majority of the data presented here reflect the response to initial pathogen exposure, as those who are already fully protected by acquired immunity will not be included as cases, and those who are partially protected may have mild disease and not be diagnosed. The primary immune response may be particularly affected by ageing. The very high case fatality rates in elderly people observed in response to novel pathogens such as COVID-19, MERS, SARS, St Louis encephalitis, and Ebola, may result from restriction of the naïve T-cell repertoire.

Studies of immune senescence have focussed largely on those over 60 years, but our data indicate that important changes in immune function are apparent at a much younger age. This could be explained by relative 'senescence' of the immune system starting in young adulthood or even earlier[165,166]. Peak immune function in childhood and adolescence, at around age 5–14 years, might represent the intersection of improved function due to maturity and increasing antigenic exposure, and decreasing function due to early senescence. This is supported by assessment of immune parameters, some of which change almost linearly over the lifespan[167], and by changes in the thymic cortex and medulla which peak in size at age 4–11 years[168], or earlier[169].

The severity of infectious disease depends not only on the control of the infectious agent but also on pathological damage arising from the immune response. Dengue is an example where strong immune responses in childhood are associated with increased clinical complications due to antibody dependent enhancement[170]. It has been suggested that the balance between an insufficient immune response and an over-active immunopathological response is generally more favourable in children[4]. However it seems surprising that a similar balance would be required for the wide range of viral and bacterial infections with similar age-severity patterns presented here, including infections in which protection is mainly antibody-mediated (e.g. smallpox) and those in which it is predominantly cell-mediated (e.g. tuberculosis).

The increase in infectious disease severity during adolescence suggests that puberty could have a role. Sex hormones influence immune responses (reviewed in[171,172]) but in different ways and with inconsistent findings

between studies. Overall, testosterone tends to suppress and oestrogens to enhance the immune response[172,173]. These associations may contribute to the generally higher mortality rate from infectious disease in males and higher incidence of autoimmune disease in females[173,174]. Numerous differences in immune marker concentrations and response to infection have been described between males and females, and these increase after puberty[175]. However, where data are available, the increase in severity of disease in young adults compared to children and adolescents was seen in both males and females (e.g. Ebola, tuberculosis, Spanish influenza, polio). The decreased immune response to human papilloma virus vaccine with age, and the profile of cytomegalovirus-specific immunity with age, are also similar in males and females[157,161,176].

Environmental exposures and heterologous infections may modulate the magnitude and quality of the immune response at different ages. For tuberculosis it has been argued that not only HIV but some (unknown) sexually and parenterally transmitted virus might trigger progression to disease and explain the age distribution[177].

Persistent infections, such as herpesviruses, may establish early in life, or increase with age, especially with the changing social contact patterns of adolescence. Cytomegalovirus seropositivity has been associated with immunosenescent changes, including a reduction in the naïve T-cell pool and commensurate increase in CD8 + memory T-cells with a reduced CD4:CD8 ratio[165,166,178–181], as well as with increased risk of mortality in older age[182,183]. However, the functional consequence of persistent cytomegalovirus infection appears to vary with age and cytomegalovirus may enhance responses to heterologous infections in younger people[166,184–186]. In most countries (and probably everywhere at the time when some of the data we present were collected) almost everyone is infected with cytomegalovirus in early childhood[161,187], and continuing exposure and multiple infections are likely. So if cytomegalovirus has a role in explaining the age-pattern of severity, it may reflect immunological attrition due to the burden of persistent infection or reinfection rather than simple presence or absence of infection[188].

The age pattern of severity suggests that natural selection has acted to optimise immune function in childhood and adolescence with a subsequent decline during adulthood. Peak function before reproduction seems counter-intuitive, but according to life history theory there is a trade-off between the energy required for immune response and that required for other functions, such as reproduction and growth[189,190]. Whilst the energy costs of innate immunity are spread throughout the lifetime[189,190] the adaptive immune system has high upfront but lower running energy costs. Thymic involution conserves energy when sufficient naïve T-cells have been generated[189], facilitating the metabolic demands of the adolescent growth spurt and secondary sexual development[191], but leaving the elderly more vulnerable.

SARS-CoV and SARS-CoV-2 appear to have more extreme variation in severity by age than other infections, with predominantly mild disease in children, and very high case fatality rates in the elderly. Unusually, there is no evidence of higher severity in infants. The reasons for this extreme variation are not yet known, but should be considered in the context of the findings of this review.

Appreciating how responses to infections vary by age outside the extremes of age can have major implications. Understanding the prevalence and transmission of SARS-CoV-2 in children, and how this differs from influenza, is crucial in predicting the role of school closures in the response[192]. Vaccination programmes that increase the average age of infection, can paradoxically increase the numbers of people with severe disease[193]. This has led to targeted vaccination of older children for measles and rubella. The higher vaccine response of younger children to human papilloma virus vaccine may lead to a shift in target age groups.

The finding that many infectious diseases are least severe in school-age children suggests that relative immune ageing starts far earlier than has previously been recognised. Improved understanding of the mechanisms underlying this observation may provide novel opportunities for intervention strategies. Children tend to have lower mortality than adults from sepsis, and comparison of their responses is being used in drug discovery[194]; similar approaches could be used for other infections. Comparison of immune response to infections which increase in severity in young adults and those that only increase later may give clues to drug and vaccine design. In the midst of a pandemic and with increasing antimicrobial resistance, new approaches such as these are desperately needed.

Methods

We sought information on severity of infectious disease by age for a wide range of different infections.

Defining outcome measures. The focus of this study is the host response to infection by age. We are interested in the outcome of infections or exposure. We have therefore used:

- Case fatality rates estimated directly from cohorts of individuals
- Case fatality rates estimated from surveillance data, comparing numbers of deaths and numbers of cases
- Proportion of cases with severe disease (e.g. the proportion hospitalised)
- Proportion with symptomatic disease among those with infection or known exposure

Note that mortality rates, though more readily available, would be misleading, as exposure is likely to vary by age.

Sources of data and possible biases. The most accurate data come from situations in which there is active case ascertainment to ensure mild cases are not missed, and active follow-up to ensure outcomes are recorded, ideally proving infection through serology or microbiology. But such studies are rare, and imposing such strict criteria would leave very little data.

Hospital-based data exclude mild cases, and admission criteria may vary with age, but outcomes are likely to be accurately recorded.

Surveillance data provide some of the largest studies but interpreting the age patterns assumes diagnosis and notification are not influenced by age group. Case fatality rates measured from surveillance data can also be biased because of the lag between incidence and death. This is a particular problem in ongoing epidemics, and for diseases with long duration, but will not necessarily bias the age pattern.

Strains of different virulence could affect different sections of the population. Although it is unlikely that strains would differ systematically by age group, studies of single outbreaks of disease avoid this theoretical problem, but are only available for some infections.

For infections for which effective vaccines or treatments are available there may be very few cases or few severe cases, and differential access to or efficacy of vaccines and treatment by age could influence the results. For these infections estimates from the older (e.g. pre-antibiotic or pre-vaccine) literature can be helpful.

For endemic infections leading to life-long immunity, disease in adults may be rare or atypical, so estimates from isolated (e.g. island) populations are most informative. Similarly, newly-emerged infections, or infections in areas where they have not been seen before, will not be affected by partial immunity in older individuals.

Search strategy. Finding sufficiently detailed age-specific information for different diseases is not straightforward and a conventional systematic review is not possible. Very few studies are done to look at the effect of age on disease outcomes. Most outbreak or surveillance reports do not include enough detail, giving age only as a median and range, or giving the age breakdown only for deaths or only for cases. Relevant data are occasionally included as background information in studies on other topics.

Information on different infections was found in different ways. PubMed was used to find reports on specific infections, searching with "case fatality" or "severity" or "hospital*"and "age", or looking at reports of large outbreaks. Outbreak reports were also searched through the *Morbidity and Mortality Weekly Report (MMWR)* produced by the US Centers for Disease Control.

To find suitable studies in the older literature, the journal *Public Health* was hand-searched from volume 1 (1888) to volume 59 (1946), the *Public Health Bulletin* from 1904–1949 and the issues of the *Journal of Tropical Medicine and Hygiene* available on-line (1915–19, 1921, 1923). All pamphlets and reports in the London School of Hygiene & Tropical Medicine library open shelves and books in the Wellcome collection library open shelves relating to infectious diseases were hand-searched. These include reports from the 19th century onwards. We also searched books and reports available electronically from the United States Public Health Service, US National Library of Medicine Digital Collections, the London School of Hygiene & Tropical Medicine, or Google on plague, cholera and yellow fever. The authors' literature collection was searched, and relevant references followed (snowball searching).

All identified studies that gave information on the age-pattern of severity were included if they satisfied the following criteria:

- Included children and adults
- Data stratified in 10-year age groups or less
- At least 100 cases included
- Source of data given

All eligible studies were included whatever age-severity pattern they showed. Where the number of cases and outcomes was presented, or could be derived, by age group, binomial exact 95% confidence intervals were calculated for the proportions. For each infection, the relative risk of the outcome (death or other measure of severity) in adults was compared to that in children using the largest dataset with information on numbers of individuals in the relevant age groups.

Data availability

All datasets are available in an Excel file[195]. This links to the supplementary material, and includes the origin of each dataset.

Code availability

There was no custom code for this analysis

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Author contributions

J.G.: conception, literature searching, data extraction, analysis, writing. P.M.: critical review and contribution to writing.

Competing interests

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Additional information

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Influence of protective isolation on outcome of allogeneic bone marrow transplantation for leukemia

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Summary:

Various isolation strategies are used to prevent infections during bone marrow transplantation; data on their efficacy are lacking. We studied whether use of high efficiency particulate air filtration (HEPA) and/or laminar airflow (LAF) units affect transplant-related mortality (TRM) or survival in the first year after allogeneic transplantation. 5065 patients with leukemia receiving bone marrow transplants from an HLA identical sibling ($n = 3982$) or alternative related or unrelated donors ($n = 1083$) between 1988 and 1992 were reported to the International Bone Marrow Transplant Registry by 222 teams. Two types of isolation were considered: (1) conventional protective isolation with single patient room and any combination of hand-washing, gloves, mask and gown; and (2) HEPA and/or LAF. Cox proportional hazards regression models were used to determine the relative risks (RRs) of transplant-related mortality (TRM) and of deaths from any cause in patients treated in HEPA/LAF units compared to patients treated in conventional isolation. HLA-identical sibling and alternative donor transplants were analyzed separately. Risks of TRM and overall mortality in the first 100 days post-transplant were significantly lower among patients treated in HEPA/LAF units than in those treated conventionally. RRs of TRM were 0.76 ($P = 0.009$) for recipients of HLA-identical sibling transplants and 0.65 ($P = 0.003$) for recipients of alternative donor transplants. Correspondingly RRs of overall mortality were 0.80 ($P = 0.02$) and 0.65 ($P = 0.0006$). Decreased risks of TRM and of death in the first 100 days post-transplant resulted in significantly higher 1-year survival rates in patients treated in HEPA/LAF rather than in conventional isolation units. Use of HEPA and/or LAF to prevent infections decreases TRM and increases survival after allogeneic bone marrow transplants for leukemia.

Keywords: allogeneic bone marrow transplantation; infection prophylaxis; protective isolation

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Bone marrow transplant recipients have a high risk of infections early after transplant. Data from the International Bone Marrow Transplant Registry (IBMTR) indicate that infections are the primary cause in about 15%, and a contributory cause in another 35%, of deaths occurring in the first post-transplant year.

Several strategies are used to prevent infections during transplants including physical barriers to infectious agents (protective isolation) and prophylactic antibiotics.[1–17] Approaches differ in costs and psychosocial effects on patients.[18,19] There is controversy whether these strategies effectively decrease infections. This study focused on whether use of high efficiency particulate air filtration (HEPA) and/or laminar airflow (LAF) units rather than conventional isolation techniques influenced transplant outcome.

Patients and methods

Population

The study included 5065 patients receiving allogeneic bone marrow transplants between 1988 and 1992 and reported to the IBMTR with the following: (1) chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL); (2) graft-versus-host disease (GVHD) prophylaxis with cyclosporine with or without other drugs or T cell depletion. We excluded patients treated without protective isolation ($n = 63$) or with isolation strategies other than the categories described below ($n = 193$), those receiving other types of GVHD prophylaxis ($n = 246$), those with insufficient HLA data to determine donor–recipient histocompatibility ($n = 153$), and

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those without information on pretransplant disease state or age ($n = 77$). Patients were reported by 222 teams.

Isolation strategies

Two isolation strategies were studied: (1) conventional protective isolation using single rooms, and any combination of hand-washing, gloves, mask, and gown to prevent infection transmission (conventional isolation, $n = 827$); and (2) protective isolation in rooms designed to lower exposure to airborne infectious agents using HEPA filtration systems with or without LAF equipment (HEPA/LAF, $n = 4238$).

Outcomes

Transplant outcomes analyzed included acute and chronic GVHD, fungal pneumonia, 1-year transplant-related mortality (TRM), and 1-year survival. Acute GVHD was defined as moderate to severe (grade II–IV) disease using published criteria;[20] patients surviving $\geqslant$21 days with engraftment were considered at risk. Chronic GVHD was determined by clinical criteria in patients surviving $\geqslant$90 days with engraftment.[21] TRM was defined as death in patients without leukemia relapse, with censoring at time of relapse or last follow-up.[22] Follow-up to 100 days post-transplant was available for 100% of patients; follow-up to 1 year was available for 84%.

Statistical methods

Patient-, disease- and transplant-related variables of the two study groups were compared using the χ^2 statistic for categorical and the Mann–Whitney U test for continuous variables. Probabilities of transplant outcomes were calculated using the Kaplan–Meier estimator; the log-rank test was used for univariate comparisons. Associations of isolation-type with TRM and survival were evaluated using Cox proportional hazards regression.[23] Since recovery of immune function differs after HLA-identical sibling and alternative donor transplants, these cohorts were analyzed separately. Potential confounding variables considered in the analysis were: donor–recipient relationship and histocompatibility, recipient age, donor–recipient sex-match, donor and recipient cytomegalovirus (CMV) serology, pretransplant Karnofsky performance score, disease, remission state, white blood cell count (WBC) at diagnosis, conditioning regimen, GVHD prophylaxis, year of transplant and the prophylactic use of systemic antibiotics, oral nonabsorbable antibiotics, cotrimoxazole, acyclovir, intravenous immune globulins, hematopoietic growth factors including G-CSF and GM-CSF, transplant center size (number of transplants done annually) and transplant center site. Forward stepwise variable selection at a 0.05 significance level was used to identify covariates associated with outcome. In each step of the analysis, the covariate for isolation type was included in the model. For each model the assumption of proportional hazards was tested using a time-dependent covariate; this indicated a differential effect of isolation type in the early and late post-transplant period. Models were therefore constructed breaking the post-transplant course into two time periods using the maximized partial likelihood method to find the most appropriate breakpoint. First order interactions between type of isolation and all significant covariates were considered.

Results

Patient-, disease- and transplant-related variables are shown in Tables 1 and 2. Among patients receiving HLA-identical sibling transplants, there were statistically significant differences between those treated in conventional *vs* HEPA/LAF isolation in patient age, pretransplant performance score, year of transplant, conditioning regimen, GVHD prophylaxis, use of systemic and oral nonabsorbable antibiotics, use of cotrimoxazole, hematopoietic growth factors and transplant center site (Table 1). Among those receiving alternative donor transplants, the cohorts differed for disease, disease state, pretransplant performance score, CMV status, year of transplant, conditioning regimen, GVHD prophylaxis, use of cotrimoxazole, acyclovir, intravenous immune globulins and transplant center site (Table 2).

Table 3 shows actuarial probabilities ($\pm$ 95% confidence interval) of acute and chronic GVHD, fungal pneumonia, TRM and 1-year survival by donor type and isolation technique in univariate analysis. Rates of acute or chronic GVHD were similar in patients treated in conventional *vs* HEPA/LAF isolation. Among patients receiving alternative donor transplants, the probability of fungal pneumonia was lower in the HEPA/LAF cohort. TRM was lower and 1-year survival higher for patients treated with HEPA/LAF isolation, whether the transplant was from an HLA-identical sibling or alternative donor (Table 3, Figure 1).

Table 4 shows the relative risk of TRM for patients treated in HEPA/LAF *vs* conventional isolation in multivariate analyses, adjusting for the effect of other significant variables. The relationship between isolation and TRM is time-dependent. Patients treated with HEPA/LAF had lower risks of TRM in the first 100 days post-transplant with no effect thereafter.

Table 5 shows the relative risk of mortality in the first post-transplant year for patients treated in HEPA/LAF *vs* conventional isolation. Again, HEPA/LAF isolation decreased overall mortality in the first 100 days post-transplant. There were no interactions between the effect of isolation and prophylactic use of antibiotics, cotrimoxazole or immune globulins in either model. Transplant center site, categorized as high or low geographic latitude, but not transplant center size, was significantly associated with TRM and mortality in the first post-transplant year. HEPA/LAF decreased TRM and mortality regardless of transplant center site.

There were no significant differences in the primary causes of death, for patients dying within 1 year post-transplant, between patients treated in HEPA/LAF *vs* conventional isolation.

Discussion

Isolation techniques used for allogeneic bone marrow transplant recipients vary.[3,6,12,17] Several studies comparing

Table 1 Characteristics of patients receiving HLA-identical sibling bone marrow transplants for leukemia in conventional isolation or HEPA/LAF units

	Conventional isolation	*HEPA/LAF*	*P*
Disease, n/n eval[a] (%)			NS
Chronic myelogenous leukemia	212/627 (34)	1294/3355 (38)	
Acute lymphoblastic leukemia	184/627 (29)	891/3355 (27)	
Acute myelogenous leukemia	231/627 (37)	1170/3355 (35)	
Leukemia state pretransplant, n/n eval (%)			0.03
1st CR or 1st chronic phase	366/627 (58)	2125/3355 (63)	
⩾2nd CR or accelerated phase	145/627 (23)	727/3355 (22)	
Not in remission or blast phase	116/627 (19)	503/3355 (15)	
Median age, years (range)	28 (1–62)	30 (<1–60)	0.005
Karnofsky score ⩾90%, n/n eval (%)	473/621 (76)	2731/3337 (82)	0.001
Male sex, n/n eval (%)	379/627 (60)	1976/3355 (59)	NS
CMV seropositive, n/n eval (%)	386/617 (63)	2036/3282 (62)	NS
High WBC at diagnosis[b], n/n eval (%)	218/566 (39)	1250/3060 (41)	NS
Year of transplant ⩾1990, n/n eval (%)	341/627 (54)	2048/3355 (61)	0.002
Donor–recipient sex-match, n/n eval (%)			NS
Male/male	224/627 (36)	1130/3348 (34)	
Male/female	139/627 (22)	735/3348 (22)	
Female/male	155/627 (25)	843/3348 (25)	
Female/female	109/627 (17)	640/3348 (19)	
Donor CMV seropositive, n/n eval (%)	316/582 (54)	1752/3186 (55)	NS
Conditioning regimen n/n eval (%)			0.0001
Bu/Cy	292/615 (47)	1111/3311 (33)	
Cy/TBI	157/615 (26)	1081/3311 (33)	
Cy/TBI/other drugs	102/615 (17)	683/3311 (21)	
TBI/other drugs	58/615 (9)	427/3311 (13)	
Other conditioning regimens	6/615 (1)	9/3311 (<1)	
GVHD prophylaxis, n/n eval (%)			0.0001
CsA + MTX	434/627 (69)	2024/3355 (60)	
CsA	56/627 (9)	467/3355 (14)	
CsA + other	73/627 (12)	459/3355 (14)	
T cell depletion	64/627 (10)	405/3355 (12)	
Systemic antibiotics[c,d], n/n eval (%)	378/619 (61)	2338/3310 (71)	0.0001
Oral nonabsorbable antibiotics[c], n/n eval (%)	337/626 (54)	2226/3336 (67)	0.0001
Cotrimoxazole[c], n/n eval (%)	527/617 (85)	2608/3301 (79)	0.0001
Acyclovir[c], n/n eval (%)	447/611 (73)	2444/3305 (74)	NS
i.v. Immune globulins[c], n/n eval (%)	332/618 (54)	1823/3306 (55)	NS
Growth factors[e], n/n eval (%)	54/627 (9)	213/3355 (6)	0.04
Geographic latitude[f], n/n eval (%)			0.0001
Transplant team 0°–29°	55/627 (9)	214/3355 (6)	
Transplant team 30°–39°	316/627 (50)	891/3355 (27)	
Transplant team 40°–49°	65/627 (10)	1373/3355 (41)	
Transplant team ⩾50°	191/627 (31)	877/3355 (26)	
Geographic region, n/n eval (%)			0.0001
North America	163/627 (26)	1059/3355 (32)	
Europe	205/627 (33)	1865/3355 (56)	
Australia/New Zealand	166/627 (26)	74/3355 (2)	
Other	93/627 (15)	357/3355 (10)	

[a]n eval refers to number of patients for whom data regarding the specified variable were available.
[b](>50×10^9/l for acute leukemia; >100×10^9/l for chronic leukemia).
[c]Used prophylactically.
[d]Excluding cotrimoxazole.
[e]G-CSF or GM-CSF, starting within 1 week of transplant.
[f]North or south of equator.
CR = complete remission; WBC = white blood cell count; CMV = cytomegalovirus; Bu = busulfan; Cy = cyclophosphamide; TBI = total body irradiation; CsA = cyclosporin A; MTX = methotrexate; i.v. = intravenous.

different techniques are reported with contradictory results.[24–31] This study represents the largest group of patients analyzed for associations between isolation technique and outcome.

Our major finding is that survival is higher in patients treated in HEPA/LAF units than in those treated with conventional isolation. This benefit persists after adjusting for other differences in patient-, disease- and transplant-related variables. Higher survival results from less TRM in the first 100 days post-transplant. The effect is seen in both HLA-identical sibling and alternative donor transplants and appears to be stronger in the latter. This effect of isolation technique is independent of use of prophylactic antibiotics or immune globulins.

Previous studies of isolation techniques analyzed small and/or heterogenous patient groups,[24–26] but three prospec-

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Table 2 Characteristics of patients receiving alternative donor bone marrow transplants for leukemia in conventional isolation or HEPA/LAF units

	Conventional isolation	*HEPA/LAF*	*P*
Disease, n/n eval[a] (%)			0.02
Chronic myelogenous leukemia	91/200 (46)	459/883 (52)	
Acute lymphoblastic leukemia	46/200 (23)	230/883 (26)	
Acute myelogenous leukemia	63/200 (31)	194/883 (22)	
Leukemia state, n/n eval (%)			0.01
1st CR or 1st chronic phase	68/200 (34)	384/883 (43)	
⩾2nd CR or accelerated phase	70/200 (35)	302/883 (34)	
Not in remission or blast phase	62/200 (31)	197/883 (22)	
Median age, years (range)	28 (3–53)	29 (1–61)	NS
Karnofsky score ⩾90%, n/n eval (%)	122/198 (62)	669/881 (76)	0.0001
Male sex, n/n eval (%)	122/199 (61)	541/883 (61)	NS
CMV seropositive, n/n eval (%)	116/199 (58)	415/864 (48)	0.009
High WBC at diagnosis[b], n/n eval (%)	90/175 (51)	372/788 (47)	NS
Year of transplant ⩾1990, n/n eval (%)	120/200 (60)	623/883 (71)	0.004
Donor–recipient sex-match, n/n eval (%)			NS
Male/male	60/195 (31)	270/864 (31)	
Male/female	43/195 (22)	150/864 (17)	
Female/male	60/195 (31)	258/864 (30)	
Female/female	32/195 (16)	186/864 (22)	
Donor CMV seropositive, n/n eval (%)	90/185 (49)	363/833 (44)	NS
Donor–recipient relationship and			NS
Histocompatibility, n/n eval (%)			
HLA-matched related donor	4/200 (2)	39/883 (4)	
1-Ag mismatched related donor	16/200 (8)	66/883 (7)	
⩾2-Ag mismatched related donor	76/200 (38)	286/883 (32)	
HLA-matched unrelated donor	80/200 (40)	358/883 (41)	
HLA-mismatched unrelated donor	24/200 (12)	134/883 (15)	
Conditioning regimen, n/n eval (%)			0.0001
Bu/Cy	60/200 (30)	161/877 (18)	
Cy/TBI	28/200 (14)	212/877 (24)	
Cy/TBI/other drugs	99/200 (49)	438/877 (50)	
TBI/other drugs	3/200 (2)	62/877 (7)	
Other conditioning regimens	10/200 (5)	4/877 (1)	
GVHD prophylaxis, n/n eval (%)			0.0001
CsA + MTX	140/200 (70)	475/883 (54)	
CsA	5/200 (2)	43/883 (5)	
CsA + other	30/200 (15)	81/883 (9)	
T cell depletion	25/200 (13)	284/883 (32)	
Systemic antibiotics[c,d], n/n eval (%)	135/199 (68)	639/876 (73)	NS
Oral nonabsorbable antibiotics[c], n/n eval (%)	119/200 (59)	529/882 (60)	NS
Cotrimoxazole[c], n/n eval (%)	177/199 (89)	652/876 (74)	0.0001
Acyclovir[c], n/n eval (%)	122/199 (61)	737/876 (84)	0.0001
i.v. Immune globulins[c], n/n eval (%)	107/199 (54)	543/874 (62)	0.03
Growth factors[e], n/n eval (%)	21/200 (11)	64/883 (7)	NS
Geographic latitude[f], n/n eval (%)			0.0001
Transplant team 0°–29°	4/200 (2)	27/883 (3)	
Transplant team 30°–39°	129/200 (64)	237/883 (27)	
Transplant team 40°–49°	22/200 (11)	338/883 (38)	
Transplant team ⩾50°	45/200 (23)	281/883 (32)	
Geographic region, n/n eval (%)			0.0001
North America	93/200 (47)	412/883 (47)	
Europe	40/200 (20)	408/883 (46)	
Australia/New Zealand	53/200 (26)	30/883 (3)	
Other	14/200 (7)	33/883 (4)	

[a]n eval refers to number of patients for whom data regarding the specified variable were available.
[b]($>50 \times 10^9$/l for acute leukemia; $>100 \times 10^9$/l for chronic leukemia).
[c]Used prophylactically.
[d]Excluding cotrimoxazole.
[e]G-CSF or GM-CSF, starting within 1 week of transplant.
[f]North or south of equator.
CR = complete remission; WBC = white blood cell count; CMV = cytomegalovirus; Bu = busulfan; Cy = cyclophosphamide; TBI = total body irradiation; CsA = cyclosporin A; MTX = methotrexate; i.v. = intravenous.

Table 3 Probabilities (95% confidence interval) of transplant outcomes by donor type, age and isolation technique (univariate analysis[a])

	Age (years)	*HLA-identical sibling transplant*			*Alternative donor transplant*		
		Conventional isolation	*HEPA/LAF*	*P*[b]	*Conventional isolation*	*HEPA/LAF*	*P*[b]
N	<25	261	1196	0.005	83	360	NS
	⩾25	366	2159		117	523	
Grade II–IV acute GVHD (at 100 days)	<25	23 ± 5%	25 ± 3%	NS	47 ± 12%	46 ± 6%	NS
	⩾25	37 ± 5%	35 ± 2%	NS	47 ± 10%	50 ± 5%	NS
Chronic GVHD (at 1 year)	<25	31 ± 7%	29 ± 3%	NS	45 ± 16%	47 ± 7%	NS
	⩾25	53 ± 7%	46 ± 3%	0.04	52 ± 15%	55 ± 6%	NS
Fungal pneumonia (at 1 year)	<25	3 ± 2%	4 ± 1%	NS	23 ± 13%	6 ± 3%	0.0001
	⩾25	7 ± 3%	7 ± 1%	NS	14 ± 9%	10 ± 3%	NS
Transplant-related mortality (at 1 year)	<25	18 ± 5%	21 ± 3%	NS	56 ± 13%	43 ± 6%	0.0008
	⩾25	40 ± 6%	31 ± 2%	0.0005	55 ± 11%	53 ± 5%	NS
Survival (at 1 year)	<25	64 ± 6%	66 ± 3%	NS	36 ± 11%	45 ± 6%	0.02
	⩾25	50 ± 5%	61 ± 2%	0.0001	33 ± 9%	38 ± 4%	0.09

[a]No adjustment for other potentially confounding variables.
[b]Log rank test.

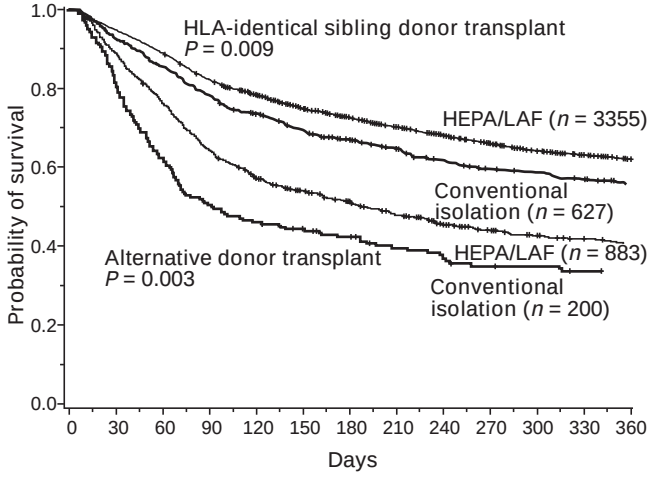

Figure 1 Probability of survival in the first year post-transplant after HLA-identical sibling transplant and alternative donor transplant by type of protective isolation.

tive studies from one single center are reported.[27,30,31] Two studied patients with leukemia. The first, involving 99 recipients of HLA-identical sibling transplants between 1981 and 1984, compared conventional to LAF isolation;[30] both groups received prophylactic systemic antibiotics. The second study, done between 1984 and 1987, included 342 recipients of HLA-identical sibling and alternative donor transplants and compared four strategies: conventional isolation, conventional isolation and prophylactic systemic antibiotics, LAF isolation, and LAF isolation and prophylactic systemic antibiotics.[31] Both studies evaluated events up to the time of engraftment and showed lower probabilities of bacteremia and other clinically significant infections with more intensive isolation; there was no survival benefit. A third study analyzed 100 patients with aplastic anemia treated with conventional isolation, conventional isolation and granulocyte transfusions or LAF isolation.[27] Compared to patients in conventional isolation, patients in LAF isolation had less bacteremia and other documented infections, less and delayed acute GVHD and higher probabilities of 100-day survival. Differences in GVHD and survival were of borderline statistical significance. Overall, results from the above studies are similar in direction and magnitude as those in the current IBMTR analysis. Although survival advantages in the prospective studies cited were not statistically significant, the large numbers of patients analyzed and longer follow-up in this study substantially increased our power to detect such differences. Our study did not elucidate the reasons for improved survival of patients in the HEPA/LAF cohort. In patients receiving transplants from donors other than an HLA-identical sibling, who are at high risk of invasive infections,[32,33] we found fewer fungal pneumonias in the HEPA/LAF cohort. We lacked sufficient data to examine relative rates of bacterermia and other bacterial infections. Distribution of causes of death did not differ by isolation technique for either HLA-identical sibling or alternative donor transplants. We did not detect any difference in incidence or severity of GVHD. Experimental data show that GVHD may be reduced by a strictly sterile environment[34] but this is rarely attained in the clinical setting.

Our study found an inverse association between distance from the equator, ie geographic latitude, and TRM and 1-year mortality independent of the effect of isolation. Patients treated in warm climates may be at higher risk of exposure to airborne fungal spores.[35] However, the effect of HEPA/LAF was similar at warm and temperate latitudes.

The data analyzed have several limitations. First, we were unable to compare HEPA with LAF. This would be desirable since costs of these two techniques differ. We also did not have data on duration of isolation and insufficient data to assess influence of gut sterilization. However, neither oral nonabsorbable nor systemic antibiotics other than cotrimoxazole, both of which decrease gastrointestinal flora, seemed to impact TRM or survival. Finally, this was a non-randomized study. Although we carefully considered

Table 4 Relative risk of transplant-related mortality (death in continuous complete remission) during the first post-transplant year

	HLA-identical sibling transplant		*Alternative donor transplant*	
	Relative risk	*P*	*Relative risk*	*P*
Conventional isolation	1.00	—	1.00	—
HEPA/LAF ⩽100 days post-transplant	0.76	0.009	0.65	0.003
HEPA/LAF, ⩾100 days post-transplant	0.88	NS	1.37	NS
Other significant variables				
Intermediate/advanced *vs* early leukemia	1.47	0.0001	1.52	0.0001
CML *vs* acute leukemia	—	NS	1.41	0.003
Age (continuous, *n* = age increment in years)	1.03[n]	0.0001	1.01[n]	0.03
Karnofsky performance score <90% *vs* ⩾90%	1.29	0.002	—	NS
CMV seropositive recipient *vs* negative	1.14	0.05	1.38	0.001
Donor–recipient sex-mismatch	1.25	0.0005	—	NS
Cotrimoxazole prophylaxis	0.61	0.0001	0.50	0.0001
i.v. Immune globulin prophylaxis	0.86	0.02	—	NS
Unrelated *vs* related donor	NA	NA	1.39	0.002
GVHD prophylaxis		0.0001		0.0001
CsA alone *vs* CsA + MTX	1.26		1.54	
CsA + other drugs *vs* CsA + MTX	1.52		1.62	
T-cell depletion *vs* CsA + MTX	1.17		1.57	
Transplant center geographic latitude:		0.0001		0.002
30°–39° *vs* < 30°	0.64		0.78	
⩾40° *vs* < 30°	0.56		0.55	

HEPA/LAF = laminar airflow/high efficiency particulate air filtration units; CML = chronic myelogenous leukemia; early leukemia = 1.CR or 1. chronic phase; intermediate leukemia = ⩾2.CR or accelerated phase; advanced leukemia = not in remission or blastic phase; i.v. = intravenous; CsA = cyclosporin A; MTX = methotrexate; NS = not significant; NA = not applicable.

Table 5 Relative risk of overall mortality (death from all causes) during the first post-transplant year

	HLA-identical sibling transplant		*Alternative donor transplant*	
	Relative risk	*P*	*Relative risk*	*P*
Conventional isolation	1.00	—	1.00	—
HEPA/LAF ⩽100 days post-transplant	0.80	0.02	0.65	0.0006
HEPA/LAF ⩾100 days post-transplant	0.84	NS	1.22	NS
Other significant variables				
Intermediate *vs* early leukemia	1.72	0.0001	1.67	0.0001
Advanced *vs* early leukemia	2.56	0.0001	2.37	0.0001
CML *vs* acute leukemia	0.80	0.0003	1.21	0.04
Age (continuous, *n* = age increment in years)	1.02[n]	0.0001	1.01[n]	0.01
Karnofsky score <90% *vs* ⩾90%	1.43	0.0001	—	NS
CMV seropositive recipient *vs* negative	—	NS	1.28	0.003
Cotrimoxazole prophylaxis	0.73	0.0001	0.46	0.0001
i.v. Immune globulin prophylaxis	—	NS	0.85	0.04
Unrelated *vs* related donor	NA	NA	1.31	0.002
CsA + MTX *vs* other GVHD prophylaxis	0.83	0.0006	0.61	0.0001
Transplant center geographic latitude:		0.0001		0.004
30°–39° *vs* < 30°	0.71		0.91	
⩾40° *vs* < 30°	0.63		0.68	

HEPA/LAF = laminar airflow/high efficiency particulate air filtration units; CML = chronic myelogenous leukemia; early leukemia = 1.CR or 1. chronic phase; intermediate leukemia ⩾2.CR or accelerated phase; advanced leukemia = not in remission or blastic phase; i.v. = intravenous; CsA = cyclosporin A; MTX = methotrexate; NS = not significant; NA = not applicable.

potential confounding by a large number of patient and treatment variables, it is possible that the observed effects are due to other unmeasured factors. The effects noted, however, are sufficiently strong to merit further study.

In conclusion, HEPA and/or LAF isolation appears to decrease TRM and increase survival after allogeneic bone marrow transplant. This benefit is stronger in patients receiving transplants from donors other than HLA-identical siblings. Further study is needed to determine whether HEPA and LAF differ, and to better define impact of prophylactic antibiotic regimens. As centres come under increasing pressure to control costs by eliminating costly supportive care measures, it is important to study carefully the impact of such changes on patient outcome.

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Cystic Fibrosis Upper Airways Primary Colonization with *Pseudomonas aeruginosa*: Eradicated by Sinonasal Antibiotic Inhalation

To the Editor:

Chronic pulmonary infection with pathogens like *Pseudomonas aeruginosa (P. aeruginosa)* is the major cause of premature death in cystic fibrosis (CF). The sinonasal area has been discussed as a reservoir for lung infection. Since the upper airways (UAW) are not assessed microbiologically according to current CF standard care, evidence (1–4) of their role in disease progression remains scarce (5). Furthermore, therapeutic options have been insufficiently evaluated and sinonasal deposition of aerosols was not effective with common nebulizers.

A 12 1/2–year-old patient (genotype DeltaF508/CFTRdele17a-18) suffered from chronic mucopurulent rhinosinusitis as predominant CF symptom (6). Otorhinological assessment revealed abundant secretions and mucosal inflammation but no morphologic changes requiring surgery. He was treated with oral antibiotic courses, nasal topical steroids, and frequent therapeutic nasal lavages (NL), applying 250 ml of isotonic saline (6), to remove mucus and crusts. Routine sputum samples were taken every 1 to 3 months. In addition, we sampled UAW by diagnostic NL as described previously (3). In brief, 10 ml of sterile isotonic saline was applied to each nostril using a 10-ml syringe, with reclined head and occluded soft palate. Then, nasal material was drained into a cup by anteflection and any material from the mouth was discarded.

First evidence of nonmucoid *P. aeruginosa* of the head was found in the patient's NL in June 2006 (Figure 1). Despite a 2-week intravenous course of tobramycin and ceftazidime, *P. aeruginosa* was detected by NL again in August 2006.

The patient subsequently inhaled tobramycin (Gernebcin 80 mg/d) off-label for 4 weeks using the Pari Sinus nebulizer (Pari Corp, Starnberg, Germany). This device, which uses the new principle of superimposing 44-Hz vibration upon nebulization, has been shown by *in vitro* (7) and *in vivo* (8) experiments to deposit aerosol into paranasal sinuses. We recently published results of the first clinical trial with the device, a double-blind, placebo-controlled pilot crossover study, assessing sinonasal inhalation of dornase alpha in five patients with CF. Dornase alpha significantly improved Quality of Life assessed by the Sinonasal Outcome Test 20 (9).

Sinonasal inhalation was performed as described previously (9). In brief, patients inhaled into each nostril consecutively while occluding the soft palate and semi-occluding the contralateral nostril (duration 4 min/nostril). Therapy was well tolerated.

During the subsequent 4.5 years, more than 40 samples from the patient's upper and lower airways and serum antibodies remained negative for *P. aeruginosa*.

Discussion

The possibility of bacterial spread from the upper to the lower airways has been suspected (1), as in initially *P. aeruginosa*–free transplanted lungs the *P. aeruginosa* genotype of the explanted CF lungs was found, implying sinonasal persistence. Besides, *P. aeruginosa* biofilms were detected intraoperatively in paranasal sinuses of patients with CF who had only intermittent lower airway colonization (2). Concordantly, we found 96% identical genotypes of *P. aeruginosa* sample pairs in patients with colonization of both airway levels (3).

In our case the novel therapeutic approach of sinonasal antibiotic administration using Pari Sinus vibrating aerosol technology led to eradication of *P. aeruginosa*.

Conclusions

Current standards of CF care would not have detected first colonization with *P. aeruginosa* in our patient. Upper airway sampling by nasal lavage can facilitate detection of pathogen colonization, and its therapy may delay pulmonary infection with *P. aeruginosa*, the leading cause of mortality in patients with CF.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript. However, we are now performing a prospective DBPC pilot study on sinonasal inhalation of tobramycin supported by InfectoPharm and Pari as well as a pilot study on sinonasal inhalation of Colistin supported by Gruenenthal, Germany.

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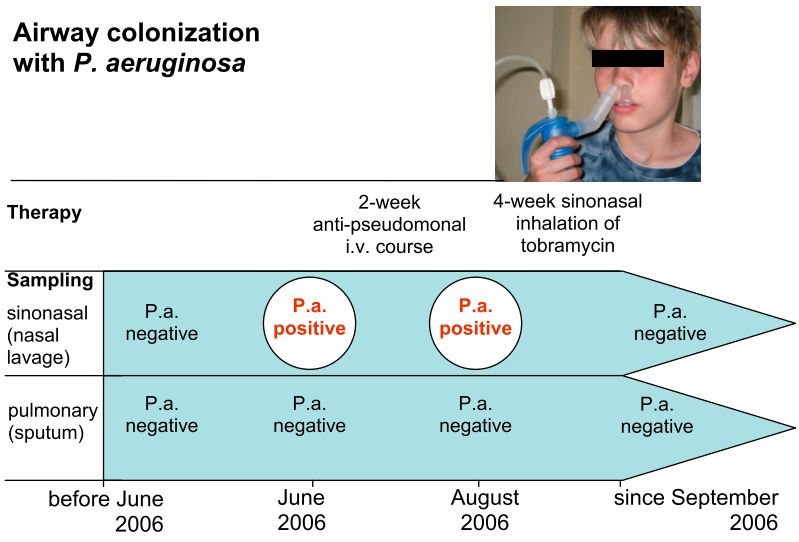

Figure 1. Chronological sequence of diagnostic findings and therapeutic measures in a 12 1/2–year-old patient with CF, showing *Pseudomonas aeruginosa* (P.a.) cultures from nasal lavage fluid and sputum as well as therapeutic measures for *Pseudomonas* eradication, including sinonasal inhalation of tobramycin via the novel Pari Sinus nebulizer.

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Erratum: Lipid Metabolism: A New Frontier in Sleep Apnea Research

Because of a typesetting error, the middle initials were inadvertently omitted for both authors in the publication of "Lipid metabolism: A new frontier in sleep apnea research" (1), an editorial published in the August 1, 2011 issue of *AJRCCM*. The names should have appeared as Luciano F. Drager, M.D., Ph.D and Vsevolod Y. Polotsky, M.D., Ph.D;

Reference

1. Drager LF, Polotsky VY. Lipid metabolism: A new frontier in sleep apnea research [editorial]. *Am J Respir Crit Care Med* 2011;184:288–290.

Erratum: Totally Implantable Vascular Access Devices in Adult Patients for Cystic Fibrosis Management

A letter published in the January 1, 2011 issue, "Totally implantable vascular access devices in adult patients for cystic fibrosis management" (1), incorrectly the order of the authors. The last author listed should have been Dr. Adriano Peris.

Reference

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original reports

A Population-Based Study of the Long-Term Risk of Infections Associated With Hospitalization in Childhood Cancer Survivors

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abstract

PURPOSE Infections pose a significant risk during therapy for childhood cancer. However, little is known about the risk of infection in long-term survivors of childhood cancer.

METHODS We performed a retrospective observational study of children and adolescents born in Washington State diagnosed with cancer before age 20 years and who survived at least 5 years after diagnosis. Survivors were categorized as having a hematologic or nonhematologic malignancy and were matched to individuals without cancer in the state birth records by birth year and sex with a comparator:survivor ratio of 10:1. The primary outcome was incidence of any infection associated with a hospitalization using diagnostic codes from state hospital discharge records. Incidence was reported as a rate (IR) per 1,000 person-years. Multivariate Poisson regression was used to calculate incidence rate ratios (IRR) for cancer survivors versus comparators.

RESULTS On the basis of 382 infection events among 3,152 survivors and 771 events among 31,519 comparators, the IR of all hospitalized infections starting 5 years after cancer diagnosis was 12.6 (95% CI, 11.4 to 13.9) and 2.4 (95% CI, 2.3 to 2.6), respectively, with an IRR 5.1 (95% CI, 4.5 to 5.8). The survivor IR during the 5- to 10-year (18.1, 95% CI, 15.9 to 20.5) and > 10-year postcancer diagnosis (8.3, 95% CI, 7.0 to 9.7) periods remained greater than comparison group IRs for the same time periods (2.3, 95% CI, 2.1 to 2.6 and 2.5, 95% CI, 2.3 to 2.8, respectively). When potentially vaccine-preventable infections were evaluated, survivors had a greater risk of infection relative to comparators (IRR, 13.1; 95% CI, 7.2 to 23.9).

CONCLUSION Infectious complications continue to affect survivors of childhood cancer many years after initial diagnosis. Future studies are needed to better understand immune reconstitution to determine specific factors that may mitigate this risk.

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INTRODUCTION

In the past five decades, the 5-year survival rate for all pediatric cancer has steadily increased to exceed 80%.[1] This improvement in survival is primarily attributed to advancements in therapy, risk stratification, and supportive care.[2,3] Although these treatment protocols are lifesaving, they have undesirable consequences, including prolonged delays in immune reconstitution leaving patients vulnerable to infection well beyond completing chemotherapy.[4-6] The Childhood Cancer Survivor Study reported that survivors of all cancer were 1.6-2.7 times more likely to be hospitalized for infection > 5 years following the completion of therapy compared with age- and sex-matched individuals in the general population.[7] As a result, infection remains a significant cause of morbidity and mortality for the estimated 500,000 survivors of childhood cancer in the United States.[5,7,8]

Although prior studies highlight the overall infection risk for children after completing therapy for any cancer, there is a paucity of data documenting the incidence of specific infections in long-term cancer survivors, including potentially vaccine-preventable illnesses severe enough to require hospitalization.[9] In the absence of these data, clinicians, in particular, pediatricians in the community who care for these children following completion of therapy, are unable to have informed discussions regarding infection risk with parents and their patients in this post-treatment period. The aim of the current study is to describe the overall incidence rate (IR) of infections requiring hospitalization for survivors of childhood and adolescent cancers at least 5 years from initial cancer diagnosis. IRs will also be calculated for specific infection types and by subgroups of survivors of hematologic and nonhematologic malignancies. Evaluating infections separately in survivors of hematologic and nonhematologic cancers allows for a better understanding of malignancy-specific risks and addresses the specific knowledge gap of immune

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

There is a paucity of data documenting the incidence of specific infections in long-term cancer survivors, specifically differentiating risk in patients with hematologic versus nonhematologic malignancies. This population-based retrospective observational follow-up analysis sought to determine whether survivors of pediatric cancer still have an elevated risk of infection > 5 years from initial diagnosis.

Knowledge Generated

Survivors of pediatric cancer remain at increased risk of infections associated with a hospitalization or death long after their initial diagnosis. This risk is elevated in survivors of both hematologic and nonhematologic malignancies for infections across most of the organ systems and pathogen groups assessed.

Relevance

It is vital for patients, caregivers, and their providers to appreciate this long-term infection risk assessment. This allows for better counseling by physicians and recommendations of care for survivors that will reduce the morbidity of infection throughout the survivorship period.

impairment after treatment in nonhematologic malignancies.[9] As therapeutic options improve and the number of childhood cancer survivors increases, it is important to define infection risk in the post-therapy period to guide counseling and encourage investigations aimed at developing effective preventative strategies.

METHODS

Study Design and Patient Population

We conducted a population-based retrospective observational follow-up analysis using linked cancer-birth-hospital discharge records from Washington State–born children and adolescents with and without cancer. We calculated IRs of infections associated with a hospitalization or death overall and during different follow-up time periods that each started 5 years after cancer diagnosis. These rates were separately calculated for children with hematologic and nonhematologic malignancies and compared with rates for children and adolescents without cancer.

Identification of Cancer Survivors

Cancer survivors were identified from two population-based cancer registries in Washington State: the Cancer Surveillance System (part of the National Cancer Institute's Surveillance, Epidemiology and End Results Program) and the Washington State Cancer Registry (part of the Centers for Disease Control and Prevention's North American Association of Central Cancer Registries). The former registry began in 1974 and includes > 70% of the state's population; the latter began in 1995. Cancer registry records of individuals age < 20 years at the time of primary cancer diagnosis were linked to Washington State birth records to identify those born in state (N = 5,605). Only children diagnosed between 1982 and 2008 and who survived at least 5 years from diagnosis were included in this analysis (N = 3,152), with last date of follow-up through December 2013. An index date of 5 years from initial cancer diagnosis was assigned to each survivor.

Comparator Subjects

Each cancer survivor was randomly matched by birth year and sex to 10 children identified in the remaining Washington State birth records. Comparator subjects representing the general population were not known to have died before the survivor's index date on the basis of further linkage to the state death records.

Outcome

Record linkage occurred in 2014-2015. Records of all subjects were linked to state hospital discharge and death records beginning at the index date, as described previously.[8] Subjects without a death or hospitalization record identified during this time were assumed to be alive and event-free through December 2013. The primary outcome was incidence of any infection associated with a hospitalization or death occurring 5 or more years after the cancer diagnosis through December 2013. Time to event was measured from index date, with median potential follow-up[10] of 9.3 years (interquartile range [IQR], 4.4-14.5 years) for both cases and comparators. Infections were defined using International Classification of Disease, Version 9 and 10 (ICD-9/-10), codes according to Medicare-Medicaid billing standards (Appendix Table A1, online only). State hospital discharge data include records of all inpatient discharges (full and observation admissions) at non-Federal facilities and contain up to 25 discharge diagnoses. State death records contain one underlying primary and up to 10 contributing causes of death. Death because of infection without an associated hospitalization was extremely rare, and thus the primary outcome is referred to more simply as infection associated with a hospitalization. As done previously, infections were classified by organism type (bacterial, fungal, or virus), by organ

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system/infection type (nervous system, respiratory, GI, genitourinary, musculoskeletal, skin, or sepsis), and by whether it is potentially vaccine-preventable (influenza A and B virus, varicella zoster virus, *H. influenzae*, hepatitis B, or *S. pneumoniae*).[11] Subjects may have had more than one hospitalization associated with infection.

Covariate Data

Race/ethnicity, birth year, and sex for cancer survivors and comparator subjects were available from the state birth records. Cancer-specific variables including International Classification of Diseases for Oncology (ICD-O) morphology and topography codes, date of diagnosis, and age at diagnosis were available from the cancer registries. Cancer types were classified per the International Classification of Childhood Cancer, 3rd edition.[12] Cancer treatment duration and relapse status were not available from these registries.

Statistical Analysis

IRs of infection associated with a hospitalization per 1,000 person-years (PY) were calculated. Poisson regression was used to estimate the incidence rate ratios (IRR) and associated 95% CIs comparing infection rates between children with and without cancer.[13] For each infection type, the count of infections (ranging from 0 to 15) was the dependent variable, with years under observation included as an offset term in the Poisson model. Birth year (continuous) and sex were included as covariates. Survivors were categorized as having a hematologic (n = 1,244) or nonhematologic (n = 1,908) malignancy, with calculation of respective IRs and IRRs. We examined the infection-related IRs and IRRs overall for 5+ years after diagnosis, and more specifically during 5 to < 10 years after diagnosis, and ≥ 10 years after diagnosis. IRs and IRRs for infections associated with a hospitalization were also calculated by infection type, organ system, and status as potentially vaccine-preventable. We used Stata version 17 (College Station, TX) for all analyses.[14]

RESULTS

The assembled cohort included 3,152 cancer survivors: 1,244 (39.5%) survivors of hematologic malignancy and 1,908 (60.5%) survivors of nonhematologic malignancy (Table 1). Leukemia (25.9%), CNS tumors (19.8%), and lymphoma (13.6%) were the most common cancers. Sex and age distribution of cancer survivors and comparators were similar by design. Each group consisted of 48.1% females and 51.9% males. The median age at cancer diagnosis was 7 years (range, 0-19 years). Median follow-up after index date was 8.8 years (range, 0.05-27.0 years; IQR, 4.1-14.2 years) for cancer survivors and 9.3 years (range, 0.1-27.0 years; IQR, 4.4-14.5 years) for age- and sex-matched comparators.

Overall and Specific Inpatient Infection Rates

In the follow-up period, there were 382 infections diagnosed during a hospitalization among 3,152 cancer survivors and 771 infections diagnosed in the inpatient setting among 31,519 comparators. This resulted in an IR of 12.6 inpatient infections per 1,000 PY (95% CI, 11.4 to 13.9) in cancer survivors and 2.4 inpatient infections per 1,000 PY rate (95% CI, 2.3 to 2.6) in the comparator subjects. Among survivors of hematologic and nonhematologic malignancy, the IR for all infections associated with a hospitalization was 18.5 (95% CI, 16.2 to 21.1) and 8.7 (95% CI, 7.5 to 10.2), respectively. Among infection types, bacterial was the most common in both survivors of hematologic and nonhematologic malignancy (IR, 7.3; 95% CI, 5.9 to 9.0; and IR, 2.7; 95% CI, 2.1 to 3.6, respectively). By organ system, respiratory infections were most common in survivors of hematologic malignancy (IR, 4.1; 95% CI, 3.5 to 4.9), whereas survivors of nonhematologic malignancy had a very similar incidence of genitourinary and respiratory infections (IR, 2.6; 95% CI, 2.0 to 3.5; and IR, 2.6; 95% CI, 1.9 to 3.4, respectively). Sepsis was also among the most frequent inpatient infection in both survivors of hematologic and nonhematologic malignancy (IR, 2.2; 95% CI, 1.5 to 3.2; and IR, 0.8; 95% CI, 0.5 to 1.4, respectively; Table 2).

The incidence of infections associated with a hospitalization per 1,000 PY declined for cancer survivors, both of hematologic and nonhematologic malignancy, in the > 10-year postdiagnosis follow-up period (IR, 8.3; 95% CI, 7.0 to 9.7) as compared with 5- to 10-year follow-up period (IR, 18.1; 95% CI, 15.9 to 20.5; Figs 1 and 2). However, these IRs were higher for cancer survivors in both follow-up periods relative to rates of infection associated with a hospitalization for age- and sex-matched comparators during the same follow-up windows (IR, 2.5; 95% CI, 2.3 to 2.8; and IR, 2.3; 95% CI, 2.1 to 2.6, respectively; Appendix Tables A2 and A3 [online only]).

Relative Risk of Infections Associated With Hospitalization

The IRR of infections associated with hospitalization in all cancer survivors was 5.1 (95% CI, 4.5 to 5.8). Although bacterial infections were more numerous in survivors of both hematologic and nonhematologic malignancy, the relative risk was highest for fungal infections in both groups (IRR, 25.2; 95% CI, 14.7 to 43.2; and IRR, 10.2; 95% CI, 5.5 to 18.9, respectively) followed by bacterial (IRR, 10.7; 95% CI, 8.3 to 13.7; and IRR, 4.0; 95% CI, 3.0 to 5.5, respectively). When evaluating organ systems, the IRR of survivors of hematologic malignancy relative to comparators was highest for infections of the nervous system (IRR, 13.9; 95% CI, 6.2 to 31.1) followed by respiratory infections (IRR, 10.1; 95% CI, 7.8 to 13.1), whereas relative risk for infections of the genitourinary followed by nervous system (IRR, 8.1; 95% CI, 5.7 to 11.4; and IRR, 5.0; 95% CI, 1.9 to 13.7, respectively) were the highest among survivors of nonhematologic malignancy (Table 3, Fig 3).

Both survivors and comparators experienced relatively low rates of inpatient vaccine-preventable infections associated with hospitalization (Table 2); however, rates were significantly higher in cancer survivors versus comparator

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TABLE 1. Characteristics of Child Cancer Survivors and Age- and Sex-Matched Comparators

Characteristic	Comparator Subjects (N = 31,519), No. (%)	All Cancer Survivors (N = 3,152), No. (%)	Survivors of Hematologic Malignancy (n = 1,244), No. (%)	Survivors of Nonhematologic Malignancy (n = 1,908), No. (%)
Sex				
Female	15,160 (48.1)	1,516 (48.1)	555 (44.6)	961 (50.4)
Male	16,359 (51.9)	1,636 (51.9)	689 (55.4)	947 (49.6)
Race/ethnicity				
Asian	1,675 (5.3)	163 (5.2)	68 (5.5)	95 (5.0)
Black	1,123 (3.6)	87 (2.8)	37 (3.0)	50 (2.6)
Hispanic	2,051 (6.5)	183 (5.8)	87 (7.0)	96 (5.0)
Native American	622 (2.0)	47 (1.5)	15 (1.2)	32 (1.7)
White	25,349 (80.4)	2,617 (83.0)	1,014 (81.5)	1,603 (84.0)
Other/unknown	699 (2.2)	55 (1.7)	23 (1.8)	32 (1.7)
Age at diagnosis, years				
< 5		1,305 (41.4)	508 (40.8)	797 (41.8)
5-9		559 (17.7)	256 (20.6)	303 (15.9)
10-14		539 (17.1)	213 (17.1)	326 (17.1)
15-19		749 (23.8)	267 (21.5)	482 (25.3)
Index year				
1987-1989		91 (2.9)	29 (2.3)	62 (3.2)
1990-1994		270 (8.6)	111 (8.9)	159 (8.3)
1995-1999		497 (15.8)	197 (15.8)	300 (15.7)
2000-2004		759 (24.1)	305 (24.5)	454 (23.8)
2005-2009		815 (25.9)	314 (25.2)	501 (26.3)
2010-2013		720 (22.8)	288 (23.2)	432 (22.6)
Cancer type				
Leukemias		815 (25.9)	815 (65.5)	
Lymphomas		429 (13.6)	429 (34.5)	
CNS		625 (19.8)		625 (32.8)
Neuroblastoma		211 (6.7)		211 (11.1)
Retinoblastoma		95 (3.0)		95 (5.0)
Renal		173 (5.5)		173 (9.1)
Hepatic		39 (1.2)		39 (2.0)
Malignant bone		110 (3.5)		110 (5.8)
Soft tissue		203 (6.4)		203 (10.6)
Germ cell		167 (5.3)		167 (8.8)
Other malignant epithelial		269 (8.5)		269 (14.1)
Other/unspecified malignancy		16 (0.5)		16 (0.8)

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TABLE 2. Incidence Rates per 1,000 Person-Years With 95% CI of Hospitalized Infections Overall and by Organism and Organ System of Child Cancer Survivors > 5 Years From Diagnosis and Comparators

	Comparator Subjects (N = 31,519)		All Cancer Survivors (N = 3,152)		Survivors of Hematologic Malignancy (n = 1,244)		Survivors of Nonhematologic Malignancy (n = 1,908)	
Infection Classification	**Events**	**Rate**	**Events**	**Rate**	**Events**	**Rate**	**Events**	**Rate**
Infections, all	771	2.4 (2.3 to 2.6)	382	12.6 (11.4 to 13.9)	221	18.5 (16.2 to 21.1)	161	8.7 (7.5 to 10.2)
Bacterial	214	0.7 (0.6 to 0.8)	137	4.5 (3.8 to 5.3)	87	7.3 (5.9 to 9.0)	50	2.7 (2.1 to 3.6)
Fungal	27	0.1 (0.1 to 0.1)	42	1.4 (1.0 to 1.9)	26	2.2 (1.5 to 3.2)	16	0.9 (0.5 to 1.4)
Viral	83	0.3 (0.2 to 0.3)	39	1.3 (0.9 to 1.8)	30	2.5 (1.8 to 3.6)	9	0.5 (0.3 to 0.9)
By organ system								
Sepsis	69	0.2 (0.2 to 0.3)	41	1.3 (1.0 to 1.8)	26	2.2 (1.5 to 3.2)	15	0.8 (0.5 to 1.4)
Nervous system	17	0.1 (0.0 to 0.1)	14	0.5 (0.3 to 0.8)	9	0.8 (0.4 to 1.4)	5	0.3 (0.1 to 0.7)
Respiratory system	202	0.6 (0.6 to 0.7)	125	4.1 (3.5 to 4.9)	78	6.5 (5.2 to 8.1)	47	2.6 (1.9 to 3.4)
GI system	230	0.7 (0.6 to 0.8)	30	1.0 (0.7 to 1.4)	19	1.6 (1.0 to 2.5)	11	0.6 (0.3 to 1.1)
Genitourinary system	100	0.3 (0.3 to 0.4)	62	2.0 (1.6 to 2.6)	14	1.2 (0.7 to 2.0)	48	2.6 (2.0 to 3.5)
Musculoskeletal system	21	0.1 (0.0 to 0.1)	< 5		< 5		< 5	
Skin	114	0.4 (0.3 to 0.4)	42	1.4 (1.0 to 1.9)	19	1.6 (1.0 to 2.5)	23	1.2 (0.8 to 1.9)
Vaccine-preventable infections, all[a]	19	0.1 (0.0 to 0.1)	24	0.8 (0.5 to 1.2)	20	1.7 (1.1 to 2.6)	< 5	
Influenza A and B virus	< 5		5	0.2 (0.1 to 0.4)	5	0.4 (0.2 to 1.0)	0	—
Varicella zoster virus	5	0.0 (0.0 to 0.0)	15	0.5 (0.3 to 0.8)	13	1.1 (0.6 to 1.9)	< 5	

NOTE. In cells with < 5, incidence rates ranged from 0.0 to 0.2.

[a]See Appendix tables for incidence rates of other specific vaccine-preventable infections.

subjects. Specifically, survivors of both hematologic malignancy and nonhematologic malignancy have an increased risk of varicella zoster virus infections relative to comparator subjects (IRR, 69.7; 95% CI, 24.8 to 195.4; and IRR, 6.7; 95% CI, 1.3 to 34.5, respectively). Survivors of hematologic malignancy also had a significantly

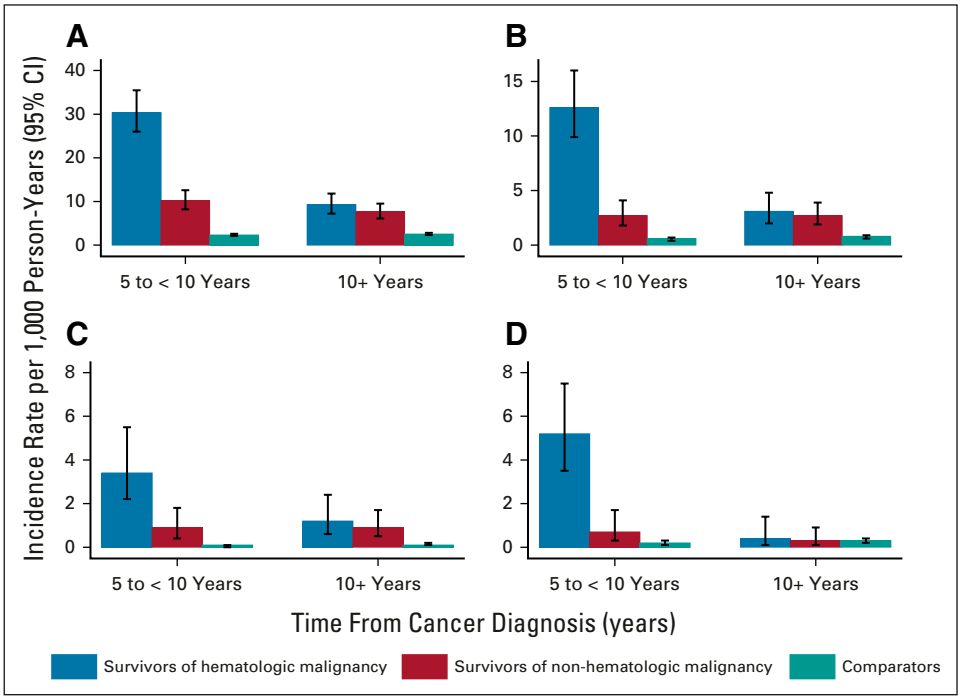

FIG 1. Rates of infection by organism type: (A) all infections, (B) bacterial infections, (C) fungal infections, and (D) viral infections.

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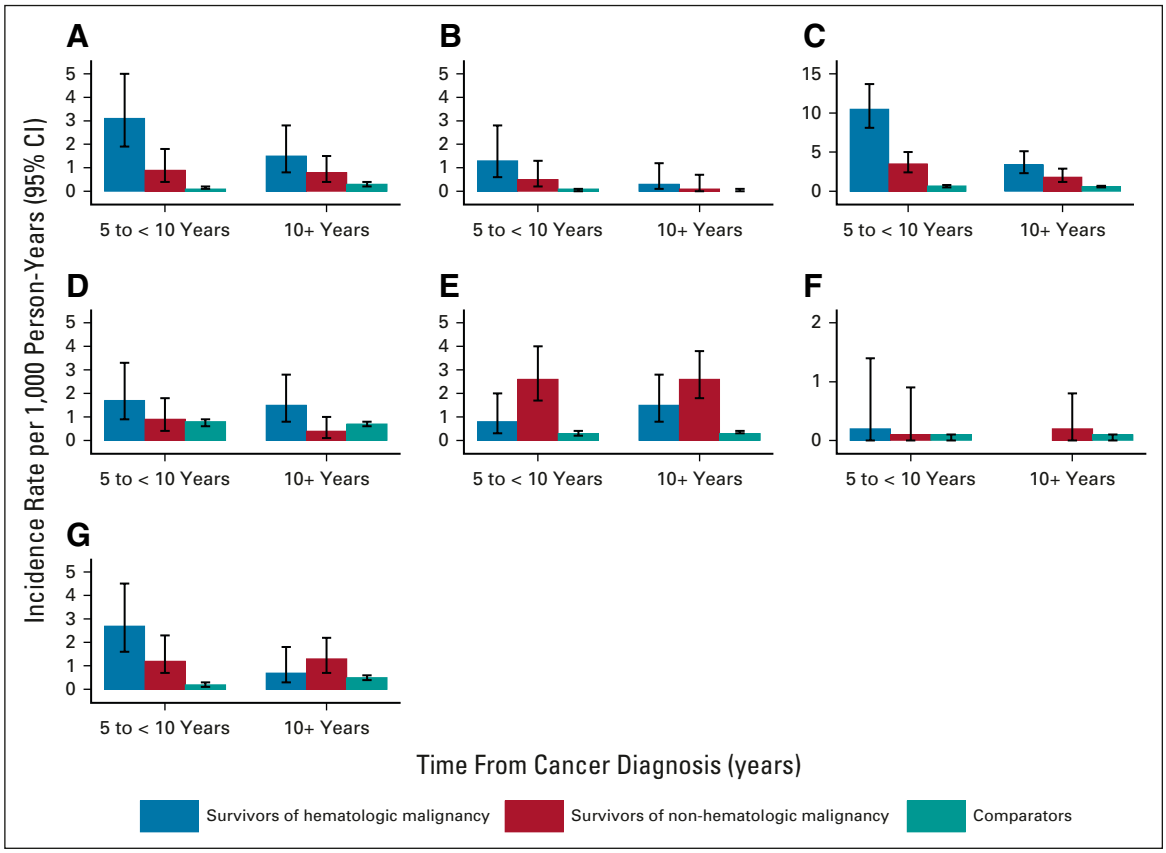

FIG 2. Rates of infection by organ system: (A) sepsis, (B) nervous system, (C) respiratory, (D) gastrointestinal, (E) genitourinary, (F) musculoskeletal, and (G) skin.

increased risk of influenza A and B infection associated with hospitalization (IRR, 32.9; 95% CI, 8.8 to 122.7; Table 3).

DISCUSSION

In this observational comparative study, infections diagnosed in the inpatient setting were consistently and significantly higher in cancer survivors versus age- and sex-matched comparators. Although higher rates of infections diagnosed during a hospitalization were documented in the 5- to 10-year period, the increased risk for such infections persisted for survivors of hematologic malignancy and nonhematologic malignancy > 10 years from cancer diagnosis. These results are consistent with prior literature. In 2011, Lorenzi et al found that pediatric cancer survivors in a British Columbia registry had an increased risk of infections diagnosed during hospitalization for up to 5 years after their diagnosis. This risk was particularly evident among the leukemia survivor subgroup.[15] A separate Canadian study from Pelland-Marcotte et al[6] leveraged administrative data from the Pediatric Oncology Group of Ontario Networked Information System to show that leukemia survivors had an increased relative risk of infections diagnosed in the inpatient and outpatient settings at 5 years and > 5 years after the completion of chemotherapy compared with a control group. Data from our US-based study, in conjunction with these Canadian reports, highlight the potential long-lasting impact that cancer therapy has on infection risk.

Although survivors of hematologic malignancy tended to have higher risk of infection than survivors of nonhematologic malignancy relative to comparators, both groups had significantly increased relative risks for inpatient infections across most of the organ systems and pathogen groups assessed. Given that the increased risk for infections associated with hospitalization existed across most infection subtypes, clinicians caring for pediatric cancer survivors need to remain vigilant for a wide range of infections in these patients compared with their healthy counterparts. This is particularly true for bacterial infections, which were the most numerous. However, it is also important to maintain awareness for fungal infections, which yielded the highest IRR of all infection types, as they were far less common in the comparators. Among organ systems, the increased risk of infections of the respiratory tract, nervous system, and genitourinary system, and sepsis were particularly profound. This higher index of suspicion may warrant lower thresholds for bringing patients into the clinic for evaluation, pursuing diagnostic testing, or referring to subspecialists for additional evaluation. Furthermore, it is imperative that patients and their caregivers are appropriately counseled about this long-term risk so that they may seek timely medical attention for infectious symptoms, rather than assuming that their infectious

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TABLE 3. IRR of Infections in Cancer Survivors > 5 Years From Diagnosis Relative to Comparators

	All Cancer Survivors		Survivors of Hematologic Malignancy		Survivors of Nonhematologic Malignancy	
Infection Classification	**IRR**	**95% CI**	**IRR**	**95% CI**	**IRR**	**95% CI**
Infections, all	5.1	4.5 to 5.8	7.5	6.5 to 8.7	3.6	3.0 to 4.3
Bacterial	6.7	5.4 to 8.2	10.7	8.3 to 13.7	4.0	3.0 to 5.5
Fungal	16.2	10.0 to 26.2	25.2	14.7 to 43.2	10.2	5.5 to 18.9
Viral	4.9	3.3 to 7.1	9.5	6.3 to 14.5	1.9	0.9 to 3.7
By organ system						
Sepsis	6.2	4.2 to 9.1	9.9	6.3 to 15.5	3.7	2.1 to 6.6
Nervous system	8.5	4.2 to 17.3	13.9	6.2 to 31.1	5.0	1.9 to 13.7
Respiratory system	6.4	5.1 to 8.0	10.1	7.8 to 13.1	4.0	2.9 to 5.5
GI system	1.3	0.9 to 2.0	2.2	1.3 to 3.4	0.8	0.4 to 1.5
Genitourinary system	6.4	4.7 to 8.8	3.8	2.2 to 6.7	8.1	5.7 to 11.4
Musculoskeletal system	2.0	0.7 to 5.7	1.2	0.2 to 9.1	2.5	0.7 to 8.3
Skin	3.8	2.7 to 5.5	4.3	2.7 to 7.0	3.5	2.2 to 5.5
Vaccine-preventable infections, all[a]	13.1	7.2 to 23.9	27.6	14.7 to 51.8	3.7	1.2 to 10.8
Influenza A and B virus	12.9	3.5 to 48.2	32.9	8.8 to 122.7	0.0	—
Varicella zoster virus	31.0	11.3 to 85.3	69.7	24.8 to 195.4	6.7	1.3 to 34.5

Abbreviation: IRR, incidence rate ratio.

symptoms can be managed with a similar approach used for otherwise healthy children and adolescents.

Survivors of both hematologic and nonhematologic malignancy also had significantly increased risk for vaccine preventable illnesses diagnosed during a hospitalization. The magnitude of risk relative to comparators was noted be higher among the survivors of hematologic malignancy. Varicella zoster virus was the most common vaccine-preventable illness reported followed by influenza. It is important to note, however, that many of these patients (approximately 70%) were born before incorporation of the varicella vaccine into the routine childhood vaccination series, and thus may not represent a failure of prior vaccine receipt. The increased risk of influenza infections in the

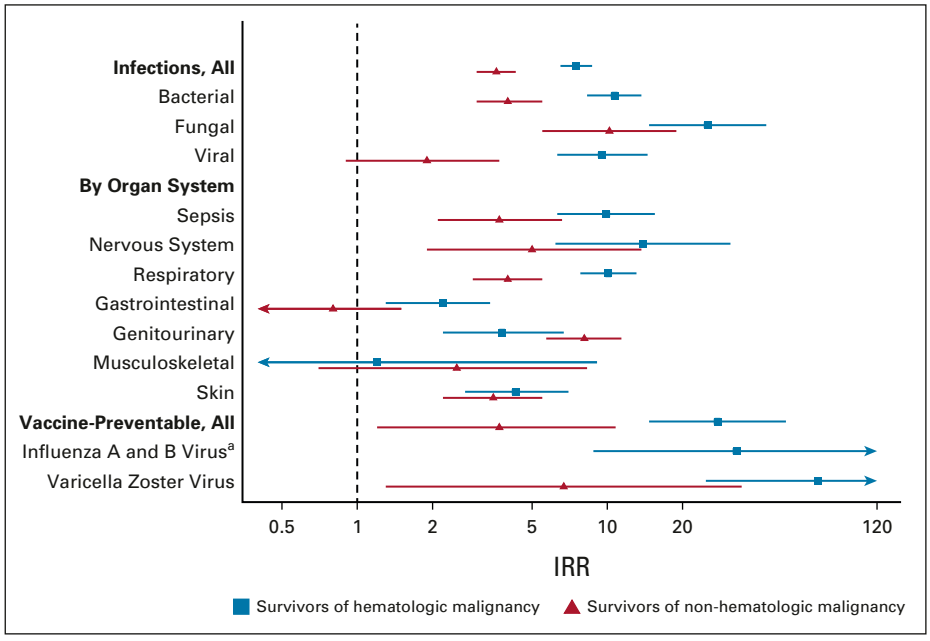

FIG 3. IRRs (Poisson regression) of infections by organism and organ system for survivors versus comparators. [a]No influenza hospitalizations in nonheme survivors. IRR, incidence rate ratio.

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survivors of hematologic malignancy underscores the importance of annual immunization for this population. Further investigation is necessary to determine whether revaccination after completion of chemotherapy would prevent some of these vaccine-preventable illnesses that present in the immediate years after completing chemotherapy. Importantly, there were few diagnosed vaccine-preventable infections associated with hospitalization in the period beyond 10 years from cancer diagnosis suggesting that the increased risk of infection from these pathogens declines with time. At present, international guidelines from the Children's Oncology Group recommend that all survivors of childhood cancer make up missed doses of regularly scheduled vaccines.[9] For survivors who have received all scheduled vaccinations, it is recommended that either (1) boosters be given or (2) titers be measured (if available) and a booster be given if a survivor lacks seroprotection for a given vaccine.[9] These guidelines generally align with practice in the United Kingdom and published recommendations from the European Conference on Infection in Leukemia, where boosters are recommended.[16-18]

Major strengths of this study include a large cohort of pediatric cancer survivors with a variety of cancer diagnoses and a large comparator group with a 10:1 ratio of age- and sex-matched subjects. Additionally, data came from robust and substantiated population-based data sources that are prospectively coded, thus helping to circumvent response bias.[19] Furthermore, this study contributes to a knowledge gap regarding long-term risk of infections diagnosed during hospitalizations in survivors of nonhematologic malignancy, specifically the high rates of fungal and genitourinary infections and significantly increased risk of sepsis relative to comparators, even > 10 years from cancer diagnosis. Overall, there is a paucity of published data reporting on late-onset (> 5 years and 10 years after diagnosis) inpatient infections in cancer survivors and a lack of results specific to certain organ systems and pathogen types, notably vaccine-preventable illnesses. The results from this study contribute to our knowledge regarding the extent of these types of late effects of treatment.

There are several limitations of this study. First, non-Hispanic Whites accounted for > 80% of the subjects in both groups. Although the population of Washington state is reported to have higher percentages of non-Hispanic White and Asian and a slightly lower percentage of Hispanic and Black people than the US population as a whole,[20] this cohort spans a diagnosis period dating back to the 1980s when the US population, especially children, was less diverse than today. Second, the data source for patients with cancer in this study did not identify whether additional treatment for relapse, secondary malignancy, or allogeneic hematopoietic cell transplantation occurred. It is possible that some of the identified infections were attributable to this additional immune suppression rather than residual effect of initial cancer therapy. However, this should be less of a concern among survivors analyzed > 10 years from initial cancer diagnosis, when significantly increased rates of infection were still observed. Third, given that this analysis only assessed inpatient infections and was dependent on diagnostic codes for infection event detection, we likely underestimated the burden of infections in the observed follow-up period. However, by restricting outcomes to those associated with hospitalization, our results should have captured the most clinically relevant serious infections.

Advancement in treatment protocols has yielded survival rates of > 80% at 5 years from diagnosis of childhood cancer; however, these patients are still at risk of significant long-term risk from infection secondary to their cancer diagnosis and therapy.[21,22] Our data show that the risk of an infection associated with hospitalization continues to be significantly increased in survivors of childhood cancer compared with age-matched controls many years to decades after initial diagnosis, with an even higher burden on survivors of hematologic malignancies. It is vital for patients, caregivers, and their providers to have an awareness of this longer-term infection risk to allow for better counseling by physicians and recommendations of care for survivors that will reduce the morbidity of infection during the survivorship period. Additional investigation is necessary to optimize strategies aimed at preventing these infections altogether.

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Final approval of manuscript: All authors
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

A Population-Based Study of the Long-Term Risk of Infections Associated With Hospitalization in Childhood Cancer Survivors

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APPENDIX

TABLE A1. Outcome Categories Derived From the Washington State Hospital Discharge and Death Registries

Category	ICD-9 [ICD-10 equivalent] Codes[a]
Infections, all	001-134.9, 136-139.9, 790.7-790.8 [A_, B_]
Sepsis, any	038 [A40-A41]
Bacterial infections	001-041.9, 079.88,079.98, 080-109.9, 320.2, 320.3, 481, 482.3, 482.4, 790.7 [A00-A05, A15-A59, A65-A79, B95-B96, B98, G00.0-G00.3, J13-J16.0, J17.0, J17.8, J20.1]
Staphylococcus	005.0, 008.41, 038.1, 041.1, 320.3, 482.4 [A41.0-A41.2, A49.0, B95.6-B95.8, G00.3]
Streptococcus	034, 038.0, 038.2, 041.0, 041.2, 320.2, 481, 482.3 [A39-A40, A49.1, B95.0-B95.1, B95.3-B95.5, G00.1-G00.2]
Gram-negative	036, 038.4, 041.3-041.7, 041.82-041.86 [A39, A41.3-A41.5, B96.1-B96.7]
Fungal infections	110-118, 136.3, 484.6 [B35-B49, B59]
Candida	112 [B37]
Non-Candida	110-111, 113-118, 136.3 [B35-B36, B38-B49, B59]
Aspergillus	117.3, 484.6 [B44]
Viral infections	042-079.83, 079.89, 079.99, 790.8 [A08.0-A08.4, A60, A63, A80-A99, B00-B34, B97]
Cytomegalovirus	078.5 [B25]
Herpes simplex virus and varicella zoster virus	052-054.9 [B00-B02]
Organ system	
Nervous	320-321.9, 323-323.4, 323.6, 324, 326, 360-360.09, 373.1, 373.4-373.6, 376.01-376.04, 376.11-376.13, 380.1, 382, 383, 384.0-384.1 [G00-G02, G04.1-G04.2, G06-G07, G53.0-G53.1, G63.0, G73.4, H00.0, H03.0-H03.1, H05.0-H05.1, H06.1, H10.0, H13.0-H13.1, H19.0-H19.2, H45.1, H48.0-H48.1, H58.8, H60.0-H60.3, H62.0-H62.4, H66-H67.1, H70, H73.0-H73.1, H75.0, H94.0]
Respiratory	011-011.2, 011.6, 011.8, 011.9, 020.3, 020.4, 020.5, 021.2, 022.1, 031.0, 039.1, 055.1, 073.0, 095.1, 112.4, 114.0, 114.4, 114.5, 136.3, 460-469.9, 472-474.09, 475-476.9, 478.21-478.24, 478.71, 480-489.9, 510, 513, 518.6 [A15.0, A15.1, A15.2, A15.3, A15.6, A15.7, A15.9, A16.0, A16.1, A16.2, A16.5, A16.7, A16.9, A20.2, A21.2, A22.1, A31.0, A42.0, A43.0, A48.1, B01.2, B05.2, B25.0, B37.1, B38-B38.2, B39-B39.2, B40.0, B40.1, B40.2, B41.0, B42.0, B44.0, B44.1, B45.0, B46.0, B59, J00-J22, J31-J32.9, J34.0, J35.0, J36-J37.1, J40-J42, J85-J86.9]
Bacterial pneumonia	011-011.2, 011.6, 011.8, 011.9, 020.3, 020.4, 020.5, 021.2, 022.1, 031.0, 039.1, 073.0, 095.1, 481, 482-482.49, 482.8, 482.9, 483.0, 483.1, 484.3, 484.5 [A15.0, A15.1, A15.2, A15.3, A15.6, A15.7, A15.9, A16.0, A16.1, A16.2, A16.5, A16.7, A16.9, A20.2, A21.2, A22.1, A31.0, A42.0, A43.0, A48.1, J13, J14, J15, J16.0, J17.0, J17.8,]
Fungal pneumonia	112.4, 114.0, 114.4, 114.5, 136.3, 484.6, 484.7 [B37.1, B38-B38.2, B39-B39.2, B40.0, B40.1, B40.2, B41.0, B42.0, B44.0, B44.1, B45.0, B46.0, B59, J17.2]
Viral pneumonia	055.1,480-480.3, 480.8, 480.9, 484.1 [B01.2, B05.2, B25.0, J10.0, J11.0, J12.0, J12.1, J12.2, J12.3, J12.8, J12.9, J17.1]
Pneumonia, NOS	483.8, 485, 486 [J16.8, J18]
GI	521.0, 522.0-522.1, 522.4-522.7, 523.0-523.4, 526.4-526.5, 527.2-527.4, 528.2-528.3, 528.5, 540-542, 566, 567.0-567.7, 569.5, 569.61, 572.0-572.1, 573.1-573.2, 575.0, 576.1 [K02, K04.1-K04.2, K04.5-K04.7, K05.0-K05.3, K10.2-K10.3, K11.2-K11.3, K12.2, K23.0-K23.1, K35-K37, K61, K63.0, K65.0, K67, K75.0, K77.0, K81.0, K83.0, K87.1, K93.0-K93.1]

(continued on following page)

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TABLE A1. Outcome Categories Derived From the Washington State Hospital Discharge and Death Registries (continued)

Category	ICD-9 [ICD-10 equivalent] Codes[a]
Genitourinary	590-590.9, 595-595.0, 599.0, 601-601.9, 604-604.9, 607.2, 608.0, 611.0, 614-616.1, 616.3-616.89 [N10-N12, N30-N30.0, N30.9, N34.0, N39.0, N41-41.9, N43.1, N45-45.9, N48.2, N49-N49.9, N61, N70-N73.9, N75.1-N75.8, N76-N76.8]
Musculoskeletal	711-711.09, 711.4-711.99, 728.86, 730-730.99 [M00-M00.9, M46.2-M46.3, M46.5, M60.0, M65.1, M71.0-M71.1, M86-M86.9]
Skin	680-684, 685.0, 686-686.9 [L00-L04.9, L05.0, L08-L08.9]
Vaccine-preventable	033.0, 036, 038.41, 041.2, 041.5, 052, 053, 055, 056, 070.0, 070.1, 070.2, 070.3, 072, 078.11, 320.0, 481, 482.2, 487 [A37.0, A39, A40.3, A41.3, A49.2, A63.0, B01, B02, B05, B06, B15, B16, B18.0, B18.1, B26, B95.3, B96.3, G00.0, G00.1, J10, J11, J13, J14, J20.1]

Abbreviations: ICD, International Classifications of Diseases (9th or 10th revisions); NOS, not otherwise specified.
[a]Hospital discharge codes on the basis of ICD-9; death registry codes on the basis of ICD-9 and ICD-10.

TABLE A2. Incidence Rates per 1,000 Person-Years with 95% CI of Inpatient Infections Overall and by Pathogen and Organ System of Cancer Survivors 5 to <10 Years From Diagnosis and Comparators

	Comparator Subjects (n = 31,519)		All Cancer Survivors (n = 3,152)		Survivors of Hematologic Malignancy (n = 1,244)		Survivors of Nonhematologic Malignancy (n = 1,908)	
Infection Classification	Events	Rate	Events	Rate	Events	Rate	Events	Rate
Infections, all	317	2.3 (2.1 to 2.6)	241	18.1 (15.9 to 20.5)	159	30.3 (26.0 to 35.5)	82	10.1 (8.2 to 12.6)
Bacterial	76	0.6 (0.4 to 0.7)	88	6.6 (5.4 to 8.1)	66	12.6 (9.9 to 16.0)	22	2.7 (1.8 to 4.1)
Fungal	10	0.1 (0.0 to 0.1)	25	1.9 (1.3 to 2.8)	18	3.4 (2.2 to 5.5)	7	0.9 (0.4 to 1.8)
Viral	26	0.2 (0.1 to 0.3)	33	2.5 (1.8 to 3.5)	27	5.2 (3.5 to 7.5)	6	0.7 (0.3 to 1.7)
By organ system								
Sepsis	20	0.1 (0.1 to 0.2)	23	1.7 (1.1 to 2.6)	16	3.1 (1.9 to 5.0)	7	0.9 (0.4 to 1.8)
Nervous system	9	0.1 (0.0 to 0.1)	11	0.8 (0.5 to 1.5)	7	1.3 (0.6 to 2.8)	< 5	
Respiratory system	91	0.7 (0.5 to 0.8)	83	6.2 (5.0 to 7.7)	55	10.5 (8.1 to 13.7)	28	3.5 (2.4 to 5.0)
GI system	104	0.8 (0.6 to 0.9)	16	1.2 (0.7 to 2.0)	9	1.7 (0.9 to 3.3)	7	0.9 (0.4 to 1.8)
Genitourinary system	39	0.3 (0.2 to 0.4)	25	1.9 (1.3 to 2.8)	< 5		21	2.6 (1.7 to 4.0)
Musculoskeletal system	10	0.1 (0.0 to 0.1)	< 5		< 5		< 5	
Skin	27	0.2 (0.1 to 0.3)	24	1.8 (1.2 to 2.7)	14	2.7 (1.6 to 4.5)	10	1.2 (0.7 to 2.3)
Vaccine-preventable infections, all	8	0.1 (0.0 to 0.1)	19	1.4 (0.9 to 2.2)	16	3.1 (1.9 to 5.0)	< 5	

NOTE. In cells with < 5, incidence rates ranged from 0.1 to 0.8.

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TABLE A3. Incidence Rates per 1,000 Person-Years with 95% CI of Inpatient Infections Overall and by Pathogen and Organ System of Cancer Survivors >10 Years From Diagnosis and Comparators

	Comparator Subjects (n = 22,392)		All Cancer Survivors (n = 2,179)		Survivors of Hematologic Malignancy (n = 855)		Survivors of Nonhematologic Malignancy (n = 1,324)	
Infection Classification	**Events**	**Rate**	**Events**	**Rate**	**Events**	**Rate**	**Events**	**Rate**
Infections, all	454	2.5 (2.3 to 2.8)	141	8.3 (7.0 to 9.7)	62	9.2 (7.2 to 11.8)	79	7.6 (6.1 to 9.5)
Bacterial	138	0.8 (0.6 to 0.9)	49	2.9 (2.2 to 3.8)	21	3.1 (2.0 to 4.8)	28	2.7 (1.9 to 3.9)
Fungal	17	0.1 (0.1 to 0.2)	17	1.0 (0.6 to 1.6)	8	1.2 (0.6 to 2.4)	9	0.9 (0.5 to 1.7)
Viral	57	0.3 (0.2 to 0.4)	6	0.4 (0.2 to 0.8)	< 5		< 5	
By organ system								
Sepsis, any	49	0.3 (0.2 to 0.4)	18	1.1 (0.7 to 1.7)	10	1.5 (0.8 to 2.8)	8	0.8 (0.4 to 1.5)
Nervous system	8	0.0 (0.0 to 0.1)	< 5		< 5		< 5	
Respiratory system	111	0.6 (0.5 to 0.7)	42	2.5 (1.8 to 3.3)	23	3.4 (2.3 to 5.1)	19	1.8 (1.2 to 2.9)
GI system	126	0.7 (0.6 to 0.8)	14	0.8 (0.5 to 1.4)	10	1.5 (0.8 to 2.8)	< 5	
Genitourinary system	61	0.3 (0.3 to 0.4)	37	2.2 (1.6 to 3.0)	10	1.5 (0.8 to 2.8)	27	2.6 (1.8 to 3.8)
Musculoskeletal system	11	0.1 (0.0 to 0.1)	< 5		0	—	< 5	
Skin	87	0.5 (0.4 to 0.6)	18	1.1 (0.7 to 1.7)	5	0.7 (0.3 to 1.8)	13	1.3 (0.7 to 2.2)
Vaccine-preventable infections, all	11	0.1 (0.0 to 0.1)	5	0.3 (0.1 to 0.7)	< 5		< 5	

NOTE. In cells with < 5, incidence rates ranged from 0.1 to 0.6.

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Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer

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In 1882, Robert Koch suggested four postulates that establish causation between an infectious agent and a particular disease: (1) the infectious organism must be found in abundance in all diseased, but not in healthy, organisms, (2) the infectious organism must be isolated from the diseased host and grown in culture, (3) the disease must be reproduced when the cultured organism is introduced into a healthy organism and (4) the same organism must be reisolated from the experimentally diseased host (Tabrah, 2011; Breitschwerdt et al., 2013). In **Figure 1** we suggest an adaptation to the original postulates of Koch to be used as a framework to assess the causation between intestinal *Pseudomonas aeruginosa* and intestinal disease in patients with cancer. In the following sections we describe the prevalence of *P. aeruginosa* in cancer and the immunosuppressive and stress-inducing conditions of cancer that facilitate the growth, dissemination and virulence of intestinal *P. aeruginosa*. In addition, we describe work showing that *P. aeruginosa* promotes intestinal epithelium cancer-related phenotypes when introduced in tumor prone model hosts.

CANCER AND OTHER IMMUNOSUPPRESSIVE CONDITIONS PROMOTE THE PREVALENCE OF *P. aeruginosa*

Bacteremia is a major cause of life-threatening complications in patients with cancer, especially those who receive anticancer chemotherapy. Cancer patients are more vulnerable to invasive infection, due to ulcerative lesions in mucosal surfaces and immune suppression secondary to chemotherapy (Safdar and Armstrong, 2001). Many studies associate bloodstream infections in cancer patients with Gram-negative bacteria (Oliveira et al., 2007; Bos et al., 2013; Montassier et al., 2013).

P. aeruginosa is a Gram-negative opportunistic bacterium that causes various infections. Common community-acquired infections with *P. aeruginosa* are skin and soft tissue infections, ulcerative keratitis and otitis externa, while hospital-acquired infections include bloodstream infections, pneumonias and urinary tract infections (Driscoll et al., 2007). Infections may be associated with a high rate of morbidity and mortality in immunocompromised hosts, such as those suffering from chemotherapy-induced neutropenia, patients with cystic fibrosis or severe burns and individuals who receive intensive care (Driscoll et al., 2007; Kerr and Snelling, 2009; Worth and Slavin, 2009; Stuart et al., 2010; Rafla and Tredget, 2011).

P. aeruginosa intestinal carriage increases from ∼3% in normal people to ∼20% in hospitalized patients (Stoodley and Thom, 1970). In a case-control study the intestinal colonization by *P. aeruginosa* in cancer patients was 10% before and 31% after hospitalization (Andremont et al., 1989). Studies conducted in oncology–hematology units, found an overall intestinal carriage of *P. aeruginosa* between 11.7 and 37% (Thuong et al., 2003). In another case-control study *P. aeruginosa* intestinal colonization was identified in 17% of controls and 60% of blood cancer patients (Vuotto et al., 2013). These epidemiology data suggest that intestinal colonization by *P. aeruginosa* is prominent among hospitalized cancer patients (Andremont et al., 1989; Vuotto et al., 2013).

The intestinal carriage of *P. aeruginosa* is likely a consequence of the opportunistic nature of this species. Most *P. aeruginosa* infections appear secondary to a breach in host defences. In addition to compromised host immunity, intestinal microbiota play a major role in intestinal defence to infection (Levison, 1973). Thus systemic exposure to antibiotics, which alters intestinal microbiota by reducing the abundance of certain microbes creates the opportunity for intestinal growth of *P. aeruginosa* and other pathogenic bacteria (Hentges et al., 1985).

INTESTINAL *P. aeruginosa* AS A SOURCE OF SYSTEMIC AND REMOTE INFECTIONS

The translocation of endogenous intestinal *P. aeruginosa* extraluminally is an important pathogenic phenomenon and a cause of systemic infections, especially in neutropenic patients with hematological malignancies (Okuda et al., 2010). During the translocation process, bacteria and their products cross the intestinal barrier by traveling between or through the cells of the intestinal epithelium, causing infection and massive inflammation (Alexander et al., 1990; Papoff et al., 2012). Lung infections caused by *P. aeruginosa* are frequent in patients and can occur by direct contamination of the lungs by gastrointestinal flora or through hematogenous spread from the intestine to the lungs. Sepsis and mortality in immunocompromised patients are the results of the presence of highly virulent strains of *P. aeruginosa* within the intestinal tract and the pathogen's ability to adhere to the

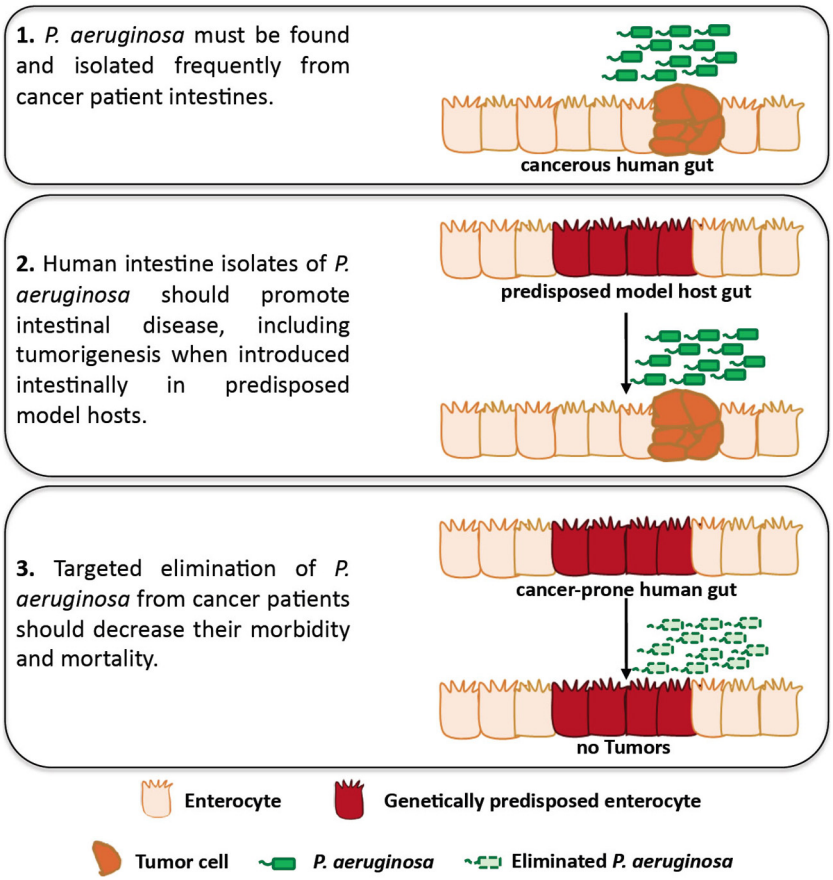

FIGURE 1 | An adaptation of Koch's postulates assessing causation between intestinal *P. aeruginosa* and disease in patients with cancer. Studies using *Drosophila* and mammalian hosts may assess the role of *P. aeruginosa* in facilitating intestinal disease, including intestinal *P. aeruginosa* growth, dissemination, virulence and tumorigenesis, in predisposed hosts. In addition, clinical studies can be designed to assess the presence of *P. aeruginosa* in cancer patients and its role in intestinal disease, including tumorigenesis for which clinical data are lacking.

intestinal epithelial barrier (Marshall et al., 1993; Alverdy et al., 2000; Osmon et al., 2004; Zaborina et al., 2006; Okuda et al., 2010).

P. aeruginosa uses different virulence factors that can damage epithelial cells, such as enzymes (proteases and elastases), toxins, adhesins, flagella and protein secretion systems (Sundin et al., 2004). The T3SS enables the injection of at least four effector proteins (ExoS, ExoT, ExoU, and ExoY) into the host cell. ExoS injected into the host epithelial cell migrates to the membrane where it binds to the mammalian factor FXYD3, expressed specifically in the colon and stomach (Okuda et al., 2010). Thus, ExoS may assist the penetration of *P. aeruginosa* through the intestinal epithelial barrier, impairing the defence function of tight junctions against bacterial penetration (Okuda et al., 2010). Moreover, gut inflammation and apoptosis–which can be initiated by the *Pseudomonas* quinolone signal (PQS)–lead to tight junction disruption and an increase of epithelial barrier permeability (Alverdy et al., 2005; An, 2008). Similarly, *P. aeruginosa* lectin PA-I, which is associated with adhesion to epithelial cell layer, is produced after intestinal ischemia and secreted into the intestinal lumen, causing tight junction interruption, epithelial barrier dysfunction and increase of its permeability (Seal et al., 2011).

INTESTINAL *P. aeruginosa* EXHIBITS ENHANCED VIRULENCE UPON STRESS, SURGERY, TRAUMA, AND MAYBE CANCER

Cohort studies show that infections are more frequent, severe and lethal among surgical patients (Craven et al., 1988; Sax et al., 2011). Surgical injury can shift the dynamics of the host-pathogen interaction leading to phenotype transformation or phase variation that develops as microbes adapt and respond to novel environments, causing morbidity and mortality (Babrowski et al., 2013). *P. aeruginosa* escalates its virulence and promotes systemic inflammation during various conditions of host stress (Seal et al., 2011). In patients colonized by *P. aeruginosa*, the prolonged surgical injury releases stress-related host factors that can trigger the otherwise dormant colonizers, making them invasive and lethal (Babrowski et al., 2013). Defence mechanisms such as degradative proteases and lipases, exopolysaccharide capsule and outer membrane-derived vesicles (OMVs), which serve as a secretion mechanism for virulence factors,

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help the pathogen to survive in the host environment (Macdonald and Kuehn, 2013). OMVs are induced in response to physiological stressors and secreted during infection serving multiple roles in bacterial pathogenesis (Macdonald and Kuehn, 2013). In surgically stressed hosts interferon-gamma, endogenous opioids and the hypoxic end-products adenosine and inosine are released into the intestinal lumen where they bind bacteria and activate the expression of PA-I lectin and other virulence factors of *P. aeruginosa*. The PA-I lectin alters the tight junction permeability of the intestinal epithelium to exotoxin A, leading to lethal gut derived sepsis (Long et al., 2008). Moreover, local intestinal depletion of extracellular phosphate (hypophosphatemia), which occurs after surgical injury, can activate virulent pathways due to bacterial sensing of low phosphate, shifting the phenotype of *P. aeruginosa* to that of a lethal strain (Long et al., 2008). Because interferon-gamma, opioids and hypoxia are part of the host response and the therapeutic regiments administered to cancer patients (Dunn et al., 2005; Vaupel and Mayer, 2007), the conditions that accompany cancer may also provide the signals for *P. aeruginosa* virulence induction.

CAN *P. aeruginosa* SIMILARLY TO OTHER GASTROINTESTINAL BACTERIA FACILITATE CANCER?

Bacteria may initiate oncogenesis because they can induce inflammation and produce cell damaging toxins that facilitate tumorigenesis (Collins et al., 2011; Tjalsma et al., 2012). The characteristic single polar flagella and type 4 pili of *P. aeruginosa* function as initiators of inflammation and adhesins, respectively (Gellatly and Hancock, 2013). *P. aeruginosa* induces Toll-like receptors to activate cytokines, chemokines and COX-2 and recruit cells of the innate and adaptive immune system (Hussain et al., 2003; Holt et al., 2008; de Lima et al., 2012). Epithelial adherence is a property of various bacteria associated with gastrointestinal disease and cancer, such as *Bacteroides fragilis, Streptococcus bovis, Escherichia coli* and *Helicobacter pylori* (Toprak et al., 2006; Selgrad et al., 2008; Abdulamir et al., 2011; Arthur et al., 2012). Cell wall antigens of *S. bovis* induce overexpression of COX-2 and NF-κB *in vitro*, which promote cellular proliferation and angiogenesis (Tafe and Ruoff, 2007; Abdulamir et al., 2009).

E-cadherin, a cell adhesion molecule serves as an antagonist of invasion and metastasis and is found mutated in human carcinomas (Cavallaro and Christofori, 2004; Berx and van Roy, 2009). *B. fragilis* secreted factor BFT cleaves E-cadherin, which is usually bound inside the plasma membrane to β-catenin. The cleavage releases catenin in the cytosol leading to the transcription of the oncogene *c-myc* (Hardy et al., 2000). Similarly, *P. aeruginosa* secreted factor LasI can disrupt adherens junctions and reduce the expression and distribution of E-cadherin and β-catenin in the cell membrane, resulting in changes in cell junction associations and enhanced paracellular permeability (Vikström et al., 2009).

Interestingly, intestinal innate immune responses and stem cells may drive tumor initiation, maintenance and metastasis (Schwitalla et al., 2013). Cancer development is assisted by apoptotic programmed cell death in the tumor microenvironment (Evan and Littlewood, 1998; Lowe et al., 2004; Adams and Cory, 2007) and *P. aeruginosa* uses many virulence factors that induce epithelial cell apoptosis. Intestinal infection with *P. aeruginosa* in *Drosophila* activates the c-Jun N-terminal kinase (JNK) pathway, which causes apoptosis of enterocytes and leads to proliferation of intestinal stem cells (Apidianakis et al., 2009). Importantly, genetic predisposition via an oncogenic form of Ras1/K-Ras oncogene, can synergize with inflammatory signals to induce stem cell-originating tumors characterized by alterations in cell polarity and tissue architecture. Moreover, sustained intestinal infection with *P. aeruginosa* in *Drosophila* induces the Imd/NF-κB pathway, which synergizes with the oncogene $Ras1^{V12}$ to activate the JNK pathway. This leads to invasion and dissemination of oncogenic hindgut cells to distant sites (Bangi et al., 2012; Christofi and Apidianakis, 2013).

CONCLUSIONS

P. aeruginosa is a common colonizer of the human intestine upon hospitalization, immunosuppression, antibiotic treatment, surgery, severe trauma and other conditions that cancer patients may face. Not only is *P. aeruginosa* carriage increased in the aforementioned conditions, but also bacteria become more virulent and damaging to the intestinal epithelium upon surgery, injury, and severe stress. Moreover, human isolates of *P. aeruginosa* can induce intestinal pathology and cancer-related epithelial phenotypes in genetically predisposed model hosts. Thus, *P. aeruginosa* appears to have the opportunity and the ability to promote intestinal disease in predisposed hosts, although further proof on the ability of this bacterium to promote tumorigenesis in mammalian models of infection is needed. The lack of epidemiological data linking *P. aeruginosa* to intestinal disease and potentially tumorigenesis in cancer patients may reflect the lack of clinical studies assessing bacterial growth and virulence in relation to cancer recurrence. Because the titter, distribution and virulence of *P. aeruginosa* in the intestine may be very dynamic (Tjalsma et al., 2012), future studies should be designed to repeatedly assess intestinal *P. aeruginosa* abundance and virulence in cancer patients versus healthy individuals. Clinical samples can be assessed for the presence of *P. aeruginosa* via classical microbiology, and next-generation sequencing may offer the chance to assess *P. aeruginosa* transcriptome during infection. Importantly, definite proof of causation of *P. aeruginosa* in morbidity and mortality of cancer patients can only be achieved if targeted elimination of *P. aeruginosa* from these patients improves the outcome of their disease. In **Figure 1** we illustrate a roadmap to specifically assess the role of *P. aeruginosa* in intestinal disease and tumorigenesis. It is conceivable that similar principles can be used to assess causality between intestinal disease and many other opportunistic pathogens harbored by the human gut.

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MAJOR ARTICLE

Surveillance Cultures Growing Carbapenem-Resistant *Acinetobacter baumannii* Predict the Development of Clinical Infections: A Retrospective Cohort Study

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Background. We aimed to determine the effect of the presence of carbapenem-resistant *Acinetobacter baumannii* in accordance with surveillance cultures on the subsequent development of clinical infections by this organism.

Methods. This retrospective cohort study was conducted at a tertiary hospital from January 2010 to November 2011. We included all consecutive patients admitted to the trauma intensive care unit, who had weekly surveillance cultures performed (from rectum, and if intubated, respiratory secretions), and without evidence of *A. baumannii* infections prior to the collection of the first surveillance culture. Univariable and multivariable analyses were performed using log-binomial regression. Survival analyses were performed using Cox proportional hazards.

Results. Three hundred sixty-four patients were included, of whom 49 (13.5%) had carbapenem-resistant *A. baumannii* on surveillance cultures. Patients with positive surveillance cultures had 8.4 (95% confidence interval [CI], 5.6–12.7; $P < .0001$) times the risk of developing a subsequent *A. baumannii* infection compared with patients who remained negative on surveillance cultures. Multivariable analysis showed significant associations between clinical infection and both positive surveillance cultures (relative risk [RR], 5.9 [95% CI, 3.8–9.3]; $P < .0001$) and mechanical ventilation (RR, 4.3 [95% CI, 1.03–18.2]; $P = .05$). On survival analyses, the only variable associated with the development of clinical infections was the presence of positive surveillance cultures (hazard ratio, 16.3 [95% CI, 9.1–29.1]; $P < .001$).

Conclusions. Presence of carbapenem-resistant *A. baumannii* on surveillance cultures is strongly associated with subsequent development of carbapenem-resistant *A. baumannii* infections. Prevention efforts should be focused at limiting the acquisition of this organism during hospitalization.

Keywords. *Acinetobacter baumannii*; carbapenem-resistant; surveillance cultures; clinical infections; intensive care unit.

Acinetobacter baumannii is a gram-negative coccobacillus, frequently identified as resistant to most commercially available antibiotics, including carbapenems [1–3]. In 2013, the Centers for Disease Control and Prevention (CDC) named carbapenem-resistant *A. baumannii* as one of the serious health threats for the US population. *Acinetobacter baumannii* is especially problematic in intensive care units (ICUs), and is associated with high morbidity and mortality as well as longer hospital stays [4,5]. Therefore, it is imperative to prevent the development of *A. baumannii* infections by identifying and modifying risk factors for these infections.

Patient-to-patient transmission of *A. baumannii* among hospitalized cases has been described in the literature and linked to contaminated hospital environment, suboptimal disinfection of communal objects,

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and contaminated healthcare workers' hands [1, 6, 7]. Patients colonized with *Acinetobacter* carry this organism on their body surfaces and contaminate their immediate environment [8, 9]. Gowns, gloves, and hands of healthcare workers become contaminated after contact with either an *Acinetobacter*-positive patient or their contaminated environment [10]. Finally, being exposed to an environment contaminated with *A. baumannii* has been associated with a higher risk of acquiring this pathogen [11]. Therefore, interventions aimed at containing *Acinetobacter* outbreaks consist of a combination of surveillance cultures, hand hygiene, heightened environmental cleaning, and contact precautions [12].

In 2009, as part of a bundle of interventions designed to limit the spread of carbapenem-resistant *A. baumannii*, we implemented weekly surveillance cultures among consecutive admissions to our adult ICUs [12]. Subsequently, the trauma ICU (TICU) was identified as one of the units with the highest rates of acquisition of *A. baumannii* [13]. We used surveillance cultures to cohort *Acinetobacter*-positive patients, and the staff caring for them, within a section of an inpatient unit. However, we did not know the clinical implications of being an asymptomatic carrier of this multidrug-resistant pathogen. Thus, the primary aim of this study was to evaluate a cohort of patients admitted to TICU in order to characterize their differential risk of developing carbapenem-resistant *A. baumannii* infections based on the presence of this organism on surveillance cultures. Secondarily, we aimed to determine the effect of other covariates (comorbidities, antibiotic exposure, and invasive procedures) on the primary association, and explore the effect of time to positive surveillance cultures on primary and secondary associations.

METHODS

This was a retrospective cohort study conducted at a 1500-licensed-bed public teaching hospital in South Florida with a documented carbapenem-resistant *A. baumannii* endemicity [13]. Over the years, the unit with highest prevalence of this organism was the TICU, a 25-bed open-layout unit located in a freestanding trauma center. We evaluated all consecutive patients admitted to TICU from 1 November 2010 to 30 November 2011. During this period, the TICU had a monthly hospital acquired rate of carbapenem-resistant *A. baumannii* of 55.9 ± 8.95 cases per 10 000 patient-days [12]. This study was reviewed and approved, and informed consent was waived by the Institutional Review Board of the University of Miami Miller School of Medicine.

Since 2009, as part of a bundle of interventions, all adult ICUs performed surveillance cultures to rule out carriage of carbapenem-resistant gram-negative bacilli, including carbapenem-resistant *A. baumannii*. As previously described [14], surveillance cultures were performed on admission to the unit and weekly thereafter throughout ICU admission. Surveillance cultures included rectal swab cultures and, if mechanically ventilated, lower respiratory cultures. Samples were streaked in MacConkey agar plates with a 10-μg ertapenem disk and incubated overnight at 37°C. Colonies were selected based on color and morphology and final identification was performed using Vitek II [14].

A patient was considered to be *Acinetobacter*-positive on surveillance cultures if either rectal or respiratory secretions grew carbapenem-resistant *A. baumannii*. Patients excluded from the analysis included all subjects who failed to have at least 1 surveillance culture during their ICU stay or who failed to have surveillance cultures prior to the development of an *A. baumannii* infection. Carbapenem-resistant *A. baumannii* infections were defined using the CDC definitions [15]; these determinations were ascertained by 2 independent reviewers using the hospital's electronic medical records. Any discrepancy on determination of clinical infection was reviewed and discussed by the team to achieve a resolution. Additional information collected included demographics, comorbidities, and the use of mechanical ventilation.

Antibiotic exposures were obtained both as a composite of any antibiotic use ("any antibiotic") and as the exposure to individual antibiotic classes (eg, cephalosporins, carbapenems). For patients who remained negative on surveillance cultures, antibiotic exposures were collected from hospital admission until unit discharge, death, or development of first positive clinical culture, whichever occurred first. For patients with positive surveillance cultures, the initial exposure period expanded from admission to the hospital to the date of collection of first positive surveillance culture. The second period encompassed the time from first positive surveillance culture until unit discharge, death, or development of a carbapenem-resistant *A. baumannii* infection, whichever occurred first.

Statistical Analysis

This study was designed to test the association between surveillance cultures growing carbapenem-resistant *A. baumannii* (independent variable) and the subsequent development of clinical infections caused by the same organism (dependent variable). Baseline characteristics of the groups (age, sex, comorbidities, antibiotic exposure, invasive procedures) based on surveillance status were compared using χ^2 and Fisher exact tests for proportions, and Student *t* tests for continuous variables.

To obtain relative risks (RRs) rather than odds ratios, univariable and multivariable analyses were performed using log-binomial regressions. Variables included in the multivariable model were selected based on statistical significance obtained in univariable analyses ($P \leq .5$). The presence of multicollinearity

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was explored for all significant antibiotic exposures prior to the building of multivariable models [16].

Cox proportional regression was used to include time-to-positive surveillance cultures. For these analyses, "time zero" was the time of admission to TICU. *Acinetobacter baumannii* infections were considered the "events." Patients who either died or were discharged from TICU before the development of the "event" were censored. Additionally, to account for the different risks of a patient to develop clinical infections during the periods before and after positive surveillance cultures, a time-dependent covariate was created using the date of first positive surveillance culture [17]. All analyses were performed using SAS software, version 9.3 (Cary, North Carolina).

RESULTS

During 13 months, 479 consecutive patients were admitted to TICU (Figure 1). Ninety-three patients were excluded due to lack of surveillance cultures; these 93 patients either failed to have orders for surveillance cultures or orders were entered incorrectly in the system (eg, "rule out vancomycin-resistant enterococci" rather than "rule out carbapenem-resistant gram-negative

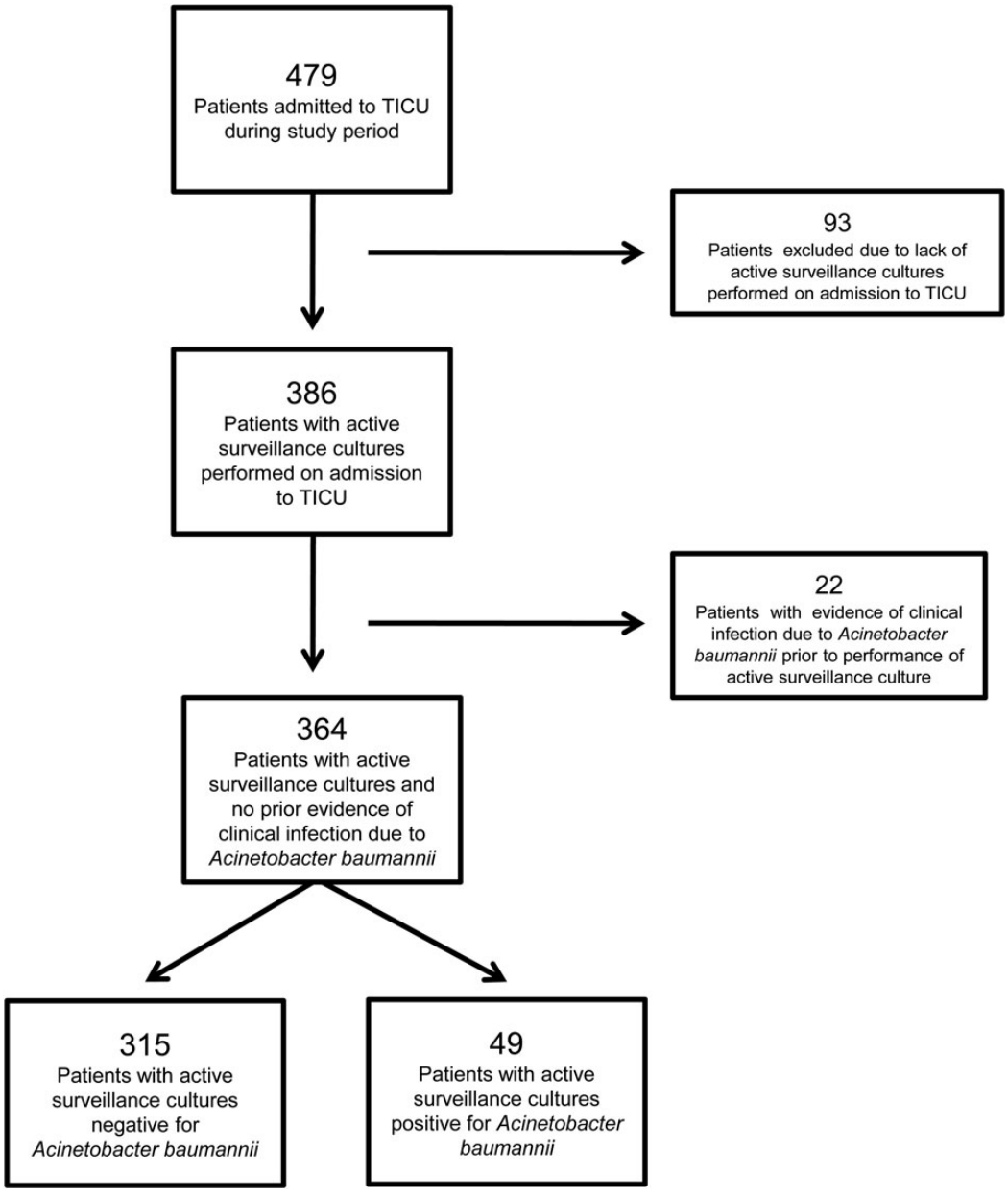

Figure 1. Patient selection flow algorithm. Abbreviation: TICU, trauma intensive care unit.

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rods"). Twenty-two patients were excluded due to evidence of *A. baumannii* infections prior to their first surveillance culture. Three hundred sixty-four patients were included in the final analysis: 49 patients (13.5%) had positive surveillance cultures for carbapenem-resistant *A. baumannii*, and the remaining 315 patients (86.5%) had negative surveillance cultures throughout their ICU stay. Baseline characteristics of the groups based on the status of their surveillance cultures are displayed in Table 1. Variables found to be statistically different at baseline included sex (group with positive surveillance cultures had higher proportion of females; $P = .005$) and mechanical ventilation (all patients with positive surveillances were mechanically ventilated; $P < .0001$). Additionally, the group with positive surveillance cultures had greater exposure to antibiotics than the group that remained negative on surveillances ($P = .04$). In regard to specific antibiotic classes, the group with positive surveillance cultures had higher exposures to both vancomycin and carbapenems ($P = .003$ and $P < .0001$, respectively).

Carbapenem-resistant *A. baumannii* infections occurred in 60 of the 364 (16.5%) patients who underwent surveillance cultures. Twenty-eight patients (47%) developed ventilator-associated pneumonia, 22 (37%) had either primary or secondary bloodstream infection, 7 (12%) had urinary tract infection, 2 (3%) had intra-abdominal infection, and 10 (17%) had some combination of skin and soft tissue infection. Of the 60 patients with clinical infections, 34 (56.7%) had previously documented positive surveillance cultures. Table 2 describes the variables associated with the development of carbapenem-resistant *A. baumannii* infections. Patients with positive surveillance cultures had 8.4 times the risk (95% confidence interval [CI], 5.6–12.7; $P < .0001$) of developing carbapenem-resistant *A. baumannii* infections than patients who remained negative on surveillance cultures. Univariable analyses showed that sex, mechanical ventilation, and exposures to either carbapenem, aminoglycosides, vancomycin, or "any antibiotics" were associated with the development of clinical infection (Table 2). After controlling for other variables, only positive surveillance cultures (RR, 5.9 [95% CI, 3.8–9.3]; $P < .0001$) and being on mechanical ventilation (RR, 4.3 [95% CI, 1.03–18.2]; $P = .05$) were associated with the development of carbapenem-resistant *A. baumannii* infection (Table 2).

Cox proportional regression showed a high hazard for carbapenem-resistant *A. baumannii* infections among patients growing this organism on surveillance cultures (hazard ratio, 18.8 [95% CI, 10.8–32.8]; $P < .0001$; Table 3). Sex, mechanical ventilation, and exposure to cephalosporins were also significantly associated with the outcome. However, after adjusting for all significant variables, the only exposure that remained significant was the growth of carbapenem-resistant *A. baumannii* on surveillance cultures. At 30 days of admission to the unit,

Table 1. Characteristics of the Groups Based on Results of Surveillance Culture

Variable	Surveillance Negative (n = 315)	Surveillance Positive (n = 49)	*P* Value[a]
Demographics			
Age >50 y	111 (35.2)	20 (40.8)	.52
Age, y, mean ± SD	43.5 ± 1.08	45.8 ± 2.32	.22
Male sex	238 (75.6)	27 (55.1)	.005
Invasive procedures			
Mechanical ventilation	226 (71.8)	49 (100)	<.0001
Antibiotic exposure (before surveillance cultures)			
Penicillins	111 (35.1)	17 (34.4)	.97
Cephalosporins	163 (51.6)	18 (37.5)	.07
Quinolones	68 (21.5)	11 (22.9)	.83
Vancomycin	202 (63.9)	41 (85.4)	.003
Aminoglycosides	17 (5.4)	4 (8.3)	.50
Carbapenems	116 (36.7)	37 (77.1)	<.0001
Any antibiotic	251 (79.4)	44 (91.7)	.04
Comorbidities			
DM without end organ damage	28 (8.9)	5 (10.2)	.95
DM with end organ damage	17 (5.4)	3 (6.1)	.94
Myocardial infarction	8 (2.5)	1 (2)	1
Congestive heart failure	1 (0.3)	0	1
Chronic pulmonary disease	10 (3.2)	2 (4.1)	.85
Peripheral vascular disease	1 (0.3)	0	1
Cardiovascular disease	6 (1.9)	2 (4.1)	.53
Dementia	5 (1.6)	0	1
Connective tissue disease	0	0	1
Peptic ulcer disease	6 (1.9)	1 (2)	1
Mild liver disease	2 (0.6)	0	1
Moderate to severe liver disease	5 (1.6)	2 (4.1)	.45
Moderate to severe renal disease	10 (3.2)	2 (4.2)	.84
Hemiplegia	0	0	1
Malignancy	2 (0.6)	0	1
Leukemia	0	0	1
Lymphoma	0	0	1
Metastatic solid tumor	1 (0.3)	0	1
AIDS	4 (1.3)	1 (2)	.78
Charlson score >2	26 (8.2)	3 (6.1)	.78
Charlson score >5	6 (1.9)	1 (2)	1
Charlson score, mean ± SD	1.35 ± 0.08	0.72 ± 0.23	.21
Source of positive surveillance culture			
Rectal	NA	6 (12.2)	
Tracheal	NA	10 (20.4)	
Rectal and tracheal	NA	33 (67.3)	

Data are presented as No. (%) unless otherwise specified.

Abbreviations: DM, diabetes mellitus; NA, not applicable; SD, standard deviation.

[a] Significance was calculated using surveillance status and dichotomization of the study population based on variables at the row level.

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Table 2. Variables Associated With Development of Clinical Infections With *Acinetobacter baumannii* During Index Admission

Variable	Clinical Infection With *A. baumannii* (n = 60)	No Clinical Infection With *A. baumannii* (n = 304)	Univariable Analysis		Multivariable Analysis	
			RR (95% CI)	*P* Value	Adjusted RR (95% CI)	*P* Value
Surveillance culture, positive	34 (56.7)	15 (4.9)	8.4 (5.6–12.7)	<.0001	5.9 (3.8–9.3)[a]	<.0001
Demographics						
Age >50 y	23 (38.3)	108 (35.5)	1.1 (.7–1.8)	.68		
Male sex	35 (58.3)	230 (75.7)	0.5 (.3–.8)	.005	1.3 (.9–1.9)[a]	.2
Invasive procedures						
Mechanical ventilation	58 (96.7)	217 (71.4)	9.4 (2.3–37.6)	.002	4.3 (1.03–18.2)[a]	.05
Comorbidities						
DM without end organ damage	7 (11.7)	26 (8.6)	1.3 (.9–1.9)	.1		
DM with end organ damage	5 (8.5)	15 (4.9)	1.4 (.9–1.9)	.08		
Myocardial infarction	1 (1.7)	8 (2.6)	1.2 (.9–1.8)	.23		
Congestive heart failure	0	1 (0.3)	1.3 (.9–1.9)	.18		
Chronic pulmonary disease	1 (1.7)	11 (3.6)	1.2 (.8–1.8)	.3		
Peripheral vascular disease	0	1 (0.3)	1.3 (.9–1.8)	.18		
Cardiovascular disease	3 (5.1)	5 (1.6)	1.4 (.9–1.9)	.06		
Dementia	0	5 (1.6)	1.2 (.9–1.8)	.25		
Connective tissue disease	0	0				
Peptic ulcer disease	2 (3.4)	5 (1.6)	1.3 (.9–1.9)	.11		
Mild liver disease	0	2 (0.7)	1.3 (.9–1.8)	.2		
Moderate to severe liver disease	2 (3.4)	5 (1.6)	1.3 (.9–1.9)	.11		
Moderate to severe renal disease	3 (5.1)	9 (3)	1.3 (.9–1.9)	.10		
Hemiplegia	0	0				
Malignancy	0	2 (0.7)	1.3 (.9–1.8)	.2		
Metastatic solid tumor	0	1 (0.3)	1.3 (.9–1.9)	.18		
Leukemia	0	0				
Lymphoma	0	0				
AIDS	1 (1.7)	4 (1.3)	1.3 (.9–1.9)	.16		
Charlson score >2[b]	5 (9.4)	24 (8.3)	1.1 (.5–2.6)	.79		
Charlson score >5[b]	2 (3.8)	5 (1.7)	1.9 (.6–6.2)	.3		
Antibiotic exposures						
Penicillins	25 (41.7)	109 (35.9)	1.2 (.8–2)	.39		
Cephalosporins	32 (53.3)	155 (51)	1.1 (.7–1.7)	.74		
Quinolones	18 (30)	71 (23.4)	1.3 (.8–2.2)	.27		
Vancomycin	53 (88.3)	196 (64.5)	3.5 (1.6–7.4)	.0012	1.8 (.7–4.4)[c]	.19
Aminoglycosides	7 (11.7)	16 (5.3)	2 (1–3.8)	.05	1.3 (.8–2.2)[c]	.3
Carbapenems	41 (68.3)	117 (38.5)	2.8 (1.7–4.6)	.0001	0.8 (.4–1.6)[c]	.59
Any antibiotic	56 (93.3)	243 (79.9)	3 (1.1–8.1)	.03	1.6 (.7–3.5)[a]	.26

Data are presented as No. (%) unless otherwise specified. Antibiotic exposures spanned from hospital admission to development of clinical infection, unit discharge, or death.

Abbreviations: CI, confidence interval; DM, diabetes mellitus; RR, relative risk.

[a] Model for multivariable logistic regression included surveillance status, mechanical ventilation, sex, and exposure to any antibiotic (fit criteria Quasi-likelihood criterion [QIC] = 336).

[b] Patients with insufficient data to calculate a Charlson comorbidity score were removed for this analysis.

[c] Model for multivariable logistic regression included surveillance status, mechanical ventilation, sex, exposure to carbapenems, exposure to vancomycin, and exposure to aminoglycosides (fit criteria QIC = 336). In this model, surveillance status and mechanical ventilation remained significant ($P < .0001$ and $P = .05$, respectively).

the proportion of patients free of clinical infections among groups with positive and negative surveillance cultures was 0.50 (95% CI, .33–.76) and 0.89 (95% CI, .82–.97), respectively. Figure 2 shows the probability of remaining free of carbapenem-resistant *A. baumannii* infections based on results of surveillance cultures.

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Table 3. Hazard Ratios for the Development of a Clinical Infection Due to *Acinetobacter baumannii*

	Univariable Analysis		Multivariable Analysis	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Surveillance culture, positive	18.8 (10.8–32.8)	<.0001	16.3 (9.1–29.1)	<.0001
Mechanical ventilation	6.1 (1.5–25.1)	.01	3.4 (.8–14.6)	.09
Charlson score >2	0.9 (.4–2.5)	.99		
Charlson score >5	1.5 (.4–6.1)	.58		
Male sex	0.6 (.3–.9)	.03	0.98 (.6–1.7)	.95
Age >50 y	0.9 (.5–1.6)	.79		
Exposure to penicillin	0.8 (.5–1.3)	.41		
Exposure to cephalosporins	0.5 (.3–.9)	.03	0.9 (.5–1.7)	.87
Exposure to quinolones	0.6 (.3–1.1)	.08		
Exposure to vancomycin	1 (.5–2.4)	.90		
Exposure to aminoglycosides	1 (.4–2.2)	.96		
Exposure to carbapenems	1.3 (.7–2.2)	.40		
Exposure to any antibiotic	0.7 (.2–2)	.52		

Antibiotic exposures spanned from hospital admission to development of clinical infection, discharge from unit, or death. Differential risks for development of clinical infections (ie, before and after positive surveillance cultures) were accounted for using a time-dependent variable within the model.

Abbreviations: CI, confidence interval; HR, hazard ratio.

DISCUSSION

This study characterizes the clinical implications of detecting carbapenem-resistant *A. baumannii* on surveillance cultures. We found that patients with positive surveillance cultures were 8 times more likely to develop carbapenem-resistant *A. baumannii* infections, even after controlling for other variables. Furthermore, among the group with positive surveillances, the median time for the development of carbapenem-resistant *A. baumannii* infections was approximately 30 days from ICU admission.

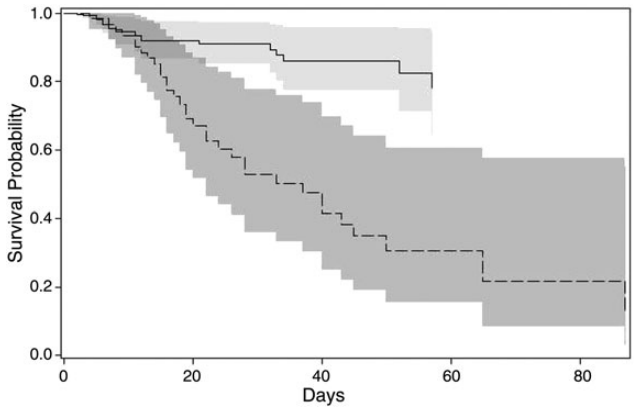

Figure 2. Survival curves with the probability to develop clinical infections with carbapenem-resistant *Acinetobacter baumannii* based on results of surveillance cultures. Curves represent the probability of staying free of clinical infection caused by carbapenem-resistant *A. baumannii*. Days represent the days from admission to the unit until the development of the outcome or censoring. Solid line represents the group that remained negative for carbapenem-resistant *A. baumannii* on surveillance cultures. Dashed line represents the group that became positive for carbapenem-resistant *A. baumannii* on surveillance cultures. Shaded area depict the 95% confidence intervals.

Prior studies have described the presence of colonization as a factor associated with the development of *A. baumannii* infections [18, 19]. Our results are in agreement with these conclusions; nevertheless, our findings are framed in the context of an active surveillance program within a retrospective cohort, thus ensuring that surveillance cultures, if positive, preceded the development of clinical infections.

Previously identified risk factors for the development of *A. baumannii* infections include mechanical ventilation [20], prior exposure to antimicrobials [20, 21], high Acute Physiology and Chronic Health Evaluation (APACHE II) score at the onset of infection [21], long duration of hospital stay, recent central venous catheter insertion [22], female sex [23], length of transducer catheter usage, and hyperalimentation [24]. In regard to antibiotic exposure, the definition of this variable is not unified across the literature, and its quantification differs across different studies [18, 19]. Our study collected antibiotic exposures as dichotomous variables across specified periods of time. Receipt of carbapenems, aminoglycosides, vancomycin, and the composite variable of any antibiotic exposure were associated with a greater risk of developing carbapenem-resistant *A. baumannii* infections. However, none of these variables were identified as independent risk factors on multivariable analyses. Previous

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studies that found antibiotic exposures as risk factors for acquisition of *A. baumannii* quantified the use of antibiotics as either grams per time period [25], "unit pressure" [18], days of exposure [22], or dichotomous variables [20]. Additionally, severity of illness has been recognized as a risk factor for the development of *A. baumannii* infections [26], as well as a marker for poor prognosis [27, 28]. The Charlson comorbidity index was used in our cohort and failed to show an association with the development of clinical infections. In a previous study [11], higher Charlson comorbidity index was shown to be associated with lower risk of acquiring *A. baumannii* in ICU patients.

Limitations of this project include being a single-center study, where surveillance cultures were performed weekly rather than on a daily basis. Additionally, surveillance cultures are an insensitive method to detect carriage, predisposing to classification bias (high number of false negatives), although this would have skewed our results toward a nonsignificant association. Another caveat of our study is the lack of molecular typing of surveillance and clinical isolates; thus, we can only assume that surveillance and clinical isolates were related within individual patients. Moreover, antibiotic exposures were evaluated as dichotomous rather than continuous variables, lacking number of days and exact onset of exposure. This lack of time-dependent covariates for antibiotic exposures predisposes our analysis to immortal time bias [29], which could have overestimated the impact of antibiotics in our associations. Even though comorbidity index was used to categorize patients' comorbidities, acuity of illness (APACHE score) was not determined. APACHE II determination upon unit admission was not included given that the outcomes occurred many days after admission. Nevertheless, we admit that the lack of serial acuity of index scores for each individual patient is a major limitation of this study.

In conclusion, this study shows that positive surveillance cultures were strongly associated with the subsequent development of carbapenem-resistant *A. baumannii* infections. Furthermore, survival analyses demonstrated 16.3 times the risk to develop carbapenem-resistant *A. baumannii* infections among patients who grew this pathogen on surveillance cultures during their ICU stay. Surveillance cultures upon admission to ICUs are a valuable infection control tool [30] that should be used not only for cohorting patients but also as a signature marker for subsequent clinical infections. We now know that we should target prevention of acquisition of carbapenem-resistant *A. baumannii* (eg, infection control bundles, antibiotic stewardship, vaccination [31]) as well as focus our efforts on patients who are known to be colonized. The latter could include not only selective digestive decolonization [32] but, most importantly, manipulation of the gut or lung microbiome to prevent the progression of colonization to clinical infections [33]. Remaining questions that should be explored are the effect of antibiotic exposures treated as continuous variables and modeled as time-dependent covariates. The effect of timing of these exposures will allow us to establish the impact of antibiotics on the acquisition of *A. baumannii*, and also their subsequent effect on the development of clinical infections by this organism, especially among previously colonized patients. Establishing the timing of these associations is important, as it will further highlight the interdependence between acquisition of multidrug-resistant organisms and antibiotic use in our inpatient population.

Note

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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A Review of the Research Literature on Evidence-Based Healthcare Design

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Abstract

Objective: This report surveys and evaluates the scientific research on evidence-based healthcare design and extracts its implications for designing better and safer hospitals.
Background: It builds on a literature review conducted by researchers in 2004.
Methods: Research teams conducted a new and more exhaustive search for rigorous empirical studies that link the design of hospital physical environments with healthcare outcomes. The review followed a two-step process, including an extensive search for existing literature and a screening of each identified study for the relevance and quality of evidence.
Results: This review found a growing body of rigorous studies to guide healthcare design, especially with respect to reducing the frequency of hospital-acquired infections. Results are organized according to three general types of outcomes: patient safety, other patient outcomes, and staff outcomes. The findings further support the importance of improving outcomes for a range of design characteristics or interventions, including single-bed rooms rather than multibed rooms, effective ventilation systems, a good acoustic environment, nature distractions and daylight, appropriate lighting, better ergonomic design, acuity-adaptable rooms, and improved floor layouts and work settings. Directions for future research are also identified.
Conclusions: The state of knowledge of evidence-based healthcare design has grown rapidly in recent years. The evidence indicates that well-designed physical settings play an important role in making hospitals safer and more healing for patients, and better places for staff to work.
Key Words: *Evidence-based design, hospital design, healthcare design, healthcare quality, outcomes, patient safety, staff safety, infection, hand washing, medical errors, falls, pain, sleep, stress, depression, confidentiality, social support, satisfaction, single rooms, noise, nature, daylight*

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Introduction

Background

A visit to a U.S. hospital is dangerous and stressful for patients, families, and staff. Hospital-acquired infections and medical errors are among the leading causes of death in the United States, each killing more people than automobile accidents, breast cancer, or acquired immune deficiency syndrome (AIDS) (Institute of Medicine [IOM], 2001; Klevens, et al., 2007a). The resulting yearly cost for U.S. hospitals is estimated to be $5 billion for hospital-acquired infections (Centers

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for Disease Control and Prevention [CDC], 2000) and $17- to $29 billion for medical errors (Kohn, Corrigan, & Donaldson, 1999). According to the IOM (2001) in its landmark *Crossing the Quality Chasm* report: "The frustration levels of both patients and clinicians have probably never been higher. Yet the problems remain. Health care today harms too frequently and routinely fails to deliver its potential benefits" (p. 1). Problems with U.S. healthcare not only negatively influence patients; they affect staff. Registered nurses have a turnover rate averaging 20% (Joint Commission, 2002).

At the same time, a major boom in hospital construction is occurring in the United States and several other countries. The U.S. healthcare system is facing the confluence of the need to replace aging 1970s' hospitals, population shifts, the graying of the Baby Boom generation, and the introduction of new medical technologies. As a result, the United States will spend more than $180 billion for new hospitals in the next 5 years alone, and healthcare construction is projected to exceed $70 billion per year by 2011 (Jones, 2007). These new hospitals will remain in place for decades.

This once-in-a-lifetime construction program provides an opportunity to rethink hospital design and especially to consider how better design can improve patient and staff outcomes. Just as medicine has increasingly moved toward *evidence-based medicine* where clinical choices are informed by research, healthcare design is increasingly guided by rigorous research linking hospitals' physical environments to healthcare outcomes, and it is moving toward *evidence-based design* (EBD) (Hamilton, 2003). For example, the Center for Health Design Pebble Project includes approximately 50 healthcare providers and manufacturers committed to using EBD for their construction projects. The Military Health System has adopted EBD for a $6 billion capital construction program for its 70 hospitals, which serve more than 9.2 million people worldwide. Kaiser Permanente and its partners in the Global Health and Safety Initiative are using EBD as a strategy to increase *triple safety* for patients, staff, and the environment. The Global Health and Safety Initiative comprises partners that provide over 100,000 hospital beds.

This report is an updated and expanded version of a 2004 report, "The Role of the Physical Environment in the Hospital of the 21st Century" (Ulrich, Zimring, Quan, Joseph, & Choudhary, 2004). Research teams from Texas A&M University and the Georgia Institute of Technology conducted a new and more extensive search for empirical studies linking the design of the physical environments of hospitals with healthcare outcomes. The following questions are explored in this study: (1) What can rigorous research tell us about "good" and "bad" hospital design? (2) Can improved design make hospitals less risky and stressful and promote more healing for patients, their families, and staff? (3) Is there scientifically credible evidence that design affects clinical outcomes and staff effectiveness in delivering care?

Methodology

This review followed a two-step process. First, we conducted key word searches to identify potentially relevant studies published in English. Thirty-two key words were used, referring to patient and staff outcomes (such as *infection*, *medical error*, *pain*, *sleep*, *depression*, *stress*, and *privacy*), physical environmental factors (*hospital*, *hospital units*, *healthcare facility*, etc.), and other healthcare-related issues (such as *patient-* and *family-centered* care). We conducted an extensive series of cross-searches using combinations of key words through the EBSCO research database, which enabled the simultaneous search of multiple databases, such as Academic Search Premier, Alt Healthwatch, MEDLINE, PsycArticles, Psychology and Behavioral Sciences Collection, PsycINFO, and CINAHL. In addition, a supplemental search was conducted through the ISI Web of Knowledge and Google Scholar. The search included any study that alluded or referred to the physical environment of healthcare buildings in the title or the abstract. We also obtained additional relevant studies from the reference lists of identified articles.

In the second stage, we screened all identified references using two criteria: First, the study should be empirically based and examine the influence of environmental characteristics on patient, family, or staff outcomes. Second, the quality of each study was evaluated in terms of its research design and methods and whether the journal was peer-reviewed.

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Summary of Key Findings

We found a growing number of rigorous studies that help establish the relationship between the physical design of hospitals and key outcomes. This report was organized according to three general types of outcomes. The first section focuses on *patient safety issues*, such as infections, medical errors, and falls. The second section examines studies related to *other patient outcomes*, such as pain, sleep, stress, depression, length of stay, spatial orientation, privacy, communication, social support, and overall patient satisfaction. The third section surveys the scientific research relevant to *staff outcomes*, such as injuries, stress, work effectiveness, and satisfaction. Although these outcomes were also discussed in the 2004 report, this new study has substantially expanded the scale of most sections. In particular, the section on hospital-acquired infections has been substantially revised and expanded, reflecting the rising severity and importance of infections, the rapid growth of infection research, and the appearance of several new studies directly relevant to hospital design. The last section of the paper, Conclusions and Design Recommendations, summarizes the findings according to design characteristics or interventions and their implications for various outcomes.

Overall, this review confirms the importance of improving the healthcare outcomes associated with a range of design characteristics or interventions, such as single-bed rooms rather than multibed rooms, effective ventilation systems, a good acoustic environment, appropriate lighting, better ergonomic design, and improved floor layouts and work settings. Compared to 2004, the body of evidence has grown rapidly and substantially in recent years. This is encouraging given that the importance of EBD has increased markedly as the need for better healthcare facilities has grown and become more urgent. It is now widely recognized that well-designed physical settings play an important role in making hospitals less risky and stressful, promoting more healing for patients, and providing better places for staff to work.

However, it is also important to address the limitations of the quality of existing evidence. In medical fields, a *randomized controlled trial* or *experiment* is considered the strongest research design for generating sound and credible empirical evidence. Our literature review, however, found relatively few randomized controlled trials linking specific design features or interventions directly to impacts on healthcare outcomes. This is not very surprising, because most changes of the physical environment in healthcare settings alter several environmental factors simultaneously. This creates confounding variables and makes it difficult to disentangle the independent effect of the environmental change of primary interest. As an example, renovating an intensive care unit (ICU) with two-bed patient rooms to create single-bed rooms would likely alter not only the number of patients per room, but also the ratio of hand-washing sinks per bed and possibly the room ventilation or air quality. However, there are certain design interventions that may alter only one environmental factor, and the intervention can be assigned randomly to some patients but not others. Examples include exposing patients to interventions such as nature distraction, art, and reduced noise. In the case of these types of interventions, particularly nature distraction, our literature search identified a number of prospective randomized clinical trials that provide strong evidence. Additionally, we identified many moderately strong quasi-experimental studies, and some well-conducted epidemiological investigations. The largest category of studies consisted of observational studies with or without control groups.

Although many studies are not well controlled, the strength of evidence is enhanced by the fact that in the case of certain environmental factors, *reliable patterns of findings* across several studies emerged with respect to outcome influences. Furthermore, these patterns were broadly consistent with predictions based on established knowledge and theory concerning environment and healthcare outcomes. For example, many studies have consistently found that high noise levels in hospitals worsen patient outcomes such as sleep quality, physiological stress, and satisfaction. It is important to note that validity is strengthened when findings tend to be reliable or consistent and are in accord with a priori hypotheses or predictions derived from previous knowledge. Thus, we believe the application of such findings in EBD should be encouraged despite

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the shortage of randomized experimental trials. On the other hand, future research should be carefully designed and controlled so that the independent role of specific environmental changes or interventions can be better understood.

Different Ways of Reading This Report

This report is written for readers from different disciplines and professions, including architects, healthcare professionals, administrators, and researchers in EBD or healthcare-related fields. It covers a wide range of topics and surveys hundreds of studies and therefore makes an exceptionally long article. It is organized in a systematic way to accommodate the needs of different readers and to facilitate reading at different levels of detail and scope, including: (1) reading the entire article; (2) reading through the article more quickly by looking at the Summary of Evidence and Recommendations that appears at the beginning of each section; (3) reviewing the subtitles and thoroughly reading one or more individual sections on specific outcomes of interest; and (4) reading the last section of the paper, Conclusions and Design Recommendations, to extract major findings and recommendations organized according to specific design issues.

In addition, readers may notice instances of redundancy, where the same study has been cited in different sections. This is necessary because some characteristics of hospital physical environments (such as nature distraction and noise) influence multiple outcomes. Therefore, certain studies are cited in multiple sections, each focusing on a different outcome. Second, as mentioned previously, some readers may choose to read individual sections related to their specific interests. Cross-references would make reading difficult for such readers. To avoid this, some studies have been cited in more than one place in the article.

Result I: Improving Patient Safety Through Environmental Measures

Reducing Hospital-Acquired Infections

Summary of Evidence and Recommendations

One critically important way that EBD improves safety is by reducing the risk of hospital-acquired infections (i.e., nosocomial infections), a leading cause of death in the United States. One general conclusion supported by the infection literature is that the design of the physical environment impacts nosocomial infection rates by affecting all three major transmission routes—air, contact, and water. This discussion addresses the three transmission routes separately and is followed by a discussion of several advantages of single-bed rooms, as compared to multibed rooms, in controlling infection.

There is a pattern across scores of studies indicating that infection rates are lower when there is very good air and water quality, and greater physical separation, isolation, or space per patient. Concerning hand washing, there is evidence that providing accessible, alcohol-based hand-rub dispensers at the bedside can increase hand-washing compliance and thereby reduce contamination spread by contact.

The large amount of research literature reviewed in this section strongly supports the following design measures for controlling and preventing infection:

- Use effective air quality control measures during construction and renovation to prevent the outbreak of airborne infections. Measures include, for example, using portable high-efficiency particulate air (HEPA) filters, installing barriers between patient-care areas and construction/renovation areas, generating negative air pressure for construction/renovation areas relative to patient-care areas, and sealing patient windows.
- Install alcohol-based hand-rub dispensers at the bedside and in other accessible locations to increase hand-washing compliance and reduce contact transmission of infection.
- Select easy-to-clean floor, wall, and furniture coverings, and employ proper cleaning and disinfection procedures.
- Design and maintain the water system at the proper temperature and adequate pressure; minimize stagnation and back flow; eliminate dead-end pipes; regularly clean point-of-use fixtures; and consider the location of decorative fountains and carefully maintain them to minimize the risk of waterborne infection.

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- Provide single-bed rooms with private toilets to enable separation or isolation of patients on admission, so that those with unrecognized infections can be tested and identified without being mixed in with uninfected individuals in multibed rooms; to reduce airborne infection transmission by increasing isolation capacity and facilitating the maintenance of good air quality through measures such as effective ventilation, filtration, and appropriate air flow direction and pressure (positive or negative); and to facilitate thorough cleaning after a patient leaves, including the use of decontamination methods such as hydrogen peroxide vapor (HPV), which may be much more effective than conventional cleaning.

Hospital-Acquired Infections: A Serious and Growing Problem

Hospital-acquired infection is one of the leading causes of death in the United States. In 2002 alone, hospital-acquired infections in U.S. hospitals numbered approximately 1.7 million, and the number of associated deaths reached 98,987 (Klevens et al., 2007a). This means that approximately 1 of every 22 hospitalized patients acquired an infection. According to the CDC (2000), the cost of treating hospital-acquired infections is estimated to be $5 billion per year. Many hospital-acquired infections are drug resistant and difficult to treat and eradicate. Patients are especially vulnerable to these infections when they are immunocompromised or otherwise weakened by age, medical or surgical treatments, or underlying disease (Weinstein, 1998). The international trend toward increasing intensity of care and patient acuity (American Hospital Association, 2005) portends a future of greater patient vulnerability to infection.

The importance of controlling hospital-acquired infection is increasingly recognized by health authorities and the general public as a crucial dimension of healthcare quality. The CDC and Healthcare Infection Control Practices Advisory Committee (HICPAC) have issued guidelines for infection control in healthcare facilities (Sehulster et al., 2004). A telephone survey of a national sample of U.S. households found that 93% of respondents indicated that if information on hospital infection rates were provided, it would influence their selection of hospitals (McGuckin, Waterman, & Shubin, 2006).

This research team identified a very large amount of scientific research pertinent to understanding the influences of the hospital physical environment on infection transmission and control. Generally speaking, infection transmission occurs via three routes: *contact*, *air*, and *water*. Contact is widely considered the principal or most frequent transmission route. In reality, these three routes may intertwine with each other in the spread of nosocomial infections. Advances in molecular detection methods and sampling techniques for viruses, bacteria, and fungi have enabled researchers to identify the exact strain and source of infections, and thereby develop a better understanding of transmission.

Reducing Infections Caused by Airborne Pathogens

Airborne transmission refers to infections that are contracted from airborne micro-organisms. Reservoirs for airborne pathogens range from dust (e.g., spores of *Clostridium Difficile* or *C. Diff.* and *Aspergillus*) to aerosol droplets (e.g., tuberculosis [TB], severe acute respiratory syndrome [SARS], influenza, chickenpox), to skin scales shed by patients infected with methicillin-resistant *Staphylococcus aureus* (MRSA) (Ulrich & Wilson, 2006). Airborne transmission has also been implicated in outbreaks of other infections such as *Acinetobacter spp*. and *Pseudomonas spp.* (Beggs, 2003; Beggs, Kerr, Snelling, & Sleigh, 2006). The relative importance of airborne transmission remains somewhat controversial (Bauer, Ofner, Just, Just, & Daschner, 1990). Brachmans's early study (1970) estimated that airborne transmission accounted for 10–20% of nosocomial infections. Beggs (2003) argued that the role of airborne transmission may have been underestimated, due to the difficulty of culturing many airborne organisms and the complexities of assessing the role such pathogens play in the contamination of environmental surfaces and subsequent contact transmission. Recently airborne infections have attracted more attention due to outbreaks of SARS in 2002–2003 and current concerns about an avian influenza (H5N1) pandemic. A few extensive research reviews have been conducted, notably Li et al.'s review (2007) on the relationship between ventilation systems and airborne transmission; Tang, Li, Eames,

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Chan, and Ridgway's report (2006) on airborne infection and ventilation control in healthcare settings; and Beggs' review (2003) on the importance of airborne transmission in healthcare-acquired infections.

This literature survey identified many studies that explicitly examine the relationships between airborne infections and environmental factors in healthcare buildings. There is a pattern of findings across these studies suggesting that hospital air quality plays a decisive role in affecting the concentration of pathogens in the air, and thereby has major effects on the frequency of airborne infectious diseases such as TB, aspergillosis, chickenpox, influenza, and SARS. The research also clearly indicates that multiple environmental approaches or interventions can be effective in controlling and preventing airborne infections. Though a sizable amount of sound research is available, data on certain aspects of air quality and infection are insufficient to permit the precise specification of, for example, minimum ventilation and filtration requirements for certain patient groups and treatment spaces (Li et al., 2007), or the maximum tolerable level of spores per cubic meter (Bouza et al., 2002) for the prevention of airborne transmission.

Sources and Environmental Routes of Airborne Infections

Airborne pathogens originate from different sources. Most pathogens in healthcare settings originate from patients, staff, and visitors within the buildings, from such sources as infected patients' respiratory tracts or skin squamae (scales). Other pathogens can enter buildings from *outside air* through dust that harbors pathogens such as *aspergillus*, *streptococci*, or *staphylococci* (Beggs, 2003). There are also less common sources of airborne infections; for example, bird droppings or aerosols from contaminated water in a warm-water therapy pool (Angenent, Kelley, St Amand, Pace, & Hernandez, 2005).

Several environmental factors and conditions have been identified as frequent sources of airborne infection outbreaks. The malfunction or contamination of ventilation systems and lack of cleaning and maintenance are commonly cited (Kumari et al., 1998; Lutz, Jin, Rinaldi, Wickes, & Huycke, 2003; McDonald et al., 1998; Schultz et al., 2003). In one MRSA outbreak, for example, the ventilation grilles in two patient bays were found to be harboring MRSA (Kumari et al., 1998). On occasions when this ventilation system was shut down, it sucked air from the ward environment into the system, contaminating the outlet grilles then it blew contaminated air back into the ward when the system was restarted. Additionally, several studies have identified hospital construction and renovation activities as the sources of airborne infection outbreaks due to dust or particulate generation (Humphreys et al., 1991; Iwen, Davis, Reed, Winfield, & Hinrichs, 1994; Loo et al., 1996; Opal et al., 1986; Oren, Haddad, Finkelstein, & Rowe, 2001).

Environmental Approaches for Reducing Airborne Infections

The research literature strongly supports implementing several environmental approaches for controlling and preventing airborne infections, including installing effective filters, specifying appropriate ventilation systems and air change rates, employing various control measures during construction or renovation, and using single-bed rooms instead of multibed rooms to increase isolation capacity and reduce transmission from infected patients. Also, limited research suggests that measures such as the use of ultraviolet irradiation can be effective in reducing airborne pathogens (Griffiths, Bennett, Speight, & Parks, 2005) and lowering the incidence of asthma in asthmatic children's homes (Bernstein et al., 2006).

High-efficiency particulate air (HEPA) filters. An effective way to control infections is to control their source. Filtration, the physical removal of particulates from air, is often the first step in ensuring good air quality. One experimental study of a commercial air purification system found that a chemical-coated filter demonstrated 61.46% efficiency in destroying pathogens and reached 99.99% efficiency when used in conjunction with ultraviolet lamps (Griffiths et al., 2005). In acute healthcare settings, a commonly used approach is the HEPA filter, which can be at least 99.97% efficient for removing particulates as small as 0.3 μm in diameter (as a reference, *Aspergillus* spores

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are 2.5 µm to 3.0 µm in diameter) (Sehulster et al., 2004). This is adequate for most healthcare settings in ambulatory care facilities and hospitals, including operating rooms (ORs) (Sehulster et al., 2004). Boswell and Fox's study (2006) revealed a significant reduction in environmental contamination by MRSA with the use of portable HEPA units in a clinical setting. In the CDC/HICPAC guidelines, the use of HEPA filtration is recommended for healthcare facilities, and it is either required or strongly recommended for all construction and renovation areas (Sehulster et al., 2004).

There is strong evidence that immunocompromised and other high-acuity patients have a lower incidence of infection when housed in HEPA-filtered isolation rooms. Bone-marrow transplant recipients in one study showed a 10-fold greater incidence of nosocomial *Aspergillus* infection when they were assigned beds outside a HEPA-filtered environment with *laminar airflow* (LAF), as compared to similar patients housed in a HEPA-filtered unit (Sherertz et al., 1987). A strong multisite study by Passweg and colleagues (1998) found that the use of HEPA and/or LAF reduced infections, decreased transplant-related mortality, and increased survival for leukemia patients after bone marrow transplant.

Ventilation systems and airflow control. After air is filtered, effective ventilation systems are needed to achieve optimal ventilation rates, airflow patterns, and humidity so that the spread of infections can be minimized. First, *ventilation rate* is an important measure to control indoor air quality. In healthcare facilities, it is usually expressed as room air changes per hour (ACH), where peak efficiency for particle removal in the air space often occurs between 12 ACH and 15 ACH. In a study of SARS infections, wards with the highest ventilation rate had a significantly lower infection rate among healthcare workers as compared with other wards (Jiang et al., 2003). A study of 17 Canadian hospitals found that the risk of healthcare workers acquiring TB was strongly linked with exposure to infected patients in rooms with low ACH rates, such as waiting areas (Menzies et al., 2000). Detailed ventilation standards are provided by the American Institute of Architects (AIA) and Facilities Guidelines Institute (FGI) in the *Guidelines for Design and Construction of Health Care Facilities* (AIA & FGI, 2006), and by the American Society of Heating, Refrigerating and Air-conditioning Engineers (ASHRAE) in *ASHRAE 62.1-2004—Ventilation for Acceptable Indoor Quality* (ASHRAE, 2004). Yet questions remain regarding the minimum ventilation requirements needed for effective prevention of infections (Li et al., 2007).

A second key aspect of ventilation is *airflow direction.* Negative pressure is preferred for rooms housing infectious patients to prevent the dispersion of pathogen-laden aerosols, dust, and skin scales from the locus of the infected patient to other spaces. Importantly, a review of 40 studies by Li et al. (2007) concluded that there is strong evidence to support and recommend the use of negatively pressurized isolation rooms. By contrast, if a care space houses an immunocompromised patient (e.g., surgical patients, patients with underlying chronic lung disease, or dialysis patients) or immunosuppressed patients (e.g., transplant patients or cancer patients), positive airflow pressure is desirable to safeguard them from aerial pathogens entering from adjacent spaces.

Finally, an exceptionally effective ventilation approach for maintaining indoor air quality is to use LAF, which is HEPA-filtered air blown into a room at a rate of 90 ± 10 feet/min in a unidirectional pattern with 100–400 ACH (Sehulster et al., 2004). When combined with HEPA filters, LAF can reduce air contamination to the lowest level; thus it is recommended for ORs and areas with ultraclean room requirements, such as those housing immunocompromised patients (Alberti et al., 2001; Arlet, Gluckman, Gerber, Perol, & Hirsch, 1989; Dharan & Pittet, 2002; Friberg, Ardnor, Lundholm, & Friberg, 2003; Hahn et al., 2002; Sherertz et al., 1987). A prospective cohort study found that the type of operating theater ventilation was an independent risk factor for the incidence of sternal surgical site infections (Yavuz et al., 2006). New theaters with LAF and automatically closing doors showed significantly better results in reducing infections than older theaters with conventional plenum ventilation.

Effective air quality control measures during construction and renovation. It is extremely important to employ effective control and prevention measures

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during construction and renovation, because such activities have been frequently implicated in outbreaks of airborne infection. Examples of such measures include using portable HEPA filters, installing barriers between patient-care areas and construction/renovation areas, generating negative air pressure for construction/renovation areas relative to patient-care areas, and sealing patient windows. Strong evidence indicates that using HEPA filters for air intakes near construction and renovation sites has positive effects on air quality and reduces the risk of infection for patients (Bouza et al., 2002; Cornet et al., 1999; Loo et al., 1996; Mahieu, De Dooy, Van Laer, Jansens, & Ieven, 2000; Opal et al., 1986; Oren et al., 2001). For example, a study conducted during extensive hospital construction and renovation documented an outbreak of invasive pulmonary aspergillosis (IPA) among acute leukemia patients housed in wards with natural ventilation, soaring to an infection rate of 50% (Oren et al., 2001). At this point some patients were moved to a hematology ward with HEPA filters. During the following 3 years, none of the patients hospitalized in the hematology ward developed IPA, although 29% of leukemia patients housed in the regular ward contracted aspergillosis. However, one strong study demonstrated that HEPA filters were not by themselves an adequate control measure during construction; they should be employed in conjunction with other measures such as sealing windows and installing barriers (Humphreys et al., 1991). It was noted earlier that the combination of LAF and HEPA filtration is capable of reducing air contamination to the lowest level. During construction or renovation activities, however, LAF is more expensive and especially difficult to achieve, because furnishings and other features can create turbulence. There is currently a lack of cost-benefit research to enable well-founded evaluations of the expense versus effectiveness of LAF for patient-care areas near construction and renovation sites.

Reducing Infections Spread by Contact

Although airborne transmission poses serious safety risks, contact contamination is generally recognized as the principal transmission route of nosocomial infections, including pathogens such as MRSA, *C. difficile*, and vancomycin-resistant enterococci (VRE), which survive well on environmental surfaces and other reservoirs (Bauer et al., 1990; IOM, 2004). The prevention of contact-spread infections is of paramount importance in healthcare settings.

Sources and Environmental Routes of Contact-Spread Infections

Environmental routes of contact-spread infections include direct person-to-person contact and indirect transmission via environmental surfaces. *Healthcare workers' hands* play a key role in both direct and indirect transmissions. A staff member may touch two patients in succession without washing his or her hands, or touch an environmental surface or feature after direct contact with an infected patient. Other staff and the patient may then acquire the pathogen by touching the same surface (Ulrich & Wilson, 2006). Research indicates that there is an inverse causal link between the hand-washing compliance rate of healthcare workers and contact transmission of infectious diseases (Larson, 1988, 1999). It is well established that hand hygiene is the most important single measure for preventing the spread of pathogens in healthcare settings (Boyce & Pittet, 2002).

In this context, the fact that hand-washing compliance rates are often low represents a very serious challenge to patient safety. Mallaret et al. (1998) reviewed 38 studies and reported that compliance rates were usually less than 40%. In more recent studies, compliance rates were still low, with most ranging between 20% and 35%; rates above 40% or 50% are the exception (Albert & Condie, 1981; Graham, 1990; Kuzu, Ozer, Aydemir, Yalcin, & Zencir, 2005; Larson, Albrecht, & O'Keefe, 2005; Randle, Clarke, & Storr, 2006; Saba et al., 2005; Sacar et al., 2006; Trick et al., 2007). Compliance rates usually are lower for indirect contact (through environmental surfaces) than for direct person-to-person contact (McArdle, Lee, Gibb, & Walsh, 2006). There is a pattern that compliance is worse in high-acuity units such as ICUs, because patient care in these units is often more demanding than in lower-acuity units (Karabay et al., 2005). Meanwhile, guidelines require staff to clean their hands more frequently when caring for sicker patients (Karabay et al., 2005). Hand hygiene tends to be especially poor in units that are busy due to understaffing and/or a high bed-occupancy

rate or patient census (Archibald, Manning, Bell, Banerjee, & Jarvis, 1997). High bed-occupancy rates have been identified as a factor contributing to higher rates of infections such as MRSA (Borg, 2003).

Furthermore, *environmental surfaces* in healthcare settings often become extensively contaminated by nearby patients or by healthcare workers' contaminated hands. Boyce, Potter-Bynoe, Chenevert, and King (1997) found that in rooms housing patients infected with MRSA, 27% of all environmental surfaces sampled were contaminated. Meanwhile, 42% of nurses who had no direct contact with MRSA patients but who had touched environmental surfaces contaminated their gloves with MRSA. Other research reports even higher levels of MRSA surface contamination (74%) in spaces previously occupied by colonized or infected patients (French et al., 2004). The same study found MRSA contamination in 100% of patient rooms sampled, regardless of whether or not the previous occupant had been infected. Furthermore, patient rooms can become contaminated with more than one type of MRSA, suggesting prolonged survival of MRSA strains from prior room occupants (French et al., 2004). It is not surprising that the risk of acquiring antibiotic-resistant infections such as MRSA and VRE is significantly increased if a patient is admitted to a room previously occupied by an infected individual (Huang, Datta, & Platt, 2006).

Because many nosocomial pathogens can survive on environmental surfaces for weeks or months (Bonilla, Zervos, & Kaufman, 1996; Kramer, Schwebke, & Kampf, 2006), such contaminated surfaces act as pathogen reservoirs and can become the source of infection outbreaks (Boyce et al., 1993; Lankford et al., 2006). Many of these environmental surfaces and features have direct relevance to architectural design, including floors (Anderson, Mackel, Stoler, & Mallison, 1982; Beyer & Belsito, 2000; Boyce et al., 1997; Skoutelis, Westenfelder, Beckerdite, & Phair, 1994), work surfaces or furniture such as chairs (Noskin, Bednarz, Suriano, Reiner, & Peterson, 2000), bed privacy curtains (Palmer, 1999), door handles (Roberts, Findlay, & Lang, 2001), sink faucets (Blanc et al., 2004; Bures, Fishbain, Uyehara, Parker, & Berg, 2000), bedside rails, over-bed tables, bed linens and patients' gowns (Boyce et al., 1997), clinical waste carts (Blenkharn, 2006), computer keyboards (Bures et al., 2000), bedside patient files (Panhotra, Saxena, & Al-Mulhim, 2005), and even toys in healthcare settings (Fleming & Randle, 2006; Merriman, Corwin, & Ikram, 2002). Other very frequently contaminated surfaces and objects include medical equipment such as infusion pumps (Aygun et al., 2002), blood pressure cuffs (Boyce et al., 1997), laryngoscope blades (Beamer & Cox, 1999), stethoscopes (Marinella, Pierson, & Chenoweth, 1997), and electronic ear-probe thermometers (Porwancher et al., 2001). The pervasiveness of such contamination underscores the importance of hand- and workplace hygiene in healthcare settings (Wilson & Ridgway, 2006; Ulrich & Wilson, 2006).

Environmental Approaches to Reduce Contact-Spread Infections

The research literature supports the effectiveness of certain environmental approaches for controlling and preventing contact-spread infections. Examples of such approaches include providing sufficient and accessible alcohol-based hand-rub dispensers, choosing easy-to-clean furniture and wall finishes, and providing single-rooms rather than multibed rooms.

Reducing contact transmissions by increasing hand-washing compliance. Education programs to increase hand-washing compliance alone have yielded, at best, mixed results (Bischoff, Reynolds, Sessler, Edmond, & Wenzel, 2000). Some investigations have found that education interventions generate no increase in hand washing. Even intensive education or training programs, such as classes and group feedback, may produce only transient increases in hand washing (Dorsey, Cydulka, & Emerman, 1996; Dubbert, Dolce, Richter, Miller, & Chapman, 1990). Recently, multifaceted interventions, in addition to education, have been more successful at increasing hand washing. These interventions include environmental measures such as providing localized availability of alcohol-rub dispensers and using posters as reminders to staff (Creedon, 2005; Gordin, Schultz, Huber, & Gill, 2005; Johnson et al., 2005; Lam, Lee, & Lau, 2004; Pittet et al., 2000; Randle et al., 2006; Trick et al., 2007).

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There is mounting evidence that the *type* of hand-washing facility influences hand-washing compliance and infection rates. Compared with traditional soap and water, alcohol-based hand-rub acts more rapidly and effectively, requires less time for staff to decontaminate their hands adequately, and has a lower risk of side-effects and recontamination (Boyce & Pittet, 2002). The CDC/HICPAC guidelines define alcohol-based hand-rub as the standard of care for hand hygiene practices in healthcare settings (Boyce & Pittet, 2002). Several studies have shown that the introduction of alcohol-based hand-rub boosted hand-washing compliance (Hugonnet, Perneger, & Pittet, 2002; Johnson et al., 2005; Trick et al., 2007). Importantly, several other studies supported the effectiveness of alcohol-based hand-rub, compared to soap and water, for improving the effectiveness of hand washing in terms of reducing microbial counts on hands (Bischoff et al., 2000; Cohen, Saiman, Cimiotti, & Larson, 2003; Girou, Loyeau, Legrand, Oppein, & Brun-Buisson, 2002; Graham, 1990; Karabay et al., 2005; Tvedt & Bukholm, 2005) and reducing infection rates (Gordin et al., 2005). MacKenzie and colleagues (2007) analyzed MRSA prevalence in more than 100 hospitals across Europe and found that the use of alcohol-based hand-rub was the single most important predictor of lower MRSA incidence after adjusting for other confounding factors. These findings have implications for designers, because alcohol-based hand-rub dispensers are small and inexpensive, and they do not require costly plumbing systems and sinks. These characteristics afford more flexibility than soap-and-water facilities, which in turn facilitates the distribution of dispensers to more locations, closer to patient-care activities and work spaces, thereby making them more accessible to busy clinicians and other staff.

The *number* and *accessibility* of hand-washing facilities also influence compliance and infection rates. In particular, the evidence suggests that installing alcohol-based hand-rub dispensers at the bedside usually improves adherence. Four studies examined the impact of multifaceted interventions that prominently included the provision of bedside alcohol-based hand-rub dispensers, and all demonstrated significant improvements in hand-washing compliance (Bischoff et al., 2000; Creedon, 2005; Pittet et al., 2000; Randle et al., 2006). Another study made a statistical adjustment for other known risk factors of poor hand-washing adherence; the positive effects of the intervention remained significant and were accompanied by decreased infection rates (Pettit et al., 2000). In an observation study, researchers compared the 3 years after the installation of alcohol-based hand-rubs in all rooms with the 3 prior years with fewer soap-and-water sinks; they observed a 21% decrease in MRSA infections and a 41% decrease in VRE infections. Although hand-washing compliance was not measured in this study, it is likely that it may have played a role in this improvement. In contrast to the effectiveness of locating hand-rub dispensers at the bedside, Muto, Sistrom, and Farr (2000) found that installing dispensers in hallway locations (near the doors to patient rooms) did not significantly increase the frequency of hand washing. Other investigations focusing on traditional sinks (soap-and-water) have obtained mixed results concerning the impact of increasing the number and accessibility of sinks, with a few studies reporting positive impacts (Kaplan & McGuckin, 1986); one finding a transient increase in compliance (Whitby & McLaws, 2004); and other studies reporting no significant changes (Lankford et al., 2000, 2003; Vernon, Trick, Welbel, Peterson, & Weinstein, 2003).

Automated technology has also been examined for its impact on hand-washing compliance for soap-and-water sinks and alcohol-based hand-rub dispensers. For traditional soap-and-water hand washing, automated sinks or faucets have shown mixed results (Larson et al., 1991; Larson, Bryan, Adler, & Blane, 1997). Simplicity of use seems to be important to the success of automation. In this regard, limited research suggests that automated touch-free alcohol-based rub dispensers are easy to use and are used more frequently than manual dispensers (Larson, Albrecht, & O'Keefe, 2005). Swoboda, Earsing, Strauss, Lane, and Lipsett (2004) examined the effect on compliance of an automatic system that monitored entries and exits from patient rooms, recorded usage of sinks and alcohol-based hand-rub dispensers, and incorporated voice-prompt devices that reminded healthcare workers and visitors to wash their hands. The system improved hand-cleaning com-

pliance from 19% to 27% and was associated with a reduction in the nosocomial infection rate.

There are some limitations, however, in current hand-washing research knowledge. Because many studies have employed multifaceted interventions, it is not clear how much of the effectiveness of increased hand washing, reduced microbial counts, or reduced infection rates can be attributed to the installation of more numerous and/or accessible alcohol-based hand-rub dispensers. Future research should include prospective controlled experiments, for example, that systematically vary the number and location of alcohol hand-rub dispensers. There is also a conspicuous need for studies that define accessible locations for hand-washing facilities in an evidence-based manner—that is, on the basis of empirical analysis of staff movement paths, visual fields, interactions with patients and families, and work processes. In this regard, the neglect of human factors and research methods are major weaknesses of hand-washing research and of the infection control literature in general. Research teams should include a human factors specialist and sometimes an environmental psychologist. The urgent need to increase hand-washing frequency underscores the high priority that should be accorded to this research direction.

Reducing contact transmission by controlling surface contamination. As previously mentioned, contaminated environmental surfaces often serve as an intermediate step in the contact spread of infections. Several design-related factors should be considered to minimize the risk of infection stemming from contaminated surfaces.

Selection of *appropriate floor and furniture coverings* is an important step, where ease of cleaning should be a key consideration. Some studies have examined flooring materials (Anderson et al., 1982; Skoutelis et al., 1994) and furniture coverings (Lankford et al., 2006; Noskin et al., 2000) as they relate to environmental contamination in healthcare settings. The use of *carpet* can be a controversial issue. On one hand, many people believe that carpet is more difficult to clean than hard floor coverings (Harris, 2000). A few studies have identified carpeting as susceptible to contamination by fungi and bacteria (Anderson et al., 1982; Beyer & Belsito, 2000; Boyce et al., 1997; Skoutelis et al., 1994). However, a recent rigorous study suggests that certain serious pathogens such as VRE survive less well or for shorter periods on carpet than on other floor coverings, including rubber tile, linoleum, vinyl sheet goods, and vinyl composition tile (Lankford et al., 2006). In addition to discovering that carpet harbors less VRE, this research found that carpeting transferred less VRE to hands via contact than rubber and vinyl flooring and performed as well in cleaning as any other floor covering tested (Lankford et al., 2006). There is limited research comparing the air above carpeted areas and hard flooring with respect to concentrations of micro-organisms, and the findings are conflicting. Anderson et al. (1982) found higher concentrations above carpeted areas, whereas Harris (2000) reported higher particulate concentrations above hard flooring.

In summary, the advantages and disadvantages of carpeting versus other floor coverings with respect to infection control are neither clear-cut nor fully resolved. However, in judging different floor coverings, it should be kept in mind that carpeting, compared to hard floorings, offers important advantages unrelated to infection control, including noise reduction (Philbin & Gray, 2002), greater ease of walking and perceived safety for the elderly (Wilmott, 1986), a possible reduction in falls (Counsell et al., 2000), longer family visits in patient rooms, and more positive evaluations and emotional responses from patients and families (Harris, 2000).

It is worth mentioning that CDC/HICPAC guidelines do not recommend against the use of carpeting in patient-care areas. However, the guidelines suggest that carpeting should be avoided in areas where spills are likely to occur or where patients are at greater risk of airborne infections (Sehulster et al., 2004). Similarly, the EBD standards for neonatal intensive care units (NICUs) of the National Perinatal Association state that suitable flooring materials "include resilient sheet flooring (medical grade rubber or linoleum) and carpeting with an impermeable backing, heat- or chemically welded seams and antimicrobial and antistatic properties. Carpeting has been shown to be an accept-

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able floor covering in the hospital and the NICU and has obvious aesthetic and noise reduction (NR) appeal, but it is not suitable in all areas (e.g., around sinks or in isolation or soiled utility/holding areas)" (White, 2006, p. S12).

The selection of *furniture-covering materials* may also influence the incidence of contamination and risk of infection. Noskin et al. (2000) identified fabric-covered furniture as a source of VRE infection in hospitals and suggested the use of easily cleanable, nonporous material. Another study compared the performance of a variety of furniture upholstery types with respect to VRE and *Pseudomonas aeruginosa* (PSAE) contamination (Lankford et al., 2006). Performance was similar across different furniture coverings in terms of reductions in VRE and PSAE after cleaning and the transfer of VRE and PSAE to hands through contact. However, for the ability to harbor pathogens, although upholstery types showed no differences with respect to PSAE, there was a difference related to VRE. Vinyl upholstery performed the best for VRE—that is, the VRE pathogen survived less well or for shorter periods on vinyl (Lankford et al., 2006). In addition, as with evaluating carpeting and other floor coverings, it is worth considering that fabric-covered furniture might foster a more home-like, less institutional feeling. The CDC/HICPAC guidelines for upholstery are broadly similar to those for carpeting in that they do not recommend against using it in patient-care areas, but they suggest minimizing its use in areas housing immunocompromised patients (Sehulster et al., 2004).

A limited amount of research has compared different *wall finishes* and *metals* with respect to their infection control properties. One study evaluated the effectiveness of copper, brass, and stainless steel surfaces in reducing the viability of air-dried deposits of MRSA (Noyce, Michels, & Keevil, 2006). The results suggested that copper had a better antimicrobial effect than stainless steel. The use of antimicrobial metals such as copper may not reduce the need for careful cleaning, however, because dirt or dust on their surfaces may diminish or eliminate their antimicrobial effects. Lankford and colleagues (2006) compared the performance of different wall finishes (latex paint with eggshell finish, microperforated vinyl, vinyl with nonwoven backing, and Xorel® wall covering), and reported that all harbored VRE and were capable of transferring the pathogen through hand contact. No reduction in VRE was found 7 days after inoculation for two of the wall products—Type II microvented vinyl with paper backing and Xorel® wall covering—indicating that harboring was a greater problem than for other wall products tested. Latex paint with eggshell finish performed worse in cleaning and disinfection than other wall finishes, indicating that cleaning produced inadequate reduction of VRE and PSAE (Lankford et al., 2006).

Proper cleaning and disinfection is another very important step in preventing the spread of infections by contact. The limited and conflicting nature of research on environmental surface materials poses a perplexing challenge to designers attempting to select materials to help control infection. It appears that for each general category of surfaces—flooring, upholstery, and wall finishes—no single material has yet been identified that consistently outperforms others across diverse performance criteria (e.g., harboring, capacity to transfer) and for different pathogens. This underscores the importance of selecting materials that are easily cleaned and of proper cleaning and disinfection procedures (Aygun et al., 2002; Barker, Vipond, & Bloomfield, 2004; Dettenkofer, Wenzler, et al., 2004; French et al., 2004; Griffiths, Fernandez, & Halcomb, 2002; Hota, 2004; Martinez, Ruthazer, Hansjosten, Barefoot, & Snydman, 2003; Neely et al., 2005; Wilson & Ridgway, 2006). As noted, some research suggests that latex paint with eggshell finish does not perform adequately in cleaning/disinfection for VRE and PSAE (Lankford et al., 2006). Detailed cleaning recommendations for environmental surfaces are available in the CDC/HICPAC Guidelines (Sehulster et al., 2004).

Notwithstanding the importance of cleaning, there is alarming evidence indicating that conventional cleaning techniques often do not adequately eliminate contamination by serious pathogens such as MRSA and *C. difficile*. This problem has led infection control researchers to investigate the effectiveness of alternative decontamination methods or technologies, notably hydrogen peroxide vapor (HPV). French and colleagues

(2004) conducted a prospective study of multibed patient rooms contaminated with MRSA in the United Kingdom, assigning six rooms to be cleaned using conventional methods and six similar rooms using HPV. Before cleaning, 70% of 359 sample swabs from the study rooms yielded MRSA. An important and disturbing finding was that following conventional cleaning, 66% of swabs taken from rooms decontaminated by traditional methods yielded MRSA, indicating that conventional cleaning failed to remove most MRSA contamination. By contrast, following HPV cleaning only 1.2% of swabs yielded MRSA, indicating that HPV was a far more effective method for decontaminating patient rooms (French et al., 2004). Another British study by Jeanes, Rao, Osman, and Merrick (2005) found that even after an exceptionally intensive three-day period of deep cleaning using traditional methods (detergent, steam cleaning, chlorine disinfectant), 16% of surfaces sampled in a Nightingale ward were still cultured with MRSA. Following HPV decontamination of the Nightingale ward, however, no MRSA at all was cultured from surfaces. These studies support the effectiveness of HPV cleaning and have implications for hospital architecture, because a key consideration in employing HPV is that no patients or staff can be in a room during the process of vapor decontamination. Accordingly, the use of HPV in multibed rooms or open bays necessitates temporarily removing all patients from the space, shutting and sealing the space for several hours, and disrupting patient care and flow. By comparison, evacuating persons from single-bed rooms following patient discharge poses little hindrance to using HPV.

Reducing Waterborne Infection Transmission

Compared with airborne and contact transmission of infection, fewer studies were identified on waterborne transmission in relation to hospital design factors. The literature nonetheless makes it clear that waterborne infections can be a serious threat to patient safety. Many bacterial and some protozoal micro-organisms can proliferate or remain viable in moist environments or aqueous solutions in healthcare settings (Sehulster et al., 2004). Anaissie, Penzak, and Dignani (2002) reviewed studies between 1966 and 2001 on waterborne nosocomial infections caused by micro-organisms other than *Legionella*. The review identified 43 reported outbreaks and an estimated 1,400 deaths each year in the United States alone resulting from waterborne nosocomial pneumonia caused by *Pseudomonas aeruginosa*. A study of 115 randomly selected dialysis facilities in the United States detected nontuberculous mycobacteria in 83% of centers (Carson et al., 1988). Contaminated water systems in healthcare settings (such as inadequately treated wastewater) may lead to the pollution of municipal water systems, enter surface or ground water, and affect residents (Iversen et al., 2004).

Sources and Environmental Routes of Waterborne Transmission

The CDC/HICPAC guidelines (Sehulster et al., 2004) identify the following categories of environmental routes or sources of waterborne transmission: (1) direct contact, such as hydrotherapy (Angenent et al., 2005); (2) ingestion of water, such as drinking water (Conger et al., 2004; Squier, Yu, & Stout, 2000); (3) inhalation of aerosols dispersed from contaminated water sources, such as improperly cleaned or maintained cooling towers, showers (Mineshita, Nakamori, Seida, & Hiwatashi, 2005), respiratory therapy equipment, and room air humidifiers; and (4) aspiration of contaminated water.

Environmental Approaches to Reduce Waterborne Infection Transmission

Based on our literature review, the following environmental approaches that aid in controlling and preventing waterborne infections were identified.

Water supply system. The water supply system should be designed and maintained with proper temperature and adequate pressure; stagnation and back flow should be minimized; and dead-end pipes should be avoided (AIA and FGI, 2006; Sehulster et al., 2004). To prevent the growth of Legionella and other bacteria, the CDC/HICPAC guidelines recommend that healthcare facilities maintain cold water at a temperature below 68°F (20°C), store hot water above 140°F (60°C), and circulate hot water with a minimum return temperature of 124°F (51°C) (Sehulster et al., 2004). When the recommended standards cannot be achieved because of inadequate facilities that cannot be renovated,

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other measures such as chlorine treatment, copper-silver ionization, or ultraviolet lights are recommended to ensure water quality and prevent infection (Sehulster et al., 2004). For example, in a university hospital where endemic nosocomial legionellosis was present and all previous disinfection measures had failed, the implementation of a copper-silver ionization system substantially decreased environmental colonization by Legionella, and the incidence of nosocomial legionellosis decreased dramatically (Modol et al., 2007). The review by Anaissie and colleagues (2002) recognized the potential severity of waterborne infections and recommended that high-risk patients should not be exposed to tap water, but should use sterile water instead.

Point-of-use fixtures. Water fixtures such as sinks, faucets, aerators, showers, and toilets have been identified as potential reservoirs for pathogenic microorganisms (Blanc et al., 2004; Conger et al., 2004; Mineshita et al., 2005; Squier et al., 2000). Such fixtures produce aerosols that can disperse microbes, and they have wet surfaces on which molds and other microorganisms can proliferate. However, empirical evidence linking these fixtures to nosocomial infections is still limited; no consensus has been reached regarding the disinfection or removal of these devices for general use (Sehulster et al., 2004). Regular cleaning, disinfection, and good maintenance should be provided, especially in areas housing immunocompromised patients.

Decorative fountains in healthcare settings. Decorative fountains increasingly are being used by designers for healthcare facilities, because they can serve as landmarks and wayfinding elements as well as positive distractions that reduce stress (Joseph, 2006). The infection control departments of some hospitals may oppose the installation of fountains out of concern for the possible generation of infectious aerosols. However, Rogers' review (2006) found no empirical study linking a waterborne infectious disease or nosocomial outbreak to the indoor placement of a water fountain or water feature in hospitals. The only related case was an outbreak of Legionnaires' disease among a group of older adults in a hotel. The source was traced to a fountain in the lobby, which was not regularly maintained and which was heated by underwater lighting (Hlady et al., 1993). Despite the absence of empirical documentation linking properly maintained fountains to hospital-acquired infections, the AIA & FGI Guidelines (2006) recommend that fountains not be installed in enclosed spaces in hospitals.

Reducing Multiroute Transmission by Means of Single-Bed Rooms and Increased Isolation

Thus far the three routes of infection transmission have been examined and discussed separately. In reality, these three routes often intertwine, and environmental approaches may influence more than one transmission route. This research team has found credible evidence for the multiroute impact of single-bed rooms and increased isolation in infection control. Therefore, we have opted to present this information in a separate section instead of within the previous sections addressing individual transmission routes.

Several literature review articles have supported the association between single-bed rooms and reduced infection rates, including Dettenkofer, Seegers, et al.'s (2004) review on the relationship between architectural design and nosocomial infections and Chaudhury, Mahmood, and Valente's review (2005) on the advantages and disadvantages of single- versus multibed accommodations. Also, Calkins and Cassella (2007) surveyed research on nosocomial infections in nursing homes and similarly concluded that private bedrooms reduce the risk of infection as compared to shared bedrooms. The present review conducted a broader, updated survey and analysis, and evaluated not only environment-infection *associations*, but also the underlying *mechanisms* that could plausibly account for these associations.

Effect of single-bed rooms in reducing airborne infection. Because infected patients carry airborne pathogens into patient rooms and nursing units, it is important to ensure sufficient isolation capacity for such patients to prevent the spread of pathogens. Providing single-bed rooms increases isolation capacity; facilitates filtration, ventilation, and airflow control (e.g., negative room pressurization); and by these well-established measures or mechanisms, it plays a key role in preventing a patient with an aerial-spread infection from infecting others and protects immunocompro-

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mised patients in nearby rooms from airborne pathogens. As might be expected, studies of cross-infection for contagious airborne diseases (such as influenza, TB, measles, and chickenpox) have revealed that placing patients in single rooms (Ben-Abraham et al., 2002), single-bed cubicles with partitions (Gardner, Court, Brocklebank, Downham, & Weightman, 1973), isolation rooms (Mulin et al., 1997), or rooms with fewer beds and more space between patients (McKendrick & Emond, 1976) is safer than housing them in multibed spaces with more patients. Vonberg and Gastmeier (2005) reviewed literature on the isolation of cystic fibrosis patients, for whom respiratory tract infections contributed markedly to morbidity and mortality. They found in 31 out of 39 studies that cross-infection of *Pseudomonas aeruginosa* had been halted by isolating patients. Research on burn patients and other vulnerable or immunosuppressed patient groups provides strong evidence that single rooms in combination with air filtration substantially reduce the incidence of infection and mortality (McManus, Mason, McManus, & Pruitt, 1994; Passweg et al., 1998; Shirani et al., 1986). In a study of nursing homes, Drinka, Krause, Nest, Goodman, and Gravenstein (2003) found that roommates of persons infected with influenza had a 3.07 higher relative risk of acquiring the illness than did individuals assigned to single-bed rooms.

Although MRSA is spread mainly by contact, it has been known for decades that patients with *Staphylococcus aureus* infections shed skin scales contaminated with the pathogen, which become suspended throughout the air in rooms and which can spread the infection to other patients sharing that space. Lidwell et al. (1970) documented a significantly reduced rate of nasal acquisition of *Staphylococcus aureus* for patients in single-bed rooms than for those in multibed rooms. Shiomori and colleagues (2002) found that in rooms with a MRSA patient, the air concentration of MRSA-contaminated skin scales reached 116 per cubic foot, representing an added risk of airborne transmission to uninfected patients.

The SARS outbreaks in Asia and Canada highlighted dramatically the failings of multibed rooms for controlling or preventing infections among both patients and healthcare workers. SARS is transmitted by droplets that can be airborne over a limited area. The point should be emphasized that SARS in Canada was predominantly a *hospital-acquired*—not a community-acquired—infection, because approximately 75% of SARS cases resulted from exposure in hospital settings (Farquharson & Baguley, 2003). In Canadian and Asian hospitals, the pervasiveness of multibed spaces in emergency departments (EDs) and ICUs worsened SARS cross-infection. Furthermore, the scarcity of isolation rooms with negative pressure was a serious obstacle to implementing effective treatment and control measures. Toronto hospitals were forced, on a crisis basis, to construct hard wall partitions with doors to replace curtain partitions between beds in multibed spaces, and to implement airflow and pressure adaptations in EDs and ICUs to create many additional negative-pressure isolation rooms with HEPA filtration (Farquharson & Baguley, 2003).

Effect of single-bed rooms in reducing contact transmission. The use of single-bed rooms instead of multibed rooms also helps to control infections spread by contact. Single-bed rooms can *facilitate cleaning and decontamination*. As discussed earlier, many surfaces and features near infected patients quickly become contaminated, creating numerous reservoirs that can transfer pathogens to patients and staff. Given the vital importance of cleaning for the removal of contamination, one advantage of single-bed rooms compared to multibed rooms is that they are easier to clean and decontaminate thoroughly after a patient is discharged. In certain countries, when a patient has been discharged from a multibed room, cleaning staff are not permitted to clean electrical equipment or anything attached to other patients remaining in the space, thus increasing the risk of cross-infection (Ulrich & Wilson, 2006). Scrupulous cleaning of double rooms, or the four-bed and six-bed spaces prevalent in many countries, often entails the disruptive and costly temporary removal of all patients from these rooms. In addition, as mentioned in an earlier section, even when conventional cleaning methods are used according to prescribed protocols or the manufacturers' instructions, extensive contamination by pathogens such as MRSA still remains on surfaces (French et al., 2004; Jeanes et al., 2005). If more

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effective cleaning techniques such as HPV are used, multibed rooms present additional challenges because all patients in the room must be transferred to other spaces during the vaporization treatment.

Single-patient rooms may also help to *improve hand-washing* compliance and thereby contribute to infection control. Some studies offer evidence that when all single-bed rooms are furnished with a conveniently located sink in each, the nosocomial infection rates in ICUs and burn units diminish, as compared to when the same staff and comparable patients are in multibed open units with few sinks (Goldmann, Durbin, & Freeman, 1981; McManus et al., 1994; McManus, McManus, Mason, Aitcheson, & Pruitt, 1985; Mulin et al., 1997). Although these studies did not measure hand-washing frequency, the investigators posited that increased hand washing was an important factor in reducing infections in the units with single-patient rooms and more sinks.

In several studies documenting the positive association between single-bed rooms and reduced infection rates, the reduction in contact transmission (such as via reduced contamination of surfaces) was not directly measured, but it might have played an important role, based on previous knowledge. For example, MRSA is spread mainly by contact. Single-bed rooms appeared to reduce or prevent MRSA infections compared to multibed rooms in various healthcare settings, including 212 ICUs across Germany (Gastmeier, Schwab, Geffers, & Ruden, 2004), 173 hospitals across Europe (MacKenzie et al., 2007), a U.K. hospital with 1,100 beds (Wigglesworth & Wilcox, 2006), and a NICU in the United States (Jernigan, Titus, Groschel, GetchellWhite, & Farr, 1996). Also, having a roommate has been identified as a risk factor for nosocomial diarrhea and gastroenteritis (Chang & Nelson, 2000; Pegues & Woernle, 1993). Ben-Abraham and colleagues (2002) found that nosocomial infection frequency was much lower in a single-bed pediatric intensive care unit (PICU) than in a unit with multibed rooms and comparable patients, and they tentatively concluded that single-bed rooms helped to limit the person-to-person spread of pathogens among patients. Although the pattern of results across studies on balance strongly suggests that single rooms reduce infection, Preston, Larson, and Stamm's (1981) finding is anomalous in that it found single-bed ICU isolation rooms were associated with only a slight, insignificant reduction in infection rates compared to multibed rooms.

Several deadly outbreaks of *C. difficile* in North American and European hospitals and thorough published investigations have underscored powerfully the threat to patient safety posed by multibed rooms. A highly virulent infection characterized by diarrhea and colitis, in several countries *C. difficile* causes more deaths than MRSA. The infection is spread mainly by contact, and *C. difficile* spores can be viable for months on environmental surfaces (Kramer, Schwebke, & Kampf, 2006). Two outbreaks in the United Kingdom at two National Health Service hospitals have caused approximately 40 deaths (Healthcare Commission, 2006) and 90 deaths (Healthcare Commission, 2007), respectively. The investigations in these hospitals identified a predominance of multibed rooms with shared toilets, and a scarcity of single rooms with private toilets as key factors that prevented the timely isolation of patients and contributed to the spread of *C. difficile* and the duration and high mortality of these outbreaks (Healthcare Commission, 2006, 2007). Another study has also reported that single-bed isolation helped prevent the spread of *C. difficile* (Malamou-Ladas, O'Farrell, Nash, & Tabaqchali, 1983).

Single rooms, admission, and proactive separation of patients. Providing a high proportion of single rooms in hospitals conveys a major safety advantage, because it enables *separation of patients upon admission* and makes it possible to prevent cross-infection from unrecognized carriers of pathogens (Ulrich & Wilson, 2006). Even if patients are screened for MRSA, *C. difficile*, or other pathogens immediately upon admission, processing test results often requires two or three days, during which time environmental surfaces in the rooms of infected patients quickly become extensively contaminated, creating pathogen reservoirs that will be touched by staff and possibly by patients (e.g., French et al., 2004). Accordingly, assigning an unidentified carrier initially to a multibed room height-

ens the risk of cross-infection. By the time test results revealing that the patient is colonized or infected are available, it may be too late to isolate the individual, because transmission to one or more roommates may already have occurred. A prospective study by Cepeda et al. (2005) screened patients for MRSA when they were admitted and placed in multibed rooms in the ICUs of two London hospitals. Patients who proved to be MRSA-positive (after a 3-day delay for testing) were assigned either to be moved into isolation or to remain in their multibed rooms. Findings indicated that moving patients to single-bed rooms after testing positive for MRSA did not reduce cross-infection to other patients (Cepeda et al., 2005), supporting the interpretation that the contamination of surfaces and/or the spread of the infection to roommates occurred in the period prior to isolation.

Single-bed rooms may also help manage the *growing problem of community-acquired infection*. MRSA and other serious multidrug resistant infections are no longer confined to healthcare settings, but are increasingly widespread and endemic in communities internationally. According to a study by the U.S. CDC, 13.7% of MRSA infections in 2005 originated in the community (Klevens et al., 2007b). Another 58.4% of MRSA infections in the United States were *community-onset*, or manifested themselves outside the hospital, but had a healthcare link, such as a patient history of surgery, hospitalization, or residence in a long-term care facility. Hospital-onset MRSA infections accounted for only 26.6% of the cases. These findings imply that mounting numbers of people admitted to the hospital as inpatients, or who visit EDs or ambulatory clinics for care, will be carriers of serious community-acquired or community-onset infections. The difficult and escalating infection control challenge for hospitals that is posed by community-acquired and community-onset infections is reflected, for example, in the fact that MRSA has become the most common cause of skin and soft-tissue infections among patients presenting to EDs in U.S. cities (Moran et al., 2006). Furthermore, the growing trend toward the spread of antibiotic-resistant pathogens in communities will inevitably continue as sicker, more vulnerable patients are cared for at home or in long-term care facilities, and as they receive frequent and prolonged courses of antibiotics (Ulrich & Wilson, 2006). Against this background, in the future hospitals may need to screen and assign all inpatients to single rooms upon admission to prevent infections from spreading to other patients. Apart from MRSA, the spread of infections such as *C. difficile* in communities implies that single rooms with toilets and good air quality will increasingly be needed in EDs and outpatient surgery clinics as well as in inpatient units.

Reducing Medical Errors

Summary of Evidence and Recommendations

Like hospital-acquired infections, medical errors pose serious threats to patient safety. This research team identified several rigorous studies linking environmental factors with medical errors. The limited literature shows that medical errors are not caused only by the mistakes of a few individuals, but by a combination of both people and the environment, and that environmental approaches can play an important role in reducing errors.

Environmental factors discussed in relation to medical errors include *noise*, *light*, and *acuity-adaptable*, *single-patient rooms*. There is limited evidence that prescription error rates increase sharply when there is an interruption or distraction from an unpredicted noise (e.g., a telephone call) (Flynn et al., 1999; Kistner, Keith, Sergeant, & Hokanson, 1994). Poor lighting levels can also affect the performance of healthcare workers and lead to medical errors. One study has shown significantly lower rates of medication-dispensing errors when the lighting level for work surfaces is sufficiently high (Buchanan, Barker, Gibson, Jiang, & Pearson, 1991). Further, as demonstrated by empirical studies (Hendrich, Fay, & Sorrells, 2002, 2004), the use of acuity-adaptable rooms can substantially reduce possible sources of medical error (such as transfers, delays, communication discontinuities among staff, loss of information, and changes in computers or systems), and thereby lower error rates. Additional research is needed to further confirm the findings of limited previous studies, and to identify ways to design better working environments that may reduce or prevent medical errors.

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Severity and General Causes of Medical Errors

The IOM (1999) estimates that 44,000 to 98,000 people die each year of preventable medical errors, based on annual percentages derived from two major studies and the annual hospital admissions rate of 1997. Even the lower estimate is more than the number of annual deaths caused by motor vehicle accidents (43,458), breast cancer (42,297), or AIDS (16,516). The report also draws attention to the total cost of medical errors in addition to the lives lost (including the expense of additional care necessitated by the errors, lost income and household productivity, and disability), which is estimated to range between $17 billion and $29 billion per year in hospitals nationwide (Kohn, et. al., 1999).

Medical errors can include a range of adverse events, including physical errors made during surgical procedures, incorrect diagnoses, and medication errors. Errors are generally triggered by a combination of active failures and latent conditions. *Active failures* are caused by the unsafe performance of caregivers or by the system through lapses, mistakes, and procedural violations. *Latent conditions* are established by designers, builders, and top level management and they make errors more likely. Examples of latent conditions caused by management include work overload, staff shortage, and inexperience with working conditions. Latent conditions related to design include noise, lack of space, and other design failures.

Impact of Noise on Medical Errors

Unpredictable loud noise can distract people and interrupt their performance. A large number of studies have documented the negative impact of noise on workers' performance in nonhealthcare settings, and unpredictable noises disrupt task performance more than predictable ones. Additionally, noise has a greater negative impact when tasks are more complicated (Leather, Beale, & Sullivan, 2003). The combination of unpredictable noise and complicated tasks can increase errors in calculation, tracking, and monitoring tasks, and lead to slower learning and poor memorization (Sundstrom & Sundstrom, 1986).

However, these findings have not been fully explored yet in healthcare settings. A number of studies investigated the contribution of auditory factors, such as high levels of ambient noise (80 dB–85 dB), different types of music (classical or rock), and auditory distractions in the occurrence of surgical and diagnostic errors (Goodell, Cao, & Schwaitzberg, 2006; Moorthy, Munz, Undre, & Darzi, 2004; Sanderson et al., 2005; Zun & Downey, 2005) and found no significant evidence of their effects. However, most of these studies have been conducted in experimental settings by carrying out simulated tasks and/or with simulated noises. Additional research is needed to test the impact of different auditory factors under real-life conditions.

There is some evidence regarding the impact of interruptions or distractions on medication dispensing errors by hospital pharmacists (Flynn et al., 1999; Kistner et al., 1994). They found that error rates for prescriptions increased sharply when there was an interruption or distraction, including unexpected noises (e.g., a telephone call).

Impact of Lighting Level on Medical Errors

Many studies in nonhealthcare settings have demonstrated that performance and errors can be affected by lighting level as well as noise. Such poor performance in healthcare settings may lead to medical errors. Sundstrom and Sundstrom (1986) found that visual inspection task performance declined when light is not bright enough. A large-scale study in pharmacy examined the effects of different illumination levels on pharmacists' prescription-dispensing errors, and it strongly suggested that the frequency of such errors was reduced when work-surface light levels were relatively high (Buchanan et al., 1991). It evaluated the error rate under three different illumination levels, including 450 lux, 1,100 lux, and 1,500 lux. Results showed that medication-dispensing error rates were significantly lower (2.6%) at an illumination level of 1,500 lux, compared to an error rate of 3.8% at 450 lux.

Reducing Patient Transfers by Means of Acuity-Adaptable Rooms

The transfer of patients between rooms or units is a source of medical error (Cook, Render, & Woods, 2000; Ulrich & Zhu, 2007). Reasons for these errors

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include delays, communication discontinuities among staff, loss of information, and changes in computers or systems. A possible solution is to create an acuity-adaptable care process and to provide patient rooms that substantially reduce transfers. When the Methodist Hospital in Indianapolis, Indiana, changed its coronary ICUs from two-bed rooms to acuity-adaptable single-bed rooms, transfers were reduced by 90% and medication errors were lowered by 67% (Hendrich, Fay, & Sorrells, 2002, 2004). Reducing transfers also saves staff time, shortens patient stays, and reduces cost (IOM, 2004). Single-patient rooms, even nonacuity-adaptable ones, have been associated with better staff communication, less patient transfer, fewer medical errors, and lower infection rates as compared to multibed patient rooms (Chaudhury, Mahmood, & Valente, 2006). Additional studies and demonstration projects are needed to ascertain the safety advantages of acuity-adaptable, single rooms for other types of units and patient categories.

Reducing Patient Falls

Summary of Evidence and Recommendations

There is a large literature that examines the causes and risk factors involved in patient falls in hospitals. This is an area of great importance, because patients who fall incur physical injuries and adverse psychological effects and have greater lengths of stay in the hospital (Brandis, 1999). Among elderly persons (more than 65 years old), most falls occur in hospitals and nursing homes, where the rate of falls reaches 1.5 per bed annually, which is almost three times the rate for community-dwelling elderly persons (American Geriatrics Society, 2001). It is estimated that the total cost of fall injuries for older people was $20.2 billion per year in the United States in 1994, and that it would reach $32.4 billion (in 1994 U.S. dollars) in 2020 (Chang, Morton, Rubenstein, & Mojica, 2004).

Although the role of the environment in causing or preventing patient falls is widely accepted, there is no conclusive evidence linking environmental interventions with reduced falls. Studies have sought to identify the design issues that might have contributed to falls (such as the placement of doorways, handrails, and toilets), but no studies have compared different design options to determine the independent impact of a single design factor on the incidence of falls. One study has provided some promising findings, suggesting that decentralized nurse stations can reduce falls; but more research in more rigorous studies is needed to confirm these findings and to identify all the variables involved. Several studies have clearly shown that despite a popular misconception, bedrails do not reduce the rate of falls and can, in fact, increase the severity of falls.

Causes and Locations of Patient Falls

Previous studies have examined the locations of fall incidents retrospectively or discussed environmental-modification programs, such as improving lighting, securing carpeting, and so on. However, a meta-analysis and systematic review of randomized controlled trials of fall-prevention interventions found that there was no clear evidence for the independent effectiveness of environmental-modification programs (Chang et al., 2004). Nonetheless, several studies have indicated that most patient falls occur in the bedroom, followed by the bathroom, and that comprehensive fall-prevention programs can have a positive effect. Brandis (1999) reported transfers to and from bed as the cause of 42.2% of inpatient falls. In another study, a group of researchers analyzed 1-year fall data (267 falls), and reported that 38% of the falls occurred during transfers to and from bed and 16.1% during toileting (Tan et al., 2005). Brandis (1999) reported design shortcomings in the bathroom and bedroom areas, including slippery floors, inappropriate door openings, poor placement of rails and accessories, and inappropriate heights of toilet and furniture. After the fall-prevention program (which included identification of high-risk patients, management strategies, environmental and equipment modification, and standardization) was implemented, there was an overall decrease in falls of 17.3%. Thus, fall-prevention strategies that include environmental modification have worked in the past. But it is not clear how much the effectiveness of such strategies can be attributed to environmental factors alone.

Unit Configuration

An innovative and promising environmental strategy for reducing falls has its origins in evidence that suggests that many falls occur when patients attempt to

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get out of bed unassisted or unobserved (Uden, 1985; Vassallo, Azeem, Pirwani, Sharma, & Allen, 2000). To facilitate the observation of patients and the provision of timely assistance, Methodist Hospital in Indianapolis, Indiana, renovated a coronary critical care unit from centralized nurse stations with two-bed rooms to decentralized nurse stations with large single-bed rooms. These changes resulted in families being present more often and therefore being available to help patients or call for aid when needed, in addition to other positive impacts (Hendrich et al., 2002, 2004). A comparison of data from 2 years prior and 3 years after the renovation showed that falls were cut by two-thirds—from six falls per thousand patients to two per thousand. Given that falls are a critical safety problem, additional research is needed to understand more completely the effectiveness of this approach and its implications for designing safe patient-care units that reduce patient falls.

Bedrails

Although there is a common conception that bedrails prevent falls and reduce injuries, there is considerable evidence demonstrating that bedrails are *ineffective* for reducing falls and may actually increase the severity of injuries caused by falls from bed (Capezuti, Maislin, Strumpf, & Evans, 2002; Hanger, Ball, & Wood, 1999; Leeuwen, Bennett, West, Wiles, & Grasso, 2001; Talerico & Capezuti, 2001; Tan et al., 2005). Examining the one-year incident reports from a 730-bed university teaching hospital, a group of researchers found that 55% of the restrained falls resulted in injury; 11.8% of bed-area falls and 27.8% of all bedside injuries were associated with bedrails (Tan et al., 2005). They also found that the restrained falls resulted in more severe injuries. In his commentary on the outcomes of bedrail use, O'Keefe (2004) cited the high rates of deaths within the bedrail-related incident reports of the U.S. Food and Drug Administration (228 deaths from 1985 to 1999), the Medical Devices Bureau of Canada (25 deaths from 1980 to 2000), and the Medical Devices Agency in the United Kingdom (15 deaths from 1995 to 2000), to further underline that bedrail use can lead to deaths.

Other studies on inpatient falls have focused on the factors associated with patients' physical, mental, medical, or cognitive conditions. Most of these studies examined the development and/or application of protocols for identifying risk factors for patient falls and corresponding interventions for their prevention, including organizational-, educational-, and practice-related interventions (Chang et al., 2004; Hendrich, 2003; Lyons, 2005; McCarter-Bayer, Bayer, & Hall, 2005; Stenvall et al., 2006; Suzuki et al., 2005; Walker, 2004).

Result II: Improving Other Patient Outcomes Through Environmental Measures

Reducing Pain

Summary of Evidence and Recommendations

Pain is a pervasive and serious problem in hospitals. However, it is encouraging that mounting scientific evidence, including that from prospective randomized controlled studies, has shown that exposing patients to nature can produce substantial and clinically important alleviation of pain (Malenbaum, Keefe, Williams, Ulrich, & Somers, 2008; Ulrich, Zimring, Quan, & Joseph, 2006; Ulrich, 2008). Limited research also suggests that patients experience less pain when exposed to higher levels of daylight in contrast to lower levels of daylight in their hospital rooms. The state of knowledge on the environment-pain relationship has grown to the point where a leading international pain research journal recently published an article that emphasizes the importance of designing healthcare facilities to harness *nature*, *light*, and other environmental factors to enhance pain control (Malenbaum et al., 2008).

Regarding design measures to reduce pain, the research implies that patient rooms should be designed with large windows so that bedridden persons suffering from pain can look out onto sunny nature spaces. Also, attention should be given to affording nature window views in procedure spaces, treatment rooms, and waiting areas where pain is a problem (Ulrich, 2008). Research also supports displaying visual art (paintings, prints, and photographs) with representational nature subject matter in healthcare settings where pain is experienced. Well-controlled randomized studies support providing technology (such as television screens and eyeglass displays) to simulate nature in spaces where patients undergo painful procedures and it is not fea-

sible to provide distraction with actual nature. Nature simulations with both visual and auditory distraction may be more diverting and engrossing and hence more effective for relieving severe pain. Furthermore, pain theories and research findings imply that patients should not be placed in rooms or treatment spaces that lack nature distraction and contain environmental stressors such as noise, because pain may thereby be exacerbated (Malenbaum et al., 2008). Finally, the evidence implies that careful attention should be given to building orientation and site planning in healthcare projects, and that plans where some buildings block pain-relieving nature views and daylight from others should be avoided.

Effects of Nature Distraction on Pain

Viewing nature may decrease pain by eliciting positive emotions, reducing stress, and distracting patients from focusing on their pain (Malenbaum et al., 2008; Ulrich et al., 2006; Ulrich, 2008). According to distraction theory, pain requires considerable conscious attention. However, if patients become diverted by or engrossed in a pleasant distraction such as a nature view, they have less attention to direct to their pain, and the experienced pain therefore will diminish. The theory predicts that the more engrossing an environmental distraction, the greater the pain reduction (McCaul & Malott, 1984). This implies that nature distractions may be more diverting and hence effective in reducing pain if they involve sound as well as visual stimulation, and induce a heightened sense of immersion (Ulrich, 2008).

A study of matched patients recovering from abdominal surgery found that those assigned to rooms with a bedside view of nature (trees) had better postoperative recovery than matched patients assigned to identical rooms with windows overlooking the wall of a brick building (Ulrich, 1984). Patients with the nature view suffered substantially less pain, as indicated by their need for far fewer doses of strong pain medication than their counterparts with the wall view. In addition, the patients exposed to nature had shorter post-surgery stays, better emotional well-being, and fewer minor complications such as persistent nausea or headache (Ulrich, 1984). Another study prospectively and randomly assigned bedridden heart-surgery patients to view color pictures mounted in their line of vision (Ulrich, Lundén, & Eltinge, 1993). Patients assigned a picture of a spatially open, well-lighted view of trees and water needed fewer doses of strong pain drugs than patients exposed to abstract images or a control condition of no picture (Ulrich et al., 1993). A well-controlled prospective study by Tse, Ng, Chung, and Wong (2002) found that healthy volunteers in a hospital setting had a higher pain threshold and greater tolerance when they looked at a videotape of nature scenery.

As noted, theory predicts that nature exposures may be more engrossing and hence pain relieving when they involve sound as well as visual distraction. Lee and colleagues (2004) conducted a randomized prospective clinical trial on the effects of nature distraction on patients undergoing colonoscopy, and they found that visual distraction alone reduced pain but did not lower the intake of sedative medications. However, a combination of nature scenery with classical music reduced both pain and self-administered sedation during colonoscopy (Lee et al., 2004). Research on burn patients suffering from intense pain found that distracting individuals during burn dressings with a nature videotape accompanied by music lessened both pain and anxiety and stress (Miller, Hickman, & Lemasters, 1992). A randomized clinical trial of patients undergoing painful bronchoscopy found that individuals assigned to look at a ceiling-mounted nature scene and listen to nature sounds (moving water, birds) reported less pain than a control group who looked at a blank ceiling during bronchoscopy (Diette, Lechtzin, Haponik, Devrotes, & Rubin, 2003). Kozarek and colleagues (1997) investigated the effects of seeing and listening to a nature travelogue on patients undergoing painful gastric procedures. Patient reports and nurse observations converged in suggesting that the combination of visual and auditory distraction improved comfort and tolerance for the procedures, as compared to a control condition without distraction (Kozarek et al., 1997). Other research suggests that a virtual reality audiovisual nature distraction (a walk through a forest with bird sounds) reduced discomfort and symptomatic distress in female chemotherapy patients (Schneider, Prince-Paul, Allen, Silverman, & Talaba, 2004).

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Effects of Daylight Exposure on Pain

The presumed pain reduction mechanism for daylight is different than for nature. Sunlight exposure increases levels of serotonin, a neurotransmitter known to inhibit pain pathways. Walch and colleagues (2005) conducted a well-controlled prospective study of the effects of daylight on pain in patients undergoing spinal surgeries, who were admitted postoperatively to rooms either on the bright or shaded side of a surgical ward. Patients in the bright rooms were exposed to 46% greater sunlight intensity than those assigned to the more shaded rooms. Findings indicated that patients in rooms with more sunlight reported less pain and stress, and took 22% less analgesic medications, resulting in a 21% reduction in medication costs. It should be mentioned that the shaded patient rooms—and associated heightened pain—resulted when a new building was constructed and blocked sunlight from reaching this side of the facility.

Improving Patients' Sleep

Summary of Evidence and Recommendations

Hospitalized patients have an increased need for sleep because of their illnesses. However, in reality, they often suffer from diminished circadian rhythms and poor sleep while hospitalized (Southwell & Wistow, 1995), which may lead to increased stress (Novaes, Aronovich, Ferraz, & Knobel, 1997; Topf & Thompson, 2001), impaired immune function, ventilatory compromise, disrupted thermoregulation, and delirium (Wallace, Robins, Alvord, & Walker, 1999). These effects may hinder the healing process and contribute to increased morbidity and mortality (Krachman, Dalonzo, & Criner, 1995; Parthasarathy & Tobin, 2004).

The research team identified more than 70 articles about sleep in healthcare settings, including descriptive, correlational, and intervention studies. The literature confirmed that sleep disruption and deprivation were very common problems in healthcare settings, especially for high-acuity patients who are more susceptible to unfavorable environmental conditions. Environmental factors such as noise and light may result in electroencephalographic arousals and awakenings, and thereby fragment sleep and prevent patients from progressing into deeper and more restorative sleep stages (BaHammam, 2006). Increased acoustic performance with reduced reverberation time and noise level increased sleep quality (Aaron et al., 1996; Topf, Bookman, & Arand, 1996).

Certain environmental approaches have shown promising results in improving patient sleep. First, *single-bed rooms* can reduce noise disturbance from roommates, visitors, and healthcare staff (Southwell & Wistow, 1995; Yinnon, Ilan, Tadmor, Altarescu, & Hershko, 1992), and thereby improve patient sleep (Gabor et al., 2003). Second, experimental studies support the installation of *high-performance sound-absorbing materials* to reduce reverberation time, sound propagation, and noise intensity levels, as well as to improve sleep (Berg, 2001; Hagerman et al., 2005; Philbin & Gray, 2002). Other noise reduction strategies such as adopting *a noiseless paging system* could also be considered. Furthermore, findings suggest that patient rooms should be carefully oriented and designed to receive *natural daylight* and maintain the normal light-dark cycle of 24-hour periods to help patients retain normal circadian rhythms and improve sleep (BaHammam, 2006; Wakamura & Tokura, 2001).

Quantity and Quality of Sleep in Healthcare Settings

Measurement of sleep. Sleep should be measured in terms of both *quantity* (such as total sleep time) and *quality* (such as the type and depth of sleep, the distribution over 24 hours, and other sleep architecture parameters) (Parthasarathy & Tobin, 2004). Even if total sleep time appears adequate, sleep quality may nonetheless be poor because of fragmentation and poor sleep architecture. Previous sleep studies have employed either subjective measures such as patients' self-reports (Sheely, 1996; Topf & Thompson, 2001; Tranmer, Minard, Fox, & Rebelo, 2003) or objective measures including direct observation (Kroon & West, 2000), polysomnography (PSG) (Freedman, Gazendam, Levan, Pack, & Schwab, 2001; Gabor et al., 2003; Singh, Mahowald, & Mahowald, 2004; Wallace et al., 1999), and bispectral index (Nieuwenhuijs, Coleman, Douglas, Drummond, & Dahan, 2002) or Actigraph (Kroon & West, 2000). Most studies have monitored sleep only at night, while some have focused on the 24-hour period and revealed that about half of the total sleep in acute care settings occurred during

the daytime (BaHammam, 2006). We found it difficult to compare findings across studies because of the different measures used.

Sleep deprivation in different healthcare settings. Patient sleep has been studied more often in *high-acuity units* than other settings (BaHammam, 2006; Parthasarathy & Tobin, 2004; Redeker, 2000). Overall, high-acuity patients show sleep fragmentation, increases in stage 1 and stage 2 sleep, and decreases in more restorative stages—slow-wave and rapid eye movement sleep—as well as reductions in sleep efficiency (BaHammam, 2006; Parthasarathy & Tobin, 2004). In a survey study of 84 neurosurgery ICU patients, 79% of patients reported sleep disturbances (Ugras & Oztekin, 2007). In another survey of randomly selected ICU patients, sleep difficulty was identified as the second most important physical stressor, following pain (Novaes et al., 1997). Parthasarathy and Tobin (2004) reported that the number of sleep arousals and awakenings ranged from 20–68 per hour and varied across different acute care settings. Findings concerning total sleep time differ considerably across studies, ranging from normal or near normal sleep time at 7–10.4 hours a day (Freedman et al., 2001; Gottschlich et al., 1994), to decreased time at 3.6–6.2 hours per day (Aurell & Elmqvist, 1985; Gabor et al., 2003; Hilton, 1976). In a study of mechanically ventilated ICU patients using continuous PSG measurements, the mean total sleep time per 24-hour period was 8.8 ± 5.0 hours, the sleep-awake cycles were fragmented, and a mean of 57 ± 18% of total sleep time occurred during the day (Freedman et al., 2001).

General wards have been studied less frequently than high-acuity settings, and most studies have employed less reliable subjective measures, such as self-report surveys (Dogan, Ertekin, & Dogan, 2005; Kuivalainen, Ryhänen, Isola, & Meriläinen, 1998; Shafiq et al., 2006). Results strongly suggest that sleep deprivation is also a widespread problem among general ward patients. In a Finnish study, 65 percent of the medical and surgical patients reported sleeping badly in the hospital (Kuivalainen et al., 1998). Another study of medical and surgical patients in Canada found moderate to high disturbance scores for awakenings and soundness of sleep (Tranmer et al., 2003).

Sleep deprivation among different patient populations. Although most sleep studies have focused on adult populations, some have examined special populations, such as older people (Béphage, 2005; Ersser et al., 1999; Vinzio, Ruellan, Perrin, Schlienger, & Goichot, 2003; Wakamura & Tokura, 2001), children and infants (Al-Samsam & Cullen, 2005; Corser, 1996; Cureton-Lane & Fontaine, 1997; Zahr & de Traversay, 1995), and specific groups, such as cardiac surgery patients (Simpson, Lee, & Cameron, 1996). In a study of PICU patients, children slept for a mean total of only 4.7 hours during the 10-hour night, with an average of 9.8 awakenings, and the mean length of a sleep episode was only 27.6 minutes (Cureton-Lane & Fontaine, 1997). In another study of 11 mechanically ventilated PICU patients, restorative sleep accounted for only 3% of total sleep time due to severe sleep fragmentation, as reflected in the high number of awakenings (Al-Samsam & Cullen, 2005).

Environmental Factors Affecting Sleep

A number of factors contribute to poor sleep in healthcare settings, including environmental factors like noise, light, and staff-patient interactions; physiological factors, such as the underlying disease and impact of medication; and the psychological characteristics of patients (BaHammam, 2006; Dogan et al., 2005; Reid, 2001). Environmental factors have been studied in several settings across different types of patients for their impact on sleep (Corser, 1996; Cureton-Lane & Fontaine, 1997; Freedman, Kotzer, & Schwab, 1999; Kuivalainen et al., 1998). In the Finnish study mentioned earlier, 80% of patients who reported poor sleep regarded environmental factors as the cause (Kuivalainen et al., 1998).

Impacts of noise. Environmental noise is one of the most important yet modifiable environmental factors affecting patients' sleep (Gabor et al., 2003; Meyer et al., 1994; Parthasarathy & Tobin, 2004; Schnelle, Ouslander, Simmons, Alessi, & Gravel, 1993; Topf & Davis, 1993; Topf & Thompson, 2001; Yinnon et al., 1992). In a survey of neurosurgery ICU patients, among those who reported sleep disturbance, 58% considered environmental noise a frequent disturbing factor (Ugras & Oztekin, 2007). Another study in an

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intermediate respiratory care unit showed a strong correlation between the number of high sound peaks (≥ 80 dBA) and arousals from sleep (Aaron et al., 1996). One study of ICUs suggested that about 20% of arousals and awakenings were related to noise, and 10% were related to patient care activities (Parthasarathy & Tobin, 2004). Topf and colleagues (1996) conducted a study with healthy volunteers in an experimental setting that replicated noise in ICUs and compared their sleep quality with that of control subjects who were not exposed to the ICU noise (Topf et al., 1996). Results showed that participants assigned to the ICU noise condition took longer to fall asleep, slept less, and experienced more awakenings and poorer sleep quality.

Impact of lighting conditions. In addition to noise, lighting is an important environmental factor affecting sleep/awake patterns (Béphage, 2005; Higgins, Winkelman, Lipson, Guo, & Rodgers, 2007). One study found that night lighting on wards was dimmed for a sleep duration that is no longer than that required by the average healthy person (Southwell & Wistow, 1995). This is a disturbing finding considering that patients need more sleep when they are ill and are more susceptible to sleep disturbances. Other research suggests that the inappropriate location, orientation, and design of patient rooms might reduce daylight exposure, diminish patients' circadian rhythms, and worsen their sleep at night (BaHammam, 2006; Wakamura & Tokura, 2001).

Environmental Approaches to Improve Sleep

Various interventions have been employed to improve patient sleep. Pharmacological assistance alone cannot achieve the desired quantity and quality of sleep in ICUs (Brown & Scott, 1998), not to mention its detrimental side effects. Environmental interventions have been developed to reduce environmental noise and disruptive staff-patient interactions at night, or to maintain the normal light-dark cycle of a day, and they have shown favorable results. Furthermore, some environmental interventions appeared to be more successful than organizational interventions like staff education or quiet hours (Gast & Baker, 1989; Moore et al., 1998; Walder, Francioli, Meyer, Lancon, & Romand, 2000).

Improving the acoustic environment. Certain environmental interventions have been found effective for reducing noise in hospital settings, including installing high-performance sound-absorbing ceiling tiles, eliminating or reducing noise sources (e.g., adopting a noiseless paging system), and providing single-bed rather than multibed rooms.

Installing high-performance sound-absorbing materials for environmental surfaces such as ceilings and walls can reduce reverberation time, sound propagation, and noise intensity levels (Berg, 2001; Hagerman et al., 2005; Philbin & Gray, 2002). Hagerman et al. (2005) examined the effects of sound-absorbing versus sound-reflecting ceiling materials in a coronary ICU by periodically changing the ceiling tiles. When the sound-absorbing tiles were in place, patient rooms showed a 5–6 dB drop in sound levels and a reduction in reverberation time from 0.8 to 0.4 second, indicating better acoustic conditions. Patients also reported fewer awakenings caused by noise. Further, Berg's research (2001) showed that even if the noise level (dB) remains almost the same, the reduction in reverberation time achieved by sound-absorbing ceiling tiles can improve sleep quality. Meanwhile, even relatively low decibel levels (27–58 dB), when coupled with longer reverberation times (sound-reflecting ceiling), significantly increased arousals in healthy volunteers sleeping in patient rooms. These findings have disturbing implications, because most hospitals have nighttime sound peaks exceeding those of the patient rooms in the study.

Providing single-bed rooms as opposed to multibed rooms can also lower noise levels and improve sleep quality. For multibed rooms in medium- and high-acuity units, most noises stem from the presence of other patients, whether caused by visitors, staff caring for other patients, or patient sounds such as coughing, crying out, and rattling bedrails (Southwell & Wistow, 1995; Yinnon et al., 1992). One study of multibed bays in a children's hospital concluded that noise levels were so high that consideration should be given to abolishing open-bay rooms (Couper et al., 1994). These findings also have important implications for patient sleep, because noises stemming from the presence of other patients can be the major cause of sleep loss in mul-

tibed rooms, (Southwell & Wistow, 1995; Yinnon et al., 1992). In the Finnish study mentioned previously, the presence of other patients was reported as one of the most disturbing factors (Kuivalainen et al., 1998). Gabor and colleagues (2003) compared the effect of open areas and single rooms on noise levels and the sleep of six healthy volunteers in an ICU. The average noise level was higher (51 dB) in the open ICU than in the single room (43 dB), as were the respective peak levels (65 dB versus 54 dB). Furthermore, total sleep time in the single-bed room (9.5 hours) was greater than that in the open ICU (8.2 hours), although the number of arousals was similar in both settings.

Avoiding light pollution. One study examined the impact of simulated bright daylight in a north-facing room with limited natural light, affixing to the bed a lamp that was turned on at 10:00 a.m. and off at 5:00 p.m. (Wakamura & Tokura, 2001). Findings suggested that hospitalized elderly patients experienced better deep sleep at night when they were exposed to the artificial diurnal daylight compared to when they had darker daytime conditions. However, not all light-related interventions are successful. Another intervention study implemented guidelines to control nighttime light levels, and this resulted in significantly lower mean light disturbance intensity and shorter periods with high light levels (Walder et al., 2000). However, these changes were accompanied by greater variation in light levels, which could disturb patients' sleep patterns. More research is needed to better understand how both daytime and nighttime light environments can be optimized to improve sleep. Design details such as flexible light controls with various lighting intensities might be considered.

Directions for Future Research

Despite emerging evidence, gaps in our knowledge still remain. To better understand the independent effect of environmental interventions, future research should control effectively for other variables that influence sleep, such as acuity of illness, sedation level, pain, and disruptive patient-care procedures. Longitudinal designs with larger numbers of patients should be developed and employ standard sleep measures over 24-hour periods rather than at night only.

Reducing Patient Stress

Summary of Evidence and Recommendations

Stress experienced by patients is an important negative outcome, which directly and adversely affects many other healthcare outcomes. If hospital physical environments contain stressful features or characteristics such as noise, patient stress and other outcomes will often be worsened. By contrast, hospital design that minimizes environmental stressors and fosters exposure to stress-reducing or restorative features should advance improved outcomes (Ulrich 1991; Ulrich et al., 2006).

Our literature review identified certain environmental features that can *reduce stress* and improve outcomes. Several well-controlled experimental studies have generated strong evidence that real or simulated views of *nature* can produce substantial restoration from psychological and physiological stress within a few minutes. Other studies using self-report methods and behavioral observation suggest that *gardens* in hospitals can reduce stress among patients and families by providing nature distraction and fostering social support. Based on these findings, it is recommended that hospital siting and design should provide restorative window views of nature and gardens from patient rooms and other interior areas where stress is a problem. Additionally, limited research on hospital *art* suggests that the great majority of patients prefers and responds positively to representational nature art, but that abstract or ambiguous art can elicit stressful reactions in many patients.

Considerable research has shown that noise is a pervasive stressor that elevates psychological and physiological stress in patients, and worsens other outcomes. Research also indicates that hospital noise levels around the world have been rising steadily since the 1960s. Therefore, a high priority should be placed on creating much quieter environments when constructing or renovating hospitals. As mentioned in another section (Improving Patients' Sleep), research has identified effective environmental approaches for quieting healthcare settings, the most important of which appears to be providing single-bed rooms. Other noise-reducing measures supported by research include insulating or eliminating noise sources (e.g., replacing overhead pag-

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ing with a noiseless system) and installing high performance sound-absorbing materials on ceiling and wall surfaces.

Stress as a Major Problem in Healthcare Facilities

Much research has confirmed that hospitalized patients experience stress, and that a large proportion suffers from acute stress. Many stressors are unavoidable accompaniments of illness and medical treatments, but others result from shortcomings in the culture of healthcare organizations. Additional stress is produced by poorly designed physical environments. In addition to afflicting patients, stress is a major burden for their families (Ulrich, 1991; Ulrich et al., 2006).

The stress experienced by a patient is an important negative outcome in itself, and it directly and adversely affects many other outcomes. These unhealthy effects are related to detrimental psychological, physiological, neuroendocrine, and behavioral changes associated with stress responses (Gatchel, Baum & Krantz, 1989; Ulrich, 1991). The neuroendocrine component, for example, elevates levels of a natural steroid, cortisol, and releases stress hormones that tax the heart and other major organs. Importantly, much research has shown that stress responses suppress immune system functioning through their effects on neuroendocrine activity and the central nervous system (Kiecolt-Glaser, et al., 1987). Stress-related immune impairment decreases resistance to infection and worsens recovery outcomes such as wound healing (Cohen, Tyrrell, & Smith, 1991; Kiecolt-Glaser et al., 1995).

Reduce Stress by Controlling Noise

Noise levels and sources in hospitals. The World Health Organization (WHO) provides guideline values for continuous background noise in hospital patient rooms, which are 35 dBA during the day and 30 dBA at night, with nighttime peaks in wards not to exceed 40 dBA (Berglund, Lindvall, & Schwela, 1999). However, much research has shown that actual background and peak noise levels fall in far higher ranges, and a review of 35 studies concluded that hospital noise levels around the world have been rising consistently since the 1960s (Busch-Vishniac et al., 2005). Background noise levels typically are 45 dB to 68 dB, with peaks frequently exceeding 85 dB to 90 dB (Aaron et al., 1996; Allaouchiche, Duflo, Debon, Bergeret, & Chassard, 2002; Balough, Kittinger, Benzer, & Hackl, 1993; Blomkvist, Eriksen, Theorell, Ulrich, & Rasmanis, 2005; Cureton-Lane & Fontaine, 1997; Falk & Woods, 1973; Guimaraes et al., 1996; Hilton, 1976; Homberg & Coon, 1999; Kent, Tan, Clarke, & Bardell, 2002; McLaughlin, McLaughlin, Elliott, & Campalani, 1996; Robertson, Cooper-Peel, & Vos, 1998). In evaluating these noise levels, it should be noted that the decibel scale for quantifying loudness or sound pressure intensity is logarithmic; each 10 dBA increase therefore represents a sound pressure level that is 10 *times* higher.

Medical equipment and staff voices often produce noise at 70–75 dB levels at the patient's head, which approach the noise level in a busy restaurant (Blomkvist et al., 2005). Noises from alarms and certain equipment (e.g., a portable X-ray machine) exceed 90 dB, which is comparable to walking next to a busy highway when a motorcycle or large truck passes. A study in a NICU measured peak levels once per minute and found that 31% of peaks exceed 90 dB (Robertson et al., 1998). One study even recorded 113 dB during shift changes at a large hospital (Cmiel, Karr, Gasser, Oliphant, & Neveau, 2004). OR noises from drills, saws, and other equipment are in the range of 100–110 dB, presenting a significant risk for noise-induced hearing loss (Hodge & Thompson, 1990; Love, 2003; Nott & West, 2003).

Our review of the research identified at least three major reasons why hospitals are excessively noisy and therefore stressful (Ulrich, 2003). First, as mentioned previously, the sources of noise are unnecessarily numerous and loud. Well-documented examples include staff voices, paging systems, alarms, bedrails moved up or down, telephones, ice machines, pneumatic tubes, and trolleys. Second, many environmental surfaces (e.g., floors, ceilings, walls) are hard and sound-reflecting, not sound-absorbing; this creates poor acoustic conditions (long reverberation times) that enable noise to echo, linger, and propagate over large areas and into patient rooms (Blomkvist et al., 2005; Ulrich, 2003). Finally, hospitals are noisy because many patients are

housed in multibed rooms in which much noise originates from other patients (Baker, 1984; Southwell & Wistow, 1995; Yinnon et al., 1992).

Effects of noise on patient stress and other outcomes. Another section in this report, "Improving Patients' Sleep," surveys research showing that noise is a major cause of awakenings and poor sleep. In addition to worsening sleep quality, noise elevates psychological and physiological stress in patients, as indicated by negative feelings such as anxiety and annoyance (Bentley, Murphy & Dudley, 1977; Haslam, 1970; Hilton, 1976; Synder-Halpern, 1985) and detrimental physiological changes such as elevated heart rate and blood pressure (Baker, 1992; Morrison, Haas, Shaffner, Garrett, & Fackler, 2003). A prospective study by Hagerman et al. (2005) examined the effects of sound-reflecting versus sound-absorbing tiles on coronary intensive care patients. When the sound-absorbing ceiling tiles were in place, patients evidenced lower physiological stress (lower sympathetic arousal), slept better, reported better care from nurses, and had a lower incidence of rehospitalization in the weeks following discharge. Other studies have focused on infants in NICUs, finding that higher noise levels elevate blood pressure, heart, and respiration rates, and decrease oxygen saturation, thereby increasing the need for oxygen support therapy (Slevin, Farrington, Duffy, Daly, & Murphy, 2000; Zahr & de Traversay, 1995).

Environmental approaches to reduce noise and stress. The foregoing discussion makes it clear that hospitals are far too noisy, and that noise in combination with acoustically poor environmental surfaces and multibed patient rooms worsens stress and other outcomes. As discussed in detail in an earlier section (Improving Patients' Sleep), there are effective environmental approaches available to quiet healthcare settings, which can be more successful than organizational interventions such as staff education or establishing quiet hours (Gast & Baker, 1989; Moore et al., 1998; Walder et al., 2000).

The most important design measure to reduce noise for inpatients appears to be single-bed rooms. In this regard, the research literature indicates that noise levels are lower in single- than multibed rooms (Gabor et al., 2003; Southwell & Wistow 1995; Yinnon et al., 1992). The major advantage of single-bed rooms is reflected in Press Ganey's national satisfaction survey, which obtained data from 2.1 million patients in 1,462 facilities during 2003. Results showed that satisfaction with noise levels was on average 11.2% higher for patients in single-bed rooms than for those in multibed rooms; this pattern held across all patient categories and for different ages, genders, and facility sizes and types (Press Ganey, 2003). This is an extremely large difference, considering that it can be difficult for hospitals to increase satisfaction scores by even two or three percentage points.

Apart from providing single rooms, another approach for quieting facilities and reducing stress is to *eliminate noise sources*, for example, by replacing overhead paging with a noiseless system and insulating pneumatic tubes and ice machines. Also, there is convincing evidence that *installing high performance sound-absorbing materials* on surfaces such as ceilings, floors, and walls can be effective in reducing noise levels, reverberation or echoing, and sound propagation (Berg, 2001; Blomkvist et al., 2005; Philbin & Gray, 2002).

Provide Nature Distraction to Reduce Stress

Biophilia theory. Wilson's biophilia hypothesis (1984) holds that humans have a partially genetic tendency to respond positively to nature. Ulrich et al. (1993) and Ulrich (2008) have developed theoretical arguments as to why a capability for rapid recovery from stress following challenging episodes was vital for the survival of early humans, and why evolution favored the selection of individuals with this partially genetic proneness for a restorative response to nature. This restoration theory implies that modern humans, as a genetic carryover of evolution, have a capacity to derive stress-reducing responses from certain nature settings and content (e.g., vegetation or water), but have no such disposition toward most built or artifact-dominated environments and materials (e.g., concrete, glass, and metal) (Ulrich, 1993, 1999, 2008). "These theoretical arguments have a practical design implication, which is that designing healthcare buildings with nature features may harness therapeutic influences that are carryovers from evolu-

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tion, resulting in more restorative and healing patient care settings" (Ulrich, 2008, forthcoming).

Research on restorative effects of nature. Upwards of a score of scientific studies of people in nonhealthcare situations as well as patients in hospitals have generated strong evidence that real or simulated views of nature can produce substantial restoration from stress. The strength of these findings is enhanced by the fact that some studies have used randomized controlled research designs and obtained physiological as well as self-report measurements of stress. Investigators have reported consistently that stress-reducing or restorative benefits of viewing nature are manifested as a constellation of positive emotional, psychological, and physiological changes. Positive feelings such as pleasantness and calm increase, while anxiety, anger, or other negative emotions diminish (Hartig, Book, Garvill, Olsson & Gärling, 1995; Ulrich, 1979; Ulrich, 1991; Van den Berg, Koole, & Van der Wulp, 2003). Also, many nature scenes sustain positive interest and thus function as pleasant distractions that may block worrisome, stressful thoughts (Ulrich, 1981.) Regarding the physiological effects of nature exposure, restoration is apparent when changes in bodily systems indicate decreased stress mobilization (for instance, reduced sympathetic nervous system activity). Physiological restoration is manifested within 3 minutes at most, or as fast as a few seconds in certain systems (Fredrickson & Levenson, 1998; Hartig, Evans, Jamner, Davis & Gärling, 2003; Joye, 2007; Laumann, Gärling, & Stormark, 2003; Parsons & Hartig, 2000; Parsons, Tassinary, Ulrich, Hebl, & Grossman-Alexander, 1998; Ulrich, 1981; Ulrich, Simons & Miles, 2003). In contrast to viewing nature-dominated settings, there is convincing evidence that looking at built environments that lack nature (e.g., parking lots, roof tops, and rooms) is significantly less effective in fostering restoration and may worsen stress (e.g., Ulrich, 1979, 1991; Van den Berg et al., 2003).

Nature and patient stress. In an interview study of the elderly in long-term care facilities, residents reported a preference for windows with prominent views of nature, but expressed dislike for window views of built content that lacked nature (Kearney & Winterbottom, 2005). Survey research on hospital patients also suggests that they prefer and attribute importance to having a bedside window view of nature (Verderber, 1986).

Furthermore, studies on patients indicate that viewing nature images for only a few minutes can promote significant restoration from stress or anxiety. One well-controlled clinical trial measured restoration from anxiety in patients waiting to undergo dental surgery in a room with or without an aquarium on different days (Katcher, Segal, & Beck, 1984). Findings suggested that anxiety was lower on days when the aquarium was present, and clinicians' ratings for patient compliance during surgery were higher. Heerwagen (1990) studied patients in a dental clinic and found that psychological and physiological markers of stress—including elevated blood pressure and heart rate—were diminished on days when a large nature mural was hung on a wall of the waiting room, in contrast with days when the wall was blank. One strong randomized prospective study of blood donors in a waiting room found that blood pressure and pulse were lower on days when a wall-mounted television displayed a nature videotape, compared to days when continuous daytime television programs or a videotape of urban areas and buildings were aired (Ulrich et al., 2003). A quasi-experimental study of patients with dementia, including Alzheimer's disease, suggested that adding large, color nature images and a nature sound track (birds, brook) to a shower room diminished stress and reduced incidents of aggressive, agitated behavior (Whall et al., 1997).

As mentioned in another section (Reducing Patients' Pain), strong studies have found that exposing patients to nature lessens stress, anxiety, and pain. A prospective clinical trial by Ulrich and colleagues (1993) found that heart-surgery patients in ICUs who were randomly assigned a picture with a landscape scene with trees and water reported less anxiety and stress and needed fewer doses of strong pain drugs than a control group that had been assigned no pictures. In the same study, another group of patients assigned an abstract picture had worse outcomes than the control group. Ulrich (1984) reported that patients recovering from abdominal surgery suffered fewer minor postsurgical complications linked to stress (e.g., headache), had better emotional well-being, required fewer doses of pain drugs, and had

shorter hospital stays if they had a bedside window view of nature (trees) rather than a brick wall. Research on burn patients suggested that exposure to a nature videotape during burn dressing changes reduced anxiety, stress, and pain intensity (Miller et al., 1992).

Gardens for reducing stress. A few studies suggest that gardens can be effective restorative settings for stressed patients, families, and staff (Marcus & Barnes, 1999; Sherman, Varni, Ulrich, & Malcarne, 2005; Ulrich, 1999; Whitehouse et al., 2001). Well-designed gardens not only can provide restorative nature views, but they also reduce stress and improve outcomes through other mechanisms, such as fostering access to social support, restorative escape, and control with respect to stressful clinical environments (Ulrich, 1999, 2008). Marcus and Barnes (1995) used behavioral observation and interview methods in postoccupancy studies of four hospital gardens and concluded that recovery from stress was the most important benefit realized by nearly all garden users. Other postoccupancy research likewise has found that patients and families who use hospital gardens report reduced stress and improved emotional well-being (Whitehouse et al., 2001). A quasi-experimental investigation of three gardens in a pediatric cancer center showed that participants (patients, families, staff) reported lower stress levels when in the gardens than inside the hospital (Sherman et al., 2005).

Limited evidence suggests that gardens tend to alleviate stress effectively for adult users when they contain green or verdant foliage, flowers, water, grassy spaces with trees or large shrubs, a modicum of spatial openness, and compatible pleasant nature sounds, such as birds and water (Marcus & Barnes, 1995, 1999; Ulrich, 1999, 2008). Broadly similar findings have emerged from research on gardens and outdoor spaces in assisted living facilities for the elderly. Rodiek (2005) surveyed elderly residents and observed their behavior in 14 assisted living facilities and reported that residents preferred outdoor spaces with greenery, flowers, birds, and water features.

Art in healthcare environments. Rigorous studies on hospital art are sparse, and most have measured patient art preferences rather than effects on outcomes such as pain. The limited findings nonetheless show similarities to results from nature studies. Results suggest a consistent pattern wherein the great majority of patients prefer and respond positively to representational nature art, but many react negatively to abstract art (Carpman & Grant, 1993; Ulrich, 1991; Ulrich & Gilpin, 2003). Nanda, Hathorn, and Neumann (2007) displayed a diverse collection of 17 paintings to patients in their hospital rooms, and asked them to rate each painting for the following questions: (1) How does the picture make you feel, and (2) Would you like to hang this picture in your hospital room? Findings indicated that patients were significantly more positive about nature paintings (landscapes with verdant foliage, flowers, and water) than they were about best-selling pictures or even works by masters such as Chagall and Van Gogh (Nanda et al., 2007). The most positively rated painting depicted a gentle waterfall with vegetation. In the same research, representational nature paintings containing human figures and harmless animals such as deer were preferred over counterparts that were somewhat abstract. Eisen (2006) studied the art preferences of schoolchildren and hospitalized pediatric patients across four age groups: 5–7, 8–10, 11–13, and 14–17 years of age. Findings suggested that, irrespective of age or gender, the great majority of hospitalized pediatric patients and schoolchildren were similar in preferring nature art (such as a forest setting with lake and deer) over abstract or cartoon-like images.

Although nature pictures elicit positive reactions, there is limited evidence that emotionally inappropriate art subject matter or styles can increase stress and worsen other outcomes (Ulrich, 1991, 1999; Ulrich & Gilpin, 2003). It may be unreasonable to expect all art to be suitable for high-stress healthcare spaces, because art varies enormously in subject matter and style, and much art is emotionally challenging or provocative. The pitfalls of displaying emotionally challenging art are revealed by a study of psychiatric patients housed in a unit extensively furnished with a diverse collection of wall-mounted paintings and prints (Ulrich, 1991). Interviews with patients suggested strongly negative reactions to artworks that were ambiguous, surreal, or could be interpreted in multiple ways. The same patients, however, reported having positive feelings and

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associations with respect to nature artwork. Additional evidence on the stressful impact of abstract art comes from a study of a sculpture installation created for cancer patients in a large university hospital (Ulrich, 1999). Prominent in the installation were several tall metal sculptures dominated by straight-edged and abstract forms, many having pointed or piercing features. A questionnaire study found that 22% of the patients reported having an overall negative emotional response to the sculpture garden (Hefferman, Morstatt, Saltzman & Strunc, 1995). Many found the sculpture ambiguous ("doesn't make any sense"), and some patients interpreted the sculptures as frightening and asked for a room change so they would not overlook the artworks (Ulrich, 1999).

Reducing Depression

Summary of Evidence and Recommendations

Depression is a serious, widespread, and costly problem in healthcare facilities. A large body of rigorous evidence indicates that exposure to bright artificial light and daylight is effective in reducing depression and improving mood, even for people hospitalized with severe depression. Artificial light is commonly used in structured or formal protocols for treating depression. A few retrospective studies suggest that hospitalized adult patients with depression can have more favorable recovery outcomes, including shorter stays, if they are assigned to sunnier rooms rather than rooms that receive less daylight or are always in shade. (See also Reducing Patients' Pain.) The credibility of the limited findings linking greater daylight or sun exposure to improved depression-related outcomes is enhanced by the fact that the many strong studies using bright artificial light have obtained broadly parallel results.

The evidence that patients' depression is diminished by daylight exposure implies the importance of the orientation and site planning of healthcare buildings (Ulrich et al., 2006). Site plans where some buildings block daylight or sun from others should be avoided. Hospitals—and in particular mental health facilities—should be designed and sited to ensure that depressed patients have abundant natural light. Providing larger windows in patient rooms and other spaces might also help alleviate depression by permitting more exposure to daylight. The use of bright artificial light warrants consideration in settings where depression is a problem and sufficient daylight is not available.

Effects of Light on Depression

The mechanisms by which *light treatment* alleviates depression are not fully understood. Light falling on the retina influences the activity of the pineal gland and by this pathway suppresses or delays secretion of melatonin, thereby reducing depression, increasing daytime alertness, and fostering better sleep quality (Martiny, 2004). A meta-analysis of 20 randomized controlled studies published in the *American Journal of Psychiatry* reached the powerful conclusion that light treatment for nonseasonal and seasonal depression is "efficacious, with effect sizes equivalent to those in most antidepressant pharmacotherapy trials" (Golden et al., 2005, p. 656). Additionally, light exposure offers the important advantage of being faster acting than antidepressant drugs. In this regard, several studies suggest that light can produce significant reduction of depression after less than 2 weeks of treatment, while antidepressant drugs require at least 4–6 weeks before effective onset. Some studies suggest that exposure to morning light may be more effective than afternoon or evening light (e.g., Lewy et al., 1998). However, exposure occurring in the middle of the day or afternoon also significantly reduces depression (Martiny, 2004).

Other research focused on *daylight* rather than artificial light. A retrospective study in a Canadian facility found that adult patients hospitalized for severe depression had shorter stays by an average of 2.6 days if they were assigned to sunny rooms rather than rooms that were always in shade (Beauchemin & Hays, 1996). Similarly, a study in an Italian hospital found that patients hospitalized for bipolar depression stayed an average of 3.7 fewer days if they were assigned east-facing rooms exposed to bright morning light, compared to patients in west-facing rooms with less sunlight (Benedetti, Colombo, Barbini, Campori, & Smeraldi, 2001). Depression is a serious problem not only for mental health patients, but also for several other categories of patients, such as those with cardiovascular disease and cancer. An investigation of myocardial infarction patients in an ICU in a Canadian

hospital suggested that female patients had shorter stays if their rooms had sunny versus shaded or dim window exposures (Beauchemin & Hays, 1998). In the same study, mortality in both sexes was lower in sunny rooms than in north-facing shaded rooms.

Reducing Length of Stay

Summary of Evidence and Recommendations

There is limited literature that directly links the physical environments of hospitals with patients' length of stay. However, the few studies conducted on light and nature views among specific types of patients have been strong, and they have consistently identified a positive impact from both. Additional studies are needed to confirm these findings and to test them among a broader range of patient types.

Sunlight

As mentioned in the section on Reducing Depression, exposure to sunlight has been reported to affect the length of patients' hospital stays. One research group studied the impact of the amount of natural light on the length of hospitalization of patients with unipolar and bipolar disorder. The researchers found that bipolar patients randomly assigned to the brighter, east-facing rooms (exposed to direct sunlight in the morning) had a 3.67-day shorter mean hospital stay than patients in west-facing rooms (Benedetti et al., 2001). Beauchemin and Hays (1996) analyzed the two-year data of psychiatric unit patients with depression and found that patients in the sunny rooms stayed an average of 2.6 fewer days than those in the sunless rooms. In another study, the researchers examined the length of stay and mortality rate of 628 myocardial infarction patients who had been randomly assigned to sunny and dull rooms. Patients in sunny rooms had shorter lengths of stay than patients in dull rooms, with a more significant difference for women patients (2.3 days in sunny rooms versus 3.3 days in dull rooms) (Beauchemin & Hays, 1998). The rate of mortality in sunny rooms was also lower than in the dull rooms (21/293 sunny versus 39/335 dull). A retrospective study of *climate* and patient data documented a correlation between climate variables and the average length of stay of psychiatric inpatients in Veterans Health Administration hospitals nationwide (Federman, Drebing, Boisvert, & Penk, 2000). Medical centers located in warmer and drier climates had shorter lengths of stay, and those in colder climates had the longest lengths of stay in winter and fall.

Views of Nature

As discussed previously, various studies have demonstrated the beneficial impact of exposure to nature views (real nature or simulated nature, such as pictures, videos, or virtual reality) in improving patient outcomes such as stress, pain, and length of stay (e.g., Diette et al., 2003; Tse et al., 2002; Ulrich, 1984, 1991). One strong study reported the relationship between exposure to nature views and length of stay, where patients recovering from abdominal surgery had a shorter stay if they had a bedside window view of nature rather than looking out onto a brick wall (Ulrich, 1984). More research is needed to examine the impact of visual exposure to nature on the overall healing process and length of stay.

Comprehensive Programs

In reality, changes in procedural or programmatic activities to improve healthcare often were accompanied by design modifications. In such cases, it is difficult to disentangle the independent effect of design interventions. For example, Good Samaritan Hospital in Cincinnati, Ohio, conducted a study in its NICU for 1 year before and 1 year after an intervention, which consisted of a major renovation and the implementation of a comprehensive developmental care program that included training and other activities (Altimier, Eichel, Warner, Tedeschi, & Brown, 2005). The design modifications focused on improving lighting and acoustics, increasing square footage per infant bed, and addressing family and staff needs (e.g., increased privacy). The sample included 852 infants—419 preintervention and 433 postintervention—grouped into three categories based on gestation (24–27 weeks, 28–30 weeks, and 31–34 weeks). The preintervention infants had lengths of stay of 79, 58, and 34 days, respectively, as compared to 58, 45, and 23 days for infants postintervention. Other health-related benefits from the environmental and programmatic changes also were observed.

Furthermore, in the section of this article called Reducing Hospital-Acquired Infections, a large body of literature indicated that the design of the physical envi-

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ronment strongly influences infection rates by affecting the airborne, contact, and waterborne transmission of infections. In this respect, EBD measures, by reducing nosocomial infection rates, play a key role in shortening hospital stays.

Reducing Spatial Disorientation

Summary of Evidence and Recommendations

Wayfinding problems in hospitals are costly and stressful and have a particular impact on outpatients and visitors, who are often unfamiliar with the hospital and are otherwise stressed and disoriented. In a study conducted at a major regional 604-bed tertiary-care hospital, the annual cost of the wayfinding system was calculated to be more than $220,000 per year in the main hospital, or $448 per bed per year in 1990. Much of this was the hidden cost of direction-giving by people other than information staff, which occupied more than 4,500 staff hours, the equivalent of more than two full-time positions (Zimring, 1990). Several other studies have also documented the high cost of wayfinding problems in hospitals (Carpman, Grant, & Simmons, 1990; Christensen, 1979; Foxall & Hackett, 1994).

A large body of literature has explored how people find their way through hospitals and other complex buildings. For example, Peponis, Zimring, and Choi (1990) found that people tend to have predictable paths when they explore and find their way in hospitals. However, space syntax analysis shows that these are often not the most direct paths or the routes that are designated as the "main paths," but rather the routes that are the most accessible to all of the other paths in the hospital. Not surprisingly, more complex overall layouts are more difficult to find one's way in (Arthur & Passini, 1992; Drinkard, 1984; O'Neill, 1992; O'Neill, 1991a and 1991b; Ortega-Andeane & Urbina-Soria, 1988). Several studies have found that characteristics such as turns other than right turns are harder to maintain (Carpman & Grant, 1993).

Whereas there is growing evidence about how people find their way, there is relatively little research that directly assesses the comparative performance of different wayfinding systems or the impact of wayfinding on other healthcare outcomes. Limited evidence shows that wayfinding problems cannot be tackled piecemeal. A wayfinding system, as the name implies, is not just about better signage or colored lines on floors. Rather, hospitals should provide integrated systems that include coordinated elements, such as visible and easy-to-understand signs and numbers; clear and consistent verbal directions; consistent and clear paper, mail-out, and electronic information; and a legible physical setting (Carpman & Grant, 1993). A well-integrated wayfinding system includes four main components that work at different levels: (1) administrative and procedural levels, (2) external building cues, (3) local information, and (4) global structure.

Administrative and Procedural Information

Certain organizational strategies can provide key information to patients and help them prepare for their hospital visit. Examples include mail-out maps, electronic information available on the Web or at kiosks, and verbal directions. These issues are not dealt with in this review because they are not directly related to the design of hospital physical environments.

External Building Cues

Signs and cues that lead to the hospital, especially to the parking lot, must be considered carefully because they are the patient's first point of contact with the hospital. For example, Carpman, Grant, and Simmons (1985) conducted a video simulation study to assess the relative role of signs and seeing a destination. The hospital wanted to direct most traffic to a parking structure rather than to a drop-off lane. When the researchers showed prospective visitors a simulated video with a design alternative that allowed arriving drivers to see the main pavilion with the drop-off lane, 37% of the respondents said that they would turn into the drop circle when they could see the entry to the garage, ignoring the signs. Consequently, the hospital chose to redesign the entry.

Local Information

Once patients find their way to the building from the parking lot, they are faced with the prospect of identifying their destination. Informational handouts, information desks, you-are-here maps, directories, and signage along the way are critical wayfinding aids (Carpman,

Grant, & Simmons, 1983; Levine, Marchon, & Hanley, 1984; Nelson-Shulman, 1983-84; Wright, Hull, & Lickorish, 1993). In an experimental study, researchers found that patients who had the benefit of an information system (including welcome sign, hospital information booklet, patient letter, and orientation aids) upon reaching the admitting area were more self-reliant and made fewer demands on staff. In contrast, uninformed patients rated the hospital less favorably and had elevated heart rates (Nelson-Shulman, 1983-84). Information provided in you-are-here maps can be useful. However, you-are-here maps should be well oriented so that the top signifies the direction of movement for ease of use. When the maps were aligned in directions other than the forward position, people not only took much longer to find their destination, but their efforts were significantly less accurate (Levine et al., 1984). Another study found that people who used signs found their destination faster than those who used only maps (Butler, Acquino, Hissong, & Scott, 1993). However, people who were given a combination of handheld maps and wall signs reached their destination more often than those who used only wall signs (Wright et al., 1993).

It is critical to design signage systems with logical room numbering and comprehensible nomenclature for departments (Carpman & Grant, 1993; Carpman, Grant, & Simmons, 1984). For example, inpatients, outpatients, and visitors to a hospital preferred simple terms such as *walkway* or *general hospital* over more complex or less familiar terms such as *overhead link*, *medical pavilion*, or *health-sciences complex*.

Contrary to the belief that fewer signs in hospital hallways means less clutter and hence less confusion, an experimental study found that patients who had access to more signs along the way were faster, less hesitant, asked for directions fewer times, and reported lower levels of stress (Carpman et al., 1984). Based on this study, the authors suggest that directional signs be placed at or before every major intersection, at major destinations, and where a single environmental cue or a series of such cues (e.g., changes in flooring material) convey the message that the individual is moving from one area into another. If there are no key decision points along a route, signs should be placed approximately every 150 to 250 feet.

Global Structure

In addition to local properties of the spaces that people move through, there are specific characteristics of the overall structure of the system of rooms and corridors that affect the paths people take (Haq & Zimring, 2003; Peponis et al., 1990). Based on observations of participants' search patterns and objective measures of spatial characteristics, researchers found that participants tended to move along more "integrated" routes—routes that are, on average, more accessible from a greater number of spaces because there are fewer turns from all other routes in the hospital. This research suggests that it may be important to identify such integrated routes in the plan when situating important facilities and key points such as the entrance (Peponis et al., 1990). In support of these findings, Baskaya, Wilson, and Ozcan (2004) found that people got lost less frequently in a hospital where the entrance is next to the main hallway than in a hospital where the entrance is not next to the main hallway. One limitation of the study, however, is that the hospitals compared in this case study were quite different; the hospital with better wayfinding systems also had an asymmetrical layout and outside views that the comparison hospital did not have, which might also have contributed to the improved wayfinding.

Some studies at the global scale have looked at properties of building layout that facilitate or impede movement. For example, when 56 study participants were given the wayfinding task of locating five targets in four synthetic office floor plans that were modeled on an actual floor plan, virtual environments with perpendicular intersecting hallways had better wayfinding performance than those with angled intersections (Werner & Schindler, 2004). Another study supports this finding by a design experiment that compared perpendicular and angled intersections. The study had the same 12 participants for both types of intersections in synthetic floor plans (Ruddle & Peruch, 2004).

The research supports the value of a systems approach to wayfinding, and it is not sufficient to consider one

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or two components separately. Well-designed signs are likely to be quite ineffective in a building that is highly complicated and does not provide simple cues that enable natural movement. For example, some hospitals with existing complex buildings tend to simply superimpose a signage system to try to make things work. This strategy is ineffective in most cases. On the other hand, while there are more than 18 studies that look at wayfinding in hospitals and other buildings (Baskaya et al., 2004; Brown, Wright, & Brown, 1997; Butler et al., 1993; Carpman & Grant, 1993; Carpman et al., 1983, 1984; Grover, 1971; Haq & Zimring, 2003; Levine et al., 1984; Nelson-Shulman, 1983-84; Ortega-Andeane & Urbina-Soria, 1988; Passini, Rainville, Marchand, & Joanette, 1995; Peponis et al., 1990; Schneider & Taylor, 1999; Weisman, 1981; Wright et al., 1993; Zimring, 1990), it is quite difficult to isolate the independent role of a single design factor on wayfinding performance or visitors' stress.

It is essential that these different pieces of information come together while designing new hospitals, when there is an opportunity to develop an effective wayfinding system at multiple levels. Additional studies are needed to ascertain the magnitude of stress that wayfinding problems may create for outpatients and family members.

Improving Patient Privacy and Confidentiality

Summary of Evidence and Recommendations

The protection of patient confidentiality and privacy has been written into U.S. law through the Health Insurance Portability and Accountability Act (HIPAA). Research has shown that inadequate privacy may lower patient satisfaction and can worsen healthcare outcomes if patients withhold personal information or refuse to be examined because of privacy concerns (Barlas, Sama, Ward, & Lesser, 2001). *Speech privacy* is one important issue addressed by HIPAA, including how well a private conversation can be overheard by an unintended listener. The *AIA/AHA Draft Interim Sound and Vibration Design Guidelines for Hospital and Healthcare Facilities* were developed in 2006, and they will be revised and enforced in professional practice (ANSI S12/WG44 [Healthcare Acoustics and Speech Privacy] and the Joint ASA/INCE/NCAC Subcommittee on Healthcare Acoustics & Speech Privacy; Skyes, 2006).

Despite the growing importance of speech privacy in healthcare settings, existing research in this area is sparse. We identified nine pertinent empirical studies that provide some evidence-based guidance regarding what designers can do to protect the privacy of patient speech. Extensive national survey data support the provision of single-bed rooms to increase patient privacy and confidentiality (Press Ganey, 2003). When single rooms are not available, as in many EDs, installing hard-wall partitions to provide speech privacy is preferable to curtains (Barlas et al., 2001; Karro, Dent, & Farish, 2005; Mlinek & Pierce, 1997). To prevent sound leakage and privacy breaches through the ceiling, hard-wall partitions should extend to the support ceiling or deck instead of stopping at the ceiling plane. Installing high-performance sound-absorbing ceiling tiles can shorten reverberation times, improve speech intelligibility, diminish propagation of voices and sounds, and lessen sound pressure intensity (Hagerman et al., 2005; Philbin & Gray, 2002). Furthermore, providing private discussion rooms near waiting, admission, and reception areas may help prevent breaches of speech privacy (Joseph & Ulrich, 2007).

Locations and Rates of Speech Privacy Incidents

Speech privacy in EDs. The ED is the most studied setting within healthcare facilities with respect to speech privacy. EDs are subject to frequent privacy breaches due to high volumes of patients and staff, the severity of patients' diseases or injuries, numerous conversations involving confidential patient information, and the widespread practice of placing patients in multibed rooms with curtain partitions. Mlinek and Pierce's (1997) observational study in EDs found that 100% of healthcare staff committed breaches of speech privacy. Karro and colleagues (2005) reported a rate of 45% for visual and auditory privacy breaches in EDs. Additionally, studies have examined specific locations within EDs for rates of privacy incidents. For triage/waiting areas, Mlinek and Pierce (1997) observed a rate of 53% for speech privacy breaches, and Karro et al. (2005) found a self-reported rate of 55% for both speech and visual privacy incidents. For

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all ED cubicle areas, Karro et al. (2005) reported a rate of 62% for speech and visual privacy breaches, and Olsen & Sabin (2003) found that 36% of patients overheard conversations. Rooms located closest to physician and nurse work areas typically had the highest rates of confidentiality breaches (Karro et al., 2005).

Speech privacy in patient rooms. No rigorous study was identified that directly observed the occurrence of speech privacy violations within patient rooms in general wards or ICUs. However, extensive survey data have shown that single-bed rooms, compared to multibed rooms, provide better protection for patient speech privacy (Press Ganey, 2003).

Environmental Approaches to Protect Speech Privacy

Speech privacy research in healthcare settings is at an early stage of development. The limited evidence suggests that some design measures can enhance speech privacy, including the use of hard-wall space partitions, single-bed rooms, high-performance sound-absorbing materials, and private discussion rooms or spaces.

Space partitions in EDs. The comparison between hard-wall and curtain partitions, especially in EDs, is the most studied architectural issue in this area (Barlas et al., 2001; Karro et al., 2005; Mlinek & Pierce, 1997; Olsen & Sabin, 2003). As might be expected, hard-wall partitions provide substantially better protection for speech privacy than curtains. Karro et al.'s survey study of EDs reported the rate for both visual and auditory privacy breaches to be 45% in areas with curtain partitions, but only 13% in hard-walled rooms. Mlinek & Pierce's (1997) direct observation research found the rate of speech privacy incidents (i.e., conversations being overheard from the next room) to be 100% for bed spaces with curtain partitions or even single-pane glass partitions, but 0% for rooms with solid walls and doors. It is noteworthy that these two studies arrived at different results, probably because of different measurement methods. In addition, an earlier study (Barlas et al., 2001) that used the same survey instrument as Karro et al. (2005) also found a much lower rate of privacy incidents in hard-walled cubicles than curtained cubicles. Different findings emerged from Olsen and Sabin's (2003) self-report surveys, where adult patients and parents of pediatric patients reported no significant differences in the overall rates of patients or parents overhearing conversations between curtained rooms and walled rooms with open doors or curtained entrances. However, the locations of overheard conversations were quite different; in hard-walled rooms, participants overheard fewer conversations from adjacent rooms or bed spaces than curtained spaces (15% versus 55%), but more conversations from hallways or nursing stations. A possible explanation is the tunneling effect of the particular hallway design and the use of open doors or curtained entrances instead of solid, closed doors.

Single-bed patient rooms. For patient rooms, it is reasonable to expect that single rooms help to protect speech privacy because fewer unintended listeners (e.g., patients and healthcare staff) are present compared with multibed rooms. Press Ganey's national data (2003) showed that patients in single-bed rooms were consistently much more satisfied with their privacy (4.5% higher) than patients in double rooms, across all major patient categories and types of units, and across different age and gender groups. Although speech privacy was not directly measured in this survey, it likely played a role in the overall sense of privacy. Other research has found that nurses perceive single-bed rooms to be more appropriate for patient examinations and collection of patient histories (Chaudhury et al., 2006). Enhanced confidentiality and privacy were among the important reasons for such evaluations.

High-performance sound-absorbing materials for environmental surfaces. As previously mentioned (in Reducing Patients' Sleep and Reducing Patient Stress), materials for surfaces such as ceilings, floors, and walls substantially affect acoustic conditions. Existing studies have demonstrated the positive effects of installing high-performance ceiling tiles and other sound-absorbing surface materials in reducing reverberation time, sound propagation, and noise pressure levels (Hagerman et al., 2005; Philbin & Gray, 2002). Such design measures also would likely enhance the speech privacy

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of environments, because the improved acoustic conditions, such as reduced sound propagation, would lessen voice travel to adjoining spaces.

Other environmental approaches to protect speech privacy. Certain other environmental approaches may also be helpful. As pointed out by Joseph and Ulrich (2007), *providing private discussion rooms* should help reduce privacy breaches in spaces such as waiting areas and admission and reception areas, where confidential information often is discussed. Such rooms are also necessary for patient floors with multibed rooms to protect speech privacy during communication between physicians and patients or their families. Additionally, the use of a *wireless communication system* may be considered so that healthcare staff, patients, and their families need not raise their voices to be heard across large spaces or down hallways. In addition, to protect speech privacy some ceiling system manufacturers recommended the use of *sound masking*, which is "the precise application of electronic background sound that blends into the environment to cover up or mask unwanted noise" (Armstrong Ceiling System, 2003). However, there appears to be a lack of solid research supporting its appropriateness and safety in healthcare settings, because sound masking in some situations might lower clinicians' ability to detect and respond to many different types of sounds, ranging from alarms to the spoken communication of staff (Joseph & Ulrich, 2007).

Directions for future research. Although the limited amount of research discussed here provides some important evidence, there is a need for future studies that use more sensitive and advanced acoustic measures, such as the privacy index (PI). PI takes into account the acoustic performance of all finishes in a space, including ceilings, floors, and furniture (Armstrong Ceiling System, 2003), and it was employed by the new *Interim Sound and Vibration Design Guidelines for Hospital and Healthcare Facilities* (ANSI S12/WG44 [Healthcare Acoustics and Speech Privacy] and the Joint ASA/INCE/NCAC Subcommittee on Healthcare Acoustics & Speech Privacy). It is important that both researchers and designers keep informed regarding these updated standards.

Improving Communication with Patients and Family Members

Summary of Evidence and Recommendations

The communication among patients, family, and staff is important because it can provide social support to patients and family members, facilitate family members' involvement in patient care, and also increase patient satisfaction with care (Laitinen & Isola, 1996; Press Ganey, 2007). Several studies have demonstrated that providing *single-patient rooms* and *private discussion areas* can facilitate communication (Astedt-Kurki, Paavilainen, Tammentie, & Paunonen-Ilmonen, 2001; Kaldenburg, 1999). Limited evidence supports the use of *dim lighting* instead of bright lighting in counseling rooms to achieve better communication and counseling results (Miwa & Hanyu, 2006). More research is needed to explore further environment-communication relationships in more diverse healthcare settings and how design measures can enhance communication.

Importance of Communication

Evidence has shown that staff-family communication can provide social support to patients, their family members, and staff, and facilitate family members' involvement in patient care. A survey comprising 369 relatives and significant others of elderly patients indicated that family-staff communication is a good source of social support for families: it helps meet family needs and reduces patient and family anxiety (Laitinen & Isola, 1996). The same study also found that better family-staff communication increased the level of family members' involvement in providing care. In fact, the lack of communication between family members and staff was the primary reason cited for family members' failure to be involved in the care of patients.

Good communication is also of the utmost importance in terms of patient satisfaction with care across different patient categories (Press Ganey, 2007). According to the data obtained from 2,359,935 patients nationally in 2006, the five top-priority issues that patients identified as affecting satisfaction are associated with communication and empathy, including (1) response to concerns/complaints made during the hospital stay; (2) staff sensitivity to the inconvenience that health problems and hospitalization can cause; (3) staff effort to include you

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in decision making about your treatment; (4) degree to which hospital staff addressed your emotional needs; and (5) how well the nurses kept you informed. These five priorities also represent specific needs of family members, such as information, assurance, proximity, support, and comfort, which were identified by several other studies (Engli & Kirsivali-Farmer, 1993; Mathis, 1984; Molter, 1979; Verhaeghe, Defloor, Van Zuuren, Duijnstee, & Grypdonck, 2005).

Environmental Factors Affecting Communication

Several studies have found that the degree of interaction and communication in healthcare settings depends on nurses' and family members' personal characteristics, as well as how the staff-family relationship has been built (Astedt-Kurki et al., 2001; Hupcey, 1998; Soderstrom, Saveman, & Benzein, 2006; Soderstrom, Benzein, & Saveman, 2003).

Providing single-patient rooms and private areas has the benefit of facilitating communication, along with other important advantages such as preventing infections, better privacy, and less noise and crowding for families. A study by Astedt-Kurki et al. (2001) identified several factors facilitating or complicating the interaction between an adult patient's family members and nursing staff. Using a questionnaire, data were collected from 155 nursing staff working on the wards and outpatient departments at a university hospital in Sweden. Interestingly enough, one of the factors complicating interaction was the absence of a peaceful place for discussion, along with the staff's haste, shift-work, and family members' shyness of approaching the staff. Semiprivate patient rooms are perceived as busy places where roommates and their families can overhear discussions. This study implies that spaces that are private and peaceful may contribute to improved communication. Kaldenburg (1999) has found that staff members in multibed rooms are reluctant to discuss patient issues or give information when they are within hearing distance of a roommate, out of respect for patient privacy. National survey data also show that patients consistently report significantly higher satisfaction with communication from nurses and physicians when they are in single rooms compared to when they have one or more roommates (Press Ganey, 2003).

It is unfortunate that there is not much research on how the built environment enhances or hinders communication, other than for single- versus multibed rooms. There is one study that examined the effect of a specific environmental factor—dim lighting—on enhancing communication (Miwa & Hanyu, 2006). Using 80 undergraduate students as subjects, this experimental study compared four different interior conditions of counseling rooms by using different decorations (with or without home-like decorations) and types of lighting (bright or dim). The researchers found that participants in the dim lighting conditions spoke longer about themselves and their identity during the interview than did participants in the bright lighting conditions, which meant more self-disclosure, more pleasant and relaxed feelings, and more favorable impressions of the interviewer. However, they found the decorations had no significant effect. Thus, this study suggested that dim lighting in counseling rooms could enhance communication.

Fostering Social Support

Summary of Evidence and Recommendations

Social support has been described as emotional, informational, and tangible support (Kahn & Antonucci, 1980), and is normally received from people in a social network and the family (McMurray, 1998). However, contacts with one's social support network are limited while the patient is hospitalized (Koivula, Tarkka, Tarkka, Laippala, & Paunonen-Ilmonen, 2002). This is unfortunate because the need for social support increases when an individual experiences changes such as an unexpected situation or stressful event (Tarkka, Paavilainen, Lehti, & Astedt-Kurki, 2003), including a hospitalization. There is strong evidence showing the benefits of social support for patients and their families (Kaunonen, Tarkka, Paunonen, & Laippala, 1999; Koivula, Paunonen-Ilmonen, Tarkka, Tarkka, & Laippala, 2002; Koivula, Tarkka et al., 2002; McMurray, 1998; Tarkka et al., 2003).

However, there is only a moderate level of evidence linking design features to the level or quality of social support. Some studies have shown that *single-patient rooms* increase the presence of family members and social support, as compared with multibed rooms (Chaudhury,

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Mahmood, & Valente, 2003; Sallstrom, Sandman, & Norberg, 1987). There is also evidence that recommends the provision of *lounges, day rooms, and waiting rooms with comfortable, movable furniture arranged in small, flexible groupings*, in order to facilitate social interactions (Holahan, 1972; Melin & Gotestam, 1981; Peterson, Knapp, & Rosen, 1977; Sommer & Ross, 1958). The use of *carpeting* instead of vinyl for floors in patient rooms appeared to increase the length of family's stay (Harris, 2000). But such measures should be applied with a comprehensive consideration, including the possible impact on infection control and cleaning, which is an area that awaits more research. (See Reducing Hospital-Acquired Infections.)

Social Support for Patients

Several studies have found that social support from nurses, families, and significant others reduces patient stress, improves patients' physiological outcomes, and has a positive influence on both patients and family members (Kaunonen et al., 1999; Koivula, Tarkka et al., 2002; McMurray, 1998; Tarkka et al., 2003). Researchers in Finland examined the impact of in-hospital social support on coronary artery bypass grafting patients' preoperative fear and anxiety, using the survey data collected from 193 inpatients (Koivula, Paunonen-Ilmonen, Tarkka, Tarkka, & Laippala, 2002). They found that when the amount of social support was high, patients experienced lower levels of fear and anxiety.

Several studies also found that increased patient-family interactions, as part of social support from families, improve patients' physiological outcomes and facilitate patient progress (Bay, Kupferschmidt, Opperwall, & Speer, 1988; Chatham, 1978; Happ et al., 2007). An experimental study by Chatham (1978) looked at the impact of social support through patient-family interaction on a patient's postoperative behaviors. An 11-item "Behavioral Checklist" was used to measure manifestations of postcardiotomy psychosis, such as the disorientation, alertness, inappropriateness, confusion, sleep, and anxiety of open-heart surgery patients. Results showed that patients with specific social interactions with families (such as eye contact, frequent touch, and verbal orientation to time, person, and place) exhibited fewer manifestations of postcardiotomy psychosis. The studies by Hendrickson (1987) and Bruya (1981) showed a decrease in intracranial pressure during family presence and patient-family interactions among patients at risk for increased intracranial pressure. A qualitative study by Happ et al. (2007) also found that family presence and their social support through touching, talking, and surveillance helped patients to deal with their treatments better and facilitated their clinical progress. The study showed that ICU and step-down patients with family members present withdrew significantly more quickly from long-term mechanical ventilation.

Support for Family Members

There is an extensive body of literature emphasizing the need for the social support of patients' families. The evidence indicates that social support reduces families' stress, anxiety, and depression, and increases their satisfaction with the hospital stay. A family member's long-term illness affects the well-being and health of the entire family. Family members also need support to cope with a long-term illness or the death of their loved one (Tarkka et al., 2003). For example, a questionnaire study of 318 Finnish widows and widowers found that social support helped in coping with grief (Kaunonen et al., 1999). The results showed that the study subjects receive social support most often from their own families and friends and that they perceived support to be helpful. Respondents who had social support were able to grieve by expressing their feelings and forgetting the demands of normal life, whereas those without support had to hold themselves up and continue the duties of normal life, which could produce excessive psychological demands without adequate sources for coping. Based on data collected through subject interviews and questionnaires, a study of 417 patient-spouse pairs found that spouse anxiety and depression were correlated with patient psychosocial distress (Moser & Dracup, 2004). They found that patients' psychosocial adjustment to illness was worse when spouses were more anxious or depressed than the patients.

Role of the Physical Environment in Encouraging Social Support

Despite the well-established importance of social support, there is only a moderate amount of research con-

cerning how hospital design can facilitate or hinder access to social support. There is strong evidence that levels of social interaction, and presumably beneficial social support as well, can be increased by providing *lounges, day rooms, and waiting rooms with comfortable, movable furniture arranged in small, flexible groupings*. A few well-designed studies in psychiatric wards and nursing homes have found that the appropriate arrangement of movable seating in dining areas enhances social interaction and also improves eating behaviors, as indicated by the increased food consumption of geriatric patients (Melin & Gotestam, 1981; Peterson et al., 1977). Much research on dayrooms and waiting areas has shown that the widespread practice of arranging seating side-by-side along room walls inhibits social interaction (Holahan, 1972; Sommer & Ross, 1958). A novel study by Harris (2000) found that family and friends stayed substantially longer during visits to rehabilitation patients when patient rooms were carpeted rather than covered with vinyl flooring.

Much evidence indicates that *single-bed rooms* are markedly better than multibed rooms for supporting or accommodating the presence of family and friends. A clear advantage of single-bed rooms in fostering social support stems from the fact that they provide more space and furniture than double rooms to accommodate family presence (Chaudhury et al., 2003). Some research suggests that open-plan multibed rooms deter family presence and therefore reduce social support (Sallstrom et al., 1987). One reason is that multibed rooms greatly reduce privacy for patient-family interactions compared to single rooms, and they are much more likely to have restricted visiting hours. A survey of staff in four hospitals, each of which had a mix of single and double rooms, found that nurses assigned higher ratings to single rooms for accommodating family members (Chaudhury et al., 2003).

Increasing Patient Satisfaction

Summary of Evidence and Recommendations

Environmental satisfaction is a significant predictor of overall satisfaction in healthcare settings (Harris, McBride, Ross, & Curtis, 2002). There is strong evidence that a satisfying environment should be designed with patients' and families' needs in mind. It should provide a comfortable and aesthetically pleasing environment (through the use of color, artwork, etc.), and provide nice window views (preferably with nature), adequate lighting or sunlight, and a helpful information guide. The provision of single-patient rooms can afford favorable environmental features, such as quiet, privacy, an accessible bathroom, and a sense of control, and thereby can improve patients' satisfaction with the healthcare experience.

Evidence From Intervention Studies and Surveys

Telephone interviews with 380 discharged inpatients have helped determine that environmental satisfaction was a significant predictor of overall satisfaction with healthcare, ranking only below perceived quality of nursing and clinical care (Harris et al., 2002). The same study also identified specific environmental factors that were perceived to be pleasing and satisfactory to patients, including: (1) color of the wall, artwork, comfortable bed, television working properly, and easy access to anything in the patient room; (2) a window with a nice view, an accessible bathroom in the room, and a room located away from noisier areas of unit; (3) adequate lighting, quiet surroundings, and a comfortable temperature; (4) a private room, environmental means for privacy (e.g., a closed door); and (5) cleanliness of the room.

Data obtained nationally by Press Ganey (2007) support the importance of and need for better room environments to improve patient satisfaction with the hospital experience. Data show that patients complained about temperatures ("too cold") and high noise levels ("so noisy") in their rooms. Another study by Hagerman et al. (2005) also found a relationship between the noise level in patient rooms and patient satisfaction by comparing patients' responses during the bad acoustic period (with sound-reflecting ceiling tiles) to those in the good acoustic period (with sound-absorbing ceiling tiles). They found that patients treated during the good acoustic period considered staff attitude to be much better, implying that good room acoustics has an effect on patient satisfaction with staff.

There is strong evidence that design changes that make the environment more comfortable, aesthetically pleas-

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ing, and informative relieve patient stress and increase satisfaction with the quality of care. Renovating a traditional waiting area in a neurology clinic (e.g., making small changes to the general layout, color scheme, furniture, floor covering, curtains, and providing informational material and information displays) resulted in more positive environmental appraisals, an improved mood, an altered physiological state, and greater reported satisfaction among waiting patients (Leather, Beale, Santos, Watts, & Lee, 2003). Patients in well-decorated and well-appointed, hotel-like rooms rated their attending physicians, housekeeping, food-service staff, food, and the hospital higher than patients in standard rooms (with typical hospital beds, inexpensive family sitting chairs, and no artwork) in the same hospital. Also, they had stronger intentions to use the hospital again and would recommend the hospital to others (Swan, Richardson, & Hutton, 2003).

It is also important to consider how the physical environment can help family members meet their needs to increase their satisfaction with hospital stays. There is a considerable body of literature identifying the needs of family members, especially in the intensive care setting (Engli & Kirsivali-Farmer, 1993; Mathis, 1984; Molter, 1979; Verhaeghe et al., 2005). Molter (1979) investigated and developed the most commonly used instrument for measuring family needs in the ICU—the Critical Care Family Needs Inventory (CCFNI). Among 45 family need statements in CCFNI, there are eight family needs that can be addressed by means of the physical environment: (1) have a waiting room near the patient; (2) see the patient frequently; (3) have a bathroom near the waiting room; (4) have comfortable furniture in the waiting room; (5) have friends nearby for support; (6) have a telephone near the waiting room (7) have a place to be alone while in the hospital; and (8) provide the ability to be alone at any time. Another study also indicated that having a place to rest, having a waiting area, and offering overnight accommodations were essential for families' satisfaction in the neonatal intensive care setting (Conner & Nelson, 1999).

The previous studies agree in predicting that single-patient rooms could significantly improve patients' and family members' satisfaction with the healthcare experience, because they can accommodate many preferred environmental features such as quiet, privacy, an accessible bathroom, and a sense of control. Several studies have focused on the impact of specific types of single-patient rooms on patient satisfaction and other clinical and financial measures (Brown & Gallant, 2006; Hendrich et al., 2004). Hendrich et al. (2004) conducted a pre/post comparison of the two years before the move to acuity-adaptable, single-patient rooms and three years after the move; they found improvements in predictive indicators of patient satisfaction. Patients were less nervous or withdrawn and treated with more respect, and nurses were regarded as more caring.There was also an improvement in quality and operational cost.

Result III: Improving Staff Outcomes Through Environmental Measures

Decreasing Staff Injuries

Summary of Evidence and Recommendations

Hospital workers experience a high rate of occupational injury, especially musculoskeletal injuries attributable to patient handling. Some injuries take healthcare workers away from their normal job duties. It is estimated that up to 38% of all nurses in the United States suffer from back injuries (American Nurses Association, 2002). Based on 2005 data, the rate of occupational injuries and illnesses for hospitals is almost double the rate for all of private industry (8.1 cases per 100 full-time workers versus 4.6 cases per 100 in industry) (Bureau of Labor Statistics, 2007). Further, as a result of these injuries, hospital workers were away from work or on restricted job duties for 3.3 days per year per 100 full-time workers.

Many studies have been undertaken to evaluate the physical stress and strain caused by patient handling. *Ceiling lifts* have been identified the most consistently as reducing the incidence of injury and the cost of injury claims. The research findings have not been as clear regarding the use of ceiling lifts for *repositioning patients*, which, because of the awkward angles required to perform this task, results in greater numbers of injuries; these injuries are not reduced as dramatically by the use of lifts. As with all physical environment interventions, to be most effective it is critical that the introduction of

lifts be accompanied by a comprehensive patient lifting program. In fact, most of the studies reviewed involved a combination of lifts and cultural change; therefore it is difficult to isolate the effect of the lifts alone.

Lifts

Musculoskeletal injuries are often caused or aggravated by patient handling. However, it is encouraging that many studies have documented reductions in staff back injuries following the installation of assistive devices and improved procedures or room design (Chhokar et al., 2005; Engst, Chhokar, Miller, Tate, & Yassi, 2005; Evanoff, Wolf, Aton, Canos, & Collins, 2003; Garg & Owen, 1992; Hignett & Evans, 2006; Keir & MacDonell, 2003; Li, Wolf, & Evanoff, 2004; Miller, Engst, Tate, & Yassi, 2006; Yassi et al., 2001). For example, using ceiling-mounted or mobile lifts may help to reduce back injuries. Several studies have found that ceiling lifts were more effective at reducing injuries and require less time and space to use, compared with mobile lifts (Hignett & Evans, 2006; Keir & MacDonell, 2003). A study comparing two long-term care facilities in Canada identified a dramatic difference in the cost of nursing injury claims for the facility that installed ceiling lifts versus the facility that had only mechanical floor lifts. For the year following the installation of the ceiling lifts, this facility experienced a 70% decrease in claims costs and 18 fewer days lost compared to the prior year. In contrast, the facility without the ceiling lifts experienced a 241% increase in claims costs and had 499 additional days lost during the same year compared with the previous year (Miller et al., 2006). Similarly, Engst et al.'s (2005) prospective study found that the unit with ceiling lifts reduced compensation costs for injuries from lifting and transferring patients by 68%, while the control unit without ceiling lifts saw a rise in cost of 68%.

Not surprisingly, lifts are more useful in facilities that require nurses to perform more patient handling activities, such as long-term care facilities. In a comparison of the effect of lifts in acute care settings versus long-term care facilities, the use of lifts was more frequent in long-term care facilities, which consequently showed greater reductions in lost staff time due to injury (Evanoff et al., 2003). Even when the upfront cost of the lifts is considered, the savings have been demonstrated to be worthwhile. Two separate studies of ceiling lifts revealed a payback of the initial cost in 2.5 years or less (Chhokar et al., 2005; Joseph & Fritz, 2006).

Repositioning

Although overall injury rates are generally found to decline with the introduction of lifts, there have been less consistent findings about the value of lifts when used for repositioning patients rather than lifting them. Two articles reported findings from a study conducted at an extended care unit of a British Columbia hospital that installed 65 ceiling lifts to support 125 beds and three tubs in 1998. The first article examined data from the 20-month period following the installation of the lifts (Ronald et al., 2002). Although there was no overall decrease in musculoskeletal injuries, there was a reduction in the rate of workforce injuries caused by lifting and transferring patients from 14.5 injuries per 100,000 hours worked preintervention to 8.1 injuries per 100,000 hours worked postintervention. A subsequent article looked at data over a three-year period and found a reduction in the overall number of claims and a decrease in the number of injuries from repositioning (Chhokar et al., 2005). Overall claims from patient handling went from 65 during the three years preintervention to 47 postintervention. The difference in repositioning injuries is explained by the fact that there were no repositioning slings available during the first year. Chhokar and colleagues further suggested that the actual reduction in injuries might be greater because back injuries can result from cumulative rather than acute stress, and therefore, some of the injuries reported in the first year after the intervention might be the result of lifting tasks from prior years. In contrast, another study in a 75-bed extended care unit of a community hospital tracked impacts for 21 months after the intervention and found no decrease in injuries from repositioning (Engst et al., 2005). In fact, more than half the staff in the intervention ward preferred to reposition patients manually with the help of other staff rather than use a lift.

More research is needed to understand whether or not ceiling lifts can reduce staff injuries from repositioning patients. An evaluation of insurance records

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(2000–2001) from seven hospitals found that 17.9% of reported injuries from hospital workers were the result of repositioning patients, which was the highest single cause of injury (Fragala & Bailey, 2003).

Comprehensive Patient Handling Programs

The majority of previous studies have focused on comprehensive patient lifting programs without isolating the independent effect of mechanical lifts. All of these studies support the importance of changing both the culture and the physical environment. A case study from a 525-bed nursing home shows impressive results from the implementation of a comprehensive strategy, which included protocol changes, training, and regular maintenance of equipment in addition to making mechanical lifting devices available (Brophy, Achimore, & Moore-Dawson, 2001). Results showed a reduction in the average annual costs attributed to low-back injuries from $201,000 to $91,000. In another nursing home study, Garg and Owen (1992) examined an ergonomic intervention strategy, which selected patient transferring devices that produced less physical stress for nursing personnel. This intervention also included training nurse assistants to use these devices and modifying toilets and shower rooms. This ergonomic intervention resulted in a reduction in back injuries of almost 50%, from 83 per 200,000 work hours to 47 per 200,000 work hours, and a reduction in physical stress and risk of low-back pain to nursing personnel.

Specialized Devices

One study found that strain on the backs of nursing staff could be reduced simply by providing slings to assist in moving patients from one seat to another, which also decreased capital outlay (Elford & Straker, 2000). However, the researchers measured only the spinal strain on nurses with and without slings; they did not estimate the impact in terms of reduced injuries or cost. Another laboratory study evaluated different pieces of equipment used to assist with bathing and how they affected the postural load on caregivers. Results showed that the adjustable shower chair, the adjustable bath, and the adjustable shower trolley caused the least stress on postural load, and the fixed shower chair and trolley caused the most postural stress (Knibbe & Knibbe, 1996).

Contradictory Evidence

It should be noted that not all of the previous studies found a conclusive link between the provision of mechanical lifting aids and the reduction of back injuries. One study in the United Kingdom did not reach significant findings, perhaps owing to the research design: the control hospital ended up implementing a patient handling program halfway through its research and the program implemented at its intervention hospital was not very robust. It was unclear what kind of hoists were used and how many of them were provided; the focus appears to be on training and other assistive device, such as sliding sheets (Smedley et al., 2003).

A Canadian study did not show differences in the rate and cost of injuries in wards with and without mechanical lift devices, but it did find that staff with access to assistive devices reported improved comfort, an increased sense of safety, and decreased fatigue (Yassi et al., 2001). In contrast, a 2005 study found that staff in the ward *without* ceiling lifts felt less stressed about their jobs and less worried about making mistakes than did the intervention group that had ceiling lifts, despite the fact that 96% of the staff members in the intervention group reported that the ceiling lifts made their jobs easier. One possible explanation for this is that using ceiling lifts to transfer patients took more time than manual lifts and transfers, and this additional time might lead to the perception of a more hectic work environment (Engst et al., 2005).

On the other hand, despite the mostly consistent positive impacts of mechanical lifts, many studies found that staff often declined to use the lifts for a variety of reasons (e.g., it takes more time and requires more space; lifts are not conveniently located, etc.) and the presence of lifts does not ensure their adoption (Chhokar et al., 2005; Evanoff et al., 2003; Joseph & Fritz, 2006; Li et al., 2004). Therefore, special attention should be paid to changing the workplace culture, such as providing training and enforcing a no-lift policy. Overall, the literature showed that comprehensive safe-patient-handling programs are important for reducing staff injuries; having mechanical lifts that are readily available and easy to use is a key component of successful programs.

Decreasing Staff Stress

Summary of Evidence and Recommendations

Healthcare providers, especially nurses, experience a high level of work stress (Jayaratne & Chess, 1984; Pines & Maslach, 1978; Siefert, Jayaratne, & Chess, 1991; Tummers, Janssen, Landeweerd, & Houkes, 2001). Several studies indicate that high workplace stress contributes to employee burnout and an intention to leave the job (Barrett & Yates, 2002; Pines & Maslach, 1978; Topf & Dillon, 1988). Registered nurses have an annual turnover rate averaging 20% (Joint Commission, 2002). Stress is a particular problem as nurses approach the possibility of retirement: 55% of nurses surveyed, predominantly managers, reported they intended to retire between 2011 and 2020 (Hader, Saver, & Steltzer, 2006). Auerbach, Buerhaus, and Staiger (2007) recently estimated that the U.S. shortage of registered nurses will increase to 340,000 by the year 2020. Although this is substantially less than the forecast made in 2000 predicting a shortfall of 800,000 registered nurses, the authors note that the nursing shortage is expected to increase to three times the size of the current shortage (Auerbach et al., 2007).

Despite convincing evidence on the negative impact of stress on healthcare workers, especially ICU nurses, relatively few studies have examined how the physical environment contributes to staff stress. Environmental factors associated with stress include noise, light, and single- versus multibed patient rooms. Noise is the most frequently studied environmental factor related to stress in hospitals, and it can be reduced by environmental approaches such as using high-performance, sound-absorbing materials. A few studies have documented the importance of light in modulating circadian rhythms and thereby improving the adjustment to night-shift work among staff. The administration of bright light in staff work areas can be useful to alleviate stress among night-shift nurses, but the specific design implications should be further examined. Finally, survey research in NICUs found that single-bed patient rooms were perceived to be less stressful for both family and staff than open-bay multibed rooms (Harris, Shepley, White, Kolberg, & Harrell, 2006).

Severity of Staff Stress

Several studies have examined the effects of stress on healthcare workers, especially nurses working in intensive care settings (Corr, 2000; Fischer, Calame, Dettling, Zeier, & Fanconi, 2000a, 2000b; Le Blanc, de Jonge, de Rijk, & Schaufeli, 2001; Sexton, Thomas, & Helmreich, 2000; Smith et al., 2001). Oehler and Davidson (1992) found acute care nurses had higher levels of job stress and *burnout* than nurses in nonacute settings. Fischer et al. (2000a, 2000b) studied stress in ICU staff by measuring levels of the stress hormone cortisol. They found that stress-related cortisol surges occurred frequently in a sample of 112 nurses and 27 physicians in NICUs and PICUs.

Stress affects *performance*, especially for novices. For example, Smith et al. (2001) examined the relationship between psychological stress and performance by studying 45 novice registered nurses in ICUs. They found that nurses in a high state of anxiety performed less well in endotracheal suctioning than their more relaxed peers. This implies that nurses with high anxiety may be at risk for medical errors and poor performance, in addition to higher burnout and attrition.

Environmental Factors Affecting Stress

Although there is considerable evidence on the negative effects of stress on healthcare workers, relatively few studies have examined how the physical environment contributes to staff stress. Several descriptive studies on staff stress have assessed the possible effects of the characteristics of intensive care environments, such as blinking lights, alarms, and equipment noise (Corr, 2000; Donchin & Seagull, 2002; Dyson, 1999). A review paper by Corr (2000) identified the healthcare physical environment as one of the causes of occupational stress, along with the job itself and the organization. Several studies of nonhealthcare workplaces such as commercial offices have found that environmental factors associated with stress include noise, crowding, poor ambient conditions (light, air quality, and temperature), and lack of control over the environment, especially the inability to regulate social conditions and achieve privacy when desired (Baum, Singer, & Baum, 1981; Evans & Cohen, 1987). However, comparatively little research has evaluated the impact of

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these various environmental factors on staff stress in healthcare settings.

Reduce staff stress by controlling noise. *Noise* is the most frequently studied environmental factor related to stress in hospitals. However, much research has examined the effects of noise on patients, and few studies have focused on its impact on healthcare staff. Survey research has found that staff perceived higher sound levels as stressful and interfering with their work (Bayo, Garcia, & Garcia, 1995; Norbeck, 1985). More importantly, Topf and Dillon (1988) found that noise-induced stress correlates with reported emotional exhaustion or burnout among critical care nurses. A quasi-experimental study by Blomkvist et al. (2005) in Sweden demonstrated the positive impact of a single environmental factor—sound-absorbing ceiling tiles (versus sound-reflecting ones)—on the perceived reduction of stress by the same group of coronary intensive care nurses over a period of months. Nurses perceived significantly lower work demands and reported less pressure and strain when the sound-absorbing tiles were in place. A survey by Harris et al. (2006) in NICUs has found that staff perceived a unit with single-patient rooms to be less stressful for both family and staff than an open-bay unit, owing to better privacy and control over the environment with respect to noise, light, temperature, and traffic.

Reduce staff stress with light. Several studies have documented the importance of light in reducing depression (see Reducing Depression), modulating circadian rhythms, and improving sleep quality (see Improving Patients' Sleep). By controlling the body's circadian system, appropriate exposure to intermittent bright light also aids the adjustment to night-shift work among staff, as demonstrated by several studies (Baehr, Fogg, & Eastman, 1999; Boivin & James, 2002; Crowley, Lee, Tseng, Fogg, & Eastman, 2003; Horowitz, Cade, Wolfe, & Czeisler, 2001; Iwata, Ichii, & Egashira, 1997; Leppamaki, Partonen, Piiroinen, Haukka, & Lonnqvist, 2003). One study with 87 female night-shift nurses examined whether repeated, brief exposure (4x 20 minutes) to bright light (over 5,000 lux) during night shifts improved subjective well-being during and after night work (Leppamaki et al., 2003). Results showed that light significantly alleviated the subjective distress associated with night-shift work, in both summer and winter. Bright light (over 2,500 lux) is used for the treatment of seasonal affective disorder in winter (Partonen & Lonnqvist, 1998). A recent study by Partonen and Lonnqvist (1998) found that bright light exposure appears to have a positive effect on mood even in healthy people.

Another study found that staff with more than three hours of *daylight* exposure during their shift had higher job satisfaction and less stress than staff with less daylight exposure. However, the findings are complicated by the factor of types of nursing activities: Nurses from ICUs, EDs, or ORs were mostly exposed to daylight for less than 3 hours, while nurses from inpatient units mostly had an exposure of more than 3 hours (Alimoglu & Donmez, 2005). More research is needed to understand the impact of natural light on staff stress.

Increasing Staff Effectiveness

Summary of Evidence and Recommendations

Jobs by nurses, physicians, and other healthcare workers often require a complex choreography of direct patient care, critical communications, charting, accessing technology and information, and other tasks. Many hospital settings have not been redesigned, although jobs have been changed, and as a result, hospital environments often increase staff stress and reduce effective care delivery. The challenge of maintaining staff effectiveness will be increasingly important as the nurse shortage mounts and the aging population increases the demand on the healthcare system. While much research in the hospital setting has been aimed at patients, there is a growing and convincing body of evidence suggesting that improved hospital design can make the jobs of staff easier. The physical environment interventions that have been shown to affect staff effectiveness include unit configuration, noise, and other distractions. Lighting levels may also have an impact on staff effectiveness, but relevant studies are still limited.

Unit Configuration

Workplace design that reflects a closer alignment of work patterns and the physical setting, such as the re-

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design of a pharmacy layout, has been shown to improve workflow, reduce waiting times, and increase patient satisfaction with service (Pierce, Rogers, Sharp, & Musulin, 1990).

According to one study of nursing staff, walking accounted for 28.9% of work time and was ranked second among various activities, following patient-care activities that accounted for 56.9% of work time (Burgio, Engel, Hawkins, McCorick, & Scheve, 1990). At least four studies have shown that the *type of unit layout* (e.g., radial, single corridor, double corridor) influences the amount of walking among nursing staff (Shepley, 2002; Shepley & Davies, 2003; Sturdavant, 1960; Trites, Galbraith, Sturdavant, & Leckwart, 1970), and two studies showed that time saved from walking was translated into patient-care activities and interaction with family members (Trites et al., 1970). Sturdavant (1960) found that fewer trips were made to patient rooms in radial units, because nurses were able to better supervise patients visually from the nursing station, although the average time spent with patients was the same in radial units as in single-corridor designs. Shepley and Davies (2003) found that nursing staff in the radial unit walked significantly less than staff in the rectangular unit (4.7 steps versus 7.9 steps per minute). However, they also noted that radial designs might provide less flexibility in managing patient loads. In addition, the majority of the staff surveyed preferred to work in the radial units.

Some studies showed that *decentralized nurse stations* reduced staff's walking time and increased patient-care time, especially when supplies were also decentralized and placed near the nurse stations (Hendrich, 2003; IOM, 2004). The location of supplies is particularly important. Centralized location of supplies could double staff walking and substantially reduce care time irrespective of whether nurses stations were decentralized (Hendrich, 2003). Other studies that compared delivery times in centralized and *decentralized pharmacy systems* found that medication delivery times were reduced by more than 50% when using decentralized distribution systems (Hibbard, Bosso, Sward, & Baum, 1981; Lomonte, Besser, & Thomas, 1983; Reynolds, Johnson, & Longe, 1978).

By shifting over to an *acuity-adaptable model* for cardiac-care patients, Clarian Health's Methodist campus reduced the number of patient transfers by 90%, and thereby reduced the amount of nursing time expended on this "nonvalue" activity (Hendrich et al., 2004). Methodist Hospital also introduced decentralized nursing stations and supply centers. Researchers observed a reduction in staff time spent walking to get supplies and a resulting increase in nursing time, although this change was not quantified in the article. Another interesting finding was that they were able to accommodate a greater number of patient days with fewer beds, with 56 acuity-adaptable beds postintervention versus a total of 63 beds preintervention (Hendrich et al., 2004).

The *co-location of similar services* can result in better coordination of caregivers. Gilleard and Tarcisius (2003) found this to be the case in a pediatric unit in Hong Kong that was redesigned to put all physical therapy needs (e.g., speech therapy, physiotherapy, occupational therapy) in one large room. Surveys showed that both staff and patients preferred the consolidated arrangement over the previous distributed model.

Noise and Distractions

Noise levels can be high in hospital environments and noise is recognized as a distraction and stressor for staff, resulting in reduced productivity. Hospital staff often complains that noise levels make their work more difficult, particularly when it comes to monitoring patients' vital signs (Sanderson et al., 2005; Zun & Downey, 2005).

ED personnel experience especially high levels of ambient noise. Previous studies have confirmed that it can be difficult for staff members to assess patients' breathing and heartbeat in noisy and moving environments such as ambulances and helicopters (Zun & Downey, 2005). Despite these complaints, a recent study tested the ability of hospital staff to measure patients' heart and lung sounds under high ambient noise conditions (90dB), and found that the majority of the staff (>90%) had no problem accurately detecting these sounds using standard equipment (Zun & Downey, 2005). The study involved 104 participants, ranging from attending physicians to technicians, and

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subjects were exposed to noise levels that were over 20 dB higher than the peak volume in a study of four EDs in Phoenix, Arizona. However, noise often has chronic rather that acute effects on performance and stress. Some studies such as those by Blomkvist and colleagues (2005) have identified long-term negative effects of noise on staff.

Loud ambient noise is the source of significant complaints in the OR (Sanderson et al., 2005). Studies have found noise levels in operating theatres to be 80–85 dB for a background level with intermittent spikes of 110–115 dB (Moorthy et al., 2004). However, not all studies indicate that a noise level of 80–85 dB is problematic. A controlled study of 12 surgeons performing laparoscopic procedures on a test module under various noise conditions (80–85 dB, both with music and under quiet conditions) found no impact of noise on the quality of performance or time taken to perform the procedure (Moorthy et al., 2004). But this study did not evaluate the impact of high peaks of intermittent noise. More research is needed to evaluate the impact of noise on communication among staff, particularly in ORs and EDs.

In addition, the typical noise produced by talking, equipment, and procedures may be compounded by noise from music, which may be played in the OR for a variety of reasons. In one U.K. survey, 72% of anesthesiologist respondents reported that music was regularly played in the OR, although 51% found music distracting and 26% found that music reduced their vigilance (Hawksworth, Asbury, & Millar, 1997). To test whether and how music plays a role in the performance of anesthesiologists, Sanderson and colleagues (2005) asked 24 participants with no medical or physiological training to monitor and report vital signs from a simulated patient using visual and auditory displays. Participants were tested under three auditory conditions: no music playing, classical music playing, and rock music playing. While participants believed that completing the task while listening to classical music was more enjoyable, a majority of participants (16 of 24) believed that the task itself was easier when completed in silence. However, the presence of music appeared to help participants to gauge correctly the direction of trends in vital signs, which were provided by the auditory display. Sanderson and colleagues speculated that this effect was because the music provided a metrical standard against which the participants could compare auditory data. It still remains to be answered whether any of these findings can be generalized to anesthetists, surgeons, or healthcare workers in general. At least one study casts doubt on the ability to generalize such findings from nonsurgeons to surgeons, and it suggests that surgeons may acquire a certain ability to block out extraneous noise while in the OR (Moorthy et al., 2004).

Surgeons are less adept when they are required to process healthcare tasks and respond to auditory distractions at the same time. In Goodell et al.'s (2006) study, 13 participants, consisting of surgical residents and medical students, were asked to perform a laparoscopic task under an uninterrupted control condition and an interrupted experimental condition, where participants were required to solve a number of arithmetic problems while performing the procedure. While the quality of the participants' work did not suffer because of cognitive distractions, the time spent on the task rose significantly during the interrupted condition. In some cases, the presence of interruptions increased the time for a given procedure by one third. Though it is not an explicit finding, this suggests the possibility that designs that minimize intrusions, particularly while surgeons are operating, may speed up a given procedure.

In short, while high noise levels are often reported as a problem by hospital staff, it appears that, with few exceptions, staff members are able to avoid the adverse effects of noise on their performance quality, although the time needed for procedures tends to increase. It is important to note that most of these studies were conducted using recorded hospital noise with constant volume; it is unclear whether staff can cope with sounds with startling volume changes (which occur frequently in hospital settings) as effectively. Overall, it is clear that the long-term effects of high levels of ambient noise on staff are troubling; although the noise may not directly hamper staff performance, the cumulative effects of stress may lead to adverse outcomes.

Impact of Other Environmental Factors on Staff Effectiveness

Other aspects of the environment, such as *lighting levels* and *auditory or visual distractions*, can also affect staff effectiveness when performing critical tasks such as dispensing medical prescriptions. There have been studies in manufacturing showing a positive effect of higher lighting levels on the speed of production (Juslen, Wouters, & Tenner, 2007). However, none that was specifically related to healthcare environments or tasks was identified. A small pilot study was conducted in a nursing home to evaluate the usefulness of providing light-emitting diode lighting triggered by motion sensors for nighttime lighting. The 17 staff members in the study reported that they found these lights convenient and useful for conducting nighttime rounds without disturbing residents' sleep (Taylor, 2005). There are several studies that have evaluated the effect of bright light (2,500 lux) and set sleep schedules on staff working the night shift (Horowitz et al., 2001). These studies report that the most significant positive effect is seen only when these factors are used in combination. Once again, such findings confirm that environmental interventions are most effective when paired with cultural or behavioral programs.

Increasing Staff Satisfaction

Summary of Evidence and Recommendations

Excellent care will hardly happen with dissatisfied hospital staff. Job satisfaction is known to be influenced by many nonphysical working conditions, such as autonomy (O'Rourke, Allgood, VanDerslice, & Hardy, 2000), compensation (Best & Thurston, 2006), and performance (Douglas, Meleis, Eribes, & Kim, 1996). Lack of support from the physical environment can make already stressful working conditions worse. Investments in the environment to increase staff satisfaction could potentially reduce the cost of staff turnover, which can cost more than $62,100 per nurse replaced (Jones, 2004). However, not many studies have examined the effects of environmental factors on job satisfaction. Natural light is one of the exceptions.

Natural Light

Mrockzek, Mikitarian, Vieira, & Rotrius (2005) conducted an Internet survey of staff working in a newly constructed facility and found that natural light in the new facility had the most positive environmental impact on work life, followed by live music in the atrium. Another study found that staff with more than 3 hours of daylight exposure during their shift had higher job satisfaction than staff with less daylight exposure. However, the findings are complicated by the types of nursing activities performed by each group (Alimoglu & Donmez, 2005). In addition, new healthcare facilities might not increase job satisfaction if they are not carefully designed. In a study comparing an old and a new ward in a mental healthcare facility, Tyson, Lambert, and Beattie (2002) concluded that the new ward resulted in no increase in job satisfaction, probably owing to the isolation of nurses caused by the larger space and separated observation wings, and understaffing in the new acute ward.

Conclusions and Design Recommendations

This study reviewed the literature linking hospital physical environments with healthcare outcomes, and identified a number of design strategies and interventions that can influence outcomes. The main body of this paper was organized by type of healthcare outcome. However, designers and healthcare workers often face the question of whether to employ specific design strategies or interventions. Therefore, the following sections discuss specific design measures and the improved outcomes that can be expected from them. Table 1 provides an overview of the relationships between design factors and healthcare outcomes. It should be noted that some of the relationships indicated in this table have not been directly tested by empirical studies, but they have been supported in an indirect way by strong available evidence.

Single-Bed Rooms

The design intervention that positively affects the largest number of outcomes in a hospital setting is the provision of single-bed patient rooms. The value of single-bed rooms has been acknowledged by the AIA after extensive research and has been included in the *2006 Guidelines for Design and Construction of Health Care Facilities* (AIA & FGI, 2006). Strong evidence indicates that single-bed rooms improve the following outcomes:

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Hospital-Acquired Infections. The use of single-patient rooms reduces airborne, contact, and waterborne transmission of hospital-acquired infections by increasing isolation capacity, facilitating the thorough cleaning of rooms and the maintenance of air quality, and also possibly increasing hand-washing compliance by healthcare workers.

Patient Sleep. Patients in single-bed rooms benefit from increased privacy and the reduction in noise from roommates, visitors, and healthcare staff. These factors improve sleep and facilitate the healing process.

Patient Privacy. Single-bed rooms help protect auditory and visual privacy compared with multibed rooms. The absence of a roommate in hospital rooms helps prevent privacy breaches during discussions between patients and care providers. Patients in single-bed rooms are more willing to provide personal information to care providers, which facilitates diagnosis and treatment.

Table 1. Summary of the Relationships Between Design Factors and Healthcare Outcomes

Healthcare Outcomes \ Design Strategies or Environmental Interventions	Single-bed rooms	Access to daylight	Appropriate lighting	Views of nature	Family zone in patient rooms	Carpeting	Noise-reducing finishes	Ceiling lifts	Nursing floor layout	Decentralized supplies	Acuity-adaptable rooms
Reduced hospital-acquired infections	**										
Reduced medical errors	*		*				*				*
Reduced patient falls	*		*		*	*			*		*
Reduced pain		*	*	**			*				
Improved patient sleep	**	*	*				*				
Reduced patient stress	*	*	*	**	*		**				
Reduced depression		**	**	*	*						
Reduced length of stay		*	*	*							*
Improved patient privacy and confidentiality	**				*		*				
Improved communication with patients & family members	**				*		*				
Improved social support	*				*	*					
Increased patient satisfaction	**	*	*	*	*	*	*				
Decreased staff injuries								**			*
Decreased staff stress	*	*	*	*			*				
Increased staff effectiveness	*		*				*		*	*	*
Increased staff satisfaction	*	*	*	*			*				

* Indicates that a relationship between the specific design factor and healthcare outcome was indicated, directly or indirectly, by empirical studies reviewed in this report.

** Indicates that there is especially strong evidence (converging findings from multiple rigorous studies) indicating that a design intervention improves a healthcare outcome.

Communication with Patients and Families. Because of enhanced auditory privacy, single-bed rooms can improve communication among patients, families, and care providers. Patients in single-bed rooms report greater satisfaction with communication from nurses and physicians compared with patients in multibed rooms.

Social Support. Compared with multibed rooms, single-bed rooms provide enhanced privacy, encourage family visits and social interaction, and are more likely to provide space to accommodate visiting relatives and friends.

Staff Stress. Staff also appreciates the benefits of single-bed rooms and reports finding them less stressful than multibed or open-bay settings.

Patient Satisfaction. Considering all the above-mentioned benefits, it is no surprise that patients are more satisfied with their hospital stays when they are placed in single-bed rooms.

Access to Daylight and Appropriate Lighting

The quality and quantity of daylight exposure and artificial lighting is associated with several patient and staff outcomes in healthcare settings. Access to daylight is important for both staff and patients. For patients, it has been found to reduce pain and the incidence of depression, and for certain types of patients, it also may reduce length of stay. For staff, access to daylight contributes to higher satisfaction. Therefore, site planning and the orientation of healthcare facilities should be carefully considered to ensure sufficient daylight and avoid situations where some buildings block light for others. Larger windows in patient rooms not only provide natural light, but they also have the potential benefit of offering views of nature and should be considered in the design process.

The amount and timing of light in healthcare settings should be tailored to the activities that take place in them. In general, sufficient lighting is beneficial to both patients and staff. Bright lighting is preferred in areas where staff performs critical tasks such as medication dispensing.

Medical Errors. Research has found that medication-dispensing errors are lower when the level of work-surface lighting is relatively high, compared to situations with lower levels of lighting. While other areas of the hospital have not been tested, it is logical to infer that bright lighting would also be useful in other places where precision is called for.

Pain. Exposure to natural light has been found to reduce patients' pain and the amount of pain medications that they use. Buildings should be carefully designed so that patient rooms can have abundant natural light.

Patient Sleep. As a major contributor to normal circadian rhythm, the amount of light that patients are exposed to at different times of day can affect sleep quality. During the day, patients should be exposed to adequate natural light or bright artificial lighting when natural light is not available. At nighttime, if possible, the light in patients' rooms should be dimmed long enough to ensure good sleep.

Depression. A considerable body of rigorous evidence indicates that exposure to light—daylight or bright artificial light—is effective in reducing depression and improving mood. These findings underline the importance of building orientation and site planning in new healthcare projects.

Length of Stay. Research on patients suffering from depression found that patients in rooms with more morning daylight had shorter lengths of stay than patients in rooms without morning sunlight.

Communication with Patients and Families. Research on counseling rooms suggests that people feel more comfortable talking and talk longer in rooms with dim lighting as compared to similar rooms with bright lighting.

Patient Satisfaction. Adequate lighting has been identified as one component affecting patients' overall satisfaction with their hospital stays.

Staff Satisfaction. Access to sufficient natural light is one of the few physical environmental attributes that

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has been linked by research with higher staff satisfaction. This finding suggests that natural light is also needed in staff working areas.

Family Zone in Patient Room

While single-patient rooms have the potential to affect the largest number of outcomes in hospital settings, some of the benefits may be facilitated by the availability of appropriate family zones within the room.

Social Support. Evidence indicates that single-patient rooms encourage family presence by providing more space and privacy and accommodating patient-family interactions, compared with multibed rooms. It is important to make sure that single-patient rooms include appropriate family zones and comfortable furniture to encourage family members to stay longer and provide more social support to patients.

Patient Falls. Providing family zones can encourage family members to remain longer in patient rooms. As a result, timely help is available from family members to assist patients with getting in and out of the bed, which may reduce the frequency of patient falls.

Noise-Reducing Finishes

Hospitals are noisy places with numerous sources of noise, and historically they have been designed with sound-reflecting surfaces that worsen acoustic conditions and enable noises to echo and propagate over large areas. Research has found that the use of noise-reducing finishes such as high-performance sound-absorbing ceiling tiles can reduce the noise in hospitals and benefit both patients and staff.

Patient Sleep. Getting a good night's sleep is very important to patients' healing processes. Studies have found that the noise level in many hospitals is quite high even at night and that noise is a major cause of awakenings and poor sleep. For this reason, measures should be taken to reduce the reverberation time, sound propagation, and noise intensity level in patient rooms.

Patient Privacy. The use of sound-absorbing materials can also enhance patient privacy by reducing sound propagation and privacy breaches. When single-bed rooms are not available, as in many EDs, hard-wall partitions rather than curtains should be used to separate bed spaces; these should be extended all the way up to the support ceiling or deck to protect speech privacy.

Patient Satisfaction. Noise is one of the factors of the ambient environment that patients complain about most frequently. Research found that a reduced noise level in patient rooms has a positive impact on patient satisfaction. Patients treated in spaces with good acoustic performance considered staff attitude and care quality to be much better than those in spaces with poor acoustics.

Patient Stress. In addition to worsening sleep quality, noise elevates psychological and physiological stress in patients. The use of sound-absorbing materials in patient rooms, in combination with reducing noise sources, can create a less stressful environment for patients.

Staff Stress. Limited research has focused on the effects of noise on staff. A recent study found that improved room acoustics (facilitated by using sound-absorbing materials) positively affected the staff's perception of work demands and lowered their work pressure and strain.

Views of Nature

Consierable research has examinined the psychological and physiological effects of viewing real and simulated nature. Most available evidence is related to the impact of nature views on patients. There is also limited evidence suggesting that staff experience restorative benefits from views of nature or exposure to gardens.

Pain. Nature has been determined to be an effective positive distraction, which can reduce the perception of pain and thereby reduce the use of pain medications. Some studies combined simulated views of nature with nature sounds or classical music; these studies demonstrated greater impact on pain reduction, compared with when auditory distraction was not available.

Length of Stay. A direct relationship between exposure to nature views and reduced length of stay in a study

of patients recovering from abdominal surgery was found in one study. More research in diverse settings with various types of patient populations is needed to examine the contribution of nature views to the overall healing process and their effect on length of stay.

Patient Stress. Strong studies have found that exposing patients to nature lessens stress and anxiety.

Minimizing Negative Distractions for Healthcare Workers

Whereas positive distractions such as nature can help alleviate patient pain and stress, negative distractions (such as noise) in healthcare staff workplaces will lower work efficiency and possibly increase the incidence of medical errors.

Staff Effectiveness. Distractions during surgery can slow the progress of procedures. Interruptions from staff can result in delays in activities and thereby reduce the productivity of an already stressed staff.

Medical Errors. Distractions during critical tasks, such as preparing medications, can result in errors, which ultimately affect patients. However, the evidence shows that design strategies such as using sound-absorbing materials to reduce noise, and providing bright lighting at work stations can help minimize distractions, reduce medical errors, and improve work efficiency.

Acuity-Adaptable Rooms

There is increasing interest in acuity-adaptable rooms, but their use in real projects is still limited. As the number of hospitals with acuity-adaptable rooms grows, there will be greater opportunity to study their impact on patients and staff. Evaluation of the concept and its implementation suggest that these rooms reduce transfers, and may reduce the incidence of patient falls and medical errors and increase patient satisfaction.

Patient Falls. Acuity-adaptable patient rooms can minimize the need for costly patient transfers.

Medical errors. Some medical errors originate from the delays, communication discontinuities, loss of information, and changes in computers or systems associated with the patient transfer process. The use of acuity-adaptable rooms can lessen the number of patient transfers and the medical errors that may occur during transfer.

Patient Satisfaction. In some recent studies, the provision of acuity-adaptable rooms was accompanied by improvements in predictive indicators of patient satisfaction. There were also improvements in quality of care and operational cost.

Looking Forward

EBD is a rapidly evolving and increasingly rigorous field. Hospital owners and designers have to make very important decisions about how hospitals will be built based on the information and knowledge available. It is clear from this review that there is a growing amount of sound research to support the application of certain specific design characteristics to improve healthcare outcomes. This paper is intended to make that evidence more accessible to practitioners, and to identify needs and directions for future research.

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The Role of the Physical Environment in the Hospital of the 21st Century: A Once-in-a-Lifetime Opportunity

by

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The Role of the Physical Environment in the Hospital of the 21st Century: A Once-in-a-Lifetime Opportunity

A visit to a U.S. hospital is dangerous and stressful for patients, families and staff members. Medical errors and hospital-acquired infections are among the leading causes of death in the United States, each killing more Americans than AIDS, breast cancer, or automobile accidents (Institute of Medicine, 2000; 2001). According to the Institute of Medicine in its landmark *Quality Chasm* report: "*The frustration levels of both patients and clinicians have probably never been higher. Yet the problems remain. Health care today harms too frequently and routinely fails to deliver its potential benefits*" (IOM, 2001). Problems with U.S. health care not only influence patients; they impact staff. Registered nurses have a turnover rate averaging 20 percent (Joint Commission on Accreditation of Healthcare Organizations, 2002).

At the same time, the United States is facing one of the largest hospital building booms in US history. As a result of a confluence of the need to replace aging 1970s hospitals, population shifts in the United States, the graying of the baby boom generation, and the introduction of new technologies, the United States will spend more than $16 billion for hospital construction in 2004, and this will rise to more than $20 billion per year by the end of the decade (Babwin, 2002). These hospitals will remain in place for decades.

This once-in-lifetime construction program provides an opportunity to rethink hospital design, and especially to consider how improved hospital design can help reduce staff stress and fatigue and increase effectiveness in delivering care, improve patient safety, reduce patient and family stress and improve outcomes and improve overall healthcare quality.

Just as medicine has increasingly moved toward "evidence-based medicine," where clinical choices are informed by research, healthcare design is increasingly guided by rigorous research linking the physical environment of hospitals to patients and staff outcomes and is moving toward "evidence-based design" (Hamilton, 2003). This report assesses the state of the science that links characteristics of the physical setting to patient and staff outcomes:

- What can research tell us about "good" and "bad" hospital design?
- Is there compelling scientifically credible evidence that design genuinely impacts staff and clinical outcomes?
- Can improved design make hospitals less risky and stressful for patients, their families, and for staff?

In this project, research teams from Texas A&M University and Georgia Tech combed through several thousand scientific articles and identified more than 600 studies—most in top peer-reviewed journals—that establish how hospital design can impact clinical

outcomes. The team found scientific studies that document the impact of a range of design characteristics, such as single-rooms versus multi-bed rooms, reduced noise, improved lighting, better ventilation, better ergonomic designs, supportive workplaces and improved layout that can help reduce errors, reduce stress, improve sleep, reduce pain and drugs, and improve other outcomes. The team discovered that, not only is there a very large body of evidence to guide hospital design, but a very strong one. A growing scientific literature is confirming that the conventional ways that hospitals are designed contributes to stress and danger, or more positively, that this level of risk and stress is unnecessary: improved physical settings can be an important tool in making hospitals safer, more healing, and better places to work.

RESEARCH PROCESS

The research teams searched through scores of databases and in libraries at Texas A&M, Georgia Institute of Technology, University of Michigan, and elsewhere. The team was looking for studies that are:

- Rigorous, in that they use appropriate research methods that allow reasonable comparisons, and discarding of alternative hypotheses. The research studies were assessed on their rigor, quality of research design, sample sizes, and degree of control.

- High impact, in that the outcomes they explore are of importance to healthcare decision-makers, patients, clinicians, and society.

In 1998, Haya Rubin and her colleagues Amanda Owens and Greta Golden found 84 studies produced since 1968 that met similar criteria (Rubin, Owens, & Golden, 1998). Reviewing the research literature six years later, the team estimated that they would find around 125 rigorous studies. We found more than 600.

RESULTS

The research team found rigorous studies that link the physical environment to patient and staff outcomes in four areas:

1. Reduce staff stress and fatigue and increase effectiveness in delivering care
2. Improve patient safety
3. Reduce stress and improve outcomes
4. Improve overall healthcare quality

I. Reduce Staff Stress and Fatigue and Increase Effectiveness in Delivering Care

There is a growing nurse shortage, and this directly threatens patient safety. And the existing hospital-based nursing force is aging. Registered nurses in the United States average more than 43 years old and will average 50 by 2010 and have a turnover rate

averaging 20 percent per year (JCAHO, 2002). The Joint Commission on Accreditation of Healthcare Organizations (JCAHO), in their 2002 report, *Health Care at the Crossroads: Strategies for Addressing the Evolving Nursing Crisis*, noted that a shortage of nurses in America's hospitals is putting patient lives in danger. JCAHO examined 1,609 hospital reports of patient deaths and injuries since 1996 and found that low nursing staff levels were a contributing factor in 24 percent of the cases. The JCAHO report and surveys of nursing found that physical working conditions, along with support and compensation, are key contributors to turnover and burnout. Environmental support for work has become more critical as the typical patient is more seriously ill, patient loads increase, technology changes, and documentation requirements increase.

While reducing staff stress and fatigue through a healing and supportive environment seems like an obvious goal, there are relatively few studies that have dealt with this issue in any detail. More attention has been given to patient outcomes. However, the following sections review several studies that do document (a) effects of healthcare environments on staff health and safety and (b) improving workplaces to increase staff effectiveness, reduce errors, and increase staff satisfaction. Each section also suggests directions for further research.

Improve Staff Health and Safety through Environmental Measures

Nurses, physicians, and other healthcare employees work under extremely stressful physical conditions. Several studies deal with healthcare employees' risk of contracting infectious diseases from patients due to airborne and surface contamination (Jiang et al., 2003; Kromhout et al., 2000; Kumari et al., 1998; Smedbold et al., 2002). A recent study conducted in the wake of the SARS epidemic in China found that isolating SARS cases in wards with good ventilation could reduce the viral load of the ward and might be the key to preventing outbreaks of SARS among healthcare workers, along with strict personal protection measures in isolation units (Jiang et al., 2003). Another study in Norway found correlations between environmental factors and nasal symptoms of 115 females who worked at 36 geriatric nursing departments. They found significant decrease in nasal inflammation in relation to presence of *Aspergillus fumigatus* in ventilation supply and elevated room temperatures (Smedbold et al., 2002). An evaluation of 17 acute-care or university hospitals in Canada shows that tuberculosis (TB) infection among healthcare workers was associated with ventilation of general or nonisolation patient rooms of less than two air exchanges per hour. The evaluation included all personnel who worked at least two days per week in the respiratory and physiotherapy departments (Menzies, Fanning, Yuan, & Fitzgerald, 2000). This study, like the others, supports the importance of adequate ventilation with good maintenance for ensuring both staff and patient safety in hospitals. Several good studies demonstrating the risk of the sick building syndrome in hospitals also have been compiled within the 2003 *Guidelines for Environmental Infection Control in Healthcare Facilities* by the US Centers for Disease Control and Prevention (CDC) and the Healthcare Infection Control Practices Advisory Committee (HICPAC) (Sehulster & Chinn, 2003).

Nursing staff members are also open to risk of injury from medical equipment such as high-intensity surgical-light sources. One study found that a light source used during surgery could potentially cause retinal damage in surgical staff (Fox & Henson, 1996). Much research has examined the effects of noise on patients, but comparatively few studies are available for healthcare staff. There is evidence that staff perceive higher sound levels as stressful (Bayo, Garcia, & Garcia, 1995; Norbeck, 1985). Importantly, noise-induced stress in nurses correlates with reported emotional exhaustion or burnout (Topf & Dillon, 1988). A recent study by Blomkvist et al. (in press, 2004) examined the effects of higher versus lower noise levels on the same group of coronary intensive-care nurses over a period of months. Lower noise levels were linked with a number of positive effects on staff, including reduced perceived work demands, increased workplace social support, improved quality of care for patients, and better speech intelligibility.

Poor ergonomic design of patient beds and nurses' stations leads to back stress, fatigue, and other injuries among nursing staff. In one nursing home study, Garg and Owen evaluated manual tasks deemed stressful by nursing staff and used the information to select patient-transferring devices and to modify toilets and shower rooms. This nursing intervention resulted in a reduction of back injuries of almost 50 percent, from 83 per 200,000 work hours to 47 per 200,000 work hours. Also, in the four months after the intervention, there were no injuries resulting in lost or restricted workdays (Garg & Owen, 1992).

Thus, reducing staff stress by ergonomic interventions, as well as careful consideration of other issues such as air quality, noise, and light, can have significant impact on staff health. In addition, it is also likely to send a message that maintaining health and safety of staff members is an important goal for the organization.

Increase Staff Effectiveness, Reduce Errors, and Increase Staff Satisfaction by Designing Better Workplaces

Jobs by nurses, physicians, and others often require a complex choreography of direct patient care, critical communications, charting, filling meds, access to technology and information, and other tasks. Many hospital settings have not been rethought as jobs have changed, and, as a result, the design of hospitals often increases staff stress and reduces their effectiveness in delivering care. While much research in the hospital setting has been aimed at patients, there is a growing and convincing body of evidence suggesting that improved designs can make the jobs of staff much easier.

Nurses spend a lot of time walking. According to one study, almost 28.9 percent of nursing staff time was spent walking (Burgio, Engel, Hawkins, McCorick, & Scheve, 1990). This came second only to patient-care activities, which accounted for 56.9 percent of observed behavior. At least four studies have shown that the type of unit layout (e.g. radial, single corridor, double corridor) influences amount of walking among nursing staff (Shepley, 2002; Shepley & Davies, 2003; Sturdavant, 1960; Trites, Galbraith, Sturdavant, & Leckwart, 1970), and two studies showed that time saved walking was translated into more time spent on patient-care activities and interaction with family

members. Sturdavant (1960) found that fewer trips were made to patient rooms in radial units as nurses were able to better supervise patients visually from the nursing station, though the average time spent with patients was the same in radial as well as single-corridor designs. Shepley and colleagues (2003) found that nursing staff in the radial unit walked significantly less than staff in the rectangular unit (4.7 steps per minute versus 7.9 steps per minute). However, Shepley and her colleagues noted that radial designs might provide less flexibility in managing patient loads. Trites and colleagues (1970) found that a decrease in the percentage of time spent walking by staff in radial units was correlated to an increase in the percentage of time spent in patient-care activities. Also, the majority of the staff surveyed preferred to work in the radial units. Hendrich's research showed that decentralized nurses stations reduced staff walking and increased patient-care time, especially when supplies also were decentralized and placed near the nurses' station (Hendrich, 2003; IOM, 2004). Centralized location of supplies, however, could double staff walking and substantially reduce care time irrespective of whether nurses stations were decentralized (Hendrich, 2003)

Workplace design that reflects a closer alignment of work patterns and the physical setting, such as redesign of a pharmacy layout, has been shown to improve work flow and reduce waiting times, as well as increase patient satisfaction with the service (Pierce, Rogers, Sharp, & Musulin, 1990). Other studies that compared delivery times in decentralized and centralized pharmacy systems found medication delivery times are reduced by more than 50 percent by using decentralized drug-dose distribution systems (Hibbard, Bosso, Sward, & Baum, 1981; Reynolds, Johnson, & Longe, 1978).

Other aspects of the environment, such as lighting levels and auditory or visual distractions, can also affect staff effectiveness while performing critical tasks such as dispensing medical prescriptions. There are relatively few studies that have examined the effect of environmental factors on medication errors, though this is clearly an area of great interest and future research potential. This is discussed in greater detail in the following section.

II. Improve Patient Safety

Hospital-Acquired Infections

One critically important way that evidence-based design improves safety is by reducing risk from hospital-acquired infections. The research team identified more than 120 studies linking infection to the built environment of the hospital. Transmission of infection to patients occurs through two general routes: airborne and contact. The research literature shows that the design of the physical environment strongly impacts hospital-acquired infection rates by affecting both airborne and contact transmission routes. The literature suggests a clear pattern wherein infection rates are lower when there is very good air quality and patients are in single-bed rather than multi-bed rooms. Also, there is some evidence that providing numerous, easily accessible alcohol-based hand-rub

dispensers or hand washing sinks can increase hand washing compliance and thereby reduce contact contamination.

Reducing Infections Caused by Airborne Pathogens

Evidence from many studies leaves no doubt that hospital air quality and ventilation play decisive roles in affecting air concentrations of pathogens such as fungal spores (*Aspergillus*) and, in this way, have major effects on infection rates.

Source of airborne infections: Well-conducted research has linked all of the following to air quality and infection rates: type of air filter, direction of airflow and air pressure, air changes per hour in room, humidity, and ventilation system cleaning and maintenance (Lutz, 2003; McDonald et al., 1998). For example, in one study where six patients and one nurse were involved with an outbreak of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-15), an environmental source was suspected, and the ventilation grilles in two patient bays were found to be harboring EMRSA-15 (Kumari et al., 1998). The ventilation system, at that time, was working on an intermittent cycle from 4:00 p.m. to 8:00 p.m. Daily shutdown of the system created negative pressure, sucking air in from the ward environment into the ventilation system and contaminating the outlet grilles. The contaminated air blew back into the ward when the ventilation system was started. In another case, the source of infection was the exhaust ducting of the adjacent isolation room ventilation system that allowed the contaminants to enter the unit via a partially open window positioned above a particular bed.

Several studies have identified hospital construction and renovation activities as the sources of airborne infection outbreaks due to dust or particulate generation (Humphreys et al., 1991; Iwen, Davis, Reed, Winfield, & Hinrichs, 1994; Loo et al., 1996; Opal et al., 1986; Oren, Haddad, Finkelstein, & Rowe, 2001). In one study, high spore counts were found within and outside construction sites in a hospital. After control measures were instituted, no further cases of disseminated aspergillosis were identified (Opal et al., 1986). In another study, a nosocomial (hospital-induced) outbreak of invasive pulmonary aspergillosis (IPA) occurred in acute leukemia patients treated in a regular ward with natural ventilation during extensive hospital construction and renovation. The observed infection rate was 50 percent. At this point, some of the patients were moved to a new hematology ward with high-efficiency particulate air (HEPA) filters. During the following three years, none of the patients hospitalized exclusively in the hematology ward developed IPA, although 29% of leukemia patients still housed in the regular ward contracted IPA (Oren et al., 2001).

Controlling and preventing airborne infection: There is convincing evidence that immunocompromised and other high-acuity patient groups have lower incidence of infection when housed in a HEPA-filtered isolation room (Passweg et al., 1998; Sherertz, et al., 1987; Sherertz & Sullivan, 1985). In one study, bone-marrow transplant recipients were found to have a tenfold greater incidence of nosocomial *Aspergillus* infection, compared to other immunocompromised patient populations, when assigned beds outside of a HEPA-filtered environment (Sherertz, et al., 1987). Air contamination is least in

laminar airflow rooms with HEPA filters, and this approach is recommended for operating-room suites and areas with ultraclean room requirements such as those housing immunocompromised patient populations (Alberti et al., 2001; Arlet, Gluckman, Gerber, Perol, & Hirsch, 1989; Dharan & Pittet, 2002; Friberg, Ardnor, & Lundholm, 2003; Hahn et al., 2002; Sherertz, et al., 1987). (Laminar flows are very even, smooth, low velocity airflows that are used in cleanrooms and other settings where high quality ventilation is critical. But laminar flows are relatively expensive and difficult to achieve because furnishings, vents and other features can create turbulence.) HEPA filters are suggested for healthcare facilities by the CDC and HICPAC, but are either required or strongly recommended in all construction and renovation areas (Sehulster & Chinn, 2003).

Effective prevention or control measures during construction and renovation activities include, for example, portable HEPA filters, installing barriers between the patient care and construction areas, negative air pressure in construction/renovation areas relative to patient-care spaces, and sealing patient windows. There is strong evidence of the impact of using HEPA filters for air intakes near construction and renovation sites (Loo et al., 1996; Mahieu, De Dooy, Van Laer, Jansens, & Ieven, 2000; Opal et al., 1986; Oren et al., 2001). A strong study by Humphreys (1991) demonstrates that HEPA filters are not *by themselves* an adequate control measure, and must be employed in conjunction with other measures such as enhanced cleaning, the sealing of windows, and barriers. Cornet et al. (1999) concludes that carefully directed airflow (e.g. laminar airflow) is important, however, we were unable to find and document cost-benefit analysis in the literature to justify the expense versus effectiveness of laminar airflow for patient-care areas near construction and renovation sites

Reducing Infections by Increasing Hand Washing

Although infection caused by airborne transmission poses a major safety problem, most infections are now acquired in the hospital via the contact pathway (Bauer, Ofner, Just, Just, & Daschner, 1990; IOM, 2004). It is well-established that the hands of healthcare staff are the principal cause of contact transmission from patient to patient (Larson, 1988). The importance of assiduous hand washing by healthcare workers, accordingly, cannot be overemphasized for reducing hospital-acquired infections. In this context, the fact that rates of hand washing by healthcare staff are low represents a very serious patient safety challenge. Several studies of hand washing in high-acuity units with vulnerable patients have found that as few as one in seven staff members wash their hands between patients: compliance rates in the range of 15 percent to 35 percent are typical; rates above 40 percent to 50 percent are the exception (Albert & Condie, 1981; Graham, 1990). Hand washing compliance tends to be consistently lower in units that are understaffed and have a high patient census or bed-occupancy rate (Archibald, Manning, Bell, Banerjee, & Jarvis, 1997).

Education programs to increase hand washing adherence have yielded disappointing or, at best, mixed results. Some investigations have found that education interventions generate no increase at all in hand washing. Even intensive education or training

programs (classes, group feedback, for example) produce only transient increases in hand washing (Conly, Hill, Ross, Lertzman, & Louie, 1989; Dorsey, Cydulka, & Emerman, 1996; Dubbert, Dolce, Richter, Miller, & Chapman, 1990). Given the tremendous morbidity and mortality associated with high rates of hospital-acquired infections, there is an urgent need to identify more effective ways for producing sustained increases in hand washing. Is there evidence suggesting improved hospital design can be effective in elevating hand washing?

Effect of number and location of sinks/dispensers on hand washing: The research team identified six studies that examined whether hand washing is improved by increasing the ratio of the number of sinks or hand-cleaner dispensers to beds and/or by placing sinks or hand-cleaner dispensers in more accessible locations (Cohen, Saiman, Cimiotti, & Larson, 2003; Graham, 1990; Kaplan & McGuckin, 1986; Muto, Sistrom, & Farr, 2000; Pittet et al., 2000; Vernon, Trick, Welbel, Peterson, & Weinstein, 2003). These studies, on balance, offer support, though limited, for the notion that providing numerous, conveniently located alcohol-rub dispensers or washing sinks can increase compliance. In particular, the evidence suggests that installing alcohol-based hand-cleaner dispensers at bedside usually improves adherence. As an example, a study by Pittet et al. (2000) found that a combination of bedside antiseptic hand-rub dispensers and posters to remind staff to clean their hands substantially increased compliance. Cohen et al. (2003) likewise reported improved adherence in association with the installation of numerous alcohol-gel dispensers. By contrast, Muto et al. (2000) reported that placing alcohol-gel dispensers next to the doors of patient rooms did not increase adherence. Two other investigations focusing on sinks (water/soap) identified a positive relationship between observed frequency of hand washing and a higher ratio of sinks to beds (Kaplan & McGuckin, 1986; Vernon et al., 2003). Providing automated water/soap sinks, however, appears not to increase hand washing rates compared to traditional non-automated sinks (Larson, et al., 1991; Larson, Bryan, Adler, & Blane, 1997).

Further, three studies offer convincing and important evidence that providing single-patient rooms with a conveniently located sink in each room reduces nosocomial infection rates in intensive care units, such as neonatal intensive care (NICU) or burn units, compared to when the same staff and comparable patients are in multibed open units with few sinks (Goldmann, Durbin, & Freeman, 1981; McManus, A. T., Mason, McManus, & Pruitt, 1994; McManus, A. T., McManus, Mason, Aitcheson, & Pruitt, 1985; Mulin et al., 1997). Although hand washing frequency was not measured in these studies, the investigators posited that increased hand washing was an important factor in reducing infections in the units with single rooms and more sinks. A comparison of an ICU converted from an open unit with few sinks to single rooms with one sink per room found a nonsignificant tendency for hand washing to increase (from 16 percent to 30 percent) but no decline in infection incidence (Preston, Larson, & Stamm, 1981). These results are perhaps explainable by the fact that several sinks in the single-bed unit were placed in comparatively inaccessible or inconvenient locations, such as behind doors or away from staff work paths.

Despite the encouraging overall pattern of findings in these studies, it is not clear how much of the effectiveness in terms of increased hand washing or reduced infection rates can be attributed to the installation of more numerous and/or accessible sinks and alcohol-gel dispensers. Future research should include controlled experiments that systematically vary the number and location of hand-cleaning stations or dispensers. There is also a conspicuous need for studies that define *accessible* locations for hand-cleaning stations in an evidence-based manner—that is, on the basis of empirical analysis of staff movement paths, visual fields, interactions with patients and families, and work processes. In this regard, the neglect of *human factors* knowledge and research methods is a major weakness of the hand washing research and, more generally, of the infection control literature. Research teams should include a human factors specialist and often an environmental psychologist. The urgent need to increase hand washing frequency underscores the high priority that should be accorded this research direction.

Reducing Infections with Single-Bed Rooms

The research team identified at least 16 studies relevant to the question of whether nosocomial infection rates differ between single-bed and multi-bed rooms. The findings collectively provide a strong pattern of evidence indicating that infection rates are usually lower in single-bed rooms. Different mechanisms or factors have been identified or implicated as contributing to lower infection incidence in single rooms. One clear set of advantages relates to reducing airborne transmission through air quality and ventilation measures such as HEPA filters, negative room pressure to prevent a patient with an aerial-spread infection from infecting others, or maintaining positive pressure to protect an immunocompromised patient from airborne pathogens in nearby rooms. A strong study by Passweg et al. (1998) found that the combination of room isolation and HEPA filtration reduced infection and mortality in bone marrow transplant patients and significantly increased their one-year survival rates. Research studying burn patients also has shown that single rooms and good air quality substantially reduce infection incidence and reduce mortality (McManus, A. T. et al., 1994; McManus, A.T., Mason, McManus, & Pruitt, 1992; Shirani et al., 1986; Thompson, Meredith, & Molnar, 2002). Studies of cross-infection for contagious airborne diseases (influenza, measles, TB, for example) have found, as would be expected, that placing patients in single rooms is safer than housing them in multibed spaces (Gardner, Court, Brocklebank, Downham, & Weightman, 1973; McKendrick & Emond, 1976).

Severe Acute Respiratory Syndrome (SARS) outbreaks in Asia and Canada dramatically highlighted the shortcomings of multibed rooms for controlling or preventing infections both for patients and healthcare workers. SARS is transmitted by droplets that can be airborne over limited areas. Approximately 75 percent of SARS cases in Toronto resulted from exposure in hospital settings (Farquharson & Baguley, 2003). The pervasiveness in Canadian and Asian hospitals of multibed spaces in emergency departments and ICUs, together with the scarcity of isolation rooms with negative pressure, severely hindered treatment and control measures. Toronto hospitals were forced to create additional negative-pressure isolation rooms by quickly constructing wall barriers to replace bed curtains and making airflow and pressure adaptations (Farquharson & Baguley, 2003).

In addition to clear advantages in reducing airborne transmission, several studies show that single-bed rooms also lessen risk of infections acquired by contact. As background for understanding how single rooms can lessen contact spread, it should first be mentioned that many environmental surfaces and features become contaminated near infected patients. Examples of surfaces found to be contaminated frequently via contact with patients and staff include: overbed tables, bed privacy curtains, computer keyboards, infusion pump buttons, door handles, bedside rails, blood pressure cuffs, chairs and other furniture, and countertops (Aygun et al., 2002; Boyce, Potter-Bynoe, Chenevert, & King, 1997; Bures, Fishbain, Uyehara, Parker, & Berg, 2000; Devine, Cooke, & Wright, 2001; Neely & Maley, 2001; Noskin, Bednarz, Suriano, Reiner, & Peterson, 2000; Palmer, 1999; Roberts, Findlay, & Lang, 2001; Rountree, Beard, Loewenthal, May, & Renwick, 1967; Sanderson & Weissler, 1992; Williams, Singh, & Romberg, 2003). These and other contaminated surfaces and features act as pathogen reservoirs that increase cross-infection risk. Boyce et al. (1997) found that in the rooms of patients infected with methicillin-resistant *Staphylococcus aureus* (MRSA), 27 percent of all environmental surfaces sampled were contaminated with MRSA

Compared to single-bed rooms, multi-bed rooms are far more difficult to decontaminate thoroughly after a patient is discharged, and therefore worsen the problem of multiple surfaces acting as pathogen reservoirs. Because different staff members who enter a room can touch the same contaminated surfaces, the risk of a nurse unknowingly becoming contaminated should be greater in multioccupancy rooms. Circumstantial support for this point is provided by research on contamination of nurses in units having patients infected by MRSA. Boyce et al. (1997) found that 42 percent of nurses who had no direct contact with an MRSA patient but had touched contaminated surfaces contaminated their gloves with MRSA.

In a study of MRSA infections in NICUs, Jernigan et al. (1996) reported that risk was lowered by isolation in single-bed rooms; high risk was associated with spatial proximity to an infected patient and shared exposure to caregivers. Ben-Abraham et al. (2002) found that nosocomial infection frequency was much lower in a single-bed pediatric intensive care unit than a unit with multi-bed rooms. The investigators tentatively concluded that single-bed rooms helped to limit person-to-person spread of pathogens between pediatric patients. Having a roommate has been identified as a risk factor for nosocomial diarrhea and gastroenteritis (Chang, V. T. & Nelson, 2000; Pegues & Woernle, 1993).

To summarize briefly, there is a convincing pattern of evidence across many studies indicating that single-bed rooms lower nosocomial infection rates. Singles appear to limit person-to-person and person-surface-person spread of infection in part because they are far easier to decontaminate thoroughly than multibed rooms after patients are discharged. Also, single rooms with a conveniently located sink or alcohol-gel dispenser in each room may heighten hand washing compliance compared to multibed rooms with few sinks. Finally, single rooms are clearly superior to multi-bed rooms with respect to reducing airborne transmission of pathogens.

Reducing Medication Errors

The research team identified three rigorous studies that link environmental factors, such as lighting, distractions, and interruptions, with errors in prescribing or dispensing medications (Booker & Roseman, 1995; Buchanan, Barker, Gibson, Jiang, & Pearson, 1991; Flynn et al., 1999). Although there are relatively few studies in this area, the findings suggest a promising research direction that merits further exploration, considering the vital importance of preventing medical errors in hospitals. Results from a large-scale study of the effects of different illumination levels on pharmacists' prescription-dispensing error rates strongly suggested that such errors are reduced when work-surface light levels are relatively high (Buchanan et al., 1991). In this study, three different illumination levels were evaluated (450 lux, 1,100 lux, 1,500 lux). Medication-dispensing error rates were significantly lower (2.6 percent) at an illumination level of 1,500 lux (highest level), compared to an error rate of 3.8 percent at 450 lux. Two investigations of medication dispensing errors by hospital pharmacists found that error rates increased sharply for prescriptions when an interruption or distraction occurred, such as a telephone call (Flynn et al., 1999; Kistner, Keith, Sergeant, & Hokanson, 1994). Thus, lighting levels, frequent interruptions or distractions during work, and inadequate private space for performing work can be expected to worsen medication errors. The process of improving systems and processes to reduce medication errors in hospitals should include an assessment of the environment in which staff members perform their activities. Additional research is required to confirm the findings from studies as well as to identify ways to design better working environments that may reduce or prevent the likelihood of such errors occurring.

There is mounting evidence that the transfer of patients between rooms or different units is a source of medication errors (Cook, Render, & Woods, 2000). Reasons why errors plague room transfers include delays, communication discontinuities among staff, loss of information, and changes in computers or systems. The solution implied is to create an acuity-adaptable care process and patient rooms that substantially reduce transfers. When Methodist Hospital in Indianapolis, Indiana, changed from two-bed rooms in coronary intensive care to acuity-adaptable single-bed rooms, transfers declined 90 percent and medication errors were lowered by 67 percent (Hendrich, Fay, & Sorrells, 2002; 2004). Reducing transfers also saves much staff time, shortens patient stays, and reduces costs (IOM, 2004). Further studies and demonstration projects are needed to ascertain the safety advantages of acuity-adaptable rooms for other types of units and patient categories.

Reduce Patient Falls

There is a very large literature that looks at the causes and risk factors involved in patient falls in hospitals. This is an area of great importance because patients who fall incur physical injuries, psychological effects, and have greater lengths of stay in the hospital (Brandis, 1999). It is estimated that the total cost of fall injuries for older people was

around $20.2 billion per year in the United States in 1994, and is projected to reach $32.4 billion (in 1994 U.S. dollars) in 2020 (Chang, 2004). While the role of the environment in causing or preventing patient falls is widely accepted, there is not yet evidence conclusively tying environmental interventions with reduced falls. Available studies usually examine the location of fall incidents retrospectively or discuss environmental-modification programs such as improving lighting, securing carpeting, and so on. However, a meta-analysis and systematic review of randomized controlled trials of fall-prevention interventions found that there was no clear evidence for the independent effectiveness of environmental-modification programs (Chang, 2004).

Nonetheless, several studies have shown that most patient falls occur in the bedroom, followed by the bathroom, and that comprehensive fall-prevention programs can have an effect. Brandis (1999) reported transfers to and from bed as the cause of 42.2 percent of inpatient falls. Design faults identified in the bathroom and bedroom areas included slippery floors, inappropriate door openings, poor placement of rails and accessories, and incorrect toilet and furniture heights. After the fall-prevention program (which included identifying high-risk patients, management strategies, environmental and equipment modification, and standardization) was implemented, there was an overall decrease of 17.3 percent in falls. Thus, fall-prevention strategies that have included environmental modification have worked in the past. However, it is not clear how much of the effectiveness of such strategies can be attributed to environmental factors alone.

An innovative and promising environmental strategy for reducing falls has its origins in evidence suggesting that many falls occur when patients attempt to get out of bed unassisted or unobserved (Uden, 1985; Vassallo, Azeem, Pirwani, Sharma, & Allen, 2000). It should be mentioned that considerable evidence has shown that bedrails are ineffective for reducing the incidence of falls and may increase the severity of fall injuries from beds (Capezuti, Maislin, Strumpf, & Evans, 2002; Hanger, Ball, & Wood, 1999; van Leeuwen, Bennett, West, Wiles, & Grasso, 2001). To increase observation and improve assistance for patients and thereby reduce falls, Methodist Hospital in Indianapolis, Indiana, changed from a coronary critical care unit with centralized nurses stations and two-bed rooms to one having decentralized nurses stations and large single-bed rooms designed to support family presence (Hendrich, et al., 2002). Comparison of data from two years prior and three years after the new unit design showed that falls were cut by 2/3—from six-per-thousand patients to two-per-thousand. Given that falls are a critical safety problem, additional research is needed to understand the effectiveness of this approach for designing patient-care units.

Improve Patient Confidentiality and Privacy

Confidentiality has emerged as a priority issue in light of research showing that physicians and nurses very frequently breach patient confidentiality and privacy by talking in spaces where they are overhead by other patients or persons (Ubel, Zell, & Miller, 1995). The seriousness of the problem is underscored, for example, by a study of an emergency department at a university hospital that showed that 100 percent of physicians and other clinical personnel committed confidentiality and privacy breaches

(Mlinek & Pierce, 1997). HIPAA, the Health Insurance Portability and Accountability Act of 1998, has further elevated the importance of providing reasonable safeguards to protect the confidentiality of staff conversations with and about patients.

Although the importance of the built environment for patient confidentiality may seem self-evident, only a few studies have directly examined the role of unit design or architecture. A study by Barlas et al. (2001) compared auditory and visual privacy for emergency department patients assigned to either multibed spaces with curtain partitions or rooms with solid walls. Those with curtains reported having far less auditory and visual privacy than patients with walls. An important finding was that 5 percent of the patients in curtained spaces reported they withheld portions of their medical history and refused parts of their physical examination because of lack of privacy (Barlas et al., 2001). None of the patients in rooms with walls reported withholding information. The fact that some emergency department patients with curtains withheld information suggests that lack of privacy can reduce patient safety. Additional convincing evidence of the importance of the emergency department physical environment comes from a study that documented frequent breaches of auditory and visual privacy and confidentiality in areas with curtains compared to rooms with solid walls (Mlinek & Pierce, 1997). A recent questionnaire study of staff in four West Coast hospitals found that nurses overwhelmingly judged single rooms to be superior to double rooms for examining a patient (85 percent) and for collecting a patient's history (82 percent) (Chaudhury, Mahmood, & Valente, 2003).

There is a clear need for additional studies that examine privacy and confidentiality breaches associated with the physical environment in single versus double rooms, multibed spaces in ICUs and other types of units, and in spaces such as waiting rooms and nurses' stations. As well, priority should be accorded to generating more research that investigates how the quality of communication and information *from* patients *to* physicians and nurses is affected by the unit architecture.

Analysis of patient satisfaction data made available by Press Ganey (2003) for this report leaves no doubt that patients in single-bed rooms, compared to those with a roommate, are consistently much more satisfied with "concern for your privacy." The satisfaction data were obtained from 2,122,439 patients who received care during 2003 in 1,462 healthcare facilities (Press Ganey, 2003). Fifty-six percent of the patients were in single rooms; 44 percent had a roommate. Greater satisfaction with privacy in single rooms was evident across all major patient categories and types of unit and across different age and gender groups. Satisfaction with privacy was 4.5 percent higher on average nationally in single rooms than doubles—a substantial difference considering that it can be difficult for hospitals to increase satisfaction scores by even 1 percent to 2 percent.

III. Reduce Stress and Improve Outcomes

Reduce Noise

The research team identified more than 130 references focusing on noise in hospitals in the research literature. Studies tend to fall into three broad categories: those that measure noise levels in hospital spaces but do not assess outcomes, studies of the effectiveness of environmental and/or organizational interventions in reducing noise, and investigations of the effects of noise on outcomes.

World Health Organization guideline values for continuous background noise in hospital patient rooms are 35 dB, with nighttime peaks in wards not to exceed 40 dB (Berglund, Lindvall, & Schwela, 1999). These guidelines notwithstanding, many studies have shown that hospital background noise levels fall in far higher ranges. Background noise levels typically are 45 dB to 68 dB, with peaks frequently exceeding 85 dB to 90 dB (Aaron et al., 1996; Allaouchiche, Duflo, Debon, Bergeret, & Chassard, 2002; Blomkvist et al., in press, 2004; Falk & Woods, 1973; Hilton, 1985; McLaughlin, McLaughlin, Elliott, & Campalani, 1996; Robertson, Cooper-Peel, & Vos, 1998). In judging these noise levels, it is worth noting that the decibel scale is logarithmic; each 10 dB increase represents approximately a doubling in the perceived sound level. A 60 dB sound, accordingly, is perceived as roughly four times as loud as a 40 dB sound. Medical equipment and staff voices often produce 70 dB to 75 dB levels measured at the patient's head, which approach the noise level in a busy restaurant (Blomkvist et al., in press, 2004). Noises from alarms and certain equipment exceed 90 dB (for example, portable X-ray machine), which is comparable to walking next to a busy highway when a motorcycle or large truck passes. A study in a NICU measured peak levels once per minute and found that 31 percent of peaks exceed 90 dB (Robertson et al., 1998). Noise peaks in hospitals can be extraordinarily loud. A recent study recorded 113 dB during shift changes at a large hospital (Cmiel, Karr, Gasser, Oliphant, & Neveau, 2004). Operating room noises from drills, saws, and other equipment are in the range of 100 dB to 110 dB, presenting a significant risk for noise-induced hearing loss (Hodge & Thompson, 1990; Love, 2003; Nott & West, 2003).

The research reviewed suggests that hospitals are excessively noisy for two general reasons (Ulrich, Lawson, & Martinez, 2003). First, noise sources are numerous, often unnecessarily so, and many are loud. Well-documented examples include paging systems, alarms, bedrails moved up/down, telephones, staff voices, ice machines, pneumatic tubes, trolleys, and noises generated by roommates. Second, environmental surfaces—floors, walls, ceilings—usually are hard and sound-reflecting, not sound-absorbing, creating poor acoustic conditions. Sound-reflecting surfaces cause noise to propagate considerable distances, traveling down corridors and into patient rooms, and adversely affecting patients and staff over larger areas. Sound-reflecting surfaces typical of hospitals cause sounds to echo, overlap, and linger or have long reverberation times (Blomkvist et al., in press, 2004; Ulrich et al., 2003).

Environmental interventions that have proven especially effective for reducing noise and improving acoustics in hospital settings include: installing high-performance sound-absorbing ceiling tiles, eliminating or reducing noise sources (for example, adopting a noiseless paging system), and providing single-bed rather than multibed rooms. In general, studies of the effectiveness of noise-reduction measures suggest that environmental or design interventions are more successful than organizational interventions such as staff education or establishing quiet hours (Gast & Baker, 1989; Moore, 1998; Walder, Francioli, Meyer, Lancon, & Romand, 2000).

A clear-cut finding in the literature is that noise levels are much lower in single-bed than multi-bed rooms. Studies of multi-bed rooms in acute care and intensive care units have shown that most noises stem from the presence of another patient (staff talking, staff caring for other patients, equipment, visitors, patient sounds such as coughing, crying out, rattling bed rails) (Baker, 1984; Southwell & Wistow, 1995; Yinnon, Ilan, Tadmor, Altarescu, & Hershko, 1992). A study of multi-bed bays in a children's hospital concluded that noise levels were so high that consideration should be given to abolishing open bay rooms (Couper et al., 1994). Further, patient satisfaction data provided for this report by Press Ganey (2003) unequivocally show that patients in single-bed rooms, compared to those with a roommate, are vastly more satisfied with the "noise levels in and around your room." The satisfaction data, as noted in an earlier section, were obtained from 2,122,439 patients who received care during 2003 in 1,462 healthcare facilities (Press Ganey, 2003). Far higher satisfaction with noise levels in single rooms was evident across all major patient categories and types of unit and across different age and gender groups. Satisfaction with noise level was 11.2 percent higher on average nationally in single rooms than doubles—a huge difference. As was noted above, it can be difficult for hospitals to achieve even 1 percent to 2 percent increases in patient satisfaction scores. The combination of findings from noise-level measurement studies and patient-satisfaction surveys highlight the great advantage of providing single rooms, compared to two-bed rooms, with respect to reducing noise.

A considerable body of research has documented negative effects of noise on patient outcomes. Several studies have focused on infants in NICUs, finding that higher noise levels, for example, decrease oxygen saturation (increasing need for oxygen support therapy), elevate blood pressure, increase heart and respiration rate, and worsen sleep (Johnson, 2001; Slevin, Farrington, Duffy, Daly, & Murphy, 2000; Zahr & de Traversay, 1995). Much research on adults and children has unequivocally shown, as might be expected, that noise is a major cause of awakenings and sleep loss (Blomkvist et al., in press, 2004; Gabor et al., 2003; Meyer et al., 1994; Parthasarathy & Tobin, 2004; Schnelle, Ouslander, Simmons, Alessi, & Gravel, 1993; Topf, 1985; Topf & Davis, 1993; Topf & Thompson, 2001; Yinnon et al., 1992). In multi-bed rooms, noises stemming from the presence of other patients often are the major cause of sleep loss. Berg (2001) found, by monitoring brain activity, that even relatively low decibel levels—38 dB to 40 dB—when coupled with longer reverberation times (sound-reflecting ceiling) significantly fragmented and worsened sleep of volunteers in patient rooms. Berg's (2001) findings have disturbing implications because most hospitals have nighttime sound peaks exceeding those of the patient rooms in his study.

Apart from worsening sleep, there is strong evidence that noise increases stress in adult patients, for example, heightening blood pressure and heart rate (Baker, 1992; Morrison, Haas, Shaffner, Garrett, & Fackler, 2003; Novaes, Aronovich, Ferraz, & Knobel, 1997; Topf & Thompson, 2001). A recent study by Blomkvist et al. (in press, 2004) examined the effects of poor versus good sound levels and acoustics on coronary intensive-care patients by periodically changing the ceiling tiles from sound-reflecting to sound-absorbing tiles. When the sound-absorbing ceiling tiles were in place, patients slept better, were less stressed (lower sympathetic arousal), and reported that nurses gave them better care. There were also indications in this study that the incidence of re-hospitalization was lower if patients had experienced the sound-absorbing rather than sound-reflecting ceiling during their hospital stay (Hagerman et al., in press, 2004). More studies are needed such as that by Blomkvist et al. (in press, 2004), which use experimental research designs and systematically vary noise conditions. Future research should also investigate the effects of noise on re-hospitalization rates and other outcomes. In sum, the main message from the research review is clear: new hospitals should be much quieter, and effective design strategies for quieting hospitals are available.

Improve Sleep

The above section reviewed many studies showing that noise levels are high in hospitals and that noise is a major cause of poorer sleep in patients. Interventions that reduce hospital noise have been found to improve sleep and reduce patient stress. As noted earlier, environmental interventions found to be most effective for reducing noise in hospital settings include: providing single-bed rather than multi-bed rooms, installing high-performance, sound-absorbing ceiling tiles, using sound-absorbing flooring where possible, and eliminating or reducing noise sources (for example, use noiseless paging, locate alarms outside patient rooms).

Reduce Spatial Disorientation

Wayfinding problems in hospitals are costly and stressful and have particular impacts on outpatients and visitors, who are often unfamiliar with the hospital and are otherwise stressed and disoriented. In a study conducted at a major regional 604-bed tertiary-care hospital, the annual cost of the wayfinding system was calculated to be more than $220,000 per year in the main hospital or $448 per bed per year in 1990. Much of this cost was the hidden costs of direction giving by people other than information staff, which occupied more than 4,500 staff hours, the equivalent of more than two full-time positions (Zimring, 1990). While almost all hospitals strongly feel the problems associated with a complicated building and poor wayfinding system, it is usually difficult to tackle this problem with a piecemeal approach. A wayfinding system, as the name implies, is not just about better signage or colored lines on floors. Rather, hospitals are seeking to provide integrated systems that include coordinated elements such as visible and easy-to-understand signs and numbers, clear and consistent verbal directions, consistent and clear paper, mail-out and electronic information and a legible physical

setting (Carpman, 1993). A wayfinding system includes four main components that work at different levels: administrative and procedural levels, external building cues, local information and global structure

Administrative and procedural information: Mail-out maps, electronic information available on the Web and at kiosks and verbal directions are organizational strategies aimed at providing key information to patients to prepare them for their hospital visit. This is not dealt with in this review.

***External building cues*:** Signs and cues that lead to the hospital, especially the parking lot, need to be considered carefully, as they are the first point of contact of the patient with the hospital (Carpman, Grant, & Simmons, 1985). For example, Carpman, Grant and Simmons conducted a video simulation study to assess the relative role of signs and seeing a destination. The hospital wanted to direct most traffic to a parking structure rather than a drop-off lane. When the researchers showed prospective visitors a simulated video showing a design alternative that allow arriving drivers to see the main pavilion with the drop-off lane, 37 percent of the respondents said that they would turn into the drop circle when they could see the entry to the garage, ignoring the signs. As a consequence, the hospital chose to redesign the entry.

***Local information*:** Once patients find their way to the building from the parking lot, they are faced with the prospect of identifying the destination. Informational handouts, information desks, you-are here maps, directories, and signage along the way are critical wayfinding aids (Carpman, Grant, & Simmons, 1983-84; Levine, Marchon, & Hanley, 1984; Nelson-Shulman, 1983-84; Wright, Hull, & Lickorish, 1993). In an experimental study, researchers found that patients who had the benefit of an information system (welcome sign, hospital information booklet, patient letter, orientation aids) upon reaching the admitting area were more self-reliant and made fewer demands on staff. In contrast, uninformed patients rated the hospital less favorably and were found to have elevated heart rates (Nelson-Shulman, 1983-84).

Information provided in you-are-here maps can be useful. However, you-are-here maps should be oriented so that the top signifies the direction of movement for ease of use. When the maps were aligned in directions other than the forward position, people not only took much longer to find their destination, but were significantly less accurate (Levine et al., 1984). Another study found that people who used signs found their destination faster than those who only used maps (Butler, Acquino, Hissong, & Scott, 1993). However, people who were given a combination of handheld maps and wall signs reached their destination more often than those who just used wall signs (Wright et al., 1993).

It is critical to design signage systems with logical room numbering and comprehensible nomenclature for departments (Carpman & Grant, 1993; Carpman, Grant, & Simmons, 1984). For example, inpatients, outpatients, and visitors to a hospital preferred simple terms such as *walkway* or *general hospital* over more complex or less-familiar terms such as *overhead link, medical pavilion* or *health-sciences complex*.

Contrary to the belief that fewer signs in hospital hallways means less clutter and hence less confusion, an experimental study in a hospital found that patients who had access to more number of signs along the way to the destination were faster, less hesitant, asked for directions a fewer number of times, and reported lower levels of stress (Carpman et al., 1984). Based on this study, the authors suggest that directional signs should be placed at or before every major intersection, at major destinations, and where a single environmental cue or a series of such cues (e.g. change in flooring material) convey the message that the individual is moving from one area into another. If there are no key decision points along a route, signs should be placed approximately every 150 feet to 250 feet.

***Global structure*:** In addition to local properties of the spaces that people move through, there are specific characteristics of the overall structure of the system of rooms and corridors that impact the paths people take (Haq & Zimring, 2003; Peponis, Zimring, & Choi, 1990). People tend to move toward spaces and through corridors that are more accessible from a greater number of spaces. Based on observations of search patterns of study participants in a hospital and use of objective measures that quantify spatial characteristics, researchers found that participants tended to move along more "integrated" routes—routes that are, on average, more accessible because they are fewer turns from all other routes in the hospital. This research suggests that it may be important to identify such integrated routes in the plan while placing important facilities and key points such as the entrance (Peponis et al., 1990).

The research supports the value of a systems approach to wayfinding. Wayfinding continues to be a pervasive problem in hospitals because it is not sufficient to consider one or two components separately. Well-designed signs are likely to be quite ineffective in a building that is highly complicated and does not provide simple cues that enable natural movement. While there are more than 17 studies that look at wayfinding in hospitals and other buildings (Brown, Wright, & Brown, 1997; Carpman & Grant, 1993; Carpman et al., 1983-84, 1984; Carpman et al., 1985; Christensen, 1979; Grover, 1971; Haq & Zimring, 2003; Levine et al., 1984; Moeser, 1988; Nelson-Shulman, 1983-84; Ortega-Andeane & Urbina-Soria, 1988; Passini, Rainville, Marchand, & Joanette, 1995; Peponis et al., 1990; Schneider, L. F. & Taylor, 1999; Weisman, 1981; Wright et al., 1993; Zimring & Templer, 1983-84), it is quite difficult to isolate single influences of design on wayfinding performance or of wayfinding on outpatient or visitor stress. The problem is exacerbated by the fact that most hospitals have existing complex buildings upon which they try to superimpose a signage system to make things work. This strategy is ineffective in most cases.

There are some very good studies that deal with designing better signage, optimal spacing and location of signage, types of information that are most effective in way finding, and so on. Similarly, other studies at the global scale have looked at the properties of the building layout that facilitate or impede movement. It is essential that these different pieces of information come together while designing new hospitals where there is opportunity to develop an effective wayfinding system at multiple levels. Additional

studies are needed to ascertain the magnitude of stress that wayfinding problems have on outpatients and family.

Reduce Depression

Several studies strongly support that bright light—both natural and artificial—can improve health outcomes such as depression, agitation, sleep, circadian rest-activity rhythms, as well as length of stay in demented patients and persons with seasonal affective disorders (SAD). At least eleven strong studies suggest that bright light is effective in reducing depression among patients with bipolar disorder or SAD. Further, seven studies show that exposure to morning light is more effective than exposure to evening light in reducing depression (Beauchemin & Hays, 1996; Benedetti, F., Colombo, C., Barbini, B., Campori, E., & Smeraldi, E., 2001; Lewy et al., 1998; Lovell, Ancoli-Israel, & Gevirtz, 1995; Terman, Terman, Lo, & Cooper, 2001; Van Someren, Kessler, Mirmiran, & Swaab, 1997; Wallace-Guy et al., 2002). An experimental study that compared the effect of morning and evening light on patients with winter depression found that morning light was *twice* as effective as evening light in treating SAD (Lewy et al., 1998). Exposure to bright morning light has been shown to reduce agitation among elderly patients with dementia. When elderly patients with dementia were exposed to 2,500 lux for two hours in the morning for two ten-day periods, their agitation reduced. Patients were significantly more agitated on non-treatment days (Lovell et al., 1995).

There is also strong evidence that exposure to bright light improves sleep and circadian rhythms. When the daytime environmental illumination level was increased in different living spaces of a dementia unit, it was found that, during increased illumination periods, the stability of the rest-activity rhythm increased in patients with intact vision, but not in visually impaired patients (Van Someren et al., 1997).

It has also been shown that patients in brightly lit rooms have a shorter length of stay compared to patients in dull rooms. Beauchemin and Hays (1996) found that patients hospitalized for severe depression reduced their stays by an average of 3.67 days if assigned to a sunny rather than a dull room overlooking spaces in shadow.

A recent randomized prospective study assessed whether the amount of sunlight in a hospital room modifies a patient's psychosocial health, quantity of analgesic medication used, and pain medication cost (Walch et al., 2004, in press). Patients undergoing elective cervical and lumbar spinal surgeries were admitted to the bright or the dim side of the inpatient surgical ward postoperatively. The outcomes measured included the standard morphine equivalent of all opioid medication used postoperatively by patients and their subsequent pharmacy cost. This strong study found that patients exposed to an increased intensity of sunlight experienced less perceived stress, less pain, took 22 percent less analgesic medication per hour and had 20 percent less pain medication costs.

Using light as an intervention to reduce depression in clinically depressed as well as non-depressed patients is a relatively inexpensive intervention that has been shown to yield consistently positive results. While many studies deal with the effects of artificial light,

natural daylight in patient rooms has also been found effective in reducing depression, reducing length of stay, and reducing intake of pain medication. Thus, an important consideration while designing hospital layouts may be to optimize exposure to morning light in patient rooms by using an east-facing orientation. This research further implies the possibility that depression might be worsened by architectural designs that block or sharply reduce natural daylight in patient rooms. A hypothetical example would be a hospital having patient-room windows looking out into a roofed atrium with few skylights and little natural daylight. In this example, deprivation of natural daylight could be extreme if patient windows were tinted to prevent users of the atrium from looking into patient rooms and violating privacy.

Provide Nature and Positive Distraction

Positive distractions refer to a small set of environmental features or conditions that have been found by research to effectively reduce stress. Distractions can include certain types of music, companion animals such as dogs or cats, laughter or comedy, certain art, and especially nature (Ulrich, 1991). The focus here is on the last, nature. (There is an additional large research literature on music, but this is not covered in this review.)

As background relevant to assessing the credibility of nature findings in healthcare environments, it should be mentioned that many studies of populations other than hospital patients have produced strong evidence that even fairly brief encounters with real or simulated nature settings can elicit significant recovery from stress within three minutes to five minutes at most (Parsons & Hartig, 2000; Ulrich, 1999). Investigators have consistently reported that stress-reducing or restorative benefits of simply viewing nature are manifested as a constellation of positive emotional and physiological changes. Stressful or negative emotions such as fear or anger diminish while levels of pleasant feelings increase. Laboratory and clinical studies have shown that viewing nature produces stress recovery quickly evident in physiological changes, for instance, in blood pressure and heart activity (Ulrich, 1991). By comparison, considerable research has demonstrated that looking at built scenes lacking nature (rooms, buildings, parking lots) is significantly less effective in fostering restoration and may worsen stress.

Questionnaire studies have found that bedridden patients assign especially high preference to having a hospital window view of nature (Verderber, 1986). Mounting research is providing convincing evidence that visual exposure to nature improves outcomes such as stress and pain. For example, a study in a Swedish hospital found that heart-surgery patients in ICUs who were assigned a picture with a landscape scene with trees and water reported less anxiety/stress and needed fewer strong doses of pain drugs than a control group assigned no pictures (Ulrich, 1991). Another group of patients assigned an abstract picture, however, had worsened outcomes compared to the control group. Ulrich (1984) found that patients recovering from abdominal surgery recovered faster, had better emotional well-being, and required fewer strong pain medications if they had bedside windows with a nature view (looking out onto trees) than if their windows looked out onto a brick wall.

Recently, strong studies using experimental designs have produced additional convincing evidence that viewing nature reduces patient pain as well as stress. These investigations also support the interpretation that nature serves as a positive distraction (Ulrich, 1991) that reduces stress and diverts patients from focusing on their pain or distress. A randomized prospective investigation found that adult patients undergoing a painful bronchosopy procedure reported less pain if they were assigned to look at a ceiling-mounted nature scene rather than a control condition consisting of a blank ceiling (Diette, Lechtzin, Haponik, Devrotes, & Rubin, 2003). Another controlled experiment that used volunteers in a hospital assessed the effect on pain of viewing a soundless nature videotape in contrast to a static blank screen (Tse, Ng, Chung, & Wong, 2002). Subjects who watched the nature scenes evidenced a higher threshold for detecting pain and had substantially greater pain tolerance. Two studies of female cancer patients have shown that taking a virtual reality nature walk while in bed or a hospital room (through a forest with bird sounds) reduced anxiety and symptomatic distress (Schneider, S. M., Prince-Paul, Allen, Silverman, & Talaba, 2004). Research on patients suffering intense pain because of severe burns found that exposing patients to a videotape of scenic nature (forest, flowers, ocean, waterfalls) during burn dressing changes significantly reduced both anxiety and pain intensity (Miller, Hickman, & Lemasters, 1992).

The possibility that nature can improve outcomes even in patients with late-stage dementia, including Alzheimer's disease, has received some support from a quasi-experimental study that found reduced levels of agitated aggressive behavior associated with a shower bath when recorded nature sounds (birds, babbling brook) and color pictures were present (Whall et al., 1997). A well-controlled study of blood donors in a waiting room found that blood pressure and pulse were lower on days when a wall-mounted television displayed a nature videotape, compared to days with continuous daytime television programs (Ulrich, Simons, & Miles, 2003). More research is needed to identify conditions under which television can either be a stress-reducing positive distraction or a stressor in hospitals.

Gardens in healthcare environments*:* Hospital gardens not only provide restorative or calming nature views, but can also reduce stress and improve outcomes through other mechanisms, for instance, fostering access to social support and providing opportunities for positive escape and sense of control with respect to stressful clinical settings (Cooper Marcus & Barnes, 1995; Ulrich, 1999). Based on postoccupancy evaluations of four hospital gardens in California, Cooper-Marcus and Barnes (1995) concluded that many nurses and other healthcare workers used the gardens for achieving pleasant escape and recuperation from stress. Other postoccupancy studies indicate that patients and family who use hospital gardens report positive mood change and reduced stress (Whitehouse et al., 2001). These reports also suggest that gardens and nature in hospitals can heighten patient and family satisfaction with overall quality of care.

Art in healthcare environments: A small number of studies on art in hospitals has yielded findings parallel to those from nature research. Results suggest a consistent pattern wherein the great majority of patients respond positively to representational

nature art, but many react negatively to chaotic abstract art (Ulrich & Gilpin, 2003). For example, Carpman & Grant (1993) studied the preferences of 300 randomly selected inpatients and concluded that the patients consistently preferred nature images but disliked abstract art. Although nature pictures and other emotionally appropriate art elicit positive reactions, there is also evidence that inappropriate art styles or image subject matter can increase stress and worsen other outcomes (Ulrich, 1991). It should not be expected that all art is suitable for high-stress healthcare spaces, as art varies enormously in subject matter and style, and much art is emotionally challenging or provocative.

The pitfalls of displaying emotionally challenging art in healthcare environments are revealed by a study of psychiatric patients (Ulrich, 1991). The unit was extensively furnished with a diverse collection of wall-mounted paintings and prints. Interviews with patients indicated strongly negative reactions to artworks that were ambiguous, surreal, or could be interpreted in multiple ways. The same patients, however, reported having positive feelings and associations with respect to nature paintings and prints.

Provide Social Support

Many studies of several different categories of patients have indicated that social support reduces stress and improves, for example, recovery outcomes in myocardial infarction patients. Considering the well-established importance of social support, it is unfortunate that there is only a moderate amount of research concerning how hospital design can facilitate or hinder access to social support. Most studies have focused on psychiatric units and nursing homes. There is strong evidence that levels of social interaction can be increased—and presumably beneficial social support as well—by providing lounges, day rooms, and waiting rooms with comfortable movable furniture arranged in small flexible groupings. A few well-designed studies in psychiatric wards and nursing homes have found that appropriate arrangement of movable seating in dining areas enhances social interaction and also improves eating behaviors, such as increasing the amount of food consumed by geriatric patients (Melin & Gotestam, 1981; Peterson, Knapp, Rosen, & al., 1977). Much research on day rooms and waiting areas has shown that the widespread practice of arranging seating side-by-side along room walls inhibits social interaction (Holahan, 1972; Sommer & Ross, 1958). A novel study by Harris (2000) found that family and friends stayed substantially longer during visits to rehabilitation patients when patient rooms were carpeted rather than covered with vinyl flooring.

Much evidence indicates that single rooms are markedly better than multi-bed rooms for supporting or accommodating the presence of family and friends. Some research suggests that open-plan multibed rooms deter family presence and accordingly reduce social support (Sallstrom, Sandman, & Norberg, 1987). Multibed rooms greatly reduce privacy for patient-family interactions compared to single rooms and are much more likely to have restricted visiting hours. A clear advantage of single rooms in fostering social support stems from the fact they provide more space and furniture than double rooms to accommodate family presence (Chaudhury et al., 2003). A survey of staff in four hospitals that each had a mix of single and double rooms found that nurses gave high

ratings to single rooms for accommodating family members but accorded double rooms low scores (Chaudhury et al., 2003). Further, patient-satisfaction data obtained from 2,122,439 patients who received care during 2003 provide overwhelming evidence that patients in single-bed rooms, compared to those with a roommate, are much more satisfied with "accommodations and comfort for family and visitors" (Press Ganey, 2003).

Do patients sharing the same room provide each other with stress-reducing social support? While some patients find roommates provide comforting social support, findings from several studies indicate that the presence of a roommate usually is a source of stress rather than social support. In most cases, roommates are linked to stressors, for example, loss of privacy or having a roommate who is unfriendly, has too many visitors, or is seriously ill (Van der Ploeg, 1988; Volicer, Isenberg, & Burns, 1977). An earlier section emphasized that noise is a much greater problem in double rooms than singles, and that noises stemming from the presence of other patients are a major cause of sleep loss.

Improve Communication to Patients

Good staff communication helps reduce patient and family anxiety, promotes better care at home after discharge, and in other ways can improve outcomes. Good communication also tends to be the single most important factor affecting overall satisfaction with care across different patient categories (Press Ganey, 2003). Data obtained from 2,122,439 patients nationally in 2003 show that patients consistently report significantly higher satisfaction with communication from nurses and physicians when they are in single rooms compared to when they have one or more roommates (Press Ganey, 2003). To explain this clear and important advantage of single rooms, Kaldenburg (1999) has proposed that staff in multibed rooms are reluctant to discuss patient issues or give information within hearing of a roommate, out of respect for privacy. Growing concern for patient confidentiality and HIPAA are certain to increase the already major advantages of single rooms with respect to communication.

IV. Improve Overall Healthcare Quality

Provide Single-Bed Patient Rooms

Based on an extremely large and varied body of research reviewed in earlier sections, there can be no question that single-bed rooms have several major advantages over double rooms and open bays. To summarize briefly, these advantages include: lower nosocomial infection rates, fewer patient transfers and associated medical errors, far less noise, much better patient privacy and confidentiality, better communication from staff to patients and from patients to staff, superior accommodation of family and consistently higher satisfaction with overall quality of care.

Reduce Length of Stay

Climate and sunlight influences length of hospital stay as well as sleep-wake patterns among hospitalized patients (Beauchemin & Hays, 1996; Benedetti, Francesco, Colombo, Cristina, Barbini, Barbara, Campori, Euridice, & Smeraldi, Enrico, 2001; Federman, Drebing, Boisvert, Penk, 2000; Hebert, Dumont, & Paquet, 1998; Kecskes et al., 2003; Kinnunen, Saynajakangs, Tuuponen, & Keistinen, 2002). One research group studied the impact of the amount of natural light on the length of hospitalization of patients with unipolar and bipolar disorder. The researchers found that bipolar patients randomly assigned to the brighter, eastern rooms (exposed to direct sunlight in the morning) had a mean 3.67-day shorter hospital stay than patients in west-facing rooms (Benedetti, Francesco et al., 2001). Patients recovering from abdominal surgery had shorter stays if they had a bedside window view of nature rather than if their windows looked out onto a brick wall (Ulrich, 1984).

The large research literature on infection reviewed in an earlier section indicated that the design of the physical environment strongly impacts hospital-acquired infection rates by affecting both airborne and contact transmission routes. Evidence-based design measures, by reducing nosocomial infection rates, play a key role in shortening hospital stays.

Increase Patient Satisfaction with Quality of Care

There is strong evidence that design changes that make the environment more comfortable, aesthetically pleasing, and informative relieve stress among patients and increases satisfaction with the quality of care provided. Renovating a traditional waiting area in a neurology clinic by making small changes to the general layout, color scheme, furniture, floor covering, curtains, and providing informational material and information displays resulted in more positive environmental appraisals, improved mood, altered physiological state, and greater reported satisfaction among waiting patients (Leather, Beale, Santos, Watts, & Lee, 2003).

Patients in well-decorated and well-appointed hotel-like rooms rated their attending physicians, housekeeping, and food-service staff, the food, and the hospital better than patients in standard rooms (typical hospital beds, inexpensive family sitting chairs, and no artwork) in the same hospital. Also, they had stronger intentions to use the hospital again and would recommend the hospital to others (Swan, Richardson, & Hutton, 2003). In another study, it was found that environmental satisfaction was a significant predictor of overall satisfaction, ranking only below perceived quality of nursing and clinical care (Harris, P. B., McBride, Ross, & Curtis, 2002). Several post-occupancy evaluation studies have examined patient and staff satisfaction with the different elements of the healthcare environment such as gardens, individual wards, and patient rooms (Brown, Wright, & Brown, 1997; Heath & Gifford, 2001; Shepley, 1995, 2002; Shepley & Davies, 2003; Shepley & Wilson, 1999; Whitehouse et al., 2001). These studies yield rich context-specific data that describe which aspects of the environment were effective. Some studies show quite clearly that tacit, as well implicit, staff and organizational practices and policies influence how an environment actually functions and is perceived

by patients and staff members. The qualitative and quantitative data provided by such studies are very important in understanding the nature of the problem (e.g. why are the gardens not being used as intended?) as well in developing solutions to tackle the problem. Future research that looks at satisfaction among hospital patients should consider using these multi-method post-occupancy evaluations that use different methods to obtain objective and subjective evaluations of use and satisfaction.

CONCLUSIONS

The research team found more than 600 rigorous studies linking a range of aspects of the built environment of hospitals to staff stress and effectiveness, patient safety, patient and family stress and healing, and improved overall healthcare quality and cost. This deep and wide base of evidence suggests that, parallel to evidence-based medicine, we can move to evidence-based design (EBD). EBD refers to a process for creating healthcare buildings, informed by the best available evidence, with the goal of improving outcomes and of continuing to monitor the success of designs for subsequent decision-making. While it is difficult to conduct rigorous research on the impacts of the healthcare environment—hospitals are complex systems where it is difficult to isolate the impact of single factors and the building industry conducts little impact-based research—The Center for Health Design and other groups have made considerable progress in developing a knowledge base of evidence.

EBD is not about hospitals that are simply nicer or fancier than traditional hospitals. Rather, the focus of evidence-based design is to create hospitals that actually help patients recover and be safer, and help staff do their jobs better. EBD is a process for creating health care buildings informed by the best available evidence concerning how the physical environment can interfere with or support activities by patients, families, and staff, and how the setting provides experiences that provide a caring, effective, safe, patient-centered environment. Many of the improvements suggested by EBD are only slightly more expensive than traditional solutions, if they are more expensive at all.

The large research literature surveyed in this report point to several actions we can take immediately:

- Provide single-bed rooms in almost all situations. Adaptable-acuity single-bed rooms should be widely adopted. Single rooms have been shown to lower hospital-induced nosocomial infections, reduce room transfers and associated medical errors, greatly lessen noise, improve patient confidentiality and privacy, facilitate social support by families, improve staff communication to patients, and increase patients' overall satisfaction with health care.
- New hospitals should be much quieter to reduce stress and improve sleep and other outcomes. Noise levels will be substantially lowered by the following combination of environmental interventions: providing single-bed rooms, installing high-performance sound-absorbing ceilings, and eliminating noise sources (for example, using noiseless paging).

- Provide patients stress reducing views of nature and other positive distractions.
- Develop wayfinding systems that allow users, and particularly outpatients and visitors, to find their way efficiently and with little stress.
- Improve ventilation through the use of improved filters, attention to appropriate pressurization, and special vigilance during construction.
- Improve lighting, especially access to natural lighting and full-spectrum lighting.
- Design ward layouts and nurses stations to reduce staff walking and fatigue, increase patient care time, and support staff activities such as medication supply, communication, charting, and respite from stress.

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Bloodstream Infections

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ABSTRACT Bacteremia and sepsis are conditions associated with high mortality and are of great impact to health care operations. Among the top causes of mortality in the United States, these conditions cause over 600 fatalities each day. Empiric, broad-spectrum treatment is a common but often a costly approach that may fail to effectively target the correct microbe, may inadvertently harm patients via antimicrobial toxicity or downstream antimicrobial resistance. To meet the diagnostic challenges of bacteremia and sepsis, laboratories must understand the complexity of diagnosing and treating septic patients, in order to focus on creating algorithms that can help direct a more targeted approach to antimicrobial therapy and synergize with existing clinical practices defined in new Surviving Sepsis Guidelines. Significant advances have been made in improving blood culture media; as yet no molecular or antigen-based method has proven superior for the detection of bacteremia in terms of limit of detection. Several methods for rapid molecular identification of pathogens from blood cultures bottles are available and many more are on the diagnostic horizon. Ultimately, early intervention by molecular detection of bacteria and fungi directly from whole blood could provide the most patient benefit and contribute to tailored antibiotic coverage of the patient early on in the course of the disease. Although blood cultures remain as the best means of diagnosing bacteremia and candidemia, complementary testing with antigen tests, microbiologic investigations from other body sites, and histopathology can often aid in the diagnosis of disseminated disease, and application of emerging nucleic acid test methods and other new technology may greatly impact our ability to bacteremic and septic patients, particularly those who are immunocompromised.

INTRODUCTION

Bloodstream infections (BSIs) and sepsis rank among the top reasons for human mortality for hospitalized patients. The full breadth of sepsis syndromes are particularly dangerous to any immunocompromised patient. Sepsis is one of the oldest of medical syndromes and dates back to the time in which Hippocrates characterized it as a clinical syndrome (1). Worldwide estimates of sepsis prevalence exceed 19 million cases per year, with over 750,000 in the United States (2). While only 2% of patients are admitted to the hospital with severe sepsis, they represent at least 10% of all ICU admissions in the U.S. (3).

In this article, we will review the human impact of BSIs in the immunocompromised host, as well as standard definitions of clinical criteria, symptoms, predisposing factors linked to the development of bacteremia and fungemia, and common modes of bacterial pathogenesis. We will review current literature to describe the presence of nonculturable BSIs, such as those caused by some viruses, parasites, and other nonculturable microbes. We will also review key aspects of the Surviving Sepsis Campaign guidelines (4) and approaches for diagnosis, therapy, and resuscitation from the septic event. Finally, we will highlight key laboratory roles and responsibilities to support the diagnosis of sepsis as part of today's interdisciplinary health care teams, detail the new Laboratory Medicine Best Practice guidelines for blood culture collection (5), and summarize the meta-analysis describing the impact of rapid diagnostics that identify bloodstreams pathogens (6). We will show that expansion of the laboratory's role, rapid diagnostics, and understanding of the complexities related to diagnosing and treating sepsis can not only improve patient survival, but also can limit the financial impact of sepsis on health care systems worldwide.

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PATHOPHYSIOLOGY OF INFECTIONS

Pathophysiology of Sepsis

Due to the widespread immune response (7) when the human immune system is faced with infectious agents in the bloodstream, symptoms are widely variable and include fever, chills, hypotension, hypothermia (especially in the elderly), diaphoresis, apprehension, change in mental status, tachypnea, tachycardia, hyperventilation, reduced vascular tone, and the possibility of organ dysfunction. Hematologic findings include neutrophilic leukocytosis, thrombocytopenia, toxic granulations of neutrophils, or disseminated intravascular coagulation. Other metabolic findings include respiratory alkalosis; renal signs, such as acute tubular necrosis, oliguria or anuria; gastrointestinal signs such as upper gastrointestinal bleeding; cholestatic jaundice, increased transaminase levels, or hypoglycemia.

Despite modern advances, sepsis is one of the leading causes of death worldwide (8). If left untreated, sepsis can transform to septic shock (sepsis complicated by either hypotension that is refractory to fluid resuscitation or by hyperlactatemia), and eventually to organ dysfunction and death (3). There are several consensus definitions that define sepsis. For all conditions, the diagnosis primarily relies upon clinical recognition of symptoms, later confirmed by laboratory testing (4).

1. Bacteremia is defined as the presence of viable bacteria in the bloodstream.
2. Systemic inflammatory response syndrome is a systemic inflammatory response to bodily harm (e.g., from infection, burns, or trauma) that requires some of the following conditions:
   a. Fever or hypothermia
   b. Tachycardia
   c. Tachypnea
   d. Leukocytosis or leukopenia
   e. Elevated C-reactive protein
   f. Elevated plasma procalcitonin
   g. Altered mental status
   h. Significant edema or positive fluid balance
   i. Hyperglycemia
   j. Arterial hypotension
   k. Organ dysfunction
   l. Hyperlactatemia
   m. Decreased capillary filling or mottling
   Nonspecific causes of inflammation can cause systemic inflammatory response syndrome and must be considered in assessment of patients who present with inflammatory symptoms.
3. Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of infection. Sepsis includes organ dysfunction (i.e., low blood pressure, reduced urine output, intestinal paralysis, reduced circulation, increased creatinine, reduced oxygen in the blood, elevated lactate, elevated bilirubin, abnormal coagulation, or metabolic acidosis).
4. Septic shock is a condition in which the patient exhibits refractory arterial hypotension or hypoperfusion despite adequate intravascular fluid resuscitation. Hypoperfusion may be manifested as lactic acidosis, oliguria, or mental status changes.

Management of sepsis and septic shock is generally driven by clinical care bundles, detailed in the International Surviving Sepsis Campaign (4). Early recognition of the disease is essential, and treatment includes stabilization of blood pressure (early fluid management and vasopressors) and treatment of infection (immediate administration of antibiotics), driven by evidence that for every hour a septic patient is not appropriately treated, mortality increases by approximately 7% (9, 10). Ideally, blood cultures are obtained from the patient prior to the administration of antimicrobials, and empiric therapy with broad-spectrum antimicrobial agents is initiated within the first hour of diagnosis.

Along with administration of antimicrobial therapy, attention is keenly focused on identifying and treating any ongoing foci of infections and/or reactivation of latent infections, a process known as source control. Identifying the source of the infection (urinary tract, respiratory, wound, etc.) is crucial to proper management. Control measures are meant to reduce or eliminate the source of infection, such as an infected device (e.g., hardware, catheters, lines, etc.), removal or drainage of an abscess, or debridement of infected tissue. Once the source of the infection is identified, microbial cultures can be obtained and targeted therapy can be administered based on the culture results. Other key definitions related to BSIs include the following:

- **Endocarditis** is an infection of a natural or prosthetic heart valve.
- **Nosocomial BSI** is defined as a BSI occurring >48 h following admission to hospital or <48 h if the patient has been hospitalized within the previous 2 weeks (11).
- **Community-acquired BSIs** are present on admission (POA).

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- **Endogenous asymptomatic bacteremia** (candidemia, fungemia) represents bacteremia that originates inside the host but that is not yet causing classic symptoms of BSI.
- **Acute bacteremia** (candidemia, fungemia) indicates the presence of microbes in the bloodstream with concomitant symptoms of bacteremia.
- **Transient bacteremia** represents sporadic and commonly asymptomatic bacteremia that is thought to occur under rare but normal circumstances. It occurs in a wide variety of procedures and manipulations, particularly those associated with mucous membrane trauma. It may also occur with such daily functions as tooth brushing and bowel movements. These intermittent and brief forms of bacteremia are especially common in tooth extraction and other dental procedures and can occasionally give rise to infective endocarditis in the susceptible patient.
- **Persistent bacteremia** is defined as bacteremia sustained for 3 days or longer, contributing to many *Staphylococcus aureus* bacteremia episodes and increasingly recognized among hospitalized patients.

Several scoring systems are commonly used to assess the severity and prognosis of a septic event. The Sequential Organ Failure Assessment (SOFA) score predicts intensive care unit (ICU) mortality based on laboratory results and clinical data. The Acute Physiological Assessment and Chronic Health Evaluation Score (Apache II Score) estimates ICU mortality based on laboratory values and patient signs, taking both acute and chronic disease into account. The use of the new "quickSOFA" (qSOFA) scores is described in the "Risk Factors and Outcomes" section of this article.

The Systemic Inflammatory Response Syndromes and Sepsis

BSIs can lead to sepsis, in the following manner. At onset, a bacterial cell triggers the host immune response. For Gram-negative pathogens, the bacterial endotoxin, a lipopolysaccharide from the cell wall, initiates the human inflammatory response. For Gram-positive pathogens, lipoteichoic acid, peptidoglycan, and extracellular products (toxins) trigger the response. An inflammatory response follows, mounting vascular, cellular, and chemical responses designed to stop injury with edema, which dilutes toxins, and with phagocytosis, which removes bacteria and cell debris.

The Role of the Human Immune Response in Sepsis

Classically immunocompromised patients have high mortality and can present with a wide variety of offending pathogens and common or unusual infections. While immunocompromised patients may sometimes die of infection due to their lack of ability to mount an effective immune response, in an immunocompetent host, the immune response is actually implicated in the disease symptoms; therefore, patient survival is dependent on whether the patient immune response to microbial pathogens is sufficient and can be ultimately balanced to regain immune homeostasis (12).

The pathogenesis of sepsis involves proinflammatory mediators, anti-inflammatory mediators, and vasoinflammatory mediators. There are several important inflammatory responses, which are linked to several different human cells that respond to bacterial invasion: 1) phagocytes (monocytes/macrophages, neutrophils, eosinophils); 2) mast cells, which are induced by lipopolysaccharide and complement (C3a and C5a) to release immune mediators; and 3) natural killer cells, which cause lysis of target cells and production of cytokines, like gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), in a process called cell-mediated cytotoxicity. Other physiological changes include reduced protein C activity, microplugging of vessels, cellular necrosis (ischemic injury), the inhibition of fibrinolysis, apoptosis, leukocyte-mediated tissue injury, endothelial dysfunction, and cytopathic hypoxia (12).

Sepsis is a complicated and variable condition and has a prolonged host immune response. During the immune response, human proinflammatory responses, aimed at eliminating pathogens, combine with the anti-inflammatory responses, aimed at limiting tissue injury. Unfortunately, the same immune responses contribute to concurrent organ injury and susceptibility to secondary infections, respectively (12), and can vary widely during the clinical course of the disease. The immune response depends on various factors, including the causative pathogen (microbial density and relative virulence), the host (genetic characteristics, immunologic condition, comorbidities, and other concurrent infection), and the therapeutic regimen(s) (Fig. 1).

The Host's Innate Immune System

As part of the host's innate immunity, pathogens activate immune cells by interacting with pattern-recognition receptors, which recognize pathogen-associated molecular patterns that are conserved among microbial species. Binding of pathogen-associated molecular patterns

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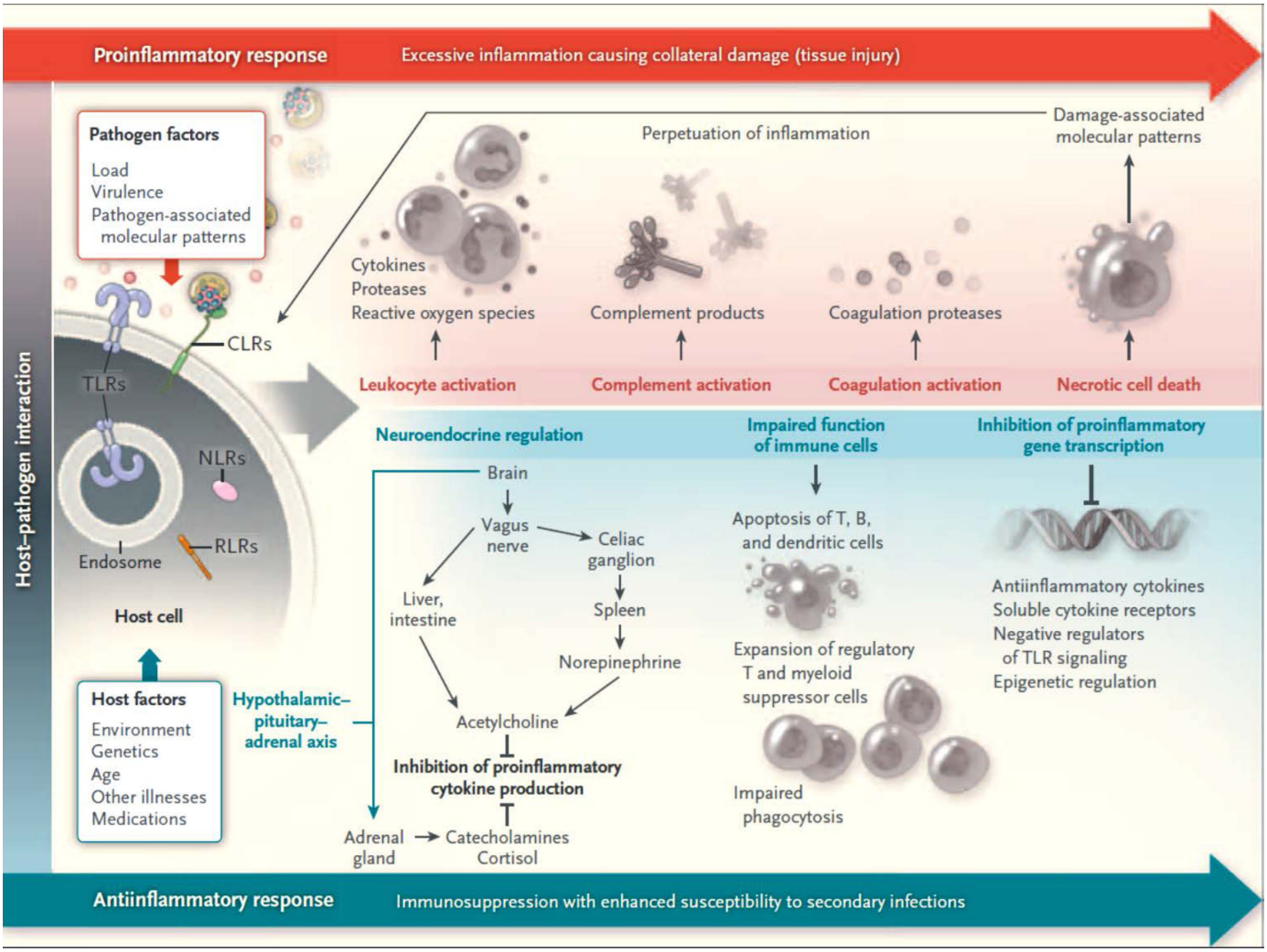

FIGURE 1 The host response in severe sepsis. The host response to sepsis is characterized by both proinflammatory responses (top of panel, in red) and anti-inflammatory immunosuppressive responses (bottom of panel, in blue). The direction, extent, and duration of these reactions are determined by both host factors (e.g., genetic characteristics, age, coexisting illnesses, and medications) and pathogen factors (e.g., microbial load and virulence). Inflammatory responses are initiated by interaction between pathogen-associated molecular patterns expressed by pathogens and pattern-recognition receptors expressed by host cells at the cell surface (toll-like receptors [TLRs] and C-type lectin receptors [CLRs]), in the endosome (TLRs) or in the cytoplasm (retinoic acid inducible gene 1-like receptors [RLRs] and nucleotide-binding oligomerization domain-like receptors [NLRs]). The consequence of exaggerated inflammation is collateral tissue damage and necrotic cell death, which results in the release of damage-associated molecular patterns, so-called danger molecules that perpetuate inflammation at least in part by acting on the same pattern-recognition receptors that are triggered by pathogens. Reprinted from reference (3), with permission.

to the human pattern-recognition receptors results in the upregulation of inflammatory gene transcription and initiation of the innate immune system. Human pattern-recognition receptors are categorized into four main classes: toll-like receptors, C-type lectin receptors, retinoic acid-inducible gene 1-like receptors, and nucleotide-binding oligomerization domain-like receptors, which partially act in protein complexes called inflammasomes (Fig. 1) (3, 13). The receptors are also capable of sensing molecules called damage-associated molecular patterns or alarmins, which are released from injured cells. Examples of alarmins include the high-mobility-group

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protein B1, S100 proteins, as well as extracellular RNA, DNA, and histones (3). Since alarmins are also released during trauma and other sterile tissue injury, the prevailing concepts that describe the pathogenesis of multiple organ failure in sepsis are not intrinsically different from those found in some noninfectious critical illnesses (3).

Organ Dysfunction

Although reduced oxygen supply to human organs is clearly an important contributor to tissue damage, inflammation can also cause dysfunction of the vascular endothelium and can contribute to cell death and loss of cell integrity, giving rise to subcutaneous and body-cavity edema (14). In parallel, injured mitochondria release alarmins, which include mitochondrial DNA and formyl peptides that can activate neutrophils and cause further tissue injury (15). Oxidative stress also damages mitochondria, and other mechanisms impair cellular oxygen use. Mechanisms that contribute to oxygen reduction include hypotension, reduced red-cell deformability, and microvascular thrombosis (16).

Coagulation Abnormalities Related to Tissue Injury

Four subtypes of protease-activated receptors (PARs) form a link between inflammation and coagulation. PAR1 is particularly important; it exerts cytoprotective effects when stimulated by activated protein C or low-dose thrombin, but it exerts disruptive effects on endothelial-cell barrier function when activated by high-dose thrombin (17, 18). Because of these interactions, human coagulation is commonly altered during sepsis (3) (Fig. 2), and the disruption can lead to disseminated intravascular coagulation. In disseminated intravascular coagulation, the coagulation cascade drives excess fibrin deposition through depression of the fibrinolytic system, which triggers fibrin removal, and via activation of a transmembrane glycoprotein, which is expressed by various cell types because of impaired anticoagulant mechanisms (including the protein C system and antithrombin) (18).

Immunosuppression and Its Association with Anti-inflammatory Processes

The immune system is composed of humoral, cellular, and neuroinflammatory mechanisms that mitigate the harmful effects of the human proinflammatory cytokines and the proinflammatory response (Fig. 1). Phagocytes can switch to an anti-inflammatory phenotype, combining with regulatory T cells, myeloid-derived suppressor cells, and neural mechanisms to promote tissue repair and reduction of inflammation (3, 12, 19). By nature of the septic event itself, patients who survive early sepsis but remain under intensive care have evidence of immunosuppression, partly from reduced expression of HLA-DR on myeloid cells (20). Reduced responsiveness of leukocytes, functional impairment of splenocytes, and immunosuppression in the spleen and lungs are all associated with sepsis (12, 20). In addition, epigenetic regulation of gene expression may also contribute to sepsis-associated immunosuppression (21) and apoptosis, especially of B cells, $CD4^+$ T cells, and follicular dendritic cells. Sepsis-associated immunosuppression has been implicated in sepsis-associated immunosuppression and death (22, 23).

Agents of Bloodstream Infections

In an immunocompromised host, the spectrum of microbes that cause BSIs has changed over the last decade, shifting from Gram-negative to Gram-positive organisms (24). Reasons for the change can be found in recent changes in clinical practice that include antimicrobial therapy, changes to immunosuppressive or chemotherapeutic regimens, and significant increases in the use of long-term indwelling devices. In addition, emerging antimicrobial resistance impacts the epidemiology of BSIs found in immunocompromised hosts (24).

Historically, patients with hematologic malignancies and febrile granulocytopenia constituted the majority of immunocompromised hosts (24, 25); however, there are currently many other types of immunocompromised hosts, including those with hematopoietic stem cell and solid organ transplants, as well as a wide variety of other immunosuppressive states, detailed later in this article. Today, many of these patients have concurrent intravascular catheters and are besieged with Gram-positive microorganisms, especially staphylococci, which have become the most frequent cause of BSIs. Additionally, BSIs caused by nontuberculous mycobacteria (NTM) and non-*Candida* yeasts are on the rise. Unfortunately, blood cultures are not a fully reliable diagnostic test—in some populations, only one-third of sepsis cases result in positive blood cultures (3).

Gram-positive and Gram-negative bacteria

Severe sepsis can be the result of either community-acquired or health care-associated infections, but it can also follow dissemination of primary infections. Primary infections such as pneumonia, urinary tract, and intra-abdominal infections are often the source of BSIs and can lead to sepsis and septic shock (8). Common causative bacterial agents of BSIs include *Staphylococcus*

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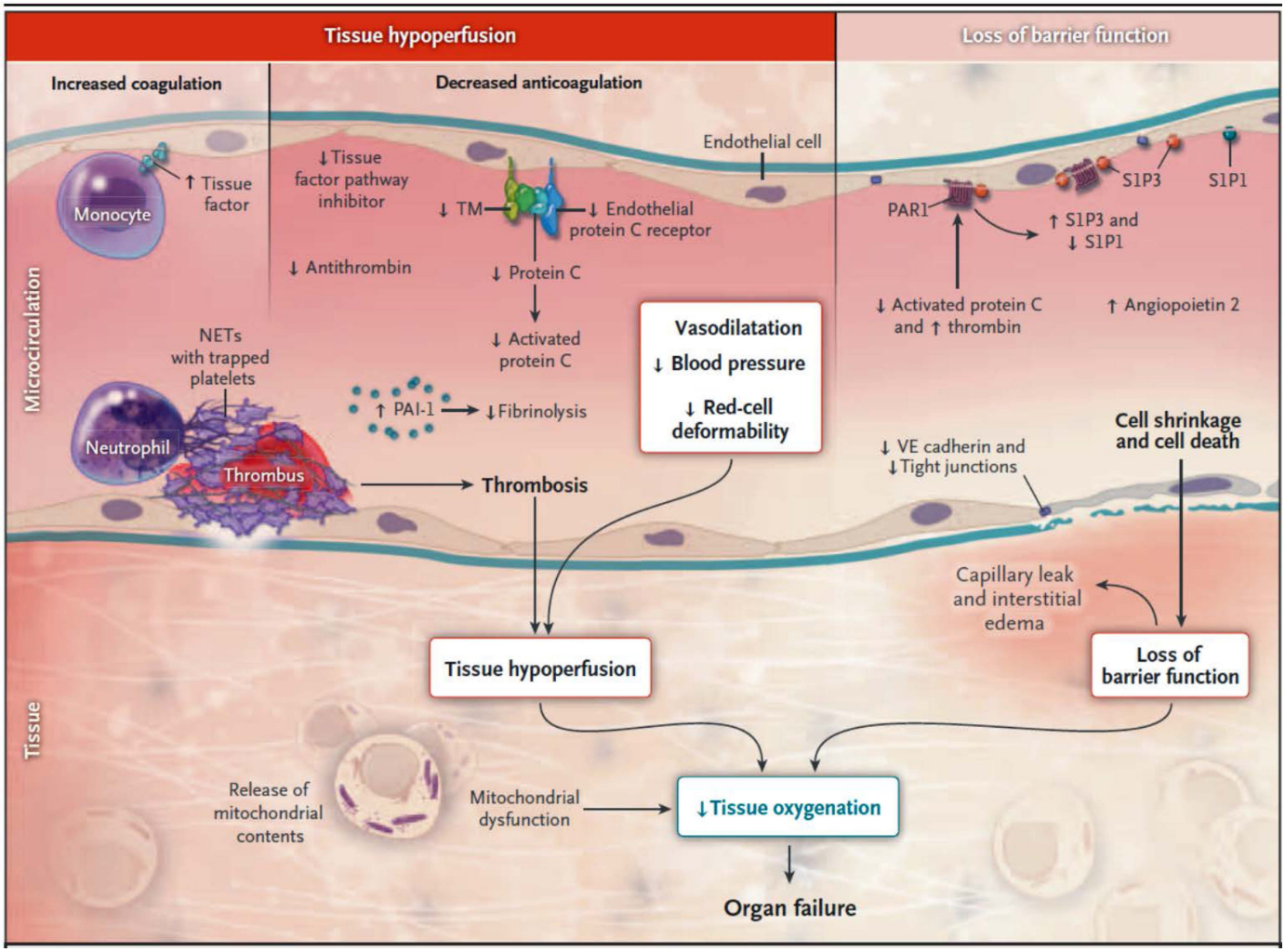

FIGURE 2 Organ failure in severe sepsis and dysfunction of the vascular endothelium and mitochondria. Sepsis is associated with microvascular thrombosis caused by concurrent activation of coagulation (mediated by tissue factor) and impairment of anticoagulant mechanisms as a consequence of reduced activity of endogenous anticoagulant pathways (mediated by activated protein C, antithrombin, and tissue factor pathway inhibitor), plus impaired fibrinolysis owing to enhanced release of plasminogen activator inhibitor type 1 (PAI-1). The capacity to generate activated protein C is impaired at least in part by reduced expression of two endothelial receptors: thrombomodulin (TM) and the endothelial protein C receptor. Thrombus formation is further facilitated by neutrophil extracellular traps (NETs) released from dying neutrophils. Thrombus formation results in tissue hypoperfusion, which is aggravated by vasodilatation, hypotension, and reduced red-cell deformability. Tissue oxygenation is further impaired by the loss of barrier function of the endothelium owing to a loss of function of vascular endothelial (VE) cadherin, alterations in endothelial cell-to-cell tight junctions, high levels of angiopoietin 2, and a disturbed balance between sphingosine-1 phosphate receptor 1 (S1P1) and S1P3 within the vascular wall, which is at least in part due to preferential induction of S1P3 through protease-activated receptor 1 (PAR1) as a result of a reduced ratio of activated protein C to thrombin. Oxygen use is impaired at the subcellular level because of damage to mitochondria from oxidative stress. Reprinted from reference (3), with permission.

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aureus, *Staphylococcus epidermidis*, *Enterococcus* spp., *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* spp.

Not only does the range of disease and pathogens vary greatly, but the way in which an individual responds to sepsis also depends on the immune condition of the host and on the pathogenic characteristics (virulence factors and bacterial load). For example, the absence of specific host immune responses such as B-cell depletion from rituximab or complement deficiency in systemic lupus erythematosus may predispose a patient to a BSI. In many instances, the underlying reason for immunosuppression provides clues to the type of microorganism causing infection. For example, a complement-depleted patient with systemic lupus erythematosus who presents with sudden onset of septic shock is at increased risk for a bloodstream infection with *Neisseria meningitidis* (26). Similarly, unusual pathogens such as *Vibrio vulnificus* may cause BSIs in patients with severe liver disease or liver transplantation. When anatomic barriers such as skin or mucosa are compromised from graft-versus-host disease or cytotoxic chemotherapy, patients are more likely to become infected with microorganisms colonizing the skin or oropharyngeal and gastrointestinal tracts. The recovery of *Leptotrichia* spp. or *Capnocytophaga* spp. from blood culture may suggest disruption of the mucosal integrity from the oropharynx or gastrointestinal tract in a granulocytopenic patient with severe mucositis.

Anaerobic bacteria

Anaerobic bacteremia is an important consideration in any patient, particularly in the immunocompromised and vulnerable populations. Anaerobic infections in the general population are most frequently related to pelvic, abdominal, and skin/soft-tissue infections, in 50 to 70%, 5 to 20%, and 5 to 20% of cases, respectively. Publications in the immunocompromised population exist but are limited in number (27–29). The *Bacteroides fragilis* group and *Clostridium* spp. are the most frequent anaerobic blood culture isolates (28), and associated conditions include cancer and malignant tumors, diabetes mellitus, post-surgery status, and advancing age. Older persons are at higher risk of mortality with anaerobic bacteremia (25 to 44%) (30, 31). In general, the risk of mortality for anaerobic BSIs is high and the prevalence of anaerobic bacteremia is increasing (30, 31). As antimicrobial resistance is also increasing among anaerobes, there is a need for more rapid and easier anaerobic antimicrobial susceptibility testing (AST) methods to be deployed.

Mycobacteria

For the diagnosis of disseminated *Mycobacterium tuberculosis* infections or NTM infections, such as *Mycobacterium chelonae* and *Mycobacterium haemophilum*, it is necessary to consider collection of specimens, like sputum or skin lesions, to improve diagnostic yield over that of blood cultures alone (32, 33). There are case reports of septicemia caused by *M. chelonae* complex, in which appropriate therapy is likely delayed, resulting in significant morbidity and multiple hospital admissions (34). Gram staining of these organisms from blood culture should not be confused with debris or with *Corynebacterium* spp. For immunocompromised individuals, the identification of a Gram-positive bacillus that is not able to be identified by phenotypic methods should prompt an acid-fast bacilli stain to distinguish between *Bacillus* spp. and the rapidly growing mycobacteria. Likewise, rapidly growing NTM, while considered rare pathogens, can cause central line-associated BSIs and have been associated with hospital-associated outbreaks in hematology–oncology wards. *Mycobacterium mucogenicum* and *Mycobacterium canariasense* were identified in patients with acute myeloid leukemia, acute lymphocytic leukemia, and aplastic anemia. In this case report, environmental surveillance cultures detected genetically similar *M. mucogenicum* and *M. canariasense* in the water supply of the hematology–oncology ward (35).

Depending on local geography and CD4 count, HIV patients commonly present with mycobacteremial infections ranging from *Mycobacterium avium-intracellulare* to *Mycobacterium tuberculosis* (36, 37). Infections caused by NTM are rapidly emerging among all hosts with altered immune responses and often are associated with an intravascular catheter (33, 38).

Fungi

Yeasts

Candidemia is frequently observed among immunocompromised hosts receiving broad-spectrum or prolonged antibiotic therapy, parenteral nutrition, intravascular catheters, or renal failure, and in those with prolonged stays in an intensive care unit (39–41). *Candida* spp. rank fourth among common causes of BSIs in some groups of hospitalized patients (42, 43). Over 95% of all candidemia is caused by 5 species: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* (43). Other yeasts include *Cryptococcus* spp., *Trichosporon* spp., *Geotrichum* spp., *Rhodotorula* spp., and *Hansenula* spp., all of which have emerged as important bloodstream pathogens in the immunocompromised host (44,

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45). All yeasts, except *Cryptococcus* spp., are often related to the presence of indwelling devices (45).

Standard blood culture bottles will recover many medically important yeasts without the need for special fungal blood bottles (46); however, performance studies show that traditional blood cultures are able to identify medically important yeasts with only varied sensitivity, which can be as low as 38 to 80% when compared to molecular methods (47). For those reasons, special broth formulations, adapted for optimal fungal recovery, are warranted for patients at high risk for candidemia and fungemia.

Nosocomial candidiasis is a life-threatening condition in both critically ill and immunocompromised patients (48). The frequency of infection and the associated mortality have not decreased over the past two decades (48). These findings highlight the difficulties encountered when diagnosing *Candida* infection in high-risk patients and the challenges in administering adequate and appropriate antifungal therapy despite the introduction of several extended-spectrum triazole and echinocandin antifungal agents (48). Moreover, the criteria for initiating antifungal therapy are not completely standardized and often result in administration of antifungal therapy with the associated risks of toxicity, development of antifungal resistance, and high costs (48).

Many yeasts are emerging as new causes of disease. *Kodameae ohmeri* (formerly *Pichia*) is a yeast that is an emerging pathogen in patients with compromised immunity, like cancer patients and premature neonates, with fatal outcomes (49, 50). A rare case of fungemia due to *Trichosporon mucoides* in a diabetes mellitus patient adds to an emerging list of trichosporonosis infections (51). *Rhodotorula* spp. are emerging opportunistic pathogens found in immunocompromised hosts (52, 53). Within the *C. glabrata* complex, *Candida nivariensis* and *Candida bracarensis* are important, since both species have lower susceptibility to fluconazole, but they may be difficult to identify (54).

Molds

Aspergillus spp. (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*), hyaline molds (*Fusarium* spp. and *Scedosporium* spp.), and a wide variety of dematiaceous fungi can cause invasive BSIs (55–58) and are associated with high mortality (59, 60). Most patients with invasive aspergillosis have underlying hematological diseases or have undergone hematopoietic stem cell transplantation. Recognized risk factors for infection are graft-versus-host disease, receipt of steroids, secondary neutropenia, age >40 years, and a stem cell source (61). In contrast, invasive mold infections are unusual in patients with human immunodeficiency virus (62).

Invasive mold infections are especially challenging among patients receiving chemotherapy for hematological malignancies, hematopoietic stem cell transplant recipients (57), HIV patients (63) and solid organ transplant recipients (64, 65). Even *Acremonium* spp. are implicated in systemic fungal diseases, with a case of *Acremonium kiliense* fungemia with proven involvement of the lungs in an allogeneic hematopoietic stem cell patient (66). *Saprochaete capitata* (formerly known as *Geotrichum capitatum* and *Blastoschizomyces capitatus*) has been known to produce serious opportunistic infections in patients with hematological malignancies (67).

Traditional blood culture methods can recover some molds (68), but depending on the species, broth culture has low sensitivity for recovering fungi when compared with molecular methods. The recovery of *Aspergillus* spp. from specific fungal blood culture bottles is infrequent despite disseminated disease (including endocarditis), owing to tissue tropism and limitations in traditional broth culture technology. Vessel invasion and skin infarcts can limit mold recovery from the bloodstream. In contrast, some molds and dematiaceous fungi such as *Fusarium* spp. (69), *Paecilomyces* spp. (70), and *Wangiella* (*Exophiala*) *dermatitidis* (71) have been successfully cultivated from patients, especially those with indwelling catheters.

Viruses

Many viruses are well-known sources of sepsis in immunocompromised hosts; however, these syndromes and their associated test methods are well covered elsewhere and therefore will not be reiterated here; rather, we provide a summary table with a list of relevant publications and rare or miscellaneous pathogens (Table 1).

Other fastidious pathogens

Immunocompromised patients are susceptible to invasive infections with fastidious pathogens that may require unique incubation procedures. Microorganisms such as HACEK (species of *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella*) bacteria can be recovered within 5 days of incubation in currently available automated blood culture systems; extended incubation is not deemed necessary (72, 73).

Legionella spp. grow poorly in routine blood culture media; they require blind subculture from blood culture bottles and special supplementation for growth on solid media (74). *Legionella bozemanae* has been reported to cause pneumonia in immunocompromised patients (75). Some *Campylobacter* spp., *Helicobacter* spp., and

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TABLE 1 Summary of interesting reviews and publications related to viral and miscellaneous pathogen bloodstream infections

Author	Year	Title
Viruses		
Agut H	2011	Acute human herpesvirus 6 (HHV-6) infections: when and how to treat.
Allain JP	2004	Occult hepatitis B virus infection: implications in transfusion.
Babakir-Mina M	2011	The novel KI, WU, MC polyomaviruses: possible human pathogens?
Berrington WR	2009	Clinical correlates of herpes simplex virus viremia among hospitalized adults.
Gerna G	2004	Pathogenesis of human cytomegalovirus infection and cellular targets.
Mazeron MC	2000	[Leukocyte depletion and infection by cytomegalovirus].
Munoz FM	1998	Disseminated adenovirus disease in immunocompromised and immunocompetent children.
Ni YH	2011	Natural history of hepatitis B virus infection: pediatric perspective.
Pantaleo G	1996	Immunopathogenesis of HIV infection.
Perez-Sola MJ	2008	Indirect effects of cytomegalovirus infection in solid organ transplant recipients.
Stratton CW	1994	Blood cultures of immunocompromised patients.
Strippoli GF	2006	Pre-emptive treatment for CMV viraemia to prevent cytomegalovirus disease in SOT recipients.
Swinnen LJ	2001	PTLDs: implications for acquired immunodeficiency syndrome-associated malignancies.
Protozoa		
Almeida EA	2011	Co-infection *Trypanosoma cruzi*/HIV: systematic review (1980-2010).
Zintl A	2003	*Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance.
Helminths		
Azira NM	2013	Review of patients with *Strongyloides stercoralis* infestation in a tertiary teaching hospital, Kelantan.
Chacin-Bonilla L	1991	Systemic strongyloidiasis. Review.
Keiser PB	2004	*Strongyloides stercoralis* in the immunocompromised population.
Link K	1999	Bacterial complications of strongyloidiasis: *Streptococcus bovis* meningitis.
Mycoplasma		
Aujard Y	2005	*U. urealyticum* and *M. hominis* infections in newborns: personal data and review of the literature.
Endresen GK	2003	*Mycoplasma* blood infection in chronic fatigue and fibromyalgia syndromes.
Endresen GK	2004	Systemic *Mycoplasma* blood infection in fibromyalgia and chronic fatigue syndrome.
Ericson JE	2015	Chorioamnionitis: implications for the neonate.
Goncalves LF	2002	Intrauterine infection and prematurity.
Llewelyn M	2002	Superantigens: microbial agents that corrupt immunity.
Narita M	2010	Pathogenesis of extrapulmonary manifestations of *M. pneumoniae* infection.
Sinkovics JG	2012	Molecular biology of oncogenic inflammatory processes.
Taylor-Robinson D	2011	Mycoplasmas in pregnancy.
Wang LJ	2013	Fatal community-acquired pneumonia: 18 years in a medical center.
Rickettsia		
Desenclos JC	2011	Louse-borne infections in humans.
Spirochetes		
Korner M	2003	Clinical significance of human intestinal spirochetosis—a morphologic approach.

Capnocytophaga spp. may also grow poorly or not at all but are important in the immunocompromised population (76, 77). Laboratories may consider performing acridine orange staining for bottles that flag positive but do not show microbes via the Gram stain (78).

Care must be taken when interpreting the Gram stain to avoid false positives that may occur due to Gram stain precipitate. Telemicroscopy is becoming feasible in microbiology and can drastically reduce the number of errors for Gram stain interpretation made by nonmicrobiologists during night shifts or by generalists, for whom competency remains a challenge due to limited exposure to the practice (79).

With improved blood culture systems, the ability to detect *Bartonella* spp. has also improved, but it still requires a prolonged incubation period of up to 6 weeks followed by subculture to freshly prepared media (80). For other microbes, the addition of supplemental agar or different incubation conditions can help support the growth. These additional considerations are well worth

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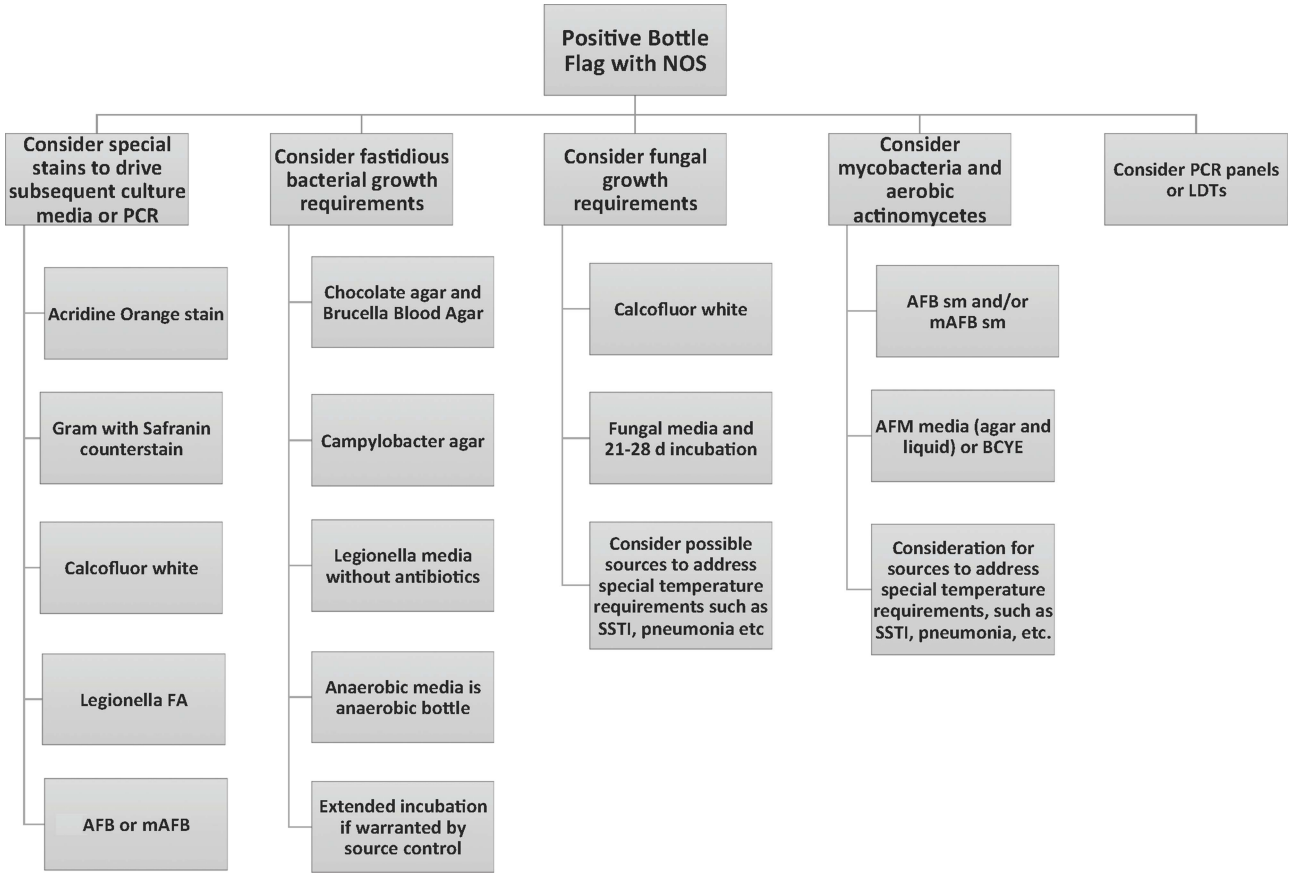

FIGURE 3 Algorithms for diagnosis, prognostication, and prediction of response to therapy. FA = fluorescent antibody stain; AFB = acid-fast bacilli; mAFB = modified AFB; SSTI = skin and soft tissue infection; LDT = Laboratory developed test.

the effort for immunocompromised patients and should be included in standard culture algorithms (Fig. 3).

PREDISPOSING FACTORS FOR BLOODSTREAM INFECTION

Risk Factors and Outcomes

Immune changes are the main risk factors for developing sepsis. It is important to assess the patient's predisposition for infection and how likely it is for a patient to develop organ dysfunction. Much is known about immunosuppression-related sepsis risk factors, and they are discussed in greater detail later in this article. Although less is known about the risk factors leading to organ dysfunction, it is important to document the patient's baseline organ function (overall health status) and any inherited risk factors (3). Age, sex, and race contribute to and influence the prevalence of sepsis. For example, severe sepsis occurs more frequently in infants and elderly individuals, is more common among males than females, and has a greater incidence rate in African Americans than in Caucasians (81). Genetic characteristics may contribute to the host's susceptibility to sepsis and are currently of great interest (82–87).

As with all medical conditions that suppress immunity, the major risk factors for acquiring a life-threatening infection, such as BSI, include two major concepts: pathogen exposure and the immune status of the individual (33). Depending on the predisposing factor (HIV, organ transplant, diabetes, etc.) different risk factors can occur. In addition to underlying immunosuppression, immunocompromised patients have increased risk for infections from unusual or multidrug-resistant (MDR) microorganisms, owing to chemoprophylaxis regimens, extended hospitalizations, or prolonged stays in intensive care units.

Improvements in the management of patients with sepsis and septic shock have increased the survival rate from 20% to over 70% in some populations (3, 88); however, as septic shock is the most severe facet of sepsis, it is not surprising that it has the highest mortality rate, still approximately 50% in some studies (88). Mortality seems to be associated with a poor prognosis when

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attributed to Gram-negative organisms or yeast, as opposed to Gram-positive organisms (88).

Even after discharge, patients who survive sepsis are less likely to live long-term, and sequelae such as impaired biological functions, exacerbation of chronic conditions (chronic kidney disease or cardiovascular disease), emergence of new chronic conditions, and neurocognitive dysfunction lower their overall quality of life (3). Refer to Table 2 for a summary of risk factors.

Critical care and surgery

Sepsis can be a fatal outcome for postsurgical patients in a variety of settings, including transplantation, wound surgery, splenectomy, intra-abdominal surgery, and cancer surgery (89–91).

Cancer

An estimated 5% of cancer patients acquire severe sepsis, and hospitalized patients with cancer are more than 5 times more likely to die (37.8%) than cancer patients without sepsis. The use of cytotoxic agents is largely responsible for immune suppression in these patients, and their therapies predispose them to BSIs. In addition, malignant neoplasms can provide entry for bacteria into the bloodstream. Even more risk comes for patients with hematologic cancers, who are 15 times more likely than the average person to suffer from severe sepsis (92).

Long-term immunosuppressive therapy for cancer is linked to development of life-threatening complications, including BSIs. Central line-associated BSIs are common among these patients with febrile neutropenia following cytotoxic chemotherapy due to translocation of intestinal organisms (*E. coli*, *E. faecium*, and *Streptococcus* spp.) (93). Further studies on enterococcal bacteremia show a high global mortality rate (42%) in patients with hematological malignancies (94). Poor outcomes are associated with age >50 years, underlying disease (leukemia, lymphoma, myeloma, etc.), pneumonia, and shock (94). Other risk factors include long hospital stays (average of 25 days), the presence of a central venous catheter, and the urinary tract as a source of infection (94). One study established that previous use of carbapenem antibiotics increased the risk of acquiring hospital-associated vancomycin-susceptible *E. faecium* BSI, which can be problematic if vancomycin resistance emerges, as that will limit therapy options (95).

TABLE 2 Predisposing factors that contribute to immunosuppression

Therapy
Immunosuppressive
Antimicrobial (i.e., broad-spectrum)
Antifungal
Bacterial colonization
Staphylococcus aureus (i.e., MRSA)
Distortions in normal bacterial microbiota (due to antimicrobial prophylaxis)
Immune deficiency
Autoimmune disease
Devitalized tissue, fluid collections
Neutropenia, lymphopenia
Medical Comorbidities
Uremia
Malnutrition
Diabetes
Alcoholism with cirrhosis
Peripheral vascular disease
Congestive heart failure
Renal and liver disease
Infection with immunomodulating viruses
CMV
EBV
HBV
HCV
HIV
Surgical and/or invasive procedure
Catheterization (i.e., urinary tract, central venous, central line-associated BSIs)

Age

Age over 40 is a risk factor for sepsis (7, 96), and the poorest outcomes occur in patients older than 85 years, with an associated mortality rate of over 38.4% (81). People over 65 years old account for only 1/8 of the U.S. population but account for 66% of all sepsis cases. At the other end of the age spectrum, children, particularly neonates, share elevated mortality rates (97). Infections are more frequent identified in elderly patients (aged ≥65 years) due to preexisting comorbidities, an impaired integumentary system, reduced mechanisms of clearance, and diminished immune response (i.e., immunosenescence, which is more marked in women than in men). Because elderly patients have a decreased immune response, the clinical presentation of infection can be atypical or subtle, which often makes infection difficult to diagnose in those with increased age. Persons over the age of 85 have the highest risk for hospitalization and mortality (98). The increased risk of hospitalization alone puts them at risk for infection (99).

In 2012, the number of elderly persons in the U.S. was 43.1 million (13.7% of the total population), mostly due to the aging of the baby boomer generation; it is estimated that that number will increase to nearly 84

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million by 2050 (20.9% of the total population) (100). As the elderly population increases, health care providers should not only be prepared for increased hospitalization rates in the years to come, but they should focus efforts to reduce infection rates in the elderly. Survival rates do appear to be positively influenced by public health campaigns and behavioral changes, e.g., reductions in smoking and obesity (100).

The most frequent cause of BSIs in the elderly population is urinary tract infection, especially with MDR uropathogens; depression of immunity plays a role in their predisposition to infection (101–103). Bacteremic urinary tract infection is defined as the isolation of the same organism from both the blood and urine within 48 h (104). The majority of urosepsis cases in the elderly are community-acquired (approximately 80%, of which nearly 90% of the Gram-negative infections are due to *E. coli*) (104). Non-*E. coli* Gram-negative organisms are more common among hospital-acquired infections. Gram-positive microbes are associated with male patients with chronic urinary catheter insertion.

At the other end of the age spectrum, at birth, infants have little immunity of their own and are susceptible to infections (105–107). Similar to elderly sepsis, the diagnosis of neonatal sepsis is difficult due to nonspecific signs and symptoms. In 2015, 45% of neonatal sepsis-related deaths (died within 28 days of birth) were due to infectious etiologies (108).

Hospitalization

Risk factors for health care-associated infections (recent hospitalization, extended-care facility, dialysis, or regular home care or hospital clinic visits) predispose patients to BSIs and sepsis. Common etiologies of health care-associated infections are methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, extended-spectrum β-lactamase-producing *K. pneumoniae*, and extended-spectrum β-lactamase-producing *E. coli* (109). Patients receiving prophylaxis with fluoroquinolones, common in hospitals, are at risk for BSIs with methicillin-resistant *S. aureus* (110) and MDR Gram-negative bacilli (111).

HIV

Advances in antiretroviral therapy (ART) have extended the life expectancy of people with HIV, and because infection is related to immunosuppression, the incidence of opportunistic infections in HIV-infected persons has been reduced. Moreover, just as the epidemic has evolved, so has the spectrum of HIV-associated opportunistic infections. For example, people with HIV are more likely to be admitted to the ICU for sepsis than for *Pneumocystis* pneumonia, as was more common in the past (112). The incidence of a community-acquired bacterial BSI is higher in HIV-positive patients (20% in adults and 30% in children) than in HIV-negative adults and children (9%) (113). Although causative agents vary by geographic region, HIV-infected persons worldwide are particularly susceptible to developing nontyphoidal salmonellae and *S. pneumoniae* bacteremia. Other pathogens such as *E. coli* and *S. aureus* are common causes of BSI among HIV-positive and HIV-negative individuals. Moreover, patients who receive ART are less likely to be hospitalized for a BSI, with an incidence rate of 0.63 pre-ART and 0.2 post-ART (113).

Regardless, a low CD4 count is the main risk factor for the development of BSIs in HIV-infected persons. Mortality rates vary from 7% to 46% worldwide, with Europe and the U.S. at 16% to 18% and 25% to 43%, respectively, and Africa at 7% to 46% (113). Although data are limited, no studies have yet shown a difference in mortality rates between HIV-positive and HIV-negative patients with community-acquired BSI (113).

Transplant

BSIs are a major cause of morbidity and mortality after solid organ transplant and hematopoietic stem cell transplantation, with the highest incidence rate (7.4% to 14%) within the first 30 days (114–116). When coupled with septic shock, the mortality rate of posttransplant BSI is approximately 50% (117). The transplantation type (i.e., kidney, kidney–pancreas, liver, etc.) also plays a role in incidence rate, and liver transplantation was associated with the highest risk of BSI (118). Although etiologic agents vary between studies, most Gram-negative BSIs are caused by *E. coli*, and the Gram-positive BSIs are largely due to *Staphylococcus* spp. (115, 116). Moreover, the Gram-negative isolates mainly consists of multidrug-resistant organisms (115, 116), thus necessitating the use of a more aggressive empiric agent, such as a carbapenem.

Risk factors for BSI posttransplantation include age >50 years, nosocomial infection, pulmonary source (117), urinary tract sources (115), and central venous catheters (119). In one study, for 27% of liver transplant BSIs, urosepsis began with catheter-related infections (115). Certain causative agents are also associated with poor outcomes. For example, *Candida* spp., enteric Gram-negative bacteria other than *E. coli*, and MDRO non-lactose-fermenting Gram-negatives (e.g., *P. aeruginosa*) (117, 119) are associated with increased mortality. Moreover, the prophylactic antimicrobials used to mini-

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mize opportunistic infection in high-risk patients inadvertently increase the rate of resistant microorganisms isolated. Increasing resistance rates of MDR organisms complicate treatment options and continue to be a concern in transplant patients.

Steroid use

Glucocorticoids, also known as corticosteroids, play an important role in regulating the immune response, and as one would expect, disruption in their levels can destabilize a sepsis patient. Glucocorticoids function to inhibit the inflammatory response, thus creating an immunosuppressive effect. During sepsis, cortisol and glucocorticoid production are reduced, which causes individuals to experience exaggerated and uncontrolled inflammatory responses. Therefore, glucocorticoids are frequently used to treat patients with severe sepsis or septic shock as a means to stabilize their immune imbalances. New data suggest that glucocorticoids serve to reprogram immune cells, which balances homeostasis and restores organ function for improved patient outcomes (120). On the other hand, high random cortisol levels are a marker of disease severity and a poor prognostic marker (120, 121). Not surprisingly, an uncontrolled immune response during sepsis disrupts homeostasis, causing metabolic chaos. For example, hyperglycemia is one clinical characteristic of sepsis that becomes an important therapeutic factor to manage in septic patients. The use of corticosteroids, such as hydrocortisone, during sepsis has the ability to stabilize blood glucose levels. However, current studies show limited to no benefit of the use of corticosteroids as sepsis therapy, and the lack of evidence-based data keep this a controversial topic (121).

Nutrition (malnourished, diabetic)

Many metabolic and nutritional deficiencies are linked to increased risk of infections and to increased severity and mortality of BSIs (122, 123). A variety of metabolic and vitamin deficiencies predispose hosts to infections and are linked to higher incidence and severity of BSIs. Vitamin D, selenium, iron, amino acids, and hormone deficiencies have all been linked to higher risks and severity of disease (124–136).

B-cell depletion

B lymphocytes produce antibodies, which function to inhibit infections by eliminating microorganisms. B-cells also regulate the immune response by producing specialized signaling molecules called cytokines. In BSIs, proinflammatory cytokines, such as TNF, interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8), function to provide response to human infection. When produced and released, the proinflammatory cytokines induce a systemic inflammatory response. Anti-inflammatory cytokines, such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and INF-γ, function to limit tissue injury.

B-cell dysfunction obviously alters the host's immune response, but so does B-cell depletion, a type of therapy utilized for the treatment of autoimmune diseases (i.e., lupus, rheumatoid arthritis, multiple sclerosis) and B-cell malignancies (i.e., Hodgkin lymphoma, chronic lymphocytic leukemia). Depletion therapy alters the host immune response, and treatment with rituximab, an anti-CD20 antibody, is associated with induced hypogammaglobulinemia, which predisposes patients to increased infections.

Neutropenia

Neutropenia predisposes patients to BSIs and sepsis, and febrile neutropenia represents one of the biggest diagnostic challenges (137–146). Like depletion therapy, treatment-related neutropenia can have severe consequences and is associated with a variety of common and rare microbes (141, 147). The situation is similar for autoimmune cytopenia (148) and other causes of granulocytopenia (107, 143, 149–153).

Intensive care unit

In critical care settings, the definitions of sepsis and septic shock were last revised in 2012. Experts from a variety of fields (154, 155) introduced changes in sepsis definitions, because previous definitions were thought to include an excessive focus on inflammation and the misleading model that sepsis follows a continuum through severe sepsis to shock. In addition, the inadequate specificity and sensitivity of systemic inflammatory response syndrome criteria was addressed and led to removal of the term "severe sepsis," which was deemed to be redundant (154, 155). In an ICU setting, sepsis is now defined as life-threatening organ dysfunction, which is caused by a dysregulated host response to infection. Organ dysfunction can be represented by an increase of 2 points or more in the SOFA score, which is associated with an in-hospital mortality of greater than 10% (154, 155). These updated definitions and clinical criteria now replace previous definitions and facilitate earlier recognition and more timely management of patients with sepsis or patients at risk of developing sepsis (154, 155).

Septic shock is now defined as a subset of sepsis in which profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mor-

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tality than with sepsis alone (154). The presence of septic shock is associated with hospital mortality rates greater than 40%. Patients with septic shock are identified by the need for use of vasopressor therapy in order to maintain their mean arterial pressure at ≥65 mm Hg, and a serum lactate level greater than 2 mmol/liter (>18 mg/dl) in the absence of hypovolemia, a decreased volume of circulating blood.

In outpatient settings, emergency departments, or general hospital ward settings, adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes typical of sepsis if they have at least 2 of the following clinical criteria, which together constitute a new bedside clinical score termed "quickSOFA" (qSOFA): respiratory rate of ≥22/min, altered mental status, or systolic blood pressure of ≤100 mm Hg.

Nosocomial BSI

Besides the classically known health care-associated infection BSIs, such as *Staphylococcus* spp. (156), *Enterococcus* spp., and MDRO Gram-negative infections, emerging pathogens are becoming more prevalent in health care-associated infection transmissions and outbreaks (156–168). Indeed, due to hospital-associated drug-resistant infections, the simple act of hospitalization increases risk for BSIs and sepsis. Therefore, clinicians and microbiologists must stay keenly aware of novel pathogens and their incidence among their patient populations to serve as a warning system for potential outbreaks and for device- and infusion-based contamination events.

Other

Introduction of novel monoclonal antibody therapy, such as anti-TNF for common conditions such as Crohn's disease, rheumatoid arthritis, and invasive procedures are all risk factors for development of sepsis (60, 88). Other conditions that can be successfully controlled with iatrogenic immunosuppression, such as diabetes, splenectomy, and congenital immunodeficiency, also impart higher risk (60, 88). In addition, rheumatic or congenital heart disease, surgery, cirrhosis, and chronic diseases (including diabetes, heart failure, chronic renal failure, and chronic obstructive pulmonary disease) add risk. Addictions, such as intravenous drug abuse and alcoholism, also represent risk factors for sepsis. Finally, other infections, such as septic abortion, pelvic infection, severe community-acquired pneumonia, abdominal infection, urinary tract infection, meningitis, and cellulitis will predispose patients to sepsis (60, 88).

DIAGNOSTIC APPROACHES AND INTERPRETATION

Unfortunately, despite the enormous human and financial impact of sepsis, this diagnosis remains largely a clinical one (169), due to the lack of rapid, sensitive, and specific laboratory tests to detect the causative pathogens. In order to provide a more accurate diagnosis, there is a significant need to improve the speed and diagnostic breadth of laboratory detection methods for the causative agents of bacteremia and sepsis and the immune aspects of bloodstream infections. There is a wide variety of diagnostic approaches for the identification and treatment of bacteremia and sepsis.

Radiographic Techniques: MRIs, CT Scans

It is important to determine the source of the infection to eliminate reseeding of the bloodstream from regions that are difficult to penetrate with antimicrobials or that may require drainage or removal. Source control is recommended within the first 12 h after a BSI is diagnosed (4) in order to prevent severe downstream morbidity and mortality. For this purpose, magnetic resonance imaging (MRI), routine radiographs, ultrasound, or computed tomography (CT) scans are useful.

Histopathology

Skin or end-organ biopsy for histopathological and microbiological investigation may be helpful in making early diagnosis of fungemia, particularly in disseminated *Fusarium* spp. or *Candida* spp. infections. *In situ* hybridization methods can be useful to identify pathogens in tissue specimens (170). Surgical pathology, autopsy services, and microbiology sections of the laboratory must work closely together and communicate findings with each other.

Microbiology Procedures

Blood cultures are among the most important cultures performed by the clinical microbiology laboratory (171). During the initial evaluation of an immunocompromised patient with a suspected BSI, blood should be routinely cultured for both aerobic and anaerobic bacteria, as well as for commonly encountered yeasts. If the clinician has a high suspicion of a dimorphic fungus or mycobacteria, then blood bottles specifically designed to recover these microorganisms should be obtained; molds may require additional test methods (172).

Routine blood cultures are collected as a set (one aerobic and one anaerobic bottle), in which the patient's blood (optimally 16 to 20 ml, and in some cases 30 ml) is split and inoculated into the broth medium of each

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bottle. The blood culture is incubated at 35°C and remains in the instrument until growth is detected, usually up to 5 days, or less if the bottle flags positive in the instrument.

Blood from the positive bottle is used to prepare a smear, and a Gram stain or other stains are performed. A variety of agar plates are then inoculated and incubated until microbial colonies can be identified and AST can be performed. The Gram stain is reviewed and resulted by a medical technologist, and this critical result is notified to the providers as soon as possible (optimally within 30 min of the blood culture flagging positive) (173). The laboratory diagnosis of bacteremia or fungemia requires urgent attention so that appropriate antimicrobial therapy can be initiated or targeted once the stain results are known (174).

Rapid detection of BSIs in the critically ill, followed by appropriate antimicrobial therapy, can have a lifesaving impact (6, 173). According to a review of more than 2,600 cases from 15 intensive care units in five U.S. and Canadian cities, the risk of death from sepsis increases by 6 to 10% with every hour that passes, from the onset of septic shock until the start of effective antimicrobial therapy (9, 175). Thus, the development of rapid, sensitive, and accurate molecular diagnostic laboratory methods to identify bacterial and fungal pathogens and characterize associated antimicrobial resistance can benefit diagnosis and therapy for septic patients and can save many lives.

While a variety of historical methods were grandfathered into laboratory protocols prior to CLIA regulations, and new methods are implemented by validating them as laboratory-defined tests, the clinical utility and accuracy of these off-label assays must be reestablished in each laboratory where the method is used. In an *ad hoc* survey conducted with members of the American Society for Microbiology's LISTSERV communities, the clinmicronet and divC in May 2009, the survey participants reported use of the following rapid methods, extrapolated from the published literature: the direct tube coagulase (176, 177), directly inoculated cefoxitin disk test with reduced zones to predict MRSA (176, 178, 179), rapid Oxoid PBP2a latex agglutination test (Thermo Fisher Scientific, Waltham, MA) (176, 180), latex agglutination for streptococci (181), direct germ tube for *Candida albicans* (182), MRSA*Select* Agar (BioRad, Hercules, CA) (183), and Pneumoslide for *S. pneumoniae* (Becton Dickinson, Cockeysville, MD) (184). All were performed directly from positive blood culture bottles. In addition, direct susceptibility testing for bacteria (185, 186) and yeast (187) has been reported.

Despite attempts to rapidly identify pathogens and their associated antimicrobial susceptibilities, these techniques are still not a common practice; patients and their physicians continue to wait for confirmatory results from blood cultures, the gold standard for diagnosis of bacterial sepsis, which can take several days before a pathogen is identified, limiting their usefulness in the management of the acutely ill patient. Moreover, blood cultures frequently remain negative even in severe cases of sepsis. For example, routine methods are relatively ineffective for detection of certain pathogens, such as *Coccidioides* spp., and some *Brucella* spp., which are examples of causes of culture-negative fungemia and bacterial endocarditis, respectively.

Clinicians should cautiously interpret the clinical relevance of recovering molds or NTM from blood cultures drawn through catheters, because of environmental contamination with mold spores or asymptomatic colonization at the site of an indwelling line. Features in the clinical history (e.g., extensive immunosuppressive therapy, prolonged fever, embolic events) may aid clinical differentiation between infection and contamination.

Semiautomated systems as well as manual blood culture systems are still available but have largely been replaced by automated continuous monitoring systems that enhance the detection of microorganisms and improve laboratory work flow (188).

Types of blood culture systems

Automated systems

There are several fully automated, continuous-monitoring broth-based blood culture systems on the market (e.g., BD Bactec FX, bioMérieux's BacT/Alert, and Trek Diagnostics VersaTrek). Aerobic and anaerobic broth formulations allow for recovery of the most common causes of BSIs. The broth contains an anticoagulant (sodium polyanethol sulfonate, SPS), an antiphagocytic agent, and an antimicrobial neutralizing agent (charcoal or resin). Using a variety of indicator systems, the bottle(s) are incubated and continuously monitored by the blood culture instrument for growth. Resin-containing media increases microorganism recovery from patients who are receiving antibiotics at the time of collection (189), and technological advancements include monitoring fill volumes.

Positive growth is detected by changes in volatiles and detected as increased CO_2 (BacT/Alert, Bactec) or by changes in pressure (VersaTrek). The bottles are monitored every 10 minutes. Blood cultures are incubated for up to 5 days (or longer for some fastidious microbes). Most, but not all, pathogen growth will trigger a positive

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instrument reading, which typically indicates the presence of viable microorganisms (false flagging can occur in some systems due to CO_2 metabolism of high white blood cell density in the bottles).

The most common Gram-negative microorganisms isolated are *E. coli* and *Klebsiella* spp.; common Gram-positive microorganisms include staphylococci (*S. aureus* and coagulase-negative staphylococci), streptococci, and enterococci (113, 190).

Interpretation of positive blood cultures

The two most useful parameters that aid in the interpretation of a positive blood culture result include the type of microorganism detected (191) and the number of sets of blood cultures from which a potential pathogen is recovered (192). Collection of two sets of blood cultures from peripheral blood before initiating antimicrobial therapy is the optimal approach for determining clinical relevance of recovered microorganisms. False-positive blood cultures, growing skin contamination introduced during collection, can lead to diagnostic confusion and unnecessary antibiotic use and costs. Therefore, blood cultures should be collected from two different sites, peripherally if possible, prior to the initiation of antimicrobial agents; however, the administration of antimicrobials should not be delayed, as early administration is critical for proper treatment.

False-positive blood cultures can lead to diagnostic confusion and unnecessary antibiotic use for immunocompromised as well as immunocompetent patients. As previously described, the common contaminating microorganisms (e.g., coagulase-negative staphylococci or viridans group streptococci) can be implicated in invasive disease, thus confounding the diagnosis. Conversely, positive blood cultures for yeasts must be assumed to represent disease, whereas negative results do not exclude it. Similarly, because of autolysis of *S. pneumoniae*, negative cultures do not exclude it as a cause of BSI (184, 193). Commercially available blood culture bottles, such as the Bactec Myco/F Lytic medium (Becton Dickinson, Franklin Lakes, NJ) and the BacT/Alert MB medium (bioMérieux, Durham, NC) provide fast and sensitive methods to detect mycobacteria (71).

Isolator

Although continuous-monitoring systems have emerged as the new gold standard, the Isolator system (Wampole Laboratories, Cranbury, NJ) is still optimal for isolating some fastidious microorganisms (*Brucella*, *Bartonella*, mycobacteria, filamentous fungi, *Histoplasma capsulatum*) by lysis and centrifugation (188, 194–196). After centrifugation, the resulting pellet is used to inoculate agar media. Contamination is a known problem due to multiple manual processing steps (197).

Principles of Blood Culture Collection

The importance of rigorously following guidelines for the proper collection of blood cultures such as those published by the Clinical and Laboratory Standards Institute cannot be overemphasized. At the initial evaluation for patients with signs and symptoms suggestive of bloodstream infection, an adequate blood volume must be cultured to achieve overall test sensitivity. For nonfastidious bacterial pathogens in patients with no prior history of antibacterial therapy, one set of blood cultures (i.e., 20 ml of blood) has been shown to detect a bacterial or yeast bloodstream infection in approximately 65 to 91% of cases; two or three sets of blood cultures improve the overall yield of detecting the pathogens, raising the yield to 80 to 99% and 96 to >99% of cases, respectively (198, 199). Medical urgency often dictates prompt initiation of empiric antibacterial therapy in immunocompromised hosts, but two to three sets of blood cultures should be obtained within 5 minutes of each other before initiating therapy. The optimal volume of blood for children can vary based on body weight, but similar to adults, higher volumes of blood increase the likelihood of recovering microorganisms.

There are many factors that contribute to clinically effective blood culture data, including disinfection of draw site and culture bottle tops prior to collection, disinfecting agent (iodine versus chlorhexidine), site of collection (peripheral versus line draw), timing of collection (transient versus persistent bacteremia and before antibiotic initiation), volume of collection (negative versus positive growth), and number of bottles/draws (contamination versus true bacteremia). Because blood cultures are susceptible to contamination, proper sample collection is essential in order to avoid culture misinterpretation and unnecessary antibiotic therapy. If the skin is not adequately cleansed prior to blood collection, then commensal skin microorganisms, such as coagulase-negative staphylococci, are likely to contaminate the culture. Coagulase-negative staphylococci are common causes of bacteremia and often associated with endocarditis (200); therefore, it is important to recognize when a positive blood culture is due to improper collection technique versus infection. One way to differentiate between a skin contaminant and a true pathogen is to begin by collecting two sets of blood cultures, each from different sites (e.g., right and left peripheral draws). Assuming proper collection technique, a contaminant

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will likely only grow in one bottle. If poor collection technique is the issue, then it is likely that more than one bottle will be positive, thus elevating the blood culture contamination rate. Data show that blood culture contamination rates decrease when draws are limited to phlebotomy staff versus nonphlebotomists (5). Likewise, if the septum of the blood culture bottle is not sufficiently decontaminated, then environmental organisms, such as *Bacillus*, can be inadvertently inoculated into the blood culture and grow, triggering a false-positive culture. Therefore, it is not only necessary to disinfect the skin prior to collection, but it is also crucial that the septa of the blood culture bottles are thoroughly disinfected prior to collection.

Aseptic technique in blood culture collection is imperative to prevent contamination from skin and other environmental microbiota. In the U.S., the positivity rate for blood cultures is approximately 10%, and many of these positive results represent contaminating skin microbiota due to inadequate skin preparation or from colonized indwelling vascular catheters (198).

Surface dirt and oils should be removed by thoroughly cleansing the skin with 70% alcohol (isopropyl or ethyl). Chlorhexidine gluconate in alcohol can then be used to disinfect the area prior to venipuncture and has become the new standard in blood culture collection. One must not contaminate the area by touching it after skin preparation (171).

Best practices and chlorhexidine skin preparation can drop the contamination rate to <1% for both phlebotomist and nursing collection rates (DM Wolk, personal communication) (201), as can use of a new skin contaminant diversion device, the SteriPath System (Magnolia Medical Technologies, Seattle, WA). One must remember that assuming a 10% positivity rate, a 3% overall contamination rate would apply to 30% of all positive cultures; therefore, successful use of new products makes a contamination rate of near 0% possible. We recommend <1% contamination as the current goal to optimize patient care and minimize needless costs.

Despite the American College of Physicians recommendation that blood cultures should be obtained through fresh venipunctures rather than indwelling intravascular devices (202), blood cultures frequently are collected from an indwelling central venous catheter in immunocompromised hosts because of restricted access to robust veins in those patients. Several studies have shown that blood cultures drawn from the indwelling catheters are less specific and have lower positive predictive value and higher rates of contamination than those obtained from a peripheral venipuncture (203, 204). The exception is a newly inserted catheter, for which investigators have shown no increase in contamination rates for these catheter-drawn blood cultures (205). The clinical significance of blood cultures collected through an indwelling catheter, particularly when only one set of blood cultures is obtained, cannot be determined reliably; therefore, true bacteremia cannot be distinguished from contaminating microbiota. For example, in a patient presenting with hypotensive shock, physicians may mistakenly infer causality from a single positive blood culture, and that may result in misuse of antibiotics and failure to investigate alternative diagnoses.

Peripheral collection

Peripheral blood is the preferred specimen for blood cultures. One set of blood cultures should be drawn from a single venipuncture, consisting of approximately 20 ml (10 ml per bottle) or such that the specimen provides a blood-to-broth ratio of 1:5 to 1:10 (171). Larger blood-to-broth ratios improve microorganism recovery (206). Additionally, microorganism recovery increases when blood cultures are collected before or during a fever spike (171). Two sets of blood cultures drawn consecutively from different anatomic sites prior to antimicrobial therapy are recommended for the diagnosis of acute sepsis (pneumonia, osteomyelitis, meningitis) (4, 171). If continuous bacteremia (endocarditis or fever of unknown origin) is suspected, then an additional set of blood cultures may improve recovery. For instances in which antimicrobial therapy was initiated, cultures should be collected when the concentration of antimicrobial agent is at its lowest.

Collection via indwelling port

Cultures drawn though an indwelling catheter are not recommended; however, if limited vein access requires the use of an indwelling catheter, then a peripheral site draw must accompany that culture in order to fully evaluate the culture results (207, 208). Comparing the time to detection, also known as time to positivity (209–218), or performing quantitative blood cultures (219, 220), may be used to diagnose catheter-related sepsis. Catheter tip cultures may also be useful in the diagnosis of a catheter-related infection when paired with blood cultures, but these have fallen from favor as a standard practice.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Laboratories utilize a combination of solid agars to promote the growth of microorganisms from positive blood cultures. There are different methods for cul-

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turing. A Gram stain-directed approach utilizes media-driven algorithms. For example, if Gram-negative rods are observed on Gram stain, then a MacConkey plate will be inoculated. When a clinically significant microorganism has been isolated from a patient with bacteremia, susceptibility testing should be performed according to the methods described by the Clinical and Laboratory Standards Institute.

In the 24 to 48 h when susceptibility results are not available, clinicians are encouraged to rely on their institution's antibiograms to help guide their selection of antibacterial therapy. In some cases, when there are enough isolates to substratify the data, a location-specific antibiogram for ICU and emergency department may be useful, as is a source-specific substratification of the data (DM Wolk, personal communication). Although some organisms such as viridans-group streptococci have predictable patterns and susceptibility testing may not be performed, clinicians should be aware that unusual resistance patterns can occur among immunocompromised hosts. For example, *Streptococcus mitis* and *Streptococcus salivarius*, two microorganisms considered to be very susceptible to penicillin, have been observed to have high MICs to penicillin when recovered from patients with febrile neutropenia (111). Multidrug-resistant Gram-negative bacillary infections are also becoming a serious problem in immunocompromised hosts (24). Depending on the institution's frequency of resistance owing to extended-spectrum beta-lactamases or *amp*C-mediated mechanisms, empirical therapy with a carbapenem may be warranted. For infections associated with intravascular devices, removal of the catheter often is necessary. Microorganisms can be embedded within a biofilm layer and thereby can be resistant to the activity of antibiotics (59, 221), which thwarts eradication of bloodstream infection without catheter removal (222). The presence of persistent bacteremia should raise suspicion of an endovascular source such as endocarditis or a metastatic focus (e.g., vertebral osteomyelitis or renal abscess). Endocarditis in granulocytopenic patients is unusual, because inflammatory-mediated processes and platelet deposition are critical for bacterial colonization of the endocardium. In other immunocompromised hosts, however, clinical suspicion for bacterial endocarditis should remain high. Further evaluation with transesophageal echocardiography may be required to assess presence or absence of valvular vegetations. Depending on the clinical context, investigations of other metastatic foci may warrant radiological imaging such as a CT scan or an MRI.

Susceptibility testing is appropriate for candidemia, particularly for questions of treatment failure or as supporting evidence that patients may be converted safely from intravenous to oral therapy. While awaiting testing results, susceptibility patterns can often be predicted based on the species of *Candida* isolated and the institution's specific antibiogram profiles. Fluconazole is appropriate as empiric therapy for institutions with low rates of non-albicans candidemia, except for patients with prior triazole exposure. Indications for voriconazole and echinocandins as empirical therapy are dictated by institution-specific trends for recovery of non-albicans yeast. Echinocandins are often very effective for patients with candidemia, but notable exceptions include *Candida guilliermondii*, the non-*Candida* yeast, and *Trichosporon* spp.

CLSI has developed and published approved methods for broth-dilution testing of caspofungin, fluconazole, and voriconazole (CLSI approved standard M27-A3) and for disk diffusion testing (CLSI approved guideline M44-A) of yeast. These methods are accurate and reproducible and are being used in clinical laboratories. Interpretative breakpoints are available for select *Candida* spp. with certain azoles and echinocandins, and the clinical relevance of testing any other organism–drug combination remains uncertain. A "susceptible–dose-dependent" category exists for species with elevated MICs to triazoles and denotes that higher doses may provide clinical efficacy. Susceptibility testing for filamentous fungi has been more challenging to standardize. CLSI has developed methods for MIC testing of filamentous fungi (CLSI document M38-A2), but more clinical data for correlating *in vitro* results with *in vivo* responses are needed. Generally speaking, for filamentous molds, the term "resistance" testing is informally used as a means to predict therapeutic failures.

For fungemia, all central venous catheters should be removed when feasible. All patients should undergo at least one ophthalmologic examination to exclude endophthalmitis, and patients with fungal endocarditis require immediate cardiothoracic surgical evaluation. Breakthrough or persistence of candidemia (or other fungi) on therapy suggests an infected intravascular device, profound immunosuppression, or microbiologic resistance. Duration of antifungal therapy is highly variable, depending on several factors, including the type of microorganism recovered (e.g., *C. albicans* versus *Fusarium* spp.), the degree of underlying immunosuppression, and the presence of metastatic foci.

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Rapid Antibiotic Therapy Saves More Lives than Any Other Intervention

In cases of sepsis, rapid intervention with appropriate antimicrobial therapy can be critical to patient survival (223, 224). For aerobes, anaerobes, and fungi, appropriate antibiotic therapy increases survival by approximately 25 to 45%. Eliminating delays in appropriate antibiotic administration increases survival by ~7 to 10% per hour (9, 175). Optimized antibiotic care requires intravenous broad-spectrum antibiotics with daily reevaluation to optimize efficacy, prevent resistance, avoid toxicity, and minimize costs, with the ultimate goal to discontinue broad-spectrum therapy within 3 to 5 days and to continue antibiotics targeted to the causative pathogen (10).

COMMERCIALLY AVAILABLE MOLECULAR METHODS

Recent developments in molecular diagnostics allow for rapid identification of microorganisms directly from positive blood culture bottles, and some can be used on samples directly from the patient.

Peptide Nucleic Acid Fluorescence *In Situ* Hybridization

Peptide nucleic acid fluorescence *in-situ* hybridization (PNA FISH) is a technology to test blood smears made directly from positive blood culture bottles. Results are provided within 2.5 h for the direct identification of Gram-positive organisms (staphylococci and enterococci), Gram-negative organisms (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) and *Candida* spp. from positive blood cultures. Probe kit utilization is driven by the Gram strain result; thus, Gram-positive cocci in clusters would utilize the *Staphylococcus* probe, yeast would utilize the *Candida* probe and Gram-positive cocci in pairs, and chains would utilize the dual *Enterococcus* probe.

PNA molecules contain the same nucleotide bases as DNA and follow standard base-pairing rules for hybridization for the following base pairs: adenine (A), thymine (T), cytosine (C), guanine (G). Differences in the PNA hybridization probes allow for stronger and more specific binding. Furthermore, PNA FISH probes target rRNA in microbial chromosomes, offering the following advantages of a small subunit rRNA (ssRNA) target: 1) sequences are known and unique between species; 2) ssRNA targets are highly abundant, multicopy targets; and 3) PNA probes, due to their small size, can bind in highly conserved regions that are not accessible to larger DNA probes. In addition, the PNA probes confer very low background, which allows the fluorescent signal to be visualized and confers greater sensitivity.

According to product inserts and clinical publications, PNA FISH performance data have demonstrated accuracy. Sensitivity and specificity are as follows: for *S. aureus*, 98.8% and 99.6%, respectively; for *C. albicans*, 99.3% and 100%; for *E. faecalis*, 96.3% and 98.3%; for other *Enterococcus* spp., 93.1% and 99.3% (225–229); for *E. coli*, 100% and 100%; for *K. pneumoniae*, 60% and 100%; for *P. aeruginosa*, 100% and 100%; and overall accuracy for *Enterobacteriaceae* is 94.3% and for non-*Enterobacteriaceae* is 100% (230). The compatibility of these probes has been proven with demonstrated accuracy for various blood culture media, including FAN and resin bottles (226, 227, 231–233).

Several studies have applied PNA FISH as an adjunct to the Gram stain from positive blood culture bottles, and it is clear that when used appropriately with a team involving laboratory, pharmacy, and physicians, PNA FISH can direct therapy, but it also reduces antibiotic and hospital costs and saves lives. Optimal outcomes for rapid intervention with PNA-FISH have been best documented when there is a strong collaborative effort between the laboratory and pharmacy. Holtzman et al. found no impact when their pharmacy was not involved (234). Others found success with physician intervention, including substantial decreases in turnaround time, mortality, and hospital expenditures (235).

The PNA FISH assay (AdvanDx, Woburn, MA) can be utilized to reduce the time required to administer targeted antibiotics therapy (153, 156, 236), to curtail unnecessary antibiotics when blood cultures are contaminated (58, 237), and to support antifungal selection for candidemia (231, 238).

GeneXpert, Xpert MRSA/SA BC Assay

The rapid detection of *Staphylococcus aureus* bacteremia and a swift determination of methicillin susceptibility have serious clinical implications affecting patient mortality. In a multicenter preclinical evaluation, Wolk et al. evaluated the performance of two Cepheid Xpert MRSA/SA assays for detection of MRSA and methicillin-susceptible *S. aureus* (MSSA) (239). Using an integrated DNA extraction process coupled to real-time PCR, MSSA and MRSA are identified directly from positive blood culture bottles in <1 h. A total of 114 wound specimens and 406 blood culture bottles were tested from study sites in the United States and Europe in order to characterize assay performance of these assays in a clinical setting. Newly positive blood culture broths with Gram's stains showing Gram-positive cocci in clusters

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were tested. Broths from each of the following continuous monitoring blood culture instruments were included: bioMérieux BacT/Alert SA standard aerobic, SN standard anaerobic; Bactec Peds Plus/F Medium, Plus Aerobic/F Medium, Plus Anaerobic/F Medium; Standard Anaerobic/F Medium, Standard/10 Aerobic/F Medium, Lytic/10 Anaerobic/F Culture Vials; and VersaTREK Redox 1 (aerobic), Redox 2 (anaerobic). BacT/Alert FAN aerobic and anaerobic broths were not included. The primers and probes in the Xpert MRSA/SA assays detect sequences within the staphylococcal protein A (*spa*) gene, the gene for methicillin resistance (*mecA*), and the staphylococcal cassette chromosome (SCC*mec*) inserted into the SA chromosomal *attB* insertion site (240, 241). Inclusion of both the *attB* insertion site and the *mec*A gene targets enables the assays to identify the presence of SCC*mec* cassette variants with *mec*A gene deletions, thus reducing false-positive results that occur in molecular tests that only target the SCC*mec* cassette (242, 243). For the Xpert MRSA/SA assay performed on the Cepheid GeneXpert system, sensitivity was 97.1% and 98.3% for MRSA in wound and blood culture specimens, respectively. Sensitivity was 100% for *S. aureus* from both specimen types (244).

SmartCycler, BD GeneOhm StaphSR Assay and BD Max

The BD GeneOhm StaphSR assay on the SmartCycler is used for detection of *Staphylococcus aureus* and MRSA directly from positive blood culture bottles. In a study by Stamper et al., a real-time PCR assay, the StaphSR assay (BD GeneOhm, San Diego, CA), was used for the identification of MSSA and MRSA from positive blood culture bottles (n = 300) (245). The sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm StaphSR assay for MSSA detection were 98.9, 96.7, 93.6, and 99.5%, respectively, when compared to phenotypic identification with a variety of reference standards, including oxacillin and cefoxitin Etests (AB Biodisk, Solna, Sweden), penicillin-binding protein 2 (Denka Seiken Co., Tokyo, Japan), and *mecA* PCR. For the detection of MRSA, the BD GeneOhm StaphSR assay was 100% sensitive and 98.4% specific. Inhibition was seen with only one sample, and the issue was resolved upon retesting.

Recently, Grobner et al. evaluated the BD GeneOhm StaphSR assay for its use in the detection of *S. aureus* and MRSA (n = 134). Of 134 samples, five discrepant results arose with this assay due to the presence of methicillin-susceptible, revertant MRSA strains (3/45) and MRSA strains that were not detected by the BD GeneOhm StaphSR assay (2/45) (246).

BioFire Film Array

The FilmArray system (BioFire; Salt Lake City, UT) is a novel and completely integrated real-time multiplex PCR instrument that incorporates multiple singleplex PCR reactions in a disposable pouch (58, 189). New additions to their FDA-cleared menu include a blood culture identification panel for the detection of relevant bacteria and yeasts, as well as antimicrobial resistance bacterial genes. The blood culture identification panel multiplexes 27 different genetic targets. All nucleic acids are extracted and purified directly from the unprocessed sample. During the nested PCR, the FilmArray performs a single, large-volume, multiplexed reaction with primers specific to several targets. Following the multiplex PCR reaction, an individual singleplex second-stage PCR reaction further amplifies the products that amplified during the first round of multiplex PCR. The FilmArray software analyzes and interprets the endpoint melting curve of the amplicons.

GenMark eSensor

The eSensor technology (XT-8) (GenMark; Carlsbad, CA) promotes high assay specificity and sensitivity owing to competitive DNA hybridization and electrochemical detection of the post-PCR target sequence. The technology is not based on fluorescence or optical detection as are the first-generation multiplex assays; thus, the protocols do not require as many of the time-consuming washing and preparation steps required by earlier multiplexing technologies. The XT-8 systems can be used in tandem with up to 3 modules per computer for a throughput of 1 to 24 samples in random access mode.

The eSensor cartridge is preprogrammed with a specific test protocol, lot number, and expiration date on an internal memory chip. In the cartridge, the preamplified target DNA is mixed with the signal probe solution and then analyzed via electrochemical detection. The GenMark pipeline includes a multiplex panel for rapid detection of common bloodstream pathogens. New adaptation of the system toward one requiring less than 60 minutes of hands-on time will make the GenMark system more easily adaptable to routine laboratory settings, and it is expected to be a fully integrated cartridge system with sample-to-answer workflows.

Nanosphere Verigene

Nanosphere's Verigene (Northbrook, IL) system uses a gold nanoparticle probe technology, typically 13 to 20 nanometers in diameter that carries a defined number of target-specific oligonucleotides or antibodies utilized in

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two methodologies, array and amplification, depending on the specific assay. The nanoparticles support increased sensitivity compared to fluorophores. Light scattered from one nanoparticle is reported to be equivalent to the light emitted from one-half million fluorophores.

Automated nucleic acid purification and amplification is performed on the Verigene Processor SP. Inside the Verigene test cartridges, nucleic acid targets are hybridized to capture oligonucleotides on a microarray containing specific oligonucleotides and gold nanoparticle probes. Finally, signal amplification of probes occurs via a silver staining process, with automated qualitative analysis performed on the Verigene Reader. Products for identification of select Gram-positive and Gram-negative pathogens from positive blood cultures, which are probe-based without target amplification (2, 213), are available.

Molecular Testing for Drug Resistance

Rapid and accurate determination of microbial drug susceptibility is actually the most critical challenge; it is essential to facilitate successful antimicrobial therapy for any person, and it is particularly useful for those with immune-function impairment. Rapid testing for genetic resistance markers is an emerging clinical practice that can not only identify the potential for drug resistance, but also help distinguish ambiguous breakpoints associated with susceptibility testing. The application of molecular diagnostic methods to detect drug resistance is evolving for some laboratories. Future methods should facilitate educated choices for therapy, which can be initiated early in diagnosis to impact patient outcomes. Evidence-based studies will be key to the adoption of these new antimicrobial testing paradigms.

Targets for genetic testing require relative genetic stability of the target sequence, a requirement that demands national and international efforts for monitoring genetic mutations in these target regions. Together, MRSA and vancomycin-resistant enterococci are the two most important resistant bacterial pathogens in U.S. hospitals, and their rapid detection remains a critical necessity as antimicrobial resistance continues to increase in the U.S. and worldwide (247, 248). Among bacteria, other useful antimicrobial resistance targets include resistance genes for β-lactams, aminocylitols, aminoglycosides, chloramphenicol, fluoroquinolones, glycopeptides, isoniazide, macrolides, mupirocin, rifampin, sulfonamides, tetracylines, and trimethoprim (249–251). For a list of PCR primers used to target such resistance markers, see Tenover and Rasheed, 2004 (251). For fungi, rapid detection of antifungal resistance is useful, primarily due to the increase in fungal infections among immunocompromised patients. Current antifungal assays rely on fungal susceptibility testing, which is dependent on growth. The practical application of antifungal molecular testing is yet to be seen, as there is still much to learn about the genetic markers which mediate resistance. The genetic information needed to examine fungal resistance at the molecular level is complex; a review of molecular mechanisms of antifungal resistance has been published (252).

The full potential of molecular diagnostics for drug resistance testing in microbiology has not been reached—its application is still in its infancy. As the molecular mechanisms of antimicrobial resistance are described, newer technologies may enhance the utility of such an approach. Furthermore, microarray technology has the promise to impact the rapid and accurate detection of multiple mutations associated with resistant bacteria, mycobacteria, viruses, and fungi. Until the full potential of drug-resistant markers is understood, rapid molecular antimicrobial testing must still be combined with traditional microbial cultivation (247, 248).

DNA Sequencing

Broad-range gene PCR and sequencing from clinical material provides an alternative, culture-independent method for detecting pathogens. The promise of this technique has remained strong, especially for the bacterial domain, but its application has not been reliably reproducible, owing in large part to issues with contamination and inadequate sensitivity (253). The gene target most commonly used to detect bacteria from a blood sample is 16S ribosomal RNA (16 rRNA gene or 16S rDNA), an approximately 1500-base-pair gene that codes for a portion of the 30S ribosome. Although investigators have demonstrated the ability to detect bacteria directly from blood with 16S rRNA gene amplification and sequencing (254), it should not be performed routinely since culture remains a more sensitive diagnostic test. Also, broad-range PCR is associated with contamination, and a positive result must be correlated with clinical history. When fastidious pathogens (*Bartonella* spp. or *Coxiella burnetii*) are suspected as the cause of bacteremia, serology and species-specific PCR from blood are optimal. The advantage of this technology in immunocompromised hosts is its ability to rapidly detect and identify bacterial and fungal pathogens from whole blood samples using broad-range primers. Its primary limitation is sensitivity, and until multicenter clinical trials are performed, these assays should be used as adjunctive tools to routine blood culture.

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Pyrosequencing (Qiagen, Germantown, MD) is a rapid method for sequencing based on the detection of pyrophosphate released during DNA synthesis. Advantages of this technology include speed and ease of use in comparison to traditional sequencing technology; disadvantages include the short lengths of sequences that can be currently analyzed. Pyrosequencing provides short sequence information of roughly 30 to 50 bases; it is useful for short-read DNA and mutation/ single nucleotide polymorphism analysis. After PCR amplification and amplicon cleanup, run times approach 1 h for 96 samples, with approximately 30 to 45 minutes for sequence analysis applications. Jordan et al. evaluated pyrosequencing directly from blood culture bottles to assess the potential of pyrosequencing to differentiate between bacteria commonly associated with neonatal sepsis (255). An informative 15 bases within the 380-bp amplicon was targeted for pyrosequencing following enrichment culture and PCR amplification. A total of 643 bacterial isolates commonly associated with neonatal sepsis, and 15 PCR-positive, culture-positive neonatal whole blood samples were analyzed by pyrosequencing. Results of DNA sequencing and culture identification were compared and were successful at using PCR and pyrosequencing together to accurately differentiate among several bacterial groups, both Gram positive and Gram negative. The system had some difficulty with viridans-group streptococci and *S. pyogenes* (255). The same group evaluated specimens from isolates from neonatal sepsis events, to support species identification that could lead to rapid deescalation or targeting of antibiotic therapy. A total of 643 bacterial isolates and 15 PCR-positive, culture-positive neonatal whole blood samples were analyzed by pyrosequencing. Pyrosequencing was able to provide useful information on the identity of species based on the amplicon generated by PCR (255).

Proteomic Tests: SepsiTyper and VitekMS

Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) methods leverage the high sensitivity of mass spectrometry with linkage to large microbial databases to provide users with species-specific spectra that can be used to reproducibly identify microorganisms, and these methods can be used for identification of pure isolates or pathogens from positive blood culture bottles (off-label use) (256). The protein biomarkers that are measured in mass spectrometry of microorganisms are highly expressed proteins responsible for housekeeping functions, such as ribosomal components, chaperones, and transcription/translation factors (38).

Relying on identification of protein profiles, (generally measuring mass-to-charge ratios [*m/z*] between 2 and 20 kDa), MALDI-TOF MS derives its information from profiles of highly conserved bacterial proteins. For MALDI-TOF MS, the protein profiles are generated from direct ionization of an intact colony or a bacterial protein extract, after manual extraction. Identification occurs after correlating an isolate's spectral signature to a database of spectra collected from reference strains. In its current form, MALDI-TOF MS requires cultivated microbes and a small quantity of matrix (typically less than 1 μg/sample). The matrix and target spotting plates/ slides are the primary consumables and are relatively inexpensive in disposable or reusable formats. Ongoing costs include the equipment maintenance agreements. MALDI-TOF MS is widely used for its high accuracy, low consumable cost and speed of analysis. A typical process consists of growth of bacteria or yeast, colony selection and placement on a target, addition of matrix, and analysis with MALDI-TOF MS. Mass spectrometry identification has diverse possibilities as it is a fast and sensitive method to measure proteins, lipids, and other targets, and it does not require prior knowledge about the organism. For a thorough review of MALDI-TOF MS technology, we refer the reader to a recent review of the technology (257).

Commercial mass spectrometry systems exist that can integrate with traditional antimicrobial susceptibility systems. Among them, the MALDI Biotyper from Bruker Daltonics is one of the most widely tested and has proven accuracy in the identification of bacteria (135, 258). The system uses a 48-spot MALDI target plate for organism identification and can be interfaced with the BD Phoenix Epicenter software or the Siemens MicroScan instrument to link antimicrobial susceptibility results that pair with bacterial identification. A few reports show promise for the identification of microorganisms using MALDI-TOF MS without subculture from blood culture bottles (66, 160, 194, 217, 240), but the methods were limited by the need for a large number of cells. These adaptations showed successful identification for only approximately 80% of blood cultures. A promising new technology, the MALDI Sepsityper system (Bruker Daltonics), aims to identify bacteria and yeast directly from positive blood culture bottles (118, 145, 217, 259).

T2Dx, Magnetic Resonance

T2 Biosystems (Lexington, MA) offers the T2Dx instrument, a miniature magnetic resonance-based platform that measures water molecules reacting in the presence

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of magnetic fields. The test is the first to use direct sample EDTA whole blood for direct detection of pathogens. The T2MR technology does not require sample preparation because the signal is not disrupted by background in the sample. For target-specific testing, nanoparticles with magnetic properties that enhance the magnetic resonance signals are used. According to the manufacturer, T2Candida detects five species of *Candida* in whole blood specimens in approximately 3 h with a limit of detection as low as 1 CFU/ml (260).

Biomarkers

Studies show that cytokines can serve as biomarkers for early diagnosis of sepsis in neonates (261, 262). Additionally, cytokines appear to be associated with disease severity. Because early diagnosis is critical for targeted therapy and better outcomes, cytokines seem like promising biomarkers for sepsis. Other biomarkers, such as C-C chemokine ligand 3 (CCL3), IL-8, heat shock protein 70 kDa 1B (HSPA1B), granzyme B, and matrix metallopeptidase 8 (MMP8), may function as reliable predictors of mortality in children with septic shock (263). C-reactive protein (CRP) and amyloid A represent other biomarkers, but their specificity is relatively poor as they are nonspecific acute-phase proteins (106).

Procalcitonin

While patients with sepsis are largely identified based upon clinical criteria, there are several biomarkers of inflammation that can help differentiate patients with true bacterial sepsis from patients who suffer from other conditions for which antibiotic therapy is not required. Traditional biomarkers such as the white blood cell count, erythrocyte sedimentation rate, CRP, and other acute-phase reactants are not sufficiently sensitive nor specific to reliably confirm or refute the diagnosis of sepsis, and in the case of erythrocyte sedimentation rate, are slow to rise. In contrast, biomarkers, such as procalcitonin (PCT), are more useful for the diagnosis of severe bacterial infections and sepsis. PCT is used to help support diagnosis, to predict complications, and for therapeutic monitoring. High plasma levels of PCT are observed in situations characterized by the onset of organ dysfunction and other symptoms of "severe sepsis" or "septic shock" (264–266).

PCT is a precursor peptide of the hormone calcitonin that varies with severity of bacterial infections, where its release is induced from parenchymal cells (lung, liver, kidney, adipocytes, and muscle) (267, 268) and contributes to the deleterious effects of systemic infections. PCT rises (1 to 10 ug/liter) early in sepsis (within 3 h) and increases in concentration for up to 6 h. It remains in the bloodstream for up to 48 h but will drop quickly when BSIs are properly treated, but not with administration of steroids, as is the case for CRP.

Procalcitonin has been extensively evaluated as a marker of infection in several populations. Fernandez-Lopez et al. demonstrated improved sensitivity and specificity of PCT compared to CRP in the emergency department to differentiate bacterial from nonbacterial illnesses in febrile infants (269, 270). Van Rossum et al. reviewed a series of studies that demonstrated superior performance of PCT over CRP for both diagnostic and prognostic applications in patients with urinary tract infection and sepsis (271). In addition, PCT has demonstrated superior performance over CRP, lactate, and IL-6 in identifying bacterial sepsis in medical ICU patients (259). Kruger and colleagues demonstrated that PCT could be used as both a diagnostic and prognostic indicator for patients with community-acquired pneumonia, with improved prognostic ability over the CRP and the WBC count (272).

Clinical use of these tests requires rapid turnaround time and places the microbiology laboratory in a position either to provide 24-7 support for these assays or to share medical guidance with a STAT laboratory where testing is performed. Uptake of this test is high in Europe but less so in the U.S., where is it more commonly used for antimicrobial stewardship than diagnosis. Since there are diagnostic limitations with all biomarkers, for example in cases where only a small quantity of PCT is induced and simultaneous induction occurs due to trauma, clinical consultation for interpretation of these results is critical. Therapy may be initiated or altered based on infectious disease biomarkers, and clinical microbiology laboratories will be challenged to expand test menus to accommodate new biomarker technology.

EMERGING TECHNOLOGY

The future of clinical diagnostics is anticipated to include a variety of rapid and multiplex methods. Review of all technologies is beyond the scope of this review; however, several will be discussed, including multiplex-PCR, DNA sequencing, liquid microarrrays, and PCR-electrospray ionization mass spectrometry. It is known that blood culture bottles, positive for bacteria, do not always support cultivation of the pathogen to agar (174, 273); therefore, new molecular methods may allow microbiology laboratories to identify fastidious pathogens

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or those damaged by antibiotics more efficiently. More detailed information about the following techniques may be found in other reviews (274, 275).

Automated Microscopy: ID/AST

A novel combination of automated microscopy paired with electrophoresis of microbes and FISH genetic probes, the Accelerate instrument (Accelerate Diagnostics, Tucson, AZ) utilizes both genomic and phenotypic detection technologies to significantly decrease time to result while sustaining high sensitivity and specificity. This process will occur on a circular cartridge. In a recent report, susceptibility of *S. aureus* was characterized by assessing immobilized live bacterial cells and differentiating them as susceptible or nonsusceptible using as few as 10 growing clones after 4 h of growth, compared to overnight growth required for traditional culture-based methods (193). In this report, 77/77 MRSA phenotypes and 54/54 MSSA phenotypes were correctly identified. In addition, 53/56 clindamycin-resistant and 75/75 clindamycin-susceptible strains were detected, and heterogeneous and inducible resistance was characterized (193).

PCR Mass Spectrometry, Genetic Profiling Directly from Blood Culture Bottles

PCR/electrospray ionization mass spectrometry from Abbott/Ibis has recently been adapted and developed for rapid identification, strain typing, and antibiotic-resistance assessment (276–278), including *S. aureus* genetic targets (239, 279). In a recent publication, Baldwin et al. provided evidence for the technology's ability to identify a broad range of pathogens, such as those that may be encountered in blood cultures (278). With accuracy listed as 91% for the genus level, there is room for improvement; however, the approach shows promise as a rapid method for identification of pathogens.

In this approach, broad-range primers are used to amplify PCR products from groupings of microbes, rather than primers targeted to a single target or species. Electrospray ionization time-of-flight mass spectrometry is used to determine the nucleic base composition (the numbers of A, C, G, and T) of the PCR amplicon, and this base composition signature is used to identify the pathogens present. Unlike nucleic acid probe assays or microarrays, mass spectrometry does not require prior knowledge of the products analyzed; rather, it simply measures the masses of the nucleic acids present in the sample. The platform is significantly different from previous technologies in its ability to detect virtually all microbes from a family and even microbes that have mutated significantly. This technology has the potential for identification of all known human microbial pathogens in 4 to 6 h from blood or sterile body fluids (280, 281). The newest version of this technology, the IRIDICA BAC BSI assay, is currently in FDA clinical trials.

Molzyme SepsiTest

Another PCR method is based on the detection of conserved sequences in whole blood for rapid diagnosis of bacteremia and fungemia. The SepsiTest is not commercially available in the United States. The test uses a universal PCR from the 16S and 18S rRNA genes, with subsequent identification of bacteria and fungi, respectively, from positive samples by sequence analysis of amplicons. The SepsiTest was evaluated in a prospective, multicenter study of 342 blood samples from 187 patients with systemic inflammatory response syndrome, sepsis, or neutropenic fever. Compared to blood culture, the diagnostic sensitivity and specificity of the PCR were 87.0 and 85.8%, respectively, achieving results with improved accuracy over the SeptiFast, and yielding results in a few hours. The concordance of PCR and blood culture for both positive and negative samples was 86.0% despite the indispensability of blood culture diagnostics (282, 283). In another study employing 20 patients with infective endocarditis and 10 patients without infective endocarditis, heart valves and preoperative blood samples were analyzed by blood culture and SepsiTest. The diagnostic sensitivity of the molecular test was 85% and that of blood culture was only 45%, remaining negative in many cases as a result of antibiotic treatment. Also, etiological agents were identified by SepsiTest in 5/10 patients without infective endocarditis, whereas blood cultures were negative (284).

Immunexpress

Immunexpress (Seattle, WA) has developed a multiplex, quantitative real-time PCR and RT-PCR combination (RT1qPCR) in a ready-to-use kit, now in clinical trials in the United States. Their SeptiCyte test was developed to be utilized in the microbiology laboratory using whole blood collected from patients soon after ICU admission. The test analyzes gene expression using RNA extracted from whole blood of patients suspected of having sepsis. The test determines the level of expression of four genes, resulting in an expression profile reflecting several key biological pathways linked to the host response to infection. These genes have been identified from a panel of previously discovered biomarkers identified in gene expression array and RT1qPCR studies. This test, with

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other laboratory findings and clinical information, is intended to aid in the detection and clinical assessment of systemic inflammation due to infection (sepsis) in critical-care patients. The SeptiCyte Lab signature and associated score was validated in a Netherlands-based clinical study, in which sepsis and infection-negative systemic inflammation cohorts were separated with a negative predictive value of 95.5% and positive predictive value of 74.7% (285).

Q-linea

Q-linea's (Uppsala, Sweden) fully automated, ASTrID instrument provides microbial identification of fungi and Gram-negative and Gram-positive bacteria together with resistance marker mapping within one working day. The technology is based on highly specific padlock probes and circle-to-circle isothermal nucleic acid amplification technology. The ASTrID platform is currently under development, with the goal to start CE-IVD validation in 2018 followed by FDA certification shortly thereafter.

SUMMARY

Bacteremia and sepsis are critically important syndromes with high mortality and associated costs. BSIs and sepsis are among the top causes of mortality in the United States, killing over 600 people per day. Many septic patients are treated in emergency medicine departments or critical care units, settings in which rapid administration of targeted antibiotic therapy drastically reduces mortality. Unfortunately, routine blood cultures are too slow to support rapid therapeutic interventions. As a result, empiric, broad-spectrum treatment is common—a costly approach that may fail to effectively target the correct microbe, may inadvertently harm patients via antimicrobial toxicity, and may contribute to the evolution of drug-resistant microbes. To meet these diagnostic challenges, laboratories must understand the complexity of diagnosing and treating septic patients, in order to focus on creating algorithms that help direct targeted antibiotic therapy and synergize with existing emergency medicine department and critical care unit clinical practices put forth in Surviving Sepsis Guidelines. Immunocompromised hosts are particularly impacted by BSIs and sepsis, and there are numerous conditions that compromise patients and increase their risk and decrease their potential for survival.

Several methods for rapid molecular identification of pathogens from blood culture bottles are available. Labs can integrate with overall care to support local Surviving Sepsis campaigns by providing rapid testing to facilitate targeted therapeutic interventions for infections with common pathogens. As a result, empiric, broad-spectrum antibiotic therapy can be shortened to improve survival, reduce health care costs, and decrease antibiotic resistance. More evidence and clinical utility studies are needed to justify the added expense of molecular methods and to determine an appropriate niche in patient populations for which the cost–benefit would be favorable. The PNA FISH methods show much promise for clinical utility and cost-effectiveness; however, the accuracy and cost–benefit of multiplex PCR remains to be fully evaluated. Pyrosequencing and PCR/ESI-MS have proven accuracy and await practical adoption to routine microbiology laboratories. MALDI-TOF MS shows real promise and is currently being adapted to rapid identification of pathogen isolates and potentially for direct detection from positive blood culture bottles.

Molecular diagnostic assays for detection of single pathogens from blood culture bottles have already been shown to have an impact by reducing mortality and decreasing costs. In the future, tests for multiple pathogens that could characterize Gram-positive, Gram-negative, and fungal infections would enable more rapid and targeted antimicrobial interventions for those with severe disease. Targeted drug-resistance gene testing of blood culture bottles will enable risk assessment and guide treatment options for sepsis. Ultimately, early intervention by molecular detection of bacteria and fungi directly from whole blood would provide the most benefit to patients and would contribute to tailored antibiotic coverage of the patient early in the course of the disease, allowing for more effective treatment and better outcomes for patients with sepsis and septic shock.

Over a century has passed since the introduction of blood cultures for the diagnosis of bloodstream infections. Significant advances have been made in improving blood culture media; as yet, no molecular or antigen-based method has proven superior for the detection of bacteremia. Our ability to make a rapid and reliable diagnosis of fungemia, however, remains elusive. Laboratorians can continue to optimize the use of blood cultures for the diagnosis of BSIs and to encourage their clinicians to obtain at least two sets of blood cultures by separate venipuncture before starting antimicrobial therapy. When blood cultures are obtained through indwelling lines or from a single venipuncture, positive blood culture results can be misleading and can result in diagnostic confusion and injudicious use of antimicrobial therapy. Although blood cultures remain the best means of diagnosing bacteremia and candidemia,

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complementary testing with antigen tests, microbiologic investigations from other body sites, and histopathology can often aid in the diagnosis of disseminated disease.

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ORIGINAL ARTICLE

Development of the Nasopharyngeal Microbiota in Infants with Cystic Fibrosis

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Abstract

Rationale: Cystic fibrosis (CF) is characterized by early structural lung disease caused by pulmonary infections. The nasopharynx of infants is a major ecological reservoir of potential respiratory pathogens.

Objectives: To investigate the development of nasopharyngeal microbiota profiles in infants with CF compared with those of healthy control subjects during the first 6 months of life.

Methods: We conducted a prospective cohort study, from the time of diagnosis onward, in which we collected questionnaires and 324 nasopharynx samples from 20 infants with CF and 45 age-matched healthy control subjects. Microbiota profiles were characterized by 16S ribosomal RNA–based sequencing.

Measurements and Main Results: We observed significant differences in microbial community composition ($P < 0.0002$ by permutational multivariate analysis of variance) and development between groups. In infants with CF, early *Staphylococcus aureus* and, to a lesser extent, *Corynebacterium* spp. and *Moraxella* spp. dominance were followed by a switch to *Streptococcus mitis* predominance after 3 months of age. In control subjects, *Moraxella* spp. enrichment occurred throughout the first 6 months of life. In a multivariate analysis, *S. aureus*, *S. mitis*, *Corynebacterium accolens*, and bacilli were significantly more abundant in infants with CF, whereas *Moraxella* spp., *Corynebacterium pseudodiphtericum* and *Corynebacterium propinquum* and *Haemophilus influenzae* were significantly more abundant in control subjects, after correction for age, antibiotic use, and respiratory symptoms. Antibiotic use was independently associated with increased colonization of gram-negative bacteria such as *Burkholderia* spp. and members of the Enterobacteriaceae bacteria family and reduced colonization of potential beneficial commensals.

Conclusions: From diagnosis onward, we observed distinct patterns of nasopharyngeal microbiota development in infants with CF under 6 months of age compared with control subjects and a marked effect of antibiotic therapy leading toward a gram-negative microbial composition.

Keywords: cystic fibrosis; healthy controls; longitudinal; microbiota; nasopharynx

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At a Glance Commentary

Scientific Knowledge on the Subject: Cystic fibrosis (CF) is characterized by early structural lung disease caused by low-grade asymptomatic pulmonary infections. Potential pathogens such as *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are most frequently cultured. In the lungs of adults with CF, community complexity and composition are correlated with CF genotype and phenotype, age, and antibiotic exposure. Little is known yet about the moment of onset of these changes in childhood.

What This Study Adds to the Field: This study shows for the first time that, starting directly from diagnosis, nasopharyngeal microbial colonization development profiles, including the commensal flora, are different in infants with CF than in control subjects. Additionally, the first antibiotic courses and prophylaxis early in life significantly alter the respiratory microbiota, with a significant reduction in potential beneficial commensals and an increase in potentially pathogenic gram-negative bacteria.

A substantial proportion of children with cystic fibrosis (CF) have pulmonary disease, which manifests starting from early in life as tissue inflammation and radiological evidence of structural lung damage, even before clinical symptoms occur (1–3). Bacterial and viral infections have been thought to drive these processes. Therefore, there has been growing interest in studying the microbial community composition of the respiratory tract in infants with CF from early in life. A multimodality approach including symptom monitoring, lung function measurements, imaging techniques, and microbial surveillance is recommended to obtain more information regarding the early pathophysiology of CF lung disease (4, 5). Interventions to alter the course of CF disease in infancy (6) include antistaphylococcal prophylaxis (7), which may be prescribed to prevent *Staphylococcus aureus* colonization from birth onward in asymptomatic infants with CF. Longer-term clinical outcomes are not clear, and currently this preventive therapy is commonly used only in the United Kingdom (7–9). The policy in the Netherlands, as well as in most other countries nowadays, is to consider antibiotic prophylaxis only in infants with CF with symptomatic airway disease.

Most of the scarce knowledge on the etiology of pulmonary infections in infants with CF is based on conventional culture. Culture-independent sequencing techniques offer an opportunity to profile whole respiratory microbial communities and to evaluate early microbial side effects of (chronic) use of antibiotic prophylaxis in infants (10, 11). Because infants are incapable of producing an expectorated sputum specimen, and because bronchoalveolar lavage is performed only when there is a compelling clinical reason, upper respiratory tract (URT) cultures are used as a surrogate for bacterial colonization and infection of the lung (12, 13). Evidence of the dynamics of URT colonization in infants with CF during the first months of life may help to improve understanding of the pathophysiology of respiratory infections and to determine ecological targets for future intervention trials. Some of the results of this study have been reported previously in the form of an abstract and poster presentation (14).

Methods

Study Population and Design

We conducted a prospective, two-center cohort study in which we enrolled 20 infants born between 2011 and 2013 who were diagnosed with CF on the basis of newborn screening and/or symptoms. The first 15 infants with CF were individually matched to 45 healthy control subjects by date of birth (range, ±6 wk) and sex (ratio, 1:3). Study population and design information can be found in the online supplement. For the purposes of the present study, we collected nasopharyngeal samples during (home) visits at ages 1, 2, 3, 4, 5, and 6 months and during acute respiratory illnesses (aRIs) from both infants with CF and control subjects. Questionnaires on respiratory symptoms, day care attendance, antibiotic use, and other environmental factors were obtained at all sampling moments.

Ethics Statement

The study (NL10/337) was approved by the pediatric medical ethics committees of the University Medical Center Utrecht and the Erasmus University Medical Center. It was carried out in accordance with European statements on Good Clinical Practice, which include the provisions of the Declaration of Helsinki.

Sampling and Storage Methods

Deep transnasal nasopharyngeal swabs were taken by trained study personnel with an eSwab 484CE nylon flocked flexible sterile swab (Copan Diagnostics, Brescia, Italy) according to standard procedure recommended by the World Health Organization (15). After sampling, swabs of patients and control subjects were immediately inoculated in 1 ml of Amies transport medium and stored within 24 hours at −80°C until analyses.

Viral and Bacterial Detection

aRI samples were tested using real-time polymerase chain reactions (PCRs) (RespiFinder SMART 22 FAST; PathoFinder, Maastricht, the Netherlands) specific for human bocavirus; respiratory syncytial virus types A and B; human influenza virus types A and B; parainfluenza virus; human rhinoviruses, adenoviruses, and bocaviruses; human coronaviruses OC43, NL63, HKU1, and 229E; and human metapneumovirus and enteroviruses (16). Culture and bacterial identification were performed according to standard procedures (12, 17, 18).

Metagenomic Analysis

Bacterial DNA isolation and quantification. DNA was isolated from a 200-μl aliquot of each sample and quantified by quantitative PCR (qPCR) specific for a conserved region of the 16S ribosomal RNA (rRNA) gene as previously described (19, 20). To avoid interference from background DNA, only samples with sufficient overall bacterial loads (i.e., samples with DNA levels ⩾0.3 pg/μl above background) were selected for further sequencing.

Amplicon library preparation. A PCR amplicon library was generated by

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amplification of the V5–V7 hypervariable region of the 16S rRNA gene as previously described (21), using a 454 GS FLX Titanium sequencer (454 Life Sciences, Hong Kong, China).

Data processing. The raw sequences obtained were processed using QIIME version 1.8 software (22). Barcodes and primers were trimmed off; chimeric sequences were identified and removed using the chimeraslayer utility; and sequences were checked for their quality. Sequences were clustered into operational taxonomic units (OTUs) using the uclust tool at 97% similarity. Aligned sequences were taxonomically annotated using the greengenes 16S rRNA database (version 13.8). In the figures and tables in this article, the numbers associated with the OTU names represent the hierarchical number of the OTU within its genus based on average relative abundance among all samples (e.g., *Corynebacterium* [6] is the sixth most dominant *Corynebacterium* observed). For each sample, α-diversity was determined by calculating diversity indexes (species richness, Shannon diversity, Chao1, and Simpson indexes) at a rarefaction depth of 1,400 sequences per sample (11). The different *Corynebacterium* OTUs were confirmed at the species level by blasting against the National Center for Biotechnology Information GenBank database. Annotation of the most abundant *Streptococcus* OTUs to species level was performed by *LytA* qPCR (23), culture (24), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (25), consecutively.

Statistical Analysis

Data analyses were performed in either IBM SPSS version 21 (IBM, Armonk, NY) or R version 3.1 software. To assess the statistical significance of differences (in baseline characteristics) between groups, we used either Student's *t* test or the Mann–Whitney *U* (MW) test for continuous data and the χ^2 test for categorical data. The statistical significance of differences in microbial composition between groups was calculated using significance analysis of microarrays in TIGR Multiexperiment Viewer (26). We used a linear multivariate regression model (multivariate analysis [MVA]) to study the independent associations between CF disease (model 1), antibiotic use (model 2), and the $\log_{10} + 1 \times 10^7$–transformed

Table 1. Baseline Characteristics of Infants with CF and Healthy Control Subjects

Population Characteristics	Infants with CF	Control Subjects	*P* Value
Number of children	20	45	
Male sex	10 (50%)	27 (60%)	NS
Date of birth, range*	Dec. 2010–Oct. 2013	Dec. 2010–Feb. 2013	
Age range (mean) at inclusion, mo	0.6–6.1 (2)	0.7–6.3 (2)	NS
CFTR mutations			
Severe: mostly F508del/F508del	16 (80%)	NA	NA
Moderate: R334W–c1132 C>T	1 (5%)	NA	NA
Mild: F508del/R117H and R117H/R117H	3 (15%)	NA	NA
Siblings	8 (40%)	35 (78%)	0.003
Use of antibiotics before inclusion	4 (20%)	0 (0%)	0.007
Day care attendance at age 3 mo†‡	1 (6%)	12 (26%)	0.08
Day care attendance at age 6 mo†	5 (25%)	39 (87%)	<0.001
Duration of exclusive breastfeeding			<0.001
<6 wk	12 (60%)	14 (31%)	
6 wk–3 mo	1 (5%)	5 (11%)	
3–6 mo	6 (30%)	12 (27%)	
>6 mo	1 (5%)	14 (31%)	
Exposure to ETS	0 (0%)	0 (0%)	NS
Born by natural delivery	18 (90%)	42 (93%)	NS
Mean gestational age, wk	39 + 2/7	39 + 6/7	NS
Vaccinated according to Dutch NIP	20 (100%)	44 (98%)	NS
Birth weight, g	3,483	3,673	NS
Presenting symptom at diagnosis			
Asymptomatic	14 (70%)	NA	NA
Symptomatic	6 (30%)	NA	NA
Failure to thrive	2 (10%)	NA	NA
Respiratory infection	2 (10%)	NA	NA
Meconium ileus	2 (10%)	NA	NA
Pancreatic insufficiency	16 (80%)	NA	NA
Delayed passage of meconium§	3 (15%)	0 (0%)	NS

Definition of abbreviations: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; ETS = environmental tobacco smoke; NA = not applicable; NIP = National Immunization Program.
Data are count (percentage) unless indicated otherwise.
P values were calculated using the χ^2 test, Fisher's exact test, or Student's *t* test. A *P* value >0.05 was considered nonsignificant and is abbreviated as NS except when borderline significant.
*Only the first 15 of 20 infants with CF were individually matched to 45 healthy control subjects by date of birth (range, ±6 wk).
†Defined as at least 4 h/wk of day care with more than one child from a different family.
‡n = 56; 3 infants with CF and 6 control subjects were not included at that age.
§More than 48 hours after birth.

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relative abundance of microbiota, corrected for other variables, including potential confounders. We used the Benjamini–Hochberg procedure to correct for multiple testing, and a q value less than or equal to 0.05 was considered statistically significant. We calculated the relative effect sizes of significant associations using the standardized regression coefficients of the multivariate model to indicate the magnitude of differences between groups (21). Nonmetric multidimensional scaling plots based on Bray–Curtis dissimilarity were used to visualize differences between groups, and the statistical significance of these differences in overall microbial community composition was calculated using permutational multivariate analysis of variance (PERMANOVA) (27). PERMANOVA is a nonparametric permutational test used to partition the variance of the Bray–Curtis dissimilarity matrix among independent variables. Hierarchical clustering using average linkage and Bray–Curtis dissimilarity was visualized using the iTol tool (28), and the optimal number of clusters was estimated using the average silhouette width and Calinski–Harabasz index (fpc R package). We created an alluvial diagram in R (package alluvial) to illustrate colonization dynamics during the first half-year of life. Using the Bray–Curtis dissimilarity, we calculated changes in microbial composition over time per time frame (e.g., month 1 to month 2, month 2 to month 3) for each individual, followed by calculation of differences in the average changes over time between groups (CF vs. control subjects; MW test). To study significant nonrandom copresence or mutual exclusion of bacterial OTUs, a network was inferred using the CoNet Cytoscape plugin (29) and the Markov clustering algorithm in the clusterMaker Cytoscape plugin and analyzed using the NetworkAnalyzer Cytoscape plugin.

Results

Study Population

We obtained 324 nasopharyngeal samples from 20 infants with CF and 45 healthy control subjects aged 1 to 6 months. Seventy percent of all infants were included in the first month of life, 15% in the second month of life, and the rest shortly thereafter. During follow-up, only five samples were missed. A total of 313 (92 CF samples and 221 control samples) of 324 nasopharyngeal samples (97%) contained sufficient amounts of DNA to be analyzed by 16S rRNA-based sequencing. The mean numbers of analyzed samples per individual were 4.6 ± 1.6 and 4.9 ± 1.6 for infants with CF and control subjects, respectively. The baseline characteristics of the study subjects are described in Table 1.

Infants with CF and control subjects differed in prior antibiotic use. Compared with control subjects, infants with CF had lower day care attendance rates and fewer siblings. Also, fewer of them were exclusively breastfed, and they had a higher frequency of antibiotic treatment. The patterns of height and weight gain over time were comparable between infants with CF and control subjects. During aRIs, although in

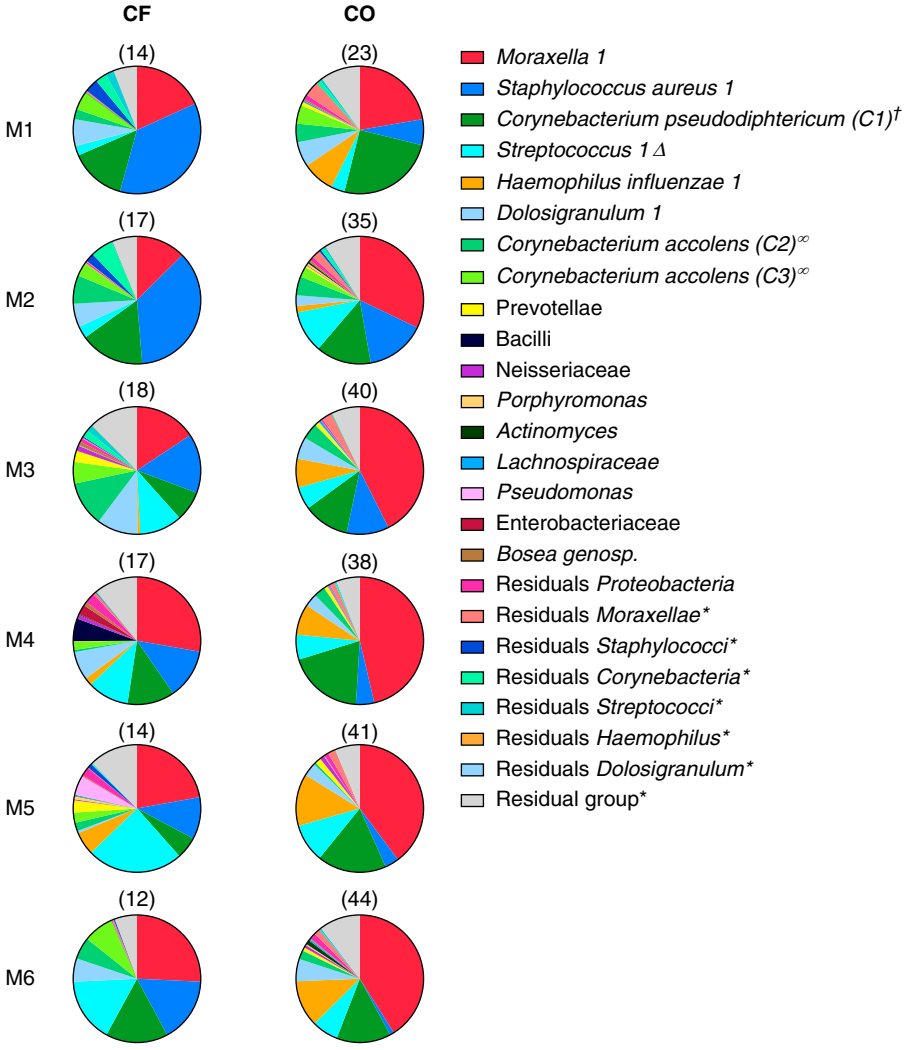

Figure 1. Overall development of microbial community composition over time in infants with cystic fibrosis (CF) and control subjects (CO). The first and second columns of the pie charts show the development of overall microbial community composition (top 35 operational taxonomic units [OTUs]) per month over the first 6 months (M) of life in infants with CF and healthy control subjects, respectively. The total number of samples per month is depicted above the respective pie charts. For ease of visualization, the OTUs of the top 35 belonging to the same genus were merged. †100% homology to *Corynebacterium pseudodiphtericum* and *Corynebacterium propinquum* after blasting. Δ*Streptococcus mitis* in CF and *Streptococcus pneumoniae* in control subjects by quantitative polymerase chain reaction, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and culture. ∞99% and 98% homology with *Corynebacterium accolens* after blasting. *All OTUs from the whole dataset belonging to this genus are included.

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both groups the aRIs were mostly diagnosed as upper respiratory infections, coughing ($P = 0.06$) and dyspnea ($P < 0.001$) were observed more often in infants with CF than in control subjects. As part of clinical practice, antibiotic prophylaxis was initiated by the treating physician in 14 (70%) of 20 infants with CF within the first 6 months of life, and this was preceded by one or more short courses of antimicrobial treatment in 6 (43%) of these 14 infants. Five short-course therapies were prescribed for in a total of five control subjects (11%).

Bacterial Density and Diversity

Sequence results, sequence depth, and phylum-level microbial composition of infants with CF and control subjects are described in the RESULTS section of the online supplement. The bacterial density was lower in samples obtained from infants with CF than those from control subjects (median, 62 vs. 197 pg/μl; $P = 0.025$). This was especially true for infants with CF who were 4 months of age and older and was related to antibiotic use (median bacterial density, 27 pg/μl in infants with CF treated with antibiotics vs. 353 pg/μl in those not treated with antibiotics; $P = 0.033$). We observed a trend toward increased density during aRIs (median, 162 vs. 136 pg/μl; $P = 0.08$). With regard to ecological diversity, no major differences in species diversity were observed between infants with CF and control subjects (Figure E1 in the online supplement). We observed no age-related changes in diversity for either group within the first 6 months of life (data not shown).

Microbial Community Composition in Infants with CF Compared with Healthy Control Subjects

Using nonmetric multidimensional scaling based on the Bray–Curtis dissimilarity matrix, we observed significant differences in microbiota composition between infants with CF and healthy control subjects ($R^2 = 0.054$, $P < 0.0002$ by PERMANOVA) (Figure E2). These differences were already explicitly present in the first months of life (i.e., for ages 1–3 mo; $R^2 = 0.052$, $P = 0.0004$).

Average microbiota differences between infants with CF and control subjects aged 1–6 months were determined using univariate analyses (significance analysis of microarrays) and characterized by higher relative abundance of several *S. aureus* OTUs and other staphylococcal species, several *Corynebacterum* spp. (mainly resembling *Corynebacterum accolens* based on BLASTN search), and *Streptococcus*, bacilli, *Acinetobacter*, Enterobacteriaceae, and *Pseudomonas* spp. in infants with CF. Contrarily, we observed a higher abundance of several *Moraxella* and several *Corynebacteria* spp. (mainly resembling *Corynebacterum pseudodiphtericum* and *Corynebacterum propinquum* based on BLASTN search), *Dolosigranulum*, *Haemophilus influenzae*, *Helcococcus*, Pasteurellaceae, and *Prevotella* spp. in control subjects. A subanalysis of samples taken from individuals during the first 3 months of life revealed a significant association already occurring between several staphylococcal OTUs, *Streptococcus*, bacilli, and *Veillonella* spp. in infants with CF, whereas several *Moraxella* spp. and *H. influenzae* were associated with control subjects. Even at this young age, *Corynebacteria* OTUs present in infants with CF were different from those in control subjects.

Microbial community development over the first 6 months of life in infants with CF was markedly different from that in control subjects (Figure 1). Whereas the nasopharyngeal microbiome in infants with CF is initially dominated by *S. aureus* and gradually replaced by *C. pseudodiphtericum* and/or *C. propinquum* and *C. accolens*, a switch to *Streptococcus* spp. and *Moraxella* spp. domination occurs after 3 months of age. In control subjects, however, *Moraxell*a spp., *C. pseudodiphtericum* and/or *C. propinquum* and *H. influenzae* are the most common microbiota members from age 1 month onward, remaining

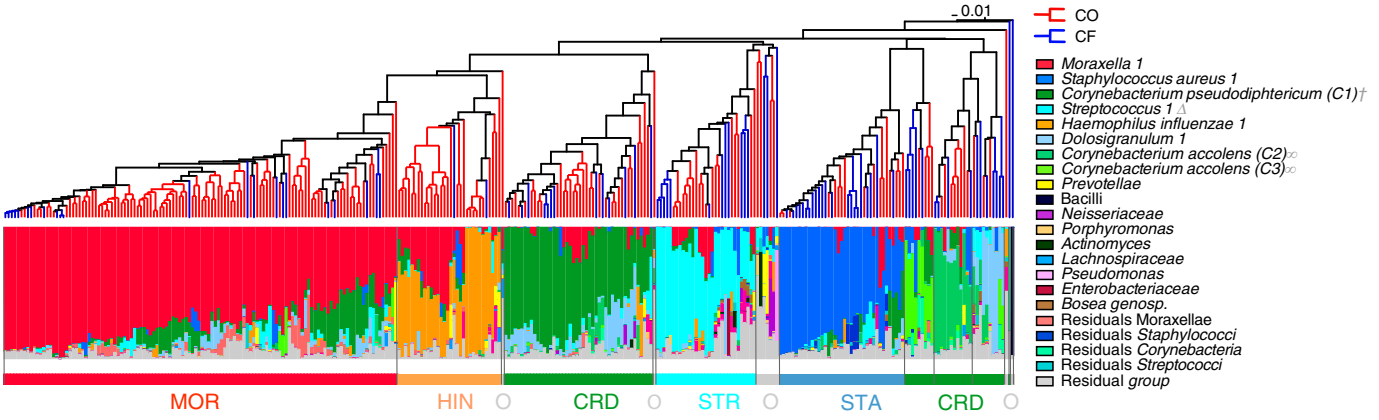

Figure 2. Clustering analysis based on Bray–Curtis dissimilarity and average linkage in infants with cystic fibrosis (CF) and healthy control subjects (CO). Average linkage hierarchical clustering based on the Bray–Curtis dissimilarity matrix is shown in a dendrogram including all 92 and 221 samples of 20 infants with CF and 45 healthy control subjects, respectively. CF samples are depicted with *blue branches* and healthy control subjects with *red branches*. Stacked bar charts show the relative abundance of the top 35 operational taxonomic units. For ease of visualization, the operational taxonomic units of the top 35 belonging to the same genus were merged. Adjacent to the dendrogram branch ends, the *colored line* depicts the clustering of the samples (clusters separated by *gray dotted lines*). Eight clusters comprising four or more samples covered, in total, 91% of all samples. †100% homology to *Corynebacterium pseudodiphtericum* and *Corynebacterium propinquum* after blasting. Δ*Streptococcus mitis* in CF and *Streptococcus pneumoniae* in control subjects by quantitative polymerase chain reaction, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and culture. ∞99% and 98% homology with *Corynebacterium accolens* after blasting. CRD = *Corynebacterium* and *Dolosigranulum*; HIN = *Haemophilus influenzae*; MOR = *Moraxella*; O = other; STA = *Staphylococcus aureus*; STR = *Streptococcus*.

predominant throughout the first 6 months of life. Because *Streptococcus* spp. cannot be annotated up to species level by 16S rRNA-based sequencing, we tested the Spearman correlation between the abundance of *Streptococcus* 1 OTU with qPCR cycle threshold values for the *Streptococcus pneumoniae*–specific gene *LytA*. We observed a positive correlation for control subjects ($\rho = 0.46$, $P < 0.001$) but not for infants with CF ($\rho = -0.09$; nonsignificant). Culturing of the streptococci, followed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and optochin testing, confirmed the high *Streptococcus* abundance detected by 16S rRNA-based sequencing to be mostly *S. pneumoniae* in control infants versus exclusively *Streptococcus mitis* in infants with CF. For the other potential respiratory pathogens, the relative abundance of most abundant *Moraxella*, *S. aureus*, and *H. influenzae* OTUs did correlate significantly to corresponding culture data in both infants with CF and control subjects ($P < 0.003$ by MW test).

Individual Microbiota Profiles of Infants with CF and Control Subjects

Clustering of microbial profiles showed eight distinct clusters or profiles containing more than four samples per cluster in total, representing 91% (301 of 331) of all samples (Figure 2). The largest cluster (n = 39), which was characterized by a high mean relative abundance of *S. aureus* (relative abundance, 75% vs. 2% in the remaining clusters), was associated with CF: 24% (22 of 92) of all CF samples and 8% (17 of 221) of control samples belonged to this cluster ($P < 0.001$ by χ^2 test). Two large clusters were strongly associated with control subjects. The first cluster (n = 32) was characterized by dominance of *H. influenzae* (relative abundance, 56% vs. 1% in the remaining clusters) and contained 2% (2 of 92) of all CF samples and 14% (30 of 221) of all control samples ($P = 0.002$). The second cluster (n = 122) was typified by predominance of *Moraxella* (relative abundance, 80% vs. 1% in the remaining clusters) and contained 24% (22 of 92) of CF and 45% (100 of 221) of all control samples ($P < 0.001$). The five remaining clusters, characterized by a high abundance of *C. pseudodiphtericum* and/or *C. propinquum*, *C. accolens*, *Dolosigranulum*, or *Streptococcus*, respectively, were not clearly enriched for either infants with CF or control subjects ($P \geq 0.05$ by χ^2 test).

The dynamics of microbiota development of the individual children over time are shown in Figure 3. In control subjects (Figure 3B), the majority of children started off with a *Corynebacterium*- and/or *Dolosigranulum*-dominated profile (any of the C or D clusters shown in Figure 2) or a *Moraxella*-dominated profile, and a smaller number started off with a *S. aureus*–dominated profile. Over time, the children with a *Moraxella*- and *Corynebacterium*- and/or *Dolosigranulum*-dominated profile remained fairly stable, whereas children with a *S. aureus*–dominated profile quickly developed a *Moraxella*-dominated profile and the *H. influenzae*–dominated profile started to emerge slowly. Children starting with or passing through a *Streptococcus*- or *H. influenzae*–dominated profile rarely kept this profile for longer than 1–2 months.

Of the infants with CF (Figure 3A), the majority started with a *S. aureus*–dominated profile and, to a lesser extent, with a *Corynebacterium*- and/or *Dolosigranulum*- or *Moraxella*-dominated profile. The majority of children in the *S. aureus*–dominated profile developed eventually toward a *Corynebacterium*- and/or *Dolosigranulum*-dominated profile. The children with a *Moraxella*-dominated profile remained fairly stable. Interestingly, compared with control subjects, significantly more infants with CF changed

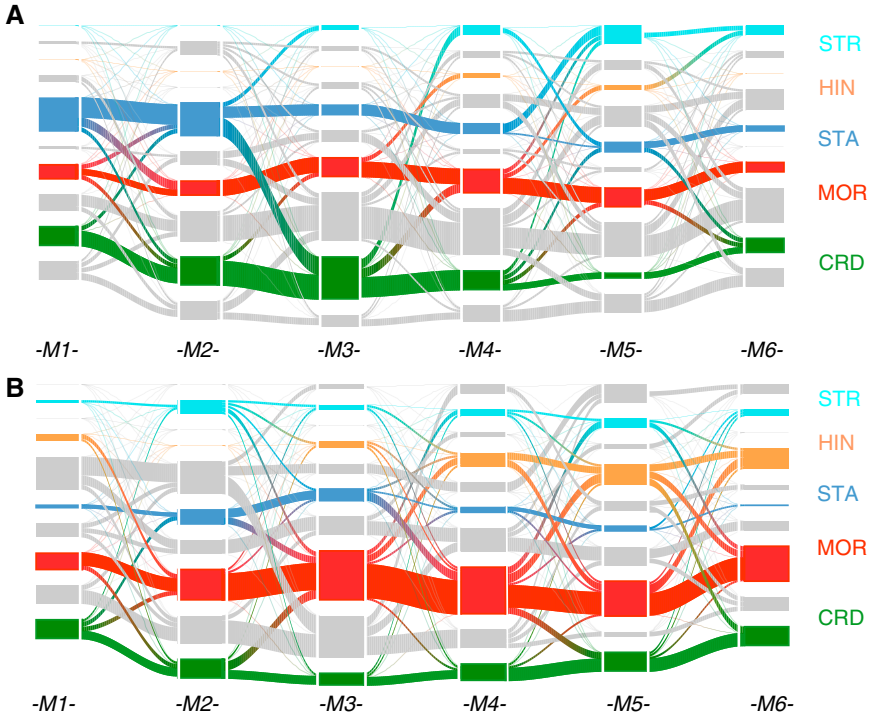

Figure 3. Microbiota colonization patterns over time. This alluvial diagram shows the development of nasopharyngeal microbiota of the individual infants over time. Movements within the eight largest clusters are depicted. Clusters are colored according to the predominant bacterial genus or genera in these clusters. For easier comparison, the diagram is presented in duplicate and colored according to the movement of (*A*) infants with cystic fibrosis (CF) and (*B*) control subjects. To simplify the diagram, we merged the four clusters dominated by *Corynebacterium* 1, 2, and 3 and *Dolosigranulum* into one cluster (*green*). *Lines* between clusters resemble individuals and the development of their microbiota between time points. Only infants with at least three consecutive samples available for analysis are included in this graph (CF, n = 18; control subjects, n = 39). Each infant with CF was given a weight of 2 to more appropriately visualize the relative attribution of colonization patterns of these infants over time. The total number of samples entering and leaving a profile can be dissimilar, owing to (*1*) study inclusion at a different age, (*2*) exclusion of samples from analysis because of low DNA yield, and/or (*3*) visualization of only the eight largest clusters. CRD = *Corynebacterium* and *Dolosigranulum*; HIN = *Haemophilus influenzae*; M = age in months; MOR = *Moraxella*; STA = *Staphylococcus aureus*; STR = *Streptococcus*.

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microbiota profiles between 4 and 5 months of age ($P < 0.023$ by MW test), resulting in an increase in children with a *Streptococcus*-dominated profile. This sudden shift in microbiota composition was preceded by a steep increase in antibiotic consumption in these children (Figure E3).

Impact of Respiratory Illness, Antibiotic Use, and Breastfeeding on Microbial Community Structure

First, because 70% of infants with CF eventually received antibiotic prophylaxis (mainly trimethoprim-sulfamethoxazole; mean starting point, approximately 4–5 months of age), which was preceded in 30% by a course of therapy (mostly amoxicillin with or without clavulanic acid) for respiratory symptoms, we studied the association between CF and microbiota composition, correcting for antibiotic use, respiratory symptoms, and age, in MVA. The bacteria that remained independently associated with CF are depicted in Figure 4 and Table E1. *Pseudomonas* was positively associated with CF, but with only borderline significane after Benjamini–Hochberg adjustment ($q = 0.057$). However, the difference in *Corynebacterium* spp. colonization between infants with CF and control subjects remained highly significant following MVA.

Second, MVA showed no differences in microbiota community structure between samples collected during aRIs (n = 11 obtained from 9 infants with CF; n = 30 obtained from 21 control subjects) and during regular visits. In addition, clinically symptomatic aRIs were not preceded by shifts in microbiota profiles within individuals (Figure E4). However, respiratory viruses were commonly detected in 78% (7 of 9) and 92% (22 of 24) of aRIs in infants with CF and control subjects, respectively. Human rhino- and coronaviruses were most commonly detected in both groups. Other viruses detected were parainfluenza virus in infants with CF and bocavirus, adenovirus, and respiratory syncytial virus in the healthy control subjects (Table E2). Multiple viruses were detected more frequently in samples of control subjects (46%) than those of infants with CF (11%).

Third, antibiotic treatment and prophylaxis in both groups were independently related to a reduction in Moraxellaceae, *Corynebacterium*, and *Dolosigranulum*, and to an increase in gram-negative bacteria, including Comamonadaceae, *Burkholderia*, and Bradyrhizobiaceae and a trend toward increased Enterobacteriaceae (Figure 5, Table E3), as shown by the MVA. Though not significant, all *S. aureus* OTUs decreased as a result of antibiotic use, whereas several other staphylococcal species increased.

In a subanalysis of the effect of antibiotic course therapy independent of antibiotic prophylaxis, we determined similar trends in microbiota changes with a decrease in *Corynebacterium* spp. ($P = 0.03$ by MW test) and *Dolosigranulum* spp. ($P = 0.03$ by MW test) 1 month after antibiotic course therapy (n = 11 samples of six infants with CF and 5 samples of five control subjects) compared with precourse microbiota (n = 11 samples).

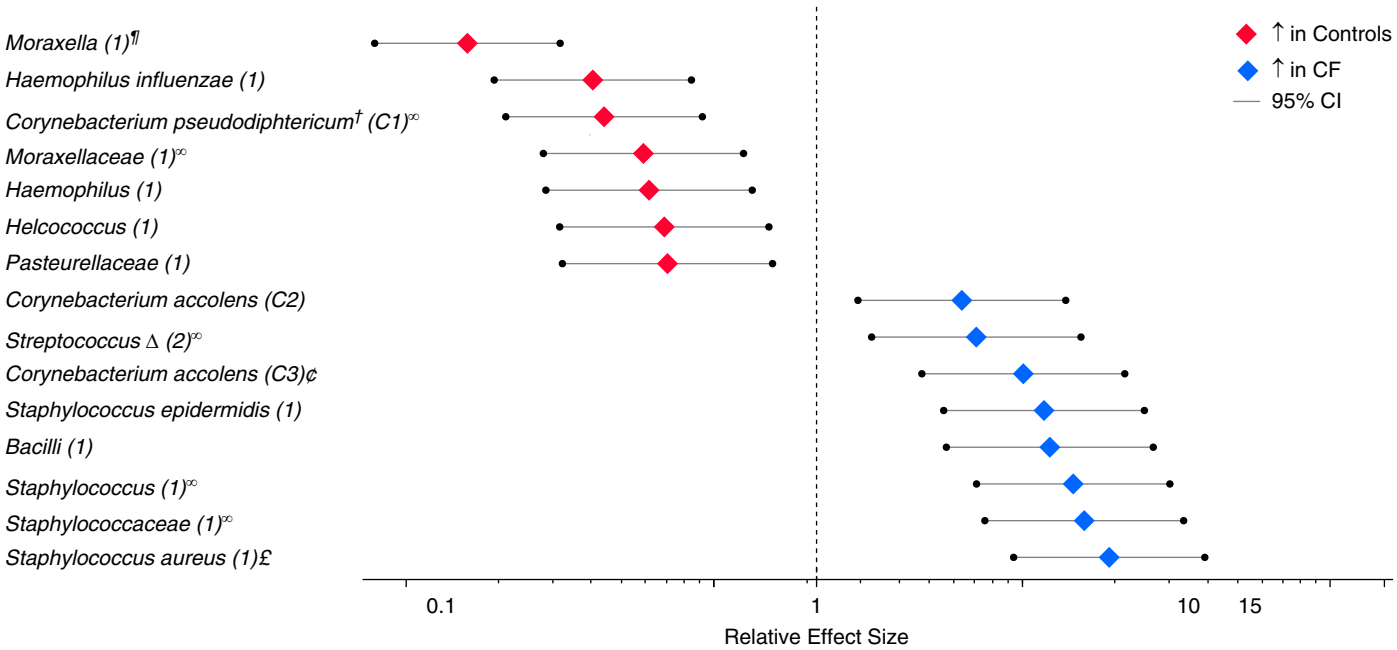

Figure 4. Relative effect size (RES) of operational taxonomic units (OTUs) that were significantly associated with cystic fibrosis (CF) in multivariate analysis. OTUs that were significantly increased (*blue*) or decreased (*red*) in infants with CF compared with control subjects are depicted. Overview of RESs with their 95% confidence intervals (CIs) of operational taxonomic units that were increased (RES >1) or decreased (RES <1) in infants with CF compared with control subjects after correction for age, antibiotic use, and respiratory health in a multivariate model after Benjamini–Hochberg correction. ∞n = 1; ¢n = 2; £n = 5; ¶n = 12; OTU(s) corresponding to the same species/genus are pooled. †100% homology to *Corynebacterium pseudodiphtericum* and *Corynebacterium propinquum* after blasting. Δ*Streptococcus mitis* in CF and *Streptococcus pneumoniae* in control subjects by quantitative polymerase chain reaction, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and culture.

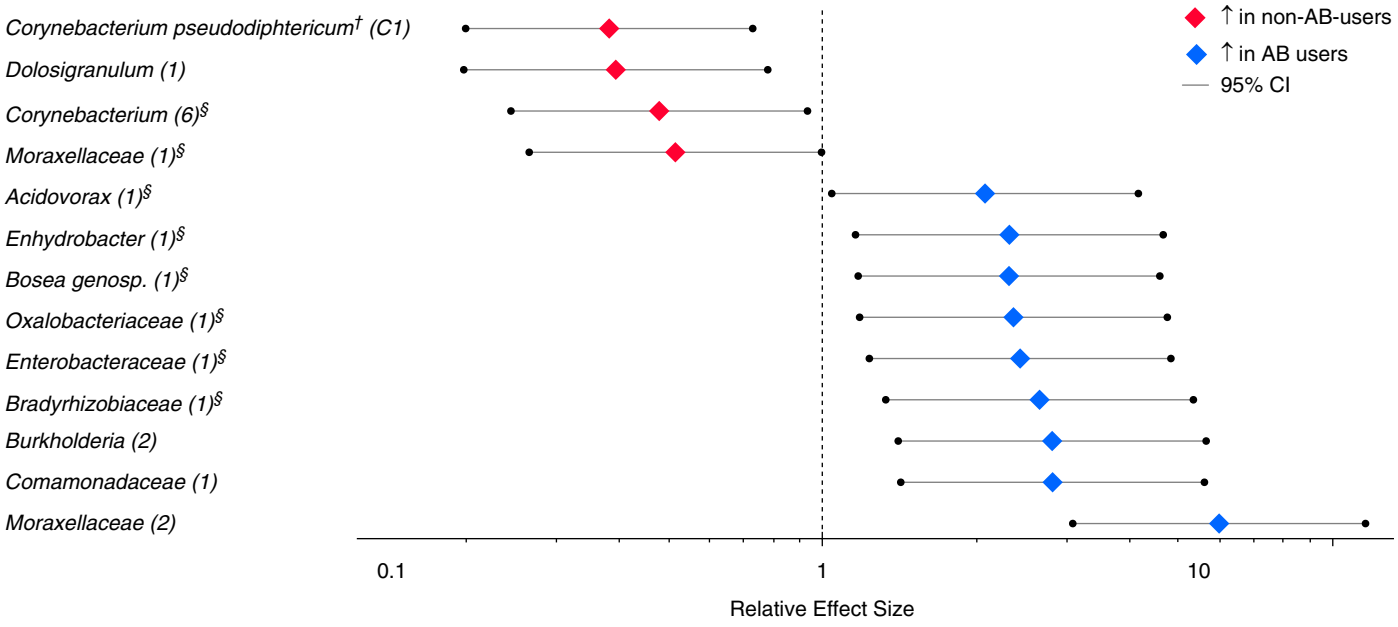

Figure 5. Relative effect size (RES) of operational taxonomic units that were significantly associated with antibiotic (AB) use in multivariate analysis. Overview of RESs with their 95% confidence intervals (CIs) of operational taxonomic units that were significantly increased (RES >1) or decreased (RES <1) in (prophylactic) AB users compared with nonusers after correction for age, cystic fibrosis disease, and respiratory health in a multivariate model following Benjamini–Hochberg correction. †100% homology to *Corynebacterium pseudodiphthericum* and *Corynebacterium propinquum*. §Borderline significant.

Finally, on the basis of previous experience (21), we investigated the role of breastfeeding (significantly lower in infants with CF) on microbiota composition in both infants with CF and control subjects, correcting for CF and age in an independent MVA excluding samples obtained during respiratory infections. A positive correlation was found between breastfeeding and *Moraxellaceae* and *Corynebacterium* during the first half-year of life in both groups. In contrast, a higher abundance of *Prevotella melaninogenica*, other *Prevotella*, *Parascardovia*, *H. influenzae*, *Porphyromonas*, and *Actinomyces* was observed in formula-fed infants.

Microbial Inference Network in Infants with CF and Control Subjects

The abundance of individual bacteria most likely affects the presence of others because of ecologic interactions (29). Therefore, a network was constructed to study the cooccurrence and exclusion of various bacteria, which helped us to interpret potential interactions within the microbial community in infants with CF and control subjects (Figure 6). We observed several distinct clusters of bacteria cooccurring with one another. Strong positive associations were observed for *Streptococcus* spp. and gram-negative bacteria such as *Burkholderia* spp. Also, we observed strong negative correlations between the *Corynebacterium* and *Moraxella* clusters and *H. influenzae*, as well as between the cluster containing *Corynebacterium* and the *S. aureus* cluster.

Discussion

The nasopharynx is the major ecological niche for potential pathogens that cause lower respiratory infections. In the present study, we found distinct patterns of nasopharyngeal bacterial colonization in infants with CF and control subjects from the first month of life onward, already starting before first antibiotic course or prophylaxis and/or day care attendance, suggesting differences in microenvironments and host antimicrobial defense between groups (30). Madan and colleagues (2, 3, 31, 32) previously characterized the oral microbiota of infants with CF, concluding that, in particular, *Streptococcus*, *Veillonella*, and *Prevotella* were the most common genera in the oropharynges of infants with CF. In our present study, we showed increased abundance of *S. aureus* in the nasopharynges of infants with CF early in life, which might be explained by a defect in early innate immune defense against *S. aureus* in infants with CF (5, 33). Furthermore, *S. aureus* is suggested to better survive in the CF ecosystem owing to its ability to grow in facultative anaerobic conditions (4, 34). Importantly, recent studies have shown that increased respiratory colonization with *S. aureus* (35), in particular small colony variants (36), is associated with worse respiratory outcome, even in infants with CF who are clinically stable (37, 38). Together, this information suggests a nonbeneficial role for this bacterium in the respiratory ecocystem of infants with CF. However, on the basis of studies of healthy infants, it is also known that *S. aureus* presence is associated with decreased cocolonization of other potential pathogens, such as *S. pneumoniae* and *H. influenzae* (24, 39). This is in line with our finding that, in parallel with increased *S. aureus* colonization, the presence of *H. influenzae* and *S. pneumoniae* were decreased in infants with CF.

Despite the fact that *H. influenzae* is often reported to be a CF pathogen of infancy and childhood (40), we observed

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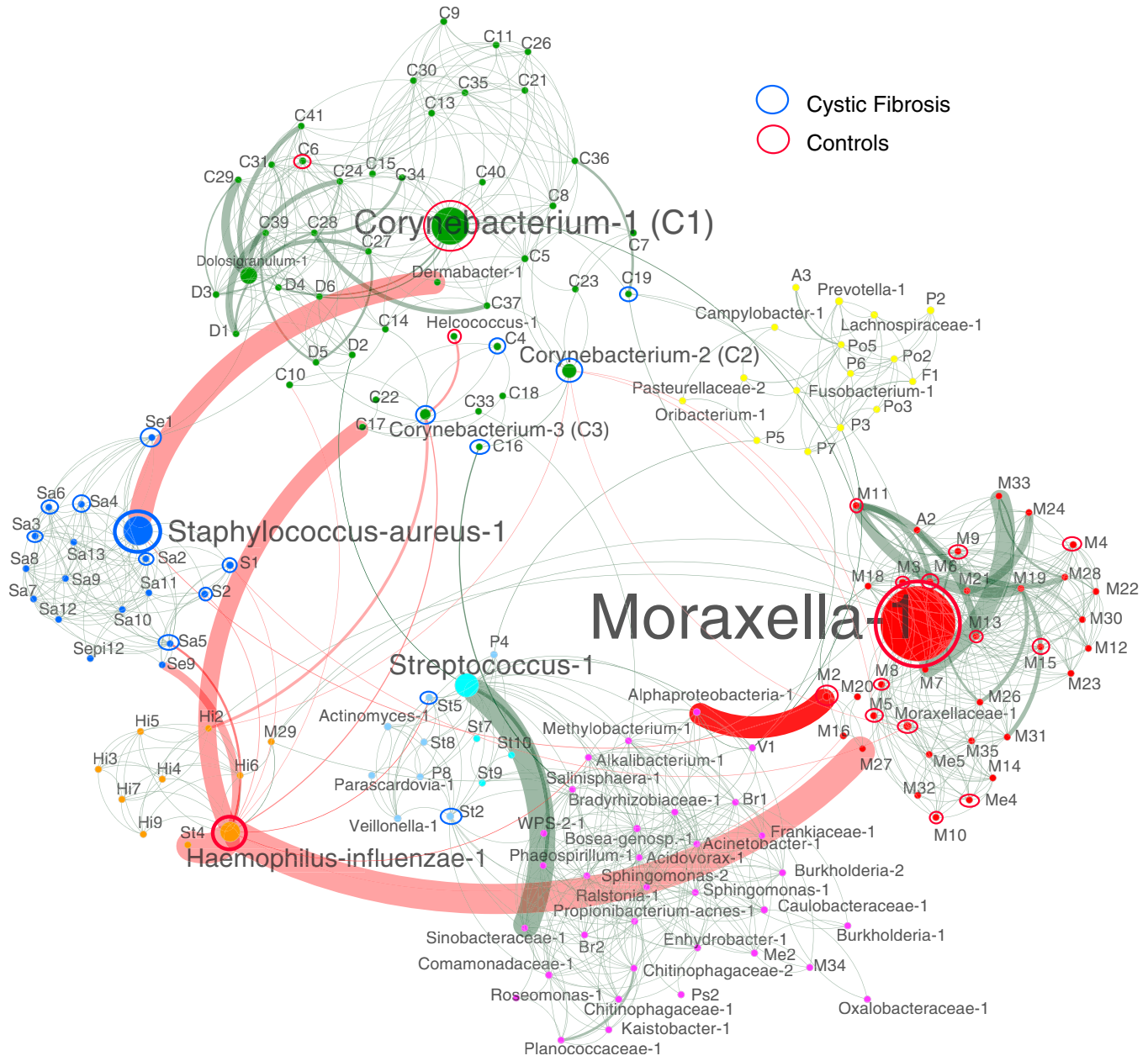

Figure 6. Network analysis of the microbial community structure in infants with cystic fibrosis and control subjects. A network was inferred to study the copresence and mutual exclusion of the top 200 operational taxonomic units (OTUs) observed in our cohort of infants. After bootstrap analysis, 190 OTUs remained as part of the network. Using the Markov clustering algorithm, 16 clusters were identified, of which the largest 8 (representing 169 OTUs in total) are depicted. The OTUs are depicted as nodes; the size of each node represents the relative abundance. Pairwise associations between nodes are depicted by *green* and *red edges*, representing positive and negative correlations, respectively. The thickness of the edges relates to the strength of the association. The OTUs positively correlated with cystic fibrosis (*blue circles*) and control subjects (*red circles*) in multivariate analysis are marked.

low colonization rates in infants with CF during their first 6 months of life.

The increased *H. influenzae*-, *S. pneumoniae*-, and *Moraxella*-dominated profiles in the healthy control subjects compared with infants with CF may also have been a consequence of increased exposure to these bacteria (41) through day care attendance and contact with older siblings, both of which were significantly lower in our CF study group.

Interestingly, on the genus level, *Streptococcus* seems to emerge in both groups from 3 months of age onward, although in contrast to control subjects, infants with CF showed outgrowth of *S. mitis* instead of *S. pneumoniae*. Whereas *S. pneumoniae* is known as a frequent respiratory tract infection (RTI) pathogen, *S. mitis* is regarded as mostly a nonpathogenic commensal of the

oropharyngeal niche. *In vitro* the latter group of streptococci has even been shown to inhibit the growth of potential pathogens such as *S. pneumoniae* (42), *H. influenzae* (43), and even *Pseudomonas* (44) by the production of hydrogen peroxide. Whether the milieu in patients with CF favors the growth of *S. mitis* remains to be shown.

A previous study of children without CF demonstrated that stable microbiota profiles in the first 6 months of life were characterized by either early dominance of *S. aureus*, *Moraxella*, or a combination of *Corynebacterium* and/or *Dolosigranulum* (33). In line with our present study, instability of microbiota dynamics was associated with *Haemophilus*- and *Streptococcus*-dominated profiles. Interestingly, in our study, *H. influenzae* acquisition and expansion over time in our control group seemed to be associated with a reduction in the commensals from the *Corynebacterium* genus, which is supported by a strong negative association between *Corynebacterium* spp. and *H. influenzae* within individuals, as shown by the bacterial interaction network, suggesting a potential negative interaction between both genera (21).

We also conclude that both *Corynebacterium* (often in combination with *Dolosigranulum*) and *Moraxella* might be important symbionts in the nasopharynx, owing to their associations with stable colonization dynamics, with health (present in 96% and 92% of control subjects, respectively), with breastfeeding (21), and with consecutive reduced risk of RTIs (45).

Furthermore, we showed that microbial community composition was highly similar in symptomatic and clinically stable periods, suggesting that respiratory symptoms at this age are still induced mostly by viral infection (46). However, we cannot rule out that symptoms might be a consequence of intrapulmonary infections or of a shift or change in strains within genera or species (47).

It is noteworthy that antibiotics, either as course treatment or as prophylaxis, are often started in cases of respiratory symptoms. Although this is obviously protective against overgrowth and dissemination of targeted species, one should be aware of the potential adverse effects (48). In the present study, we demonstrated that antibiotics prescribed before 6 months of age induced a significant shift in microbial composition, resulting in the emergence of gram-negative bacteria, such as *Burkholderia* and bacteria of the families Comamonadaceae, Bradyrhizobiaceae, and (a trend toward) Enterobacteriaceae. Because these bacteria have a natural tolerance for most antibiotics prescribed in the study population, they have the opportunity to become selectively expanded and to become pathogenic during prolonged treatment, whereas normally they are unable to compete for niche space (11). Furthermore, these gram-negative bacteria are positively associated with a more oral type of microbiota profile, including *Streptococcus mitis*, which might partially explain the emergence of this species in infants with CF from age 3 months onward.

At least as important as the increase in gram-negative bacteria is the observed reduction in the primarily highly abundant symbionts *Corynebacterium* and *Dolosigranulum* following (not preceding) antibiotic treatment; both these genera were recently found to be associated with stability of respiratory microbiota and a decreased risk of consecutive RTIs (21). This topic deserves further study to unravel whether these genera are indeed keystone species (49) of the healthy respiratory microbiota and, if so, whether eliminating these genera by antibiotic treatment should be avoided.

The strength of this study is that it is the first to describe in detail the development of the nasopharyngeal microbiota in infants with CF and birthdate-matched control subjects during the first half-year of life, starting before symptoms occurred and before treatment was initiated. We were thereby able to study the ecological effects of the first antibiotic treatment and initiation of prophylaxis this early in life.

A limitation of our study is the relatively small study population, although we partially counterbalance this by the matching 3 control subjects to 1 CF infant. Furthermore, although URT samples are commonly used as surrogates for the lower respiratory tract in routine clinical practice, we are not 100% certain that the URT samples provided an adequate representation of this niche; therefore, further research is needed to determine the intraindividual concordance of the microbiota present in the upper and lower respiratory tracts in infants with CF.

In conclusion, the development of nasopharyngeal microbial colonization profiles is different in infants with CF than in healthy control subjects from diagnosis onward. Additionally, antibiotic courses and prophylaxis early in life altered the respiratory microbiota significantly, with a reduction in potential beneficial commensals and an increase in potentially pathogenic gram-negative bacteria. This emphasizes the need for improved diagnostic tools to discriminate between viral and bacterial infections in these children and to develop targeted antimicrobial therapy. Follow-up studies are warranted to further understand the relationship between early microbiota development and respiratory disease in children with CF and to study long-term implications of different types of (prophylactic) antibiotic therapy. ■

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Commentary

Assessing the role of environment in *Pseudomonas aeruginosa* healthcare-associated bloodstream infections: a one-year prospective survey

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SUMMARY

Background: Deciphering precise sources and patterns of healthcare-associated *Pseudomonas aeruginosa* colonization/infection is crucial in defining strategies of prevention and control.
Aim: To prospectively investigate the role of hospital environment in *P. aeruginosa* nosocomial bloodstream infections (Pa-BSIs) during one year in a tertiary-care hospital.
Methods: Clinical records of patients presenting Pa-BSIs after >48 h of hospitalization were investigated to confirm the nosocomial character of BSIs and identify the routes of entry and risk factors. Environmental investigations were performed to track *P. aeruginosa* source/reservoir along the care pathway. Clinical and environmental strains were compared by whole-genome sequencing to identify the route of contamination from hospital environment to patients.
Findings: Fifty-three BSIs episodes in 49 patients were considered as nosocomial, mostly involving men (73%), with an average age of 62.4 years, immunosuppressed in >40% of cases, and after previous antibiotic therapy in almost 92% of cases. BSIs occurred after 27 days of hospitalization on average. The main routes of entry were urinary (30%, on indwelling catheters for two-thirds of cases) and cutaneous (17%, catheter-related in almost 80% of cases). *P. aeruginosa* was found in 16 out of 49 investigations, representing 34 positive samples, including 54% of sink traps, 23% of water, and 20% of tap aerators. An epidemiological link was established between environmental and clinical strains only for eight patients, representing 15% of nosocomial BSIs.
Conclusion: The hospital environment usually considered as the main source of *P. aeruginosa* healthcare-associated infections was identified as responsible for nosocomial BSIs in only 15% of patients. Since the implementation of water and hospital environment management, one may hypothesize that *P. aeruginosa* has become a community-acquired pathogen with a nosocomial expression in infection.

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Introduction

The most recent European point-prevalence survey reported a prevalence of 5.9% of healthcare-associated infections (HAIs), with bloodstream infections (BSI) ranking fourth and causing the highest mortality [1,2]. Among the causative agents, *Pseudomonas aeruginosa* is increasingly involved in HAIs in intensive care units (ICUs) and in immunocompromised patients [3]. The high level of antibiotic resistance of *P. aeruginosa* is marked by the emergence of multidrug-resistant (MDR) or extremely drug-resistant (XDR) phenotypes [4]. *P. aeruginosa* resistant to carbapenems (CRPa) represents 19.3% of overall clinical isolates and 25.5% of isolates collected in ICU [2,5]. This situation is especially concerning due to the increasing number of systemic *P. aeruginosa* infections in recent years, the 30% mortality attributable to Pa-BSI, and the risk of death in cases of CRPa-BSI increasing by at least 7% [1,6—9].

In hospital, risk factors for *P. aeruginosa* infection are linked to patients, types of care provided, and the presence of *P. aeruginosa* in the hospital environment close to the patient [10]. *P. aeruginosa* acquisition may be endogenous from the patient's intestinal or oropharyngeal microbiota, or exogenous through nursing staff, cross-transmission between patients or from the hospital environment. During *P. aeruginosa* outbreaks, an association between the presence of *P. aeruginosa* in water niches and Pa-HAI onset is suggested, but the contribution of the environment in Pa-HAI outside outbreak circumstances is rarely reported and remains controversial [11—13]. Even if *P. aeruginosa* is an opportunistic premise plumbing pathogen (OPPP) and Pa-HAIs are usually considered as nosocomial, deciphering precise sources and patterns of *P. aeruginosa* colonization/infection is crucial in defining strategies of prevention and control. The aim of this study was to characterize *P. aeruginosa* hospital-acquired (HA-)BSIs and to determine the proportion of them linked to *P. aeruginosa* environmental reservoir.

Methods

Clinical investigation

A prospective single-centre study was conducted at the University Hospital of Montpellier during 2022. Based on daily automated alert from the bacteriology laboratory, all events of Pa-BSI were included in the study, corresponding to *P. aeruginosa*-positive blood cultures at least 48 h after the patient's hospital admission. A case corresponded to a patient presenting at least one event of Pa-BSI. The onset of several Pa-BSIs in the same patient with negative blood cultures in between was considered to be recurrence. Clinical records were analysed to collect risk factors for Pa-BSIs and define the nosocomial character of the BSI (HA-BSI). A case was considered as HA as soon as the reason for hospitalization was not related to a *P. aeruginosa* infection already in progress at patient's admission. Socio-demographic and clinical criteria were collected from patients' clinical records: sex, age, immune status (aged $\geq$85 years was considered as immunosuppression), clinical ward, delay in BSI onset, length of stay, exposure to antibiotics in the previous three months, number and type of invasive devices (including devices removed $<$48 h before BSI onset), mortality at 7 and 30 days, *P. aeruginosa* antibiotic phenotype and suspected route of entry. Descriptive epidemiological data were analysed by multiple correspondence analysis (MCA) using the R packages Rcmdr and RcmdrPlugin.FactoMiner (available at https://cran.r-project.org/web/packages/RcmdrPlugin.FactoMineR/index.html) to identify potential associations between epidemiological criteria and BSI occurrence. Graphs of individuals/variables from the output of the MCA were generated using the Factoextra package (available at https://cran.r-project.org/web/packages/factoextra/index.html). The study was approved by our Ethical Committee (registration number ID RCB: 2016-A00138-43) and declared to ClinicalTrials.gov under the number ID: NCT02751658).

Environmental investigation

Pa-HA-BSIs were prospectively investigated by sampling the patient's immediate environment. After a case of BSI, the water point-of-use representing a risk for patient's exposure to *P. aeruginosa* was sampled, i.e. water point-of-use in the patient's bedroom and bathroom, or in the adjacent room used for nursing care or hand hygiene by healthcare professionals. The systematic sampling plan included shower trap, sink trap in patient's bedroom, faucet aerator of tap water in patient's bedroom, water of tap water and shower in patient's bedroom, damp surfaces around the washbasin, and support ramp near the toilets. Dry and wet surfaces were sampled with sterile cotton swabs (Copan Diagnostics Inc., Murrieta, CA, USA). Swabs were pre-incubated for 12 h at 37 °C in a soybean trypticase broth (Difco, Bordeaux, France) before streaking on to MacConkey agar media (Difco, Bordeaux, France), then incubated for 72 h at 37 °C in aerobic conditions. Volumes of 250 mL water were collected in bottles containing sodium thiosulphate (final concentration of 20 mg/L), filtered on to cellulose nitrate membrane of 0.45 μm porosity (Sartorius Stedim Biotech, Dourdan, France). The membranes were incubated on MacConkey agar plates for 72 h at 30 °C. Each colony morphotype was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Brucker, Champs sur Marne, France). If a sample was positive for *P. aeruginosa* and homogeneous, i.e. with the presence of a single morphotype, then a single *P. aeruginosa* colony was sequenced. However, if different *P. aeruginosa* morphotypes could be distinguished, a representative colony of each morphotype was sequenced for comparison with the responsible BSI strain. It should also be noted that we did not select only multidrug-resistant strains from environmental samples as we used MacConkey agar media.

Molecular typing

Clinical strains were genotyped by multi-locus sequence typing (MLST) in order to describe local *P. aeruginosa*

population structure involved in HA-BSI and to identify epidemic high-risk (EHR) genotypes, such as ST111, ST175, ST235 that are global/worldwide EHR, and ST253 and ST395 especially present in French hospitals [14—18]. When an environmental reservoir of *P. aeruginosa* was suspected, clinical and environmental isolates were compared by multiplex repetitive sequence-based polymerase chain reaction (rep-PCR) as a first step to assess their clonal relatedness [19]. Environmental and clinical isolates displaying the same profile in multiplex rep-PCR were then whole-genome sequenced using Illumina NextSeq (Illumina, San Diego, CA, USA) technology (whole-genome sequencing: WGS) and a pairwise single nucleotide polymorphism (SNP) analysis was performed to investigate potential epidemiological link, supported for isolates displaying a core-genome (cg-)SNP distance of $\leq$30 [20]. For this purpose, the 'all-in-one' variant calling tool Snippy (Galaxy Version 4.6.0+galaxy0) was used with the isolate found in the environment as a reference. The environmental acquisition of *P. aeruginosa* was considered as possible when *P. aeruginosa* was absent in water but present in aerator and/or sink traps. The presence of *P. aeruginosa* in water was considered as a strong argument for attributing HA-BSI onset to an environmental exposure and nosocomial acquisition of *P. aeruginosa* was then considered as probable in such case.

The study was registered under the Bioproject number PRJNA1083517.

Results

Clinical characteristics of Pa-HA-BSIs

During our 12-month study, 82 events of Pa-BSIs were observed at the University Hospital of Montpellier, corresponding to 53 HA-BSIs and 29 community-acquired BSIs. No cluster cases nor *P. aeruginosa* outbreaks were detected. The 53 HA-BSIs occurred in 49 patients, including four patients with recurrence. HA-BSI mainly affected men (72.7% of cases), average age 62.4 years, and 43% of patients were immunocompromised (i.e aged $\geq$85 years, or with a malignant disease, or treated with immunosuppressive drugs) (Supplementary Table S1). More than 92% of patients received antibiotic therapy in the three months preceding HA-BSI onset. The average time between admission and positive blood culture was 27.2 days (2—92 days), with an average hospital stay of 57.7 days (range: 7—345 days). There was no evidence of seasonality in Pa-HA-BSIs (Supplementary Figure S1). The prevalence of HA-BSIs by age group showed 25% of BSIs in patients aged 64—85 years, 14% aged 45—64 years, and 8% and 5% aged 15—44 and $>$85 years, respectively. Less than 1% of HA-BSIs occurred in children aged $<$14 years (Supplementary Figure S2). Patients affected by Pa-HA-BSI were mostly hospitalized in medical wards (47.2%) and critical care wards (43.4%). Surgical patients represented 9.4% of cases. When we calculated the ratio of the number of BSIs occurring in critical care wards to those occurring in medical wards, the overall risk of Pa-BSIs was increased by 5.7-fold (RR: 5.7; 95% CI: 3.18—9.42) for patients hospitalized in in critical care wards. Mortality rates were 11.3% at day 7 and 17% at day 30, and exposure to MDR-Pa did not increase the risk of mortality (RR: 1.5; 95% CI: 0.32;7.03). Suspected routes of entry for Pa-HA-BSIs were the urinary tract in 30.2% of cases, skin in 17%, digestive tract in 13.2%, respiratory tract in 9.4%, secondary to surgery in 5.7% of cases, and remained undetermined in 24.5% of cases. Of HA-BSIs originating from the urinary tract, 62.5% were linked to a urinary catheter. Intravenous catheters were involved in 77.8% of skin-originating HA-BSIs. Finally, 100% of respiratory-originating HA-BSIs occurred in intubated or tracheotomized patients.

According to MCA analyses, only 46.2% of the variance was explained by the first four axes, testifying to the complexity of predicting Pa-BSIs due to their multifactorial trait. MCA analysis showed a positive correlation between hospitalization in critical care wards, pulmonary portal of entry, maleness and mortality (day 30) (Supplementary Figure S3).

Prospective investigation of the hospital environment

Of 388 environmental samples collected during 53 investigations, 34 samples in 16 investigations were positive for *P. aeruginosa*. Sink traps accounted for 54.3% of *P. aeruginosa*-positive samples, followed by water (22.9%), tap aerators (20%) and wet surfaces (2.8%). Of *P. aeruginosa*-positive samples, 55.9% were also contaminated by one to four bacteria, mostly belonging to other OPPPs (*Pseudomonas* sp. and *Stenotrophomonas maltophilia* respectively in 38.8% and 16.1% of cases) or Enterobacteria. Eleven samples negative for *P. aeruginosa* were positive for OPPPs and/or Enterobacteria, mainly in sink traps (Supplementary Table S2). One investigation revealed *P. aeruginosa* in water sample, tap aerator, and sink trap of the same water point-of-use, despite the presence of an antimicrobial filter, highlighting a fault in effectiveness of the antimicrobial filter.

Population structure of P. aeruginosa from HA-BSIs and hospital environment

Of the 53 clinical strains typed by MLST, 41 different STs were identified, including eight EHR genotypes, responsible for 13 HA-BSIs, and one ST never yet described (Figure 1). Among the 34 *P. aeruginosa* environmental strains, 16 distinct molecular profiles were identified in multiplex rep-PCR. Subsequently, MLST analyses of 14 *P. aeruginosa* environmental strains involved in suspected epidemiological links (Supplementary Table S1) identified nine different STs, including three EHR accounting for 50% of the environmental strains. All nine environmental STs were common to clinical STs, underlying the higher diversity of clinical than environmental isolates.

Path of contamination of Pa-HA-BSIs

Among the 16 *P. aeruginosa*-positive investigations, multiplex rep-PCR and MLST comparisons of clinical and environmental strains identified nine suspected epidemiological links. Pairwise cg-SNP analysis using the genome of environmental isolate as reference corroborated eight epidemiological links with an average of 16.2 cg-SNPs difference per pair (ranging from four to 25 cg-SNPs depending on the pair considered) (Supplementary Table S3). The refuted epidemiological link identified 3991 cg-SNPs difference among clinical and environmental isolates. The nosocomial acquisition of *P. aeruginosa* was considered as possible in six situations and probable in the last two situations (Table I).

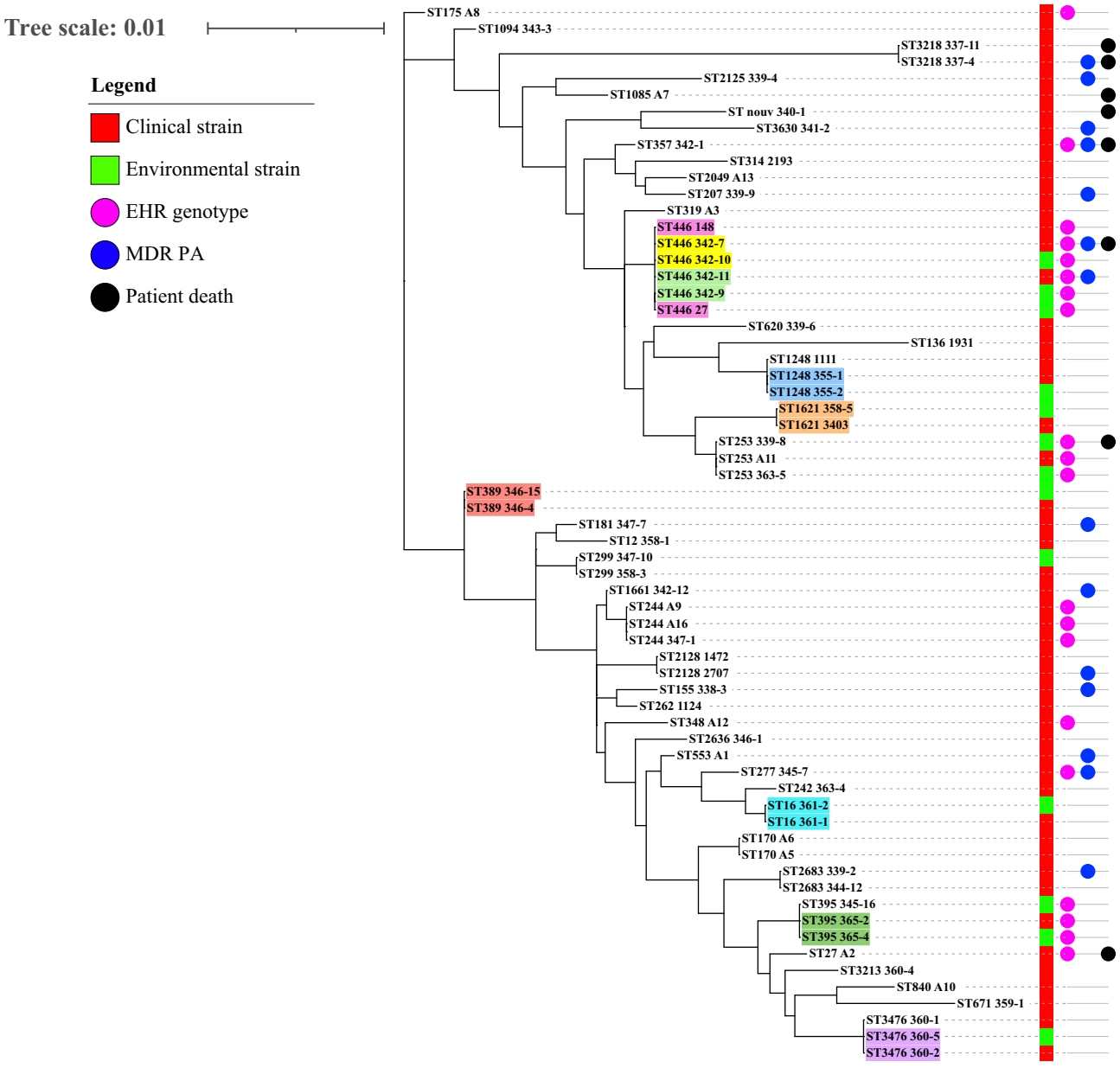

Figure 1. Maximum-likelihood tree estimation using multi-locus sequence typing (MLST) of 53 *P. aeruginosa* causing a bloodstream infection event and 14 *P. aeruginosa* collected in water environment. Epidemiological links are highlighted by coloured labels. Two *P. aeruginosa* reservoirs did not undergo MLST genotype identification, as the molecular fingerprints obtained in multiplex repetitive sequenced-based polymerase chain reaction allowing comparison of the clinical and environmental strains were clearly distinct.

Discussion

During our one-year study, Pa-HA-BSIs occurred predominantly in elderly men with a history of hospitalization, immunosuppression, and urinary tract was the most common route of entry for BSIs, as also described in France the same year [21]. Proper management of invasive devices is essential in preventing HA-BSIs, as ~40% of them were linked to invasive devices. Conversely to previous study, there was no evidence of seasonality in Pa-HA-BSIs occurring in our hospital during the one-year survey [22]. No excess mortality was observed for MDR-Pa-BSIs in contrast to the literature, but this may be explained by the small sample size, as only three MDR-Pa deaths were recorded in our study [1].

Previous studies showed the importance of the environment as a source of Pa-HAIs in outbreaks, estimated to reach 86.3% for VIM-producing *P. aeruginosa* in ICU in a recent study based on a mathematical model [11,23]. However, to date the role of the hydric environment as origin of *P. aeruginosa* sporadic HAIs remains poorly explored. Our prospective single-centre study included all Pa-HA-BSIs and performed real-time investigations of the patient's immediate environment for detecting *P. aeruginosa* environmental reservoirs. In our case, the environmental contamination of water point-of-use close to patients was responsible for 12.5%–50% of HA-BSI. Indeed, eight possible or probable epidemiological links between an environmental exposure to Pa- and HA-BSI were described in a total of 53 HA-BSIs and 16 situations in which *P. aeruginosa* was

Table I
Summary of the eight situations in which an epidemiological link was established, suggesting that the acquisition of *P. aeruginosa* secondary to hospital environmental exposure was possible

Patient	Gender	Age (years)	Immune status	Ward of care	Medical invasive device	Mortality	*P. aeruginosa* MDR	Suspected route	Exposure to antibiotics	Days between admission and BSI	Length of stay (days)	Contamination site	Filter presence	MLST (strain BSI and environmental)	No. of cg-SNPs
16	Man	66	ID	Critical care wards	CVC, CVP	No	Yes	Digestive	Yes	61	NA	Shower trap	Shower filter	ST446[a]	17
16[b]	Man	66	ID	Critical care wards	CVC, CVP	Yes	Yes	Unknown	Yes	88	91 (D)	Shower trap	Shower filter	ST446[a]	25
20	Man	55	ID	Critical care wards	CVC, CVP, nasogastric tube	No	No	Digestive	Yes	20	38	Sink and shower traps	Basin and shower filter	ST446[a]	11
23[b]	Women	84	IC	Medical wards	CVC	No	No	Unknown	Yes	36	43	Sink trap	No basin filter	ST3476	14
32[c]	Man	81	IC	Medical wards	—	No	No	Post surgery	No	10	15	Sink trap and shower traps, shower faucet aerator and shower water	No basin and shower filter	ST1621	19
36	Man	79	IC	Medical wards	Urinary catheter, CVP	No	No	Urinary	Yes	17	27	Sink and shower traps, damp surfaces	Basin and shower filter	ST16	4
42	Women	85	ID	Medical wards	—	No	No	Unknown	Yes	13	48	Sink trap	No basin filter	ST1248	23
44[c]	Man	69	ID	Critical care wards	CVC, CVP	No	No	Cutaneous	Yes	23	40	Sink trap, faucet aerator and downstream water	Basin filter	ST389	17

BSI, bloodstream infection; MLST, multi-locus sequence typing; cg-SNP, core-genome single nucleotide polymorphism; ID, immunosuppressed; CVC, central venous catheter; CVP, peripheral venous catheter; IC, immunocompetent; (D), death; MDR, multi-drug resistant bacteria.
[a] Epidemic high-risk genotypes.
[b] Recurrence.
[c] Possible epidemiological link (compared with probable epidemiological link for other patients).

retrieved from hospital environment. In our study, the intra-sample diversity of *P. aeruginosa* in environmental samples was not assessed, although when different *P. aeruginosa* morphotypes presented in the same sample, one colony of each morphotype was analysed. Therefore, it is possible that in the eight situations where a *P. aeruginosa* reservoir was identified as unrelated to the bacterium responsible for BSI, the epidemiological link could not be demonstrated due to the selection of a single colony in the case of a single morphotype. However, this bias present in most bacterial genomics studies is difficult to eliminate.

Of the eight environment HA-BSIs, three were involved the worldwide ST446 EHR genotype, described as long-term persistent in the environment and as extensively drug-resistant to antibiotics [24]. EHR genotypes overall were responsible for 24.5% of BSIs. The predominance of EHR genotypes in environmental success has already been reported [13]. However, their predominance in HA-BSIs associated with environmental contamination testifies to the extent of their adaptive capacity, supporting their environmental success and facilitating their change of niche from the environment to the patient [25].

In our study, the origin of a large majority of BSIs characterized as nosocomial on the basis of clinical criteria remained undetermined. The hypothesis of an endogenous origin is supported by the increasing prevalence of *P. aeruginosa* intestinal carriage, estimated according to studies between 10% and 50% in patients hospitalized in ICU, and up to 51% among residents of long-term care facilities [26—28]. To date, the intestinal carriage of *P. aeruginosa* in the hospitalized patients is unknown and probably underestimated since intestinal screening is not performed in hospitals and identification of *P. aeruginosa* is fortuitous, secondary to the search for carbapenemase-producing Enterobacterales. Our results challenge a recent study showing that 50% of Pa-HA infections were linked either to a persistent environmental reservoir or to a cross-transmission [12]. That study focused on all types of Pa-HA infections occurring only in ICU whereas we focused on Pa-BSIs in a whole hospital. Our conclusions were more in agreement with Volling *et al*. reporting on the basis of robust epidemiological and genotypic criteria that 7% of Pa-HAI in ICUs may be attributable to sink drains. One other study reported infrequent contamination of patients by *P. aeruginosa* from sink traps, even if these results seemed undervalued as they did not explore water as a source of *P. aeruginosa* contamination [13]. Such a low rate of environmental acquisition of *P. aeruginosa* could be partly explained by the use of clapper traps and the improved compliance with hand hygiene in ICU, which limits the risk of infection through cross-transmission from an environmental reservoir [13]. Usually, ICU nursing staff are more aware of and trained in the role of the environment in the occurrence of HAI, and the exogenous transmission of *P. aeruginosa* depends especially on compliance with preventive and hygiene measures [29]. In our study, the risk of acquiring *P. aeruginosa* from the environment in critical wards was comparable to that in other care wards, thus highlighting the effectiveness of the prevention and environmental control measures put in place in our hospital. As in Couchoud's study, we compared isolates by both WGS and SNP calling analyses. However, for each group of isolates belonging to the same ST and presenting a difference of $\leq$30 loci in cg-MLST, Couchoud *et al*. performed a cg-SNP search using an external reference [13]. As a result, genomic regions specific to the cluster of isolates but absent in the reference genome will not be studied. As it is essential to choose a reference that is closely related to the genomes analysed, in order to increase the performance and reliability of SNP calling, we selected an internal reference, i.e. the environmental isolate of the pair, in order not to underestimate the genetic distance existing between the isolates of a pair or cluster [30]. Furthermore, the threshold of seven SNPs chosen by Couchoud *et al*. to consider two isolates as clonal was very restrictive [13]. To date, there is no consensus on the threshold of SNPs permissible for epidemiologically linking isolates. The evolutionary speed of *P. aeruginosa* is not clearly defined, although a rate of one SNP every four to five months has been proposed [31]. It would have been interesting to present the results obtained with a threshold of up to 30 SNPs, as widely used in the literature to determine clonally related isolates, even more than the presence of an environmental reservoir increases the genetic distance between isolates belonging to the same cluster [20,32,33].

Our environmental investigations reported sink traps as the most frequent hospital niche colonized by *P. aeruginosa*. Sink traps constitute technological niches at the interface between drains and human activities and offer privileged locations for colonization and persistence [13,34]. The risk of patient contamination from aerosols generated by sink traps is now recognized including during outbreaks [35]. No prospective sampling of the environment (prior to reporting BSI to PA) was performed in our hospital, underlining the difficulty in determining the direction of the environment—patient or patient—environment contamination. However, the aim of our work was to determine the proportion of Pa-HA-BSIs associated with an environmental reservoir but without prejudging the causal link. Previously, it has been reported that contamination of the water environment close to the patient is an independent risk factor for Pa-HAI-BSIs [11]. To consider this difficulty in determining the direction of contamination, we defined two levels of epidemiological association: possible and probable. We can assume that sink or shower trap were easily contaminated by the infected patient due to the proximity of trap and the patient, hence the term 'possible epidemiological link'. However, in our opinion, the presence of *P. aeruginosa* in the water supplied to the patient's room is a strong argument for attributing the Pa-BSI to the existence of the environmental reservoir, since the contamination of the water network from infected patient is unlikely. Thus, despite the complexity of establishing the chronology/direction of *P. aeruginosa* contamination between patients and the hospital environment, the genetic relatedness of two strains must be put into perspective with precise spatial—temporal criteria in order to determine the probability of transmission and to predict the direction of the epidemiological link [36].

Beside *P. aeruginosa*, enterobacteria were retrieved in 25.5% of the environmental samples, especially in sink traps, underlining again their major role as reservoirs of pathogens responsible for HAIs and emphasizing the need for their chronic disinfection. However, continuous exposure of sink traps to low levels of disinfectant may favour adaptive mutations of *P. aeruginosa* to a wide range of disinfectants (beyond those to which the strains were exposed) as well as to antibiotics due to biocide/antibiotic co-selection phenomena [37,38]. Moreover, the perennial adaptation of *P. aeruginosa* and other OPPPs to wet technological niches favours the maturation of biofilms

protecting against disinfection processes and ultimately favouring the establishment and proliferation of other pathogens (meticillin-resistant *Staphylococcus aureus*, CPE) not traditionally associated with water [39]. Biofilms constitute a hot-spot for the spread of antimicrobial resistance [39]. Consequently, improving the management of *P. aeruginosa* environmental reservoirs should limit Pa-HAI, and broadly prevent HAI caused by other pathogens and the dissemination of antibiotic resistance [40].

In conclusion, for 85% of Pa-BSIs defined as HA on the basis of clinical criteria, no environmental reservoir was identified, suggesting that *P. aeruginosa* must no longer be considered as a nosocomial pathogen *sensu stricto*. Since the implementation of management measures of water risk in past decades, one can hypothesize that *P. aeruginosa* has become a community-acquired pathogen of nosocomial expression for which infectious character is revealed as a result of hospital selection pressures.

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Conflict of interest statement
None declared.

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Ethics approval
This study was approved by the local ethics committee (Comité de Protection des Personnes Sud Mediterrannee 1, Registration No. RCB: 2016-A00138-43), and reported to ClinicalTrials.gov (NCT02751658).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2024.11.009.

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PAPER

A Decade of Reduced Gram-negative Infections and Mortality Associated With Improved Isolation of Burned Patients

Albert T. McManus, PhD; Arthur D. Mason, Jr, MD; William F. McManus, MD; Basil A. Pruitt, Jr, MD

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Objective: To compare the incidence of gram-negative bacteremia (GNB) and mortality in patients with large burns (≥20% of total body surface) hospitalized in either an open ward (OW) or a single-bed isolation (IW) environment.

Design: Retrospective cohort study.

Setting: The US Army Institute of Surgical Research, Burn Center, Ft Sam Houston, Tex.

Patients: Two thousand five hundred nineteen consecutive patients with large burns divided into two 10-year cohorts. Patients in the first cohort period were treated under OW conditions; patients in the second cohort period, under IW conditions. Infection (bacteremia) data were from a laboratory database. A microbial surveillance system was used to monitor patient isolation. Mortality was compared with predicted mortality derived by logistic regression of outcome, burn size, and age of patients without bacteremia in the study.

Main Outcome Measures: Presence of GNB and survival.

Results: The incidence of GNB was higher in the OW cohort (31.2%) than the IW cohort (12.0%) ($P<.001$). The postinjury time of first GNB was delayed in the IW vs the OW cohort (28.9 days vs 11.8 days, respectively) ($P<.001$). For patients who had GNB in the OW cohort, mortality was higher than predicted (observed-predicted mortality ratio, 1.61) ($P<.001$). Such increased mortality was not present in the IW cohort. Multiple antibiotic-resistant gram-negative pathogens were endemic in the OW cohort. There was no evidence of cross infection or endemic conditions with multiple antibiotic-resistant gram-negative pathogens in the IW cohort.

Conclusion: Improvements in isolation of burned patients were associated with decreased incidence of GNB, delayed postinjury time of GNB, and improved survival. Improved survival is likely related to decreased susceptibility as a result of longer exposure to the benefits of treatment and wound closure. These results suggest that, in patients with severe burn injuries, gram-negative infections and the related mortality can largely be prevented.

(Arch Surg. 1994;129:1306-1309)

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INFECTION IS a major cause of morbidity and mortality in hospitalized burned patients. We have previously estimated the added risk for mortality due to infection in a population of more than 5500 admissions for burns and demonstrated that bacteremia was associated with a 21% increase of mortality above that predicted by logistic regression analysis of injury severity and age.[1] When the bacteremic population was subdivided by pathogen, gram-negative opportunists were found to be the principal cause of the observed increase in mortality. Mortality in the group of patients with gram-negative bacteremia (GNB) was more than 50% greater than that predicted for patients without bacteremia.[1]

The clinical significance of gram-negative pathogens in burned patients became obvious after the introduction of newer generations of penicillin and aminoglycosides in the early 1960s.[2,3] *Pseudomonas aeruginosa* and members of the Enterobacteriaceae family became predominant and, during the subsequent 20 years, caused several endemics.[4,5] Distinguishing features of these endemics were multiple antimicrobial resistance and consistent biotypes within causative strains.

In 1983, the intensive care facilities of our burn center were modified so that seriously burned patients could be cared for in individual rooms rather than the previ-

See Methods on next page

From the US Army Institute of Surgical Research, Ft Sam Houston, Tex.

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METHODS

In the present study, we have selected a 20-year period that includes the decades prior and subsequent to the opening of the new single-bed intensive care unit. We have arbitrarily used a total burn size of 20% or larger of the body surface as the inclusion criterion in an attempt to compare patients with serious injuries. Burned patients selected by this criterion represent more than 90% of those with bacteremia and 95% of those who died during the 20-year period. A comparison of patient demographics between the open-ward (1974 to 1983) and single-bed-unit (1984 to 1993) cohort periods is presented in **Table 1**.

We have used the same case definition of bacteremia (candidemia) as in our previous report.[1] Isolation of a species once or on multiple subsequent blood cultures was considered a case of infection with that organism (species). For mortality comparisons, a patient was categorized by the presence and types of organisms that caused positive findings on blood cultures.

Mortality predictions were made using logistic regression techniques described by Mason et al[1] for burned patients with no bacteremia. In the present study, a predictor was generated based on 1515 patients (with burns on ≥20% of body surface) admitted between 1974 and 1993 who did not have a positive blood culture during hospitalization. The analysis related patient outcome to burn size and age. The equation derived for this population is:

$$K = -4.985 + 0.1054(\%\ \text{Burn}) - 0.2279(\text{Age}) + 0.006122(\text{Age}^2) - 0.0000351(\text{Age}^3)$$

$$PM = e^K/(1+e^K)$$

where PM is predicted mortality. Predicted mortality for an individual is based on that individual's burn size and age. The estimate of the expected number of deaths within a patient group is the sum of the individual predicted mortalities for that group. Confidence limits for the group mortality estimate were derived by a binomial expansion technique. An example of a group prediction for the 1515 patients with no bacteremia is presented in the following tabulation:

Data	Value
No. of admissions	1515
No. (%) of predicted deaths	351(23.2)
95% confidence interval	328-374
No. of observed deaths	351
Observed-predicted mortality ratio	1.00

Based on our previous observation that gram-negative isolates from blood cultures had the greatest associated added mortality risk, we separated the population with bacteremia into patients with one or more cases of bacteremia (candidemia) and at least one culture positive for GNB and patients with cultures positive for gram-positive bacteremia only. Gram-positive cases were principally due to *Staphylococcus aureus*. Patients with one or more yeast species isolated from blood cultures were examined as an additional group. These patients were included in the gram-negative group as well if they also had GNB. Throughout the duration of this review, blood was cultured only for clinical indications of infection.

Table 1. Patient Demographics*

	Open-Ward Cohort (1974-1983)	Single-Bed Cohort (1984-1993)
No. of admissions	1605	914
Mean age, y	31.3	32.2
Mean total burn, % of body surface	47.5	42.8
Third-degree burns, %	22.6	22.5
Deaths, No. of patients	672	214
Mortality, % of patients	41.9	23.4

**Includes patients with burns of 20% or more of body surface.*

Table 2. Incidence of Blood Pathogens in Open-Ward (1974 to 1983) and Single-Bed (1984 to 1993) Cohorts

	Cohort Years, No. (%) of Patients		
Infections	1974-1983 (N=1605)	1984-1993 (N=914)	P*
Gram-negative	500 (31.2)	110 (12.0)	.001
Gram-positive	510 (31.8)	183 (20.0)	.001
Yeast	107 (6.7)	49 (5.4)	NS

**NS indicates not significant.*

ously open intensive care ward environment. We have documented that the occupation of this new facility was associated with a break in transmission of two on-going endemics. Well-characterized multiple-antibiotic-resistant strains of *Providencia stuartii* and *P aeruginosa* were eliminated by intentional cohort admission of new patients to the new unit. The break in patient-to-patient transmission is thought to have been facilitated by the move and also associated with changes in nurse staffing that limited cross trafficking between patients in their individual rooms.[6,7] Caregivers wore masks, gowns, and gloves while in contact with patients; these covers were removed and hands were washed when exiting patient rooms. A microbial surveillance system was used to document colonization by *P stuartii* and *Pseudomonas* strains, the presence of new resistant organisms, or any apparent cross contamination of patients with such organisms. This information was made available to caregivers so that possible breaks in isolation techniques could be rapidly identified and corrected. We have continued this microbial surveillance as an element of quality improvement.

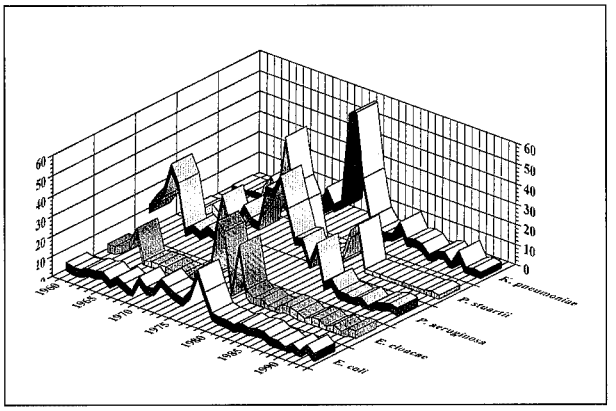
RESULTS

The predicted number of deaths in the population of 1004 patients with bacteremia was 405. The 95% confidence limits for this estimate were 381 and 429 deaths. The actual number of deaths among the patients with bacteremia was 535, a number well beyond the upper confidence limit. The ratio of observed mortality to predicted mortality was 1.32, a 32% increase above that expected on the basis of extent of injury and age. A comparison of the incidence of gram-negative, gram-positive, and yeast blood isolates for the two time periods is presented in **Table 2**.

Table 3. Incidence of Gram-negative Blood Pathogens in Patients With Burns*

Organism	Cohort Years, No. (%) of Patients 1974-1983	1984-1993	P†
Pseudomonas aeruginosa	268 (17.8)	31 (3.4)	.0001
Klebsiella pneumoniae	193 (12.0)	37 (4.0)	.0001
Enterobacter cloacae	99 (6.2)	18 (2.0)	.0001
Escherichia coli	76 (4.7)	26 (2.8)	.0206
Providencia stuartii	66 (4.1)	1 (0.1)	.0001

*Indicates patients with burns of 20% or more of body surface.
†Comparisons are between cohort years.



Distribution of the principal gram-negative pathogens for which blood cultures were positive between 1959 and 1994. K pneumoniae *indicates* Klebsiella pneumoniae; P stuartii, Providencia stuartii; P aeruginosa, Pseudomonas aeruginosa; E cloacae, Enterobacter cloacae; *and* E coli, Escherichia coli.

Table 4. Patients With Blood Cultures Positive for Gram-negative Isolates*

	Cohort Years 1974-1983	1984-1993
No. (%) of patients with gram-negative infection	500 (31)	110 (12)
Predicted mortality, No. (%) of infected patients	227 (45)†	54 (49)‡
Observed mortality, No. of patients	367	47
Observed-predicted mortality, ratio	1.61§	0.87‖

*Includes patients with burns of 20% or more of body surface.
†Confidence interval was 209 to 244.
‡Confidence interval was 46 to 63.
§P<.0001.
‖Difference was not significant.

Table 5. Patients With Blood Cultures Positive for Candidemia*

	Cohort Years 1974-1983	1984-1993
No. (%) of patients with candidemia	107 (6.7)	49 (5.4)
Predicted mortality, No. (%) of infected patients	53 (50)†	26 (53)‡
Observed mortality, No. of patients	85	27
Observed-predicted mortality, ratio	1.60§	1.04‖

*Includes patients with burns of 20% or more of body surface.
†Confidence interval was 44 to 62.
‡Confidence interval was 20 to 33.
§P<.0001.
‖Difference was not significant.

Table 6. Patients With Blood Cultures Positive for Gram-positive Isolates*

	Cohort Years 1974-1983	1984-1993
No. (%) of patients with gram-positive infections	218 (13.6)	117 (12.8)
Predicted mortality, No. (%) of patients	61 (28)†	36 (31)‡
Observed mortality, No. of patients	57	28
Observed-predicted mortality, ratio§	0.93	0.78

*Includes patients with burns of 20% of body surface.
†Confidence interval was 50 to 72.
‡Confidence interval was 28 to 44.
§Differences were not significant.

A comparison of the incidence of the five most common gram-negative species in this 20-year period is presented in **Table 3**. As can be seen, all species were significantly less frequent in the second cohort. The incidence of these organisms over a 35-year period at this institution is presented in the **Figure**. Sporadic outbreaks of these pathogens stopped with the opening of the new single-bed unit. The numbers of blood cultures positive for bacteria, and in particular GNB, have been significantly reduced since that time. The incidence of cultures positive for yeast was similar in both groups.

The actual mortality in each of the organism groups was next compared with the predicted mortality. The results for gram-negative organisms are presented in **Table 4**. The predicted mortality was similar in both groups, with that in the later group slightly greater. As can be seen, the actual number of deaths in the open-ward cohort was 61% above that predicted. This result is essentially the same as that described in our earlier report.[1] The mortality associated with GNB in the later group is within the confidence limit of the prediction for patients without blood cultures positive for GNB. The number of postburn days to the first culture positive for GNB in the single-patient-room cohort was found to be more than twice that found in the earlier cohort (28.9 vs 11.8 days, respectively; $P<.001$). Mortality assessment of patients with candidemia is presented in **Table 5**. The increase in mortality associated with candidemia during the first period is similar to our previous finding. The increase in associated mortality was absent in the later group.

The results for patients with only gram-positive bacteremia during their hospitalization are presented in **Table 6**. These results also agree with those of our previous review. Gram-positive bacteremia, when not preceded or followed by cultures positive for GNB or yeast, was not associated with an increase in mortality above that predicted on the basis of extent of burn and age.

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COMMENT

Control of infection has been a goal in burn care since the development of the germ theory. There is, however, no consensus on the most appropriate infection control program for serious burns.[8-10] The purpose of this report is not to make specific claims for the design of this burn center or for our institution's specific methods of patient isolation and infection control, but to demonstrate that reduction in the incidence of blood infections has fulfilled a prediction in our previous review. Improvement in control of GNB has been associated with an improvement in mortality comparable to the previous increment in mortality attributable to such infections.

The improvement in outcome for the fewer patients that still become infected with gram-negative organisms is less easy to explain. Early excision, improved ventilation techniques, nutritional support, and other treatments and support techniques continue to improve patient care. It seems possible that the reduction in GNB has reduced a morbid complication that has traditionally clouded the true benefit of improved techniques of physiological support. We have previously reported on the significance of *P aeruginosa* infections during the history of our burn center and have shown that the incidence of *P aeruginosa* infections was reduced and the associated mortality decreased after the use of single-patient rooms.[4,7,11] These differences were associated with a much later postinjury time of infection, which was thought to explain in part the improved survival. Patients with delayed colonization and infection appear to represent a less susceptible patient population in which response to infection is more effective. The delay in time of infection documented in this study allows patients to have a longer exposure to the benefits of such treatments and reach a stage of recovery in which response to infection is more effective. Isolation from cross contamination closes the window of susceptibility to infection and appears to shift the balance between microbial pathogenicity and host resistance in favor of the patient.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of the Army or the US Department of Defense.

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Reprint requests to US Army Institute of Surgical Research, Library Branch, 2322 Harney Rd, Ft Sam Houston, TX 78234-6315 (Dr McManus).

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DISCUSSION

David N. Herndon, MD, Galveston, Tex: The authors are to be congratulated on this huge and excellent review of infections and morbidity and mortality in burns over 20% total body surface treated at their institution over the last 20 years. Their results are incontrovertible and will serve as a landmark reference for years to come.

The marked decrease in mortality noted in the current decade vs the previous decade has been reported by other observers, including ourselves, and in a recent collection of mortality data from a variety of burn units performed by Saffle and reported at the American Burn Association [Saffle J, Davis B, Sullivan J, Williams P, The American Burn Association Patient Registry. Improved survival from thermal injury: data from the ABA Burn Patient Registry. *Proc Am Burn Assoc.* 1994;26:105]. The other units reporting improvements in survival, in contrast, had no changes in hospital design or infection control procedures in the year 1983, as described by Dr McManus. Rather, they attribute their observed decreases in mortality, which are remarkably similar to the decreases in mortality described here, to improvements in early excision of the burn wound, early enteral feeding with decreased utilization of parenteral hyperalimentation, improved ventilatory support techniques, utilization of perioperative antibiotics, and more aggressive rehabilitation. These advances have contributed, clearly, to a decrease in length of hospital stay—in these reports—for major burns of 50% in the latter decade vs the former decade. Most burn specialists would predict that the greatest contributor to decreased infections and mortality in this decade has not been change of architecture of a burn unit or infection control techniques, but has been early closure of the burn wound.

Have the authors performed a logistic equation using the rate of wound closure as a factor to determine the degree to which this revolutionary and dramatic change in burn care has affected mortality?

Environmental control or single-bed care may indeed be very important issues. However, randomized prospective studies are required to substantiate independent treatment effects.

Dr McManus: Thank you, Dr Herndon, for your questions and discussion. We certainly agree with you that burn care has improved and mortality has decreased throughout the United States during this past decade. In our own particular instance, we noted this same trend and actually a significant improvement in survival and reported it to this Society in 1986, 1 year after the opening of our new unit. Our early excision practices had not changed when that improvement in survival was reported.

I do not want to argue that the differences that we've shown you in infection are completely unrelated to early excision. I think that early excision is perhaps the greatest improvement that has occurred in the treatment of burns since the development of topical antimicrobial burn creams. But in our case, we noted an improvement in survival with the break in the two endemics that were ongoing when we opened this unit.

And as I stated, I do not propose that this paper is to argue for a specific design of a burn unit. I think it does show you that gram-negative infections, as measured by bacteremia, can be controlled by improved patient care and by isolation.

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Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination

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KEYWORDS
Methicillin-resistant *Staphylococcus aureus*; Environmental contamination; Hydrogen peroxide vapour decontamination

Summary The hospital environment can sometimes harbour methicillin-resistant *Staphylococcus aureus* (MRSA) but is not generally regarded as a major source of MRSA infection. We conducted a prospective study in surgical wards of a London teaching hospital affected by MRSA, and compared the effectiveness of standard cleaning with a new method of hydrogen peroxide vapour decontamination. MRSA contamination, measured by surface swabbing was compared before and after terminal cleaning that complied with UK national standards, or hydrogen peroxide vapour decontamination. All isolation rooms, ward bays and bathrooms tested were contaminated with MRSA and several antibiogram types were identified. MRSA was common in sites that might transfer organisms to the hands of staff and was isolated from areas and bed frames used by non-MRSA patients. Seventy-four percent of 359 swabs taken before cleaning yielded MRSA, 70% by direct plating. After cleaning, all areas remained contaminated, with 66% of 124 swabs yielding MRSA, 74% by direct plating. In contrast, after exposing six rooms to hydrogen peroxide vapour, only one of 85 (1.2%) swabs yielded MRSA, by enrichment culture only. The hospital environment can become extensively contaminated with MRSA that is not eliminated by standard cleaning methods. In contrast, hydrogen peroxide vapour decontamination is a highly effective method of eradicating MRSA from rooms, furniture and equipment. Further work is needed to determine the importance of environmental contamination with MRSA and the effect on hospital infection rates of effective decontamination.

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Introduction

Infection with methicillin-resistant *Staphylococcus aureus* (MRSA) has become endemic in hospitals around the world despite concerted efforts by infection control professionals.[1] In the UK, the proportion of blood isolates of *S. aureus* that are resistant to methicillin has risen from just a few percent in the late 1980s to over 30%, with rates of over 40% seen in tertiary referral hospitals.[2–4] Similar prevalence rates are reported from other European countries[4] and the US.[5] *S. aureus* is the commonest cause of surgical-site infection in English hospitals, and more than half the infecting strains are methicillin resistant.[6] Nearly all MRSA infections are acquired in hospital and many of them are potentially preventable.[7]

MRSA is transmitted between patients on the hands of hospital staff, and handwashing is widely recognized as the single most important factor for prevention of colonization and infection.[8,9] Nevertheless, despite the implementation of standard infection control practices, the widespread failure to control MRSA is striking.[10] The hospital environment may be another important, but neglected, factor in hospital cross-infection.[11–13]

Since the 1950s, hospital design and hygienic practices have been largely directed at controlling *S. aureus* contamination of air, hands, instruments and surfaces. By the late 1970s these practices had been so successful that the hospital environment was considered to make little contribution to the spread of hospital infection.[14,15] Nevertheless, *S. aureus* and MRSA can survive on dry surfaces for prolonged periods,[12,16–20] and many investigations since the 1980s have shown that these organisms can contaminate surfaces and equipment touched by hospital staff.[12,21–26] Standards of hospital cleaning have declined[11,13,27] and floors, furniture and medical equipment may now be sources of MRSA transmission.[11–13,22]

The current guidelines for the control of MRSA in UK hospitals recognize the potential importance of the environment and recommend terminal cleaning of isolation rooms after patient discharge.[2] In practice, manual cleaning of complex environments containing beds, furniture, medical equipment and soft furnishings is difficult. This problem may be solved by gaseous decontamination methods.

Hydrogen peroxide vapour is effective against a wide range of organisms, including bacterial endospores,[28] vegetative bacteria[29] and viruses.[30] It is safer than other disinfectant gases such as formaldehyde and ethylene oxide, and decomposes to water and oxygen. It has been used to decontaminate laboratory and medical equipment, pharmaceutical manufacturing facilities and animal houses[31–35] but has not previously been applied in healthcare settings. The present study investigated MRSA environmental contamination in a hospital where MRSA infection is common, and compared the effectiveness of standard hospital environmental cleaning with a commercial hydrogen peroxide vapour decontamination system.

Materials and Methods

Setting

This investigation was conducted in a 1200-bed London teaching hospital where MRSA colonization and infection is common. Environmental sampling was conducted on four different wards dealing with vascular, lower gastrointestinal, orthopaedic/plastic and general surgical patients. Environmental decontamination with hydrogen peroxide vapour was conducted on the vascular surgery ward only.

Surface samples were taken from the following locations: ward single side rooms that had recently been used to isolate patients infected or colonized with MRSA; four-bedded ward bays recently used to cohort MRSA patients; the area around the bed of a patient colonized with MRSA who was being barrier-nursed on the open ward; beds on an open ward that were being used to nurse non-MRSA patients; and bathrooms used for MRSA and non-MRSA patients.

Environmental surface sampling and microbiological methods

Cotton tipped sterile swabs (Bibby Sterilin, Stone, Staffordshire, UK) were moistened in sterile brain–heart infusion (BHI) broth (Oxoid, Basingstoke, Hampshire, UK) and used to sample surface areas of approximately 25 cm^2 by standardized swabbing in two directions at right angles. In intervention studies, two adjacent 25 cm^2 areas were sampled, one before and one after the intervention. Between 10 and 25 different sites, chosen to include surfaces

likely to be touched by patient and/or staff hands, were sampled in each room. These included: floor corners and floor areas beside the bed, over-bed tables, bed frames and bed-raising panels, bedside chairs and lockers, door handles, light switches, sink taps, televisions and remote controls.

Swabs were used to inoculate Baird–Parker (BP) agar plates (Oxoid, Basingstoke, Hampshire, UK) incubated at 37 °C for 36 h. Growth was recorded semi-quantitatively: if growth was only within the section of the plate directly inoculated with the swab, it was classified as '+', and growth outside the direct inoculum after streaking out was classified as '++'. Enrichment cultures were made for each sample by incubating swabs in BHI containing 4 mg/L ciprofloxacin (Bayer, Newbury, Hampshire, UK) at 37 °C for 24 h and then plating on to BP plates incubated at 37 °C for 36 h.

Presumptive *S. aureus* colonies were confirmed by coagulase production and mannitol fermentation. The susceptibility of all confirmed isolates was tested by the British Society for Antimicrobial Chemotherapy disk diffusion method[36] against: penicillin, methicillin, gentamicin, neomycin, vancomycin, erythromycin, fusidic acid, tetracycline, linezolid, rifampicin, mupirocin, trimethoprim and ciprofloxacin.

Conventional terminal cleaning

This is defined as environmental cleaning after discharge of an infectious patient. The hospital has protocols and service level agreements in place for this procedure that accord with NHS standards.[27] These do not specify details of cleaning procedures, but in this hospital a solution of detergent sanitizer (GWP 4L, containing 5–15% non-ionic surfactant and 5–15% cationic surfactant, diluted 1:500) (GWP Group, Elland, W. Yorkshire, UK) is used for surface cleaning; bed curtains and linen are removed for laundering; all horizontal surfaces are damp dusted, specifically including window architraves, high and low surfaces, curtain rails, radiators, pipes, door handles, ventilation and air-conditioning grilles and surrounds; furniture is cleaned, specifically including bed frames and mattresses; all surfaces are dried with a disposable cloth; walls are damp-mopped and left to dry; the floor is swept with a dust control mop, then damp mopped with detergent sanitizer; wash basins and taps are cleaned. The cleaning cloths and mop heads are disposable.

Hydrogen peroxide vapour decontamination equipment

The room bio-decontamination system (BIOQUELL PLC, Andover, Hampshire, UK) consists of four portable modules: the Clarus™ R hydrogen peroxide gas generator, the Clarus™ R2 aeration unit, an instrumentation module and a control computer. The Clarus™ R is placed within the room and evaporates 30% (w/w) liquid hydrogen peroxide (Fisher Chemicals, Loughborough, Leicestershire, UK). The resulting hydrogen peroxide vapour is delivered via a dual axis vapour distribution system that ensures high kinetic energy and even distribution throughout the room. The gas concentration, temperature and relative humidity are measured by the instrumentation module and monitored by a control computer situated outside the room. When decontamination is complete, Clarus™ R2 aeration units inside the room catalytically convert the hydrogen peroxide vapour into water and oxygen.

Room decontamination process

Four single ward side rooms of approximately 35.0 m^3 in volume (two with en-suite toilet cubicles of approximately 4.0 m^3), which had recently housed patients infected or colonized with MRSA, were decontaminated with hydrogen peroxide vapour after the patients had left but before terminal cleaning. The side rooms each contained a bed and other hospital furniture. A shower room dedicated to MRSA patients (13.2 m^3) and a bathroom (45.4 m^3) shared by MRSA and non-MRSA patients, were also decontaminated. The shared bathroom was split into three sections: a communal area leading to two bathrooms, one for MRSA patients and one for other patients. In the communal area there were two shared hoists but one sling was reserved for MRSA patients.

After the decontamination equipment had been set up in the room, the inlet and outlet vents of the air-conditioning system and the door were sealed with adhesive tape. A portable hydrogen peroxide sensor [Portasens II, ATI (UK) Ltd, Ashton-under-Lyne, UK] was used to monitor hydrogen peroxide gas concentrations in adjacent areas outside the room and to verify the absence of hydrogen peroxide in the room at the end of decontamination.

Biological indicators (BIs)

BIs, consisting of $>1 \times 10^6$ *Bacillus stearothermophilus* spores dried on stainless steel disks sealed in Tyvek pouches (Apex Laboratories Inc., Apex, USA), were hung at four points around the room before decontamination. After decontamination, the BI disks were removed and cultured according to the manufacturer's instructions.

Results

MRSA environmental contamination in ward side rooms, bays and bathrooms before terminal cleaning

Three hundred and fifty-nine swabs were taken before cleaning from 18 ward side rooms, two four-bedded ward bays and four bathrooms recently occupied or used by patients infected or colonized with MRSA (15 of the 18 patients in the side rooms were infected with MRSA and three were colonized). MRSA was recovered from every side room, bay and bathroom. Seventy-four percent of swabs yielded MRSA; 70% of positive cultures were by direct plating and 30% by broth enrichment (Table I).

Four (50%) of eight sites in the non-MRSA patient bathroom and six (67%) of nine sites in the communal bathroom area yielded MRSA. The communal area contained two patient hoists, both of which were positive for MRSA.

Single swabs were also taken from the frames of 23 beds on an open ward that were being used to nurse non-MRSA patients. Forty-three percent of the bed frames yielded MRSA; 50% by direct plating. A 24th bed had been removed, but a swab from the floor area of that bed space yielded MRSA on enrichment culture.

MRSA environmental contamination in ward side rooms and bays after terminal cleaning

Two hundred and forty-eight swabs were taken from adjacent sites matched before and immediately after terminal cleaning in eight side rooms and two bays. Ninety percent of swabs from the matched sites yielded MRSA before cleaning; 78% by direct plating. Sixty-six percent of swabs from the matched sites yielded MRSA after cleaning; 74% by direct plating (Table I). In addition, nine of 10 swabs taken after terminal cleaning from the bed area of a patient who had been barrier-nursed on the open ward were positive for MRSA; seven by direct plating.

Hydrogen peroxide vapour decontamination

The complete process of room decontamination took an average of 5 h, with the aeration phase often run overnight for convenience. A typical cycle maintained a concentration of approximately 500 ppm of hydrogen peroxide vapour for 40 min. The ambient temperature and relative humidity ranged from 26–28 °C and 35–50%, respectively. Hydrogen peroxide was not detected in the corridors outside the rooms being decontaminated, nor in the rooms themselves after decontamination. Gas concentrations up to 10 ppm were detected in the ceiling voids in adjacent rooms, but no more than 1 ppm at head height (data not shown). These low levels did not persist because of air circulation and gas decomposition, and are within the UK Health and Safety Executive exposure limits.[37] Exposure to hydrogen peroxide vapour within the rooms killed all *B. stearothermophilus* BIs (data not shown).

MRSA contamination in ward side rooms and bathrooms before and after decontamination with hydrogen peroxide vapour

Before decontamination, 61 (72%) of 85 sites were positive for MRSA; 72% by direct plating. After exposure to hydrogen peroxide vapour, only one (1.2%) of the 85 sites (a floor corner in one of the

Table I Results of surface swabbing

	Total before cleaning	Matched before cleaning	Matched after cleaning	Matched before H_2O_2[a]	Matched after H_2O_2[a]
No. of rooms sampled	24[b]	10[c]	10[c]	6[d]	6[d]
No. of swabs	359	124	124	85	85
Number yielding MRSA	264 (73.5)	111 (89.5)	82 (66.1)	61 (71.8)	1 (1.2)
From direct plating	185 (70.1)	87 (78.4)	61 (74.4)	44 (72.1)	0 (0.0)
++Growth	75 (40.5)	37 (42.5)	26 (42.6)	24 (54.5)	-
+ Growth	110 (59.5)	50 (57.5)	35 (57.4)	20 (45.5)	-
From enrichment only	79 (29.9)	24 (21.6)	21 (25.6)	17 (27.9)	1 (100.0)

Matched denotes rooms in which adjacent sites were sampled before and after intervention. The number in parenthesis denotes the percentage.

[a] Hydrogen peroxide vapour decontamination.

[b] Eighteen single isolation rooms, two four-bed bays, four bathrooms.

[c] Eight single isolation rooms, two four-bed bays.

[d] Four single isolation rooms, two bathrooms.

rooms) yielded MRSA, by selective broth enrichment (Table I).

Antibiotic sensitivity patterns of MRSA environmental isolates

There were 23 antimicrobial susceptibility patterns amongst the 380 MRSA environmental isolates. The most common pattern (in addition to resistance to penicillin and methicillin) was resistance only to erythromycin, ciprofloxacin and neomycin, which was seen in 159 (42%) of isolates (Table II).

Most of the environmental MRSA isolates had the same antimicrobial susceptibility pattern as the strain isolated from the previous patient occupant. However, in the eight side rooms with matched sampling sites, a mean of 2.3 different susceptibility patterns were found in rooms before conventional cleaning and 1.4 after (Table II). There was greater diversity amongst isolates from sites outside the isolation rooms: four patterns were found amongst the 10 isolates from the bed frames on the open ward, four amongst the 14 isolates from the shared bathroom, and a mean of 3.5 patterns amongst isolates from the two MRSA bays sampled before conventional cleaning and 2.5 after (data not shown).

Discussion

We found extensive surface environmental contamination with MRSA in ward areas previously occupied by colonized or infected patients. The level of contamination was much higher than that reported by Boyce *et al*. (1997)[22] who used similar culture methods and found 27% of 350 environmental surfaces around MRSA patients to be contaminated compared with 74% of 359 in our study. Boyce *et al*. (1997)[22] identified MRSA in 73% of 22 rooms of infected patients and 69% of 16 rooms of colonized patients, whereas we found MRSA in all the rooms sampled, regardless of patient status.

Contamination of isolation rooms is not surprising, and it is usually supposed that the patient contaminates the room rather than the other way around. However, in the present study we found that side rooms were often contaminated with more than one antibiogram-type of MRSA, suggesting survival of MRSA strains from previous room occupants. Similarly, four MRSA types were contaminating 43% of 23 beds in an open ward not currently being used to nurse MRSA patients and four types were found in a shared bathroom facility.

Contaminated environmental surfaces that are commonly touched by patients and/or staff may act as sources for hand transfer. In support of this, Boyce *et al*.[22] demonstrated that five (42%) of 12 nurses contaminated their gloves with MRSA while performing activities that required no direct patient contact but involved touching objects in the rooms of MRSA patients. Oie *et al*.[26] found MRSA on the door handles of 19% of 21 rooms MRSA patients but also on 7% of door handles of 175 non-MRSA rooms. In our study, MRSA was found before

Table II Antibiotic susceptibility patterns of MRSA environmental isolates (number of isolates and percentage in brackets)

Antibiotic sensitivity pattern	Total[a]	Matched before cleaning[b]	Matched after cleaning[b]
MET,E,CIP,N	159 (41.6)	51 (45.9)	28 (34.1)
MET,E,CIP	107 (28.0)	23 (20.7)	22 (26.8)
MET,E,CIP,W,CN,MUP	41 (10.7)	22 (19.8)	18 (21.9)
MET,E,CIP,N,W,CN,TE	29 (7.6)	-	6 (7.3)
MET,CIP	10 (2.6)	5 (4.5)	5 (6.1)
MET,E,CIP,N,CN	10 (2.6)	2 (1.8)	-
MET,E,CIP,W,MUP,RD	3 (0.8)	-	-
MET,E,CIP,N,W,CN,FD	3 (0.8)	-	-
MET,E,CIP,W,CN,TE,FD	2 (0.5)	-	-
MET,E,CIP,W,MUP	2 (0.5)	-	-
MET	2 (0.5)	-	-
Other patterns (one isolate each)	12 (3.1)	8 (7.2)	1 (1.2)
Total	380	111	82
Mean number of patterns: per room	-	2.3	1.4
per bay	-	3.5	2.5

MET, methicillin (methicillin resistance results in penicillin resistance); E, erythromycin; CIP, ciprofloxacin; N, neomycin; W, trimethoprim; CN, gentamicin; TE, tetracycline; MUP, mupirocin; RD, rifampicin; FD, fusidic acid.

[a] Represents all MRSA environmental isolates throughout the study period.

[b] Represents MRSA isolates from adjacent sites matched before and immediately after terminal cleaning in eight side rooms and two bays.

cleaning on 59% of 27 door handles and 33% of nine sink taps. We also found MRSA on other sites commonly touched by staff, such as bed-raising control panels, light switches and patient hoists.

In the present study, standard methods of terminal cleaning that complied with current NHS guidelines were ineffective in eliminating MRSA: 74% of environmental swabs yielded MRSA before cleaning and 66% after. All the rooms yielded MRSA from some sites. There is other published evidence that terminal cleaning is poorly effective: Blythe *et al*.[23] found that 46% of 41 isolation rooms remained contaminated with MRSA after cleaning, and Byers *et al*.[38] found that 16% of 376 environmental sites in a single room after cleaning remained contaminated with vancomycin-resistant enterococci (another organism that can contaminate the hospital environment).

In contrast, hydrogen peroxide vapour decontamination was highly effective in eradicating environmental MRSA. Before decontamination, 72% of room swabs yielded MRSA; afterwards, only one of 85 samples (1.2%) yielded MRSA, and then only by enrichment culture. Although the present decontamination cycles take 5 h in total, it is likely that this will be reduced with further optimization.

In conclusion, we found heavy and extensive environmental contamination with MRSA in a hospital caring for many infected and colonized patients. The distribution of this contamination and the isolation of multiple strains suggest that the environment may be an important source of hospital cross-infection. Standard terminal cleaning had little impact on this contamination while the hydrogen peroxide vapour decontamination system was very effective. This gaseous method is particularly useful for decontaminating complex furniture and equipment that is difficult to clean manually. The system can be used to decontaminate entire wards if necessary, but as rooms have to be vacated before gassing, it would normally be limited to the decontamination of smaller vacant rooms and bathrooms. However, furniture, bedding and medical equipment could be temporarily removed from the general ward environment and processed in a dedicated decontamination unit. Other nosocomial pathogens can survive in the hospital environment[11,12,39] and are also potential targets for hydrogen peroxide vapour decontamination.

Further investigation of the clinical significance of hospital environmental contamination and of more effective cleaning methods are required. This should include evaluation of the impact on hospital infection rates of chemical disinfectants for surface cleaning and hydrogen peroxide vapour for decontamination of rooms and equipment.

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Polymicrobial bloodstream infections: Epidemiology and impact on mortality

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ABSTRACT

The aim of this study was to investigate the impact of polymicrobial bloodstream infections (pBSIs) on the outcome of sepsis in an area where antimicrobial resistance is of concern. This was a retrospective analysis of data collected prospectively from patients developing BSI outside of an intensive care unit (non-ICU patients) or after ICU admission. Demographics and clinical characteristics were compared for patients with pBSI versus monomicrobial BSI (mBSI) and following stratification by ICU or non-ICU and severity of sepsis status. Possible risk factors for adverse outcome were explored by multivariate analysis, and outcomes were measured by Cox regression analysis. Among 412 patients with BSI, 47 patients (11.4%) with pBSI were recorded; compared with patients with mBSI, they had significantly higher APACHE II scores and presented more frequently with severe sepsis/septic shock. The all-cause 28-day mortality was significantly higher for pBSI versus mBSI (38.3% vs. 24.7%; $P = 0.033$), whereas appropriateness of treatment was comparable (78.7% vs. 86.6%). Primary bacteraemia by combinations of *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* was predominant among pBSIs; in mBSIs, urinary tract infections by *Escherichia coli*, *K. pneumoniae* or *Pseudomonas aeruginosa* predominated. Multivariate analysis demonstrated pBSI as a significant contributor to 28-day mortality (HR = 1.86; $P = 0.039$), along with presence of two or more co-morbidities (HR = 2.35; $P = 0.004$). In conclusion, pBSIs differed epidemiologically from mBSIs, with the emergence of enterococcal species, and portended an almost two-fold increased risk of 28-day mortality. Prospective studies are warranted to elucidate possibly modifiable factors.

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1. Introduction

Severe sepsis and septic shock are recognised among the leading causes of morbidity, for which the pathophysiology is still poorly understood [1,2]. Polymicrobial infections could reflect single or multiple infectious sites with only scarce data available on their epidemiology or underlying mechanisms. Furthermore, the impact of polymicrobial causality on outcome has not been fully investigated; most of the available data derive from patients with bloodstream infections (BSIs) and particularly from the intensive care unit (ICU) setting. However, no clear-cut answer is provided on whether the prognosis of polymicrobial infections is worse than that of monomicrobial infections [3–7].

Quite often, the golden hour before ICU admission is 'spent' in medical wards [8,9]. Therefore, focusing on the epidemiology of sepsis before ICU admission could have possible implications in improving the ultimate outcome of patients [8]. In the present study, we describe the epidemiology of microbiologically documented septic episodes in a multicentre, mixed cohort of ICU and non-ICU patients. The primary endpoint was to define differences in final outcome between septic bacteraemic episodes of polymicrobial origin compared with those of monomicrobial origin. As a secondary endpoint, we sought to investigate differences in epidemiology between polymicrobial and monomicrobial BSIs in this cohort in view of an established prevalence of multidrug-resistant Gram-negative pathogens in Greek hospitals [10].

2. Patients and methods

Data collected prospectively between January 2007 and May 2011 from 35 centres across Greece were analysed. Data for 2627 patients were available in the database of the Hellenic Sepsis Study Group (HSSG); analysis was restricted to patients with bacteraemic infections. Patients were divided into those developing sepsis after ICU admission (ICU patients) and those either admitted into hospital with sepsis or developing sepsis in the general ward without being transferred to the ICU (non-ICU patients).

Inclusion criteria were: (a) age $\geq$18 years; (b) sepsis, severe sepsis or septic shock due to either acute pyelonephritis, lower respiratory tract infection, intra-abdominal infection (IAI) or primary bacteraemia; and (c) recovery of the offending pathogen from blood cultures. Exclusion criteria were: (a) infection by human immunodeficiency virus (HIV); (b) neutropenia, defined as an absolute neutrophil count $<500/mm^3$; (c) chronic intake of corticosteroids, defined as any daily oral intake of $\geq$1 mg/kg of equivalent prednisone for $>$1 month; and (d) denial to inform consent.

Written informed consent was provided by the patients or by their first-degree relatives for patients unable to consent. The study protocol was submitted to and approved by the Ethics Committees of the hospitals of the participating centres. Every patient was enrolled once in the study.

2.1. Definitions

Sepsis, severe sepsis and septic shock were defined according to established criteria [2,11]. The major infections possibly leading to secondary bacteraemia, namely acute pyelonephritis, community-acquired pneumonia (CAP), hospital-acquired and ventilator-associated pneumonia (HAP/VAP) and acute intra-abdominal infection (IAI), were diagnosed according to established criteria [12–15].

Primary bacteraemia was diagnosed for patients presenting with both of the following: (a) peripheral blood culture positive for Gram-positive or Gram-negative bacteria; and (b) absence of any alternative site of infection consistent with the pathogen cultured in blood. Isolates of coagulase-negative staphylococci or of skin flora isolated from single blood cultures were not considered pathogenic [11]. Polymicrobial BSI refers to an infection in which more than one micro-organism was recovered from the same blood culture.

Patients were followed-up for 28 days and outcome was recorded. For every patient, a complete diagnostic work-up was performed at the discretion of the treating physician comprising history, thorough physical examination, blood cell counts, blood biochemistry, blood gases, blood culturing from peripheral and central lines, urine cultures, chest radiography, and chest and abdominal computed tomography or ultrasound if considered necessary. Co-morbidities captured in the database were type 2 diabetes mellitus, chronic obstructive pulmonary disease, chronic renal disease, chronic heart failure, solid tumour malignancy and chronic intake of corticosteroids; patients with the latter co-morbidity, as defined above, were excluded from the current analysis.

Definite antimicrobial therapy administered to the patient was considered appropriate when at least one of the administered agents was active on the isolated pathogen or pathogens according to the antibiogram.

2.2. Statistical analysis

The database of the HSSG was screened for clinically and microbiologically documented BSIs and the retrieved cases were grouped as either monomicrobial or polymicrobial. Analyses were performed for the basic demographic characteristics of each group, the type and adequacy of the initial empirical antibiotic regimen, and the type of hospitalisation (wards versus ICU).

Survival was estimated by Kaplan–Meier analysis; comparisons were done by the log-rank test. For quantitative variables shown to have a normal distribution by Kolmogorov–Smirnov's statistics, data were reported by mean $\pm$ standard deviation; comparisons between such groups were done by the Student's *t*-test.

Alternatively, the Wilcoxon rank-sum test was used. Comparisons between groups for qualitative data were done by χ^2 test or Fisher's exact test when appropriate. Receive operator characteristic (ROC) curve analysis was performed for numerical variables to define a threshold that significantly affected mortality. Cox regression analysis was done to disclose the impact on survival of the presence of polymicrobial BSI and other independent variables demonstrated in the univariate analysis. Hazard ratios (HRs) and 95% confidence intervals were calculated. A *P*-value of $<$0.05 was considered statistically significant.

2.3. Microbiological studies and resistance breakpoints

Identification of pathogens was performed using standard microbiological methods according to each hospital's procedures. The disk diffusion method or automated systems [VITEK® 2 (bioMérieux Inc., Durham, NC) or Phoenix (BD Diagnostics, Inc., Sparks, MD)] were used for all tested antibiotics according to the most recent recommendations of the Clinical and Laboratory Standards Institute (CLSI) [16]. When an automated system was not available, susceptibilities of pathogens to colistin and tigecycline were also tested by Etest (AB BIODISK, Solna, Sweden). Extended-spectrum β-lactamase production was determined by the double-disk test, metallo-β-lactamase production was confirmed by the ethylene diamine tetra-acetic acid (EDTA) synergy test, and KPC enzyme production was screened by the boronic acid double-disk test. Urine and respiratory cultures were performed quantitatively [17].

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Table 1
Demographic and clinical characteristics of patients enrolled in the study.

	Monomicrobial bacteraemia (*N* = 365; 88.6%)	Polymicrobial bacteraemia (*N* = 47; 11.4%)	*P*-value
Age (years) (mean ± S.D.)	68.7 ± 16.9	70.7 ± 13.3	0.450
Gender [*n* (%)]			0.812
Male	186 (51.0)	23 (48.9)	
APACHE II score (mean ± S.D.)	16.4 ± 8.8	19.3 ± 7.0	0.014
White blood cells (/mm^3) (mean ± S.D.)	16 704.6 ± 11 104.7	16 267.5 ± 11 310.9	0.888
Severity [*n* (%)]			0.009
Sepsis	193 (52.9)	15 (31.9)	
Severe sepsis/shock	172 (47.1)	32 (68.1)	
Combination of pathogens [*n* (%)]			0.000
Gram-negative	318 (87.1)	18 (38.3)	
Gram-negative + *Candida* spp.	0 (0.0)	6 (12.8)	
Candida spp.	10 (2.7)	0 (0.0)	
Gram-negative + Gram-positive	0 (0.0)	18 (38.3)	
Gram-positive	37 (10.1)	5 (10.6)	
Hospitalisation (ICU vs. non-ICU) [*n* (%)]			0.102
ICU	111 (30.4)	21 (44.7)	
Source of bacteraemia [*n* (%)]			0.228
Primary bacteraemia	137 (37.5)	18 (38.3)	
Pyelonephritis	137 (37.5)	13 (27.7)	
CAP	27 (7.4)	2 (4.3)	
IAI	30 (8.2)	7 (14.9)	
VAP	28 (7.7)	7 (14.9)	
HAP	3 (0.8)	0 (0.0)	
Soft-tissue infection	3 (0.8)	0 (0.0)	
Adequacy of administered antimicrobial regimen			0.132
Yes	316 (86.6)	37 (78.7)	
Presence of at least one co-morbidity			0.146
Yes	223 (61.1)	34 (72.3)	
All-cause 28-day mortality (%)	24.7	38.3	0.033

S.D., standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; CAP, community-acquired pneumonia; IAI, intra-abdominal infection; VAP, ventilator-associated pneumonia; HAP, hospital-acquired pneumonia.

3. Results

A total of 2627 patients with sepsis were screened and data for 412 patients with bacteraemic infections were entered into the analysis; in 47 (11.4%) of them the bacteraemic episode was polymicrobial. The studied population was distributed as 132 ICU patients and 280 non-ICU patients. Demographic characteristics of the patients stratified by the presence of monomicrobial or polymicrobial BSIs are presented in Table 1. Patients with polymicrobial bacteraemia significantly more often had severe sepsis/septic shock compared with patients with monomicrobial bacteraemia as well as higher Acute Physiology and Chronic Health Evaluation (APACHE) II scores. Adequacy of administered treatment and co-morbidities did not have statistical differences among patients with polymicrobial versus monomicrobial BSIs. Similarly, ICU or non-ICU admission was not associated with significant differences in either the frequency of polymicrobial BSIs or the appropriateness of administered antibiotics (Table 1).

The most common causative pathogens isolated from polymicrobial BSIs were *Enterococcus faecalis* (20.4%), *Klebsiella pneumoniae* and *Acinetobacter baumannii* (12.2% each), whereas *Escherichia coli* was by far the predominant pathogen in monomicrobial BSIs (32.9%) followed by *K. pneumoniae* (19.7%) and *Pseudomonas aeruginosa* (9.3%) (Table 2). The most common combinations in polymicrobial bacteraemias were as follows: *A. baumannii* + *Enterococcus* spp. (6 cases, equally distributed between *E. faecalis* and *Enterococcus faecium*), *E. faecalis* + Enterobacteriaceae (6 cases, equally distributed between *E. coli*, *Proteus mirabilis* and *Enterobacter cloacae*), combinations of Enterobacteriaceae (4 cases, *P. mirabilis* with either *E. coli* or *K. pneumoniae*), *K. pneumoniae* + *A. baumannii* (4 cases) and *K. pneumoniae* + *P. aeruginosa* (3 cases). The majority of polymicrobial bacteraemias were primary BSIs (38.3%); the urinary tract was the most common source of secondary bacteraemia (27.7%) followed by IAI and VAP. Monomicrobial BSIs where equally distributed as primary bacteraemias (37.5%) and BSI secondary to urinary tract infections (37.5%), followed by IAI (8.2%) (Table 1).

The all-cause 28-day mortality of the cohort was 26.2%; monomicrobial BSIs were associated with a mortality rate of 24.7% and the respective rate for polymicrobial BSIs was 38.3% ($P = 0.033$) (Fig. 1). Mortality varied ($P = 0.071$) according to the type of recovered pathogen (i.e. Gram-negative, Gram-positive, fungal and possible combinations), being highest when a combination of Gram-negative with Gram-positive isolate was recovered (mortality 50.0%) and lowest when a Gram-negative isolate was recovered along with a fungal pathogen (mortality 16%). Gram-negative bacteraemia was associated with lower mortality compared with pooled all other types of bacteraemia (23.8% vs. 36.8%; $P = 0.020$). However, when outcomes were stratified by the presence of polymicrobial infection, polymicrobial BSIs containing at least one Gram-negative isolate had similar mortality to all other types of bacteraemia (38.9% vs. 37.9%; not significant). No differences in adequacy of administered treatment

Table 2
Microbiology of enrolled patients with bacteraemia.

Pathogen	No. (%) of isolates	
	Monomicrobial infections	Polymicrobial infections
Escherichia coli	120 (32.9)	9 (9.2)
Klebsiella pneumoniae	72 (19.7)	12 (12.2)
Pseudomonas aeruginosa	34 (9.3)	9 (9.2)
Acinetobacter baumannii	25 (6.8)	12 (12.2)
Proteus mirabilis	15 (4.1)	8 (8.2)
Staphylococcus aureus	0 (0.0)	3 (3.1)
Enterococcus faecalis	16 (4.4)	20 (20.4)
Other Enterobacteriaceae	15 (4.1)	6 (6.1)
Candida albicans	6 (1.6)	3 (3.1)
Other	62 (17.0)	16 (16.3)
Total	365 (100)	98 (100)

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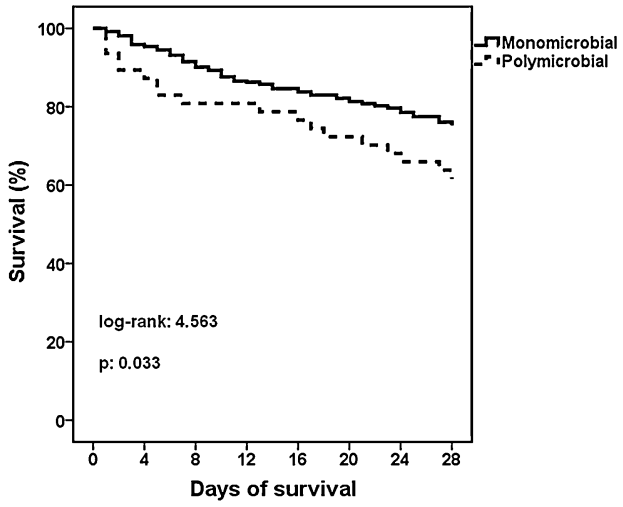

Fig. 1. Survival curves of patients with bacteraemic sepsis enrolled in the study. Patients were divided into those with bacteraemia of monomicrobial and polymicrobial causality.

were demonstrated between bacteraemias containing Gram-negative pathogens compared with all other types of bacteraemia (85.4% vs. 86.5%; not significant).

After ROC curve analysis for age, it was identified that age ≥61 years was related to unfavourable outcome at 80% sensitivity. To this end, a nominal age variable was generated with patients aged <61 years and ≥61 years.

Mortality per infectious source was distributed as follows: acute pyelonephritis, 16.3%; CAP, 35.7%; IAIs, 27.0%; primary bacteraemia, 27.7%; VAP, 48.6%; and HAP, 66.7%. To this end, another nominal variable was generated where patients with CAP, VAP and HAP were grouped together as sufferers from high-mortality infections and patients with acute pyelonephritis, primary bacteraemia and IAIs were grouped together as sufferers from low-mortality infections. In a similar way, it was found that the presence of at least two co-morbidities was linked with unfavourable outcome. Data from the univariate analysis are shown in Table 3.

Finally, all nominal variables representing independent predictors of mortality were entered into one model and Cox regression analysis for unfavourable outcome was selected. As presented in Table 4, only the presence of polymicrobial bacteraemia and the presence of at least two co-morbidities retained statistical significance in the final model and represent the most significant drivers of mortality in the studied population.

4. Discussion

This is one of the first current studies focusing on polymicrobial bacteraemic sepsis in a mixed cohort of ICU and non-ICU patients. According to the results, polymicrobial BSIs were associated with higher APACHE II score and, not unexpectedly, were more likely to express as severe sepsis and/or septic shock. In contrast to the majority of published studies, polymicrobial BSIs were associated with significantly lower 28-day survival compared with the monomicrobial counterparts. The multivariate analysis enabled us to demonstrate only two factors as major contributors to adverse outcome; polymicrobial bacteraemia (HR = 1.86) and the presence of two or more underlying co-morbidities (HR = 2.35). Notably, none of the demonstrated factors is modifiable. In this cohort, 38.3% of polymicrobial BSIs were attributed to primary bacteraemias, with *E. faecalis* being the most prevalent pathogen; these data in the absence of an intra-abdominal focus are indicative of an underlying mechanism of translocation from the gut, which again could represent the epiphenomenon of severe sepsis and not the cause [18].

The least contribution of Gram-negatives in the mortality of the studied population was an unexpected finding. Gram-negatives were predominant in the arm of monomicrobial BSIs and their rank of order was in accordance with other published articles from Greece as well as with the WHONET national registry [10,19]. Multidrug-resistant Gram-negative pathogens have been predominant in nosocomial infections in Greece for almost a decade [10]. Awareness of this particular epidemiology has probably allowed for high rates of appropriate treatment in the current cohort including ICU and no-ICU settings; therefore, it could explain why inappropriate treatment was not demonstrated as a contributor to mortality. The lower mortality of Gram-negative monomicrobial BSIs, originating in the main from urinary infections, might be attributed to an earlier administration of adequate treatment. However, timing of administration of antimicrobials was not recorded in the database, therefore such an explanation remains speculative.

The elucidation of 'other than Gram-negative' pathogens as contributors to mortality in polymicrobial BSIs, along with the high prevalence of *E. faecalis* in this group, was also unexpected. In 2010, according to the national registry WHONET, *Enterococcus* spp. ranked sixth among BSI pathogens both in ICU and medical wards, representing 4.8% and 6.8% of bacteraemic episodes, respectively. These data are in accordance with the monomicrobial BSIs registered in the current study. In this context, the high prevalence of *E. faecalis* observed in polymicrobial BSIs without a demonstrated intra-abdominal source warrants further investigation.

An originality of this study was the addition of information related to the management of septic episodes outside the ICU setting, for which the literature is limited [8,20,21]. ICU studies possess a complexity of definitions and factors affecting outcomes that preclude direct application of conclusions to the non-ICU patient. Therefore, given the expanding population of critically ill patients treated in hospital wards, studies of sepsis outside the ICU have gained importance [8].

Three large publications from the 1980s on nosocomial BSIs did not elucidate worse outcomes associated with polymicrobial infections compared with monomicrobial counterparts. They all pointed out the severity of the underlying disease and various

Table 3
Univariate analysis of independent factors affecting 28-day mortality.

	Mortality [deaths/exposed patients (%)]		*P*-value
Sex	Male: 67/209 (32.1)	Female: 42/203 (20.7)	0.020
Age	<61 years: 19/105 (18.1)	≥61 years: 82/307 (26.7)	0.031
Co-morbidities[a]	<2: 76/363 (20.9)	≥2: 27/49 (55.1)	<0.0001
Infections[b]	Low mortality: 79/346 (22.8)	High mortality: 29/66 (43.9)	<0.0001
Causality	Monomicrobial infections: 90/365 (24.7)	Polymicrobial infections: 18/47 (38.3)	0.037

[a] Type 2 diabetes mellitus, chronic obstructive pulmonary disease, chronic renal disease, chronic heart failure and solid tumour malignancy.
[b] Patients with community-acquired, ventilator-associated and hospital-acquired pneumonia were grouped together as sufferers from high-mortality infections, whereas patients with acute pyelonephritis, primary bacteraemia and intra-abdominal infections were grouped together as sufferers from low-mortality infections.

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Table 4
Cox regression analysis of independent predictors of 28-day mortality.

	HR (95% CI)	*P*-value
Female sex	0.62 (0.36–1.04)	0.070
Age ≥61 years	1.56 (0.85–2.88)	0.151
≥2 co-morbidities[a]	2.35 (1.32–4.17)	0.004
High-mortality infections[b]	1.63 (0.92–2.99)	0.093
Polymicrobial infection	1.86 (1.03–3.36)	0.039

HR, hazard ratio; CI, confidence interval.
[a] Type 2 diabetes mellitus, chronic obstructive pulmonary disease, chronic renal disease, chronic heart failure and solid tumour malignancy.
[b] Patients with community-acquired, ventilator-associated and hospital-acquired pneumonia were grouped together as sufferers from high-mortality infections.

other parameters expressing the severity of the septic episode per se (e.g. respiratory failure, haemolysis, etc.) as predictors of mortality [22,23]. The same applies for a recent study by Aliaga et al. comparing monomicrobial with polymicrobial BSIs implicating *P. aeruginosa* [24]. Interestingly, in the current study the true contribution of polymicrobial infections to 28-day mortality was clearly demonstrated only when sepsis/septic shock were omitted from the multivariate analysis. Publications supporting our observation that polymicrobial infections are associated with worse outcome are limited to two sequential studies by Pittet et al. on nosocomial BSIs in the period 1986–1991 [4,5] as well as a third study among cancer patients where polymicrobial BSIs had a 50% response rate compared with 60–94% for monomicrobial BSIs [25].

A recent study in an ICU population by Sancho et al. reported that polymicrobial BSIs had a similar frequency of species compared with monomicrobial BSIs and did not contribute to mortality, although they were associated with prolonged ICU and hospital stay [26]. The most prevalent pathogens in polymicrobial infections were 'high-risk' micro-organisms, namely non-*E. coli* Enterobacteriaceae, *P. aeruginosa* and *Candida* spp., and were strong predictors of mortality. The authors reported that APACHE II score at the onset of BSI and septic shock were predictors of related mortality, but polymicrobial BSI and inadequate empirical antimicrobial treatment were not [26]. In that study, the respiratory tract was by far the most common source of polymicrobial bacteraemia, whereas in the current study primary bacteraemia was predominant. In both studies the percentage of IAI was very low among polymicrobial bacteraemias, despite the results of other series of patients [20,22,23] (Table 1). It is worth mentioning that both studies have elucidated the increased prevalence of *E. faecalis* among polymicrobial BSIs.

A factor potentially contributing to worse outcomes in the context of polymicrobial sepsis could be the inappropriateness of the antibiotic therapy. Its significance has been clearly demonstrated in ICU studies [27,28]. In contrast, in studies with mixed ICU and non-ICU populations the significance of inappropriate antimicrobial treatment waned, probably because the non-ICU populations were less severely ill [8,29]. Only one study to our knowledge has demonstrated a clear association of inappropriate treatment with polymicrobial infection, among other factors [30]. As mentioned above, high rates of appropriate treatment encountered both in monomicrobial and polymicrobial BSIs (86.6% and 78.7%, respectively) in the current study could explain why appropriateness of treatment was not demonstrated as a contributor to mortality in the multivariate model. According to the majority of published reports, appropriateness was in the range of 30–46% [28].

However, a limitation of this study is that the database did not capture the time elapsed between the advent of sepsis and the onset of antimicrobials. Moreover, adequacy of treatment used in this analysis represents definite treatment, and institution-specific variations may have influenced the speed of distribution of antibiograms and the length of 'empirical' treatment. Another limitation is the lack of data regarding chronic liver failure and cirrhosis, a factor that could potentially be related to polymicrobial bacteraemia and worse outcomes. On the other hand, the multicentre design is probably among the strengths of this study as it allowed an estimation of a wide population of 2627 critically ill patients and a comparison between ICU and non-ICU-admitted counterparts. It also allowed for a representative estimation of the country, as reflected by the epidemiological concordance with the national registry WHONET.

5. Conclusion

The results of this analysis encompassing ICU and non-ICU patients demonstrated that the presence of polymicrobial bacteraemia almost doubled the risk of death in this cohort, whereas the presence of two or more underlying co-morbidities was associated with a more than two-fold risk. An emerging role of enterococci was also demonstrated in this setting. The true significance of these observations, as well as the possible contribution of other factors such as the adequacy and timely administration of antimicrobial treatment, warrant further investigation in prospective purpose-structured studies.

Funding

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Competing interests

HG has served as a board member and/or consultant for Pfizer, Novartis, Merck (USA) and Durata-Achaogen, has received grants/grant pending from Pfizer, Novartis, Merck (USA) and Durata-Achaogen, has received payment for lectures including service on speakers bureaus from Pfizer and Novartis, and has received travel/accommodations/meeting expenses from Novartis, Janssen and Pfizer. All other authors declare no competing interests.

Ethical approval

The study protocol was submitted to and approved by the Ethics Committees of the hospitals of the participating centres.

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Exogenous Sources of Pseudomonas aeruginosa in Intensive Care Unit Patients: Implementation of Infection Control Measures and Follow-Up With Molecular Typing

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INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY SEPTEMBER 2006, VOL. 27, NO. 9

ORIGINAL ARTICLE

Exogenous Sources of *Pseudomonas aeruginosa* in Intensive Care Unit Patients: Implementation of Infection Control Measures and Follow-Up With Molecular Typing

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BACKGROUND. In 1998, a study in the intensive care unit (ICU) of our institution suggested possible transmission of *Pseudomonas aeruginosa* from faucet to patient and from patient to patient. Infection-control measures were implemented to reduce the degree of *P. aeruginosa* colonization in faucets, to reduce the use of faucet water in certain patient care procedures, and to reduce the rate of transmission from patient to patient.

OBJECTIVE. To evaluate the effect of the control measures instituted in 1999 to prevent *P. aeruginosa* infection and colonization in ICU patients.

DESIGN. Prospective, molecular, epidemiological investigation.

SETTING. A 870-bed, university-affiliated, tertiary care teaching hospital.

METHODS. The investigation was performed in a manner identical to the 1998 investigation. ICU patients with a clinical specimen positive for *P. aeruginosa* were identified prospectively. Swab specimens from the inner part of the ICU faucets were obtained for the culture on 9 occasions between September 1997 and December 2000. All patients and environmental isolates were typed by pulsed-field gel electrophoresis (PFGE).

RESULTS. Compared with the 1998 study, in 2000 we found that the annual incidence of ICU patients colonized or infected with *P. aeruginosa* had decreased by half (26.6 patients per 1,000 admissions in 2000 vs 59.0 patients per 1,000 admissions in 1998), although the populations of patients were comparable. This decrease was the result of the decreased incidence of cases in which an isolate had a PFGE pattern identical to that of an isolate from a faucet (7.0 cases per 1,000 admissions in 2000, vs 23.6 per 1,000 admissions in 1998) or from another patient (6.5 cases per 1,000 admissions in 2000 vs 16.5 cases per 1,000 admissions in 1998), whereas the incidence of cases in which the isolate had a unique PFGE pattern remained nearly unchanged (13.1 cases per 1,000 admissions in 2000 vs 15.6 cases per 1,000 admissions in 1998).

CONCLUSIONS These results suggest that infection control measures were effective in decreasing the rate of *P. aeruginosa* colonization and infection in ICU patients, confirming that *P. aeruginosa* strains were of exogenous origin in a substantial proportion of patients during the preintervention period.

Infect Control Hosp Epidemiol 2006; 27:953-957

Pseudomonas aeruginosa is a common cause of infections in intensive care unit (ICU) patients.[1] It is ubiquitous in the environment but can also be part of the endogenous flora of hospitalized patients.[2] Recent studies have shown, using molecular typing methods, that environmental sources, such as sinks, tap water, and medical devices, can play a major role in outbreaks.[3-6] On the other hand, sporadic or endemic infections have generally been considered to originate mainly from the endogenous flora.[3,7-9]

In a previous molecular epidemiological investigation, we reported that acquisition of *P. aeruginosa* from exogenous sources was also an important cause of infection and colonization in ICU patients during a nonepidemic period.[10] The aim of the present study was to evaluate the efficacy of several control measures implemented to decrease the acquisition of *P. aeruginosa* from exogenous sources among ICU patients.

METHODS

This prospective, molecular, epidemiological investigation was performed at the University Hospital of Lausanne (Lausanne, Switzerland), which is an 870-bed tertiary care hospital that opened in 1982. The ICUs of the University Hospital of

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Lausanne are located on the same floor but have 2 distinct water distribution networks: the surgical, burn, and pediatric ICUs have one water network, and the medical ICUs have another. Only faucets used for direct patient care were investigated in this study.

Patients were included in the study if they were hospitalized in one of the ICUs and had at least 1 culture of a clinical sample that yielded *P. aeruginosa*. There was no routine bacteriological screening of the patients during the study period. Demographic and epidemiological data (unit and room of hospitalization and dates of admission and discharge) were retrieved from the hospital information system.

Investigation of the water system was performed on 9 occasions (September 1997; January, June, and December 1998; January, May, and December 1999; and July and December 2000). Each faucet was dismantled and the hot-cold water mixing chamber was swabbed. Swab specimens were plated on blood and cetrimide agar plates and incubated at 35°C for 2 days. Growth of *P. aeruginosa* was evaluated semiquantitatively by the streaking method (rare, growth in the first quadrant; few, growth in the second quadrant; moderate, growth in the third quadrant; many, growth in the fourth quadrant).

Molecular typing was performed by means of pulsed-field gel electrophoresis (PFGE) as described elsewhere.[11] In brief, plugs of genomic DNA were digested with the restriction enzyme *Spe*I, and electrophoresis was performed (with the CHEF DR III system; BioRad) at a voltage of 6 V/cm at 12°C for 24 hours with a linear pulse time of 1 to 36 seconds. The PFGE profiles were analyzed with the BioNumerics software, version 3.5 (Applied Maths). On the basis of results of a previous study on the stability of PFGE patterns of *P. aeruginosa* isolates from patients with cystic fibrosis,[11] we considered 2 isolates to be genetically similar if the number of discordant fragments was fewer than 7. In 1998, we found that typing of isolates from the same environmental specimen that had similar colony morphologic features always revealed identical genotypes. On the basis of these results, for each specimen, 1 isolate per colony morphology was typed in 2000. For 10 patients in 1998 and 9 patients in 2000, *P. aeruginosa* isolates were not available for typing.

A case of colonization or infection was defined as isolation of a given genotype of *P. aeruginosa* from a clinical specimen during a patient's stay in an ICU. Thus, recovery of isolates with 2 different genotypes from a single patient was considered to be 2 cases. The cases were categorized into 3 groups according to molecular typing data. Group A comprised cases in which the isolate had a genotype identical to that of an isolate recovered from a faucet. Group B comprised cases in which the isolate had a genotype identical to that of an isolate recovered from at least 1 other patient but not recovered from the faucets. Group C comprised cases in which the isolate had a unique genotype. Patient-to-patient transmission was considered possible if isolates with indistinguishable PFGE patterns were recovered from patients hospitalized during overlapping periods in the same ICU, and an epidemiological cluster was defined as 2 or more such cases.

In 1999, control measures were taken to reduce the reservoir and the rate of transmission of *P. aeruginosa*. These measures were as follows: (1) an attempt to eradicate *P. aeruginosa* in faucets by increasing the water temperature or by copper and silver ionization (these measures were primarily taken to control *Legionella* contamination),[10] (2) replacement of tap water by *P. aeruginosa*–free bottled water for patient use (ie, to drink and as mouthwash), and (3) reinforcement of standard precautions and hand disinfection with an alcohol solution instead of disinfectant soap. Before the intervention, the water temperature was 50°C at the distribution source and 38°C-45°C at the faucets; after the intervention, it was 65°C at the source and 50°C-58°C at the faucets.

The 1998 annual incidences of patients with clinical specimens positive for *P. aeruginosa* for cases in groups A, B, and C were compared with data from 2000 by means of the χ^2 test. Annual incidences of such patients were also recorded in wards (surgery, medicine, ear-nose-throat) supplied by other water distribution networks.

TABLE. Comparison of Epidemiological Data on Patients with *Pseudomonas aeruginosa* Colonization or Infection in the Intensive Care Unit During 3 Years

Variable	1998	1999	2000
No. of admissions	2,367	2,456	2,446
Mean duration of stay, d	4.86	4.37	4.83
Mean no. of specimens per patient	3.1	3.0	3.1
Anatomical site that yielded *P. aeruginosa,* % of specimens			
Respiratory tract	69	ND	76
Wound	14	ND	15
Other	16	ND	9
No. of patients from whom *P. aeruginosa* was isolated	139	99	68
No. of patients per 1,000 admissions	59.0	40.3	26.6
No. (%) of faucet specimens positive for *P. aeruginosa*	12/132 (9.1)	19/114 (16.7)	11/87 (12.6)

NOTE. ND, not done.

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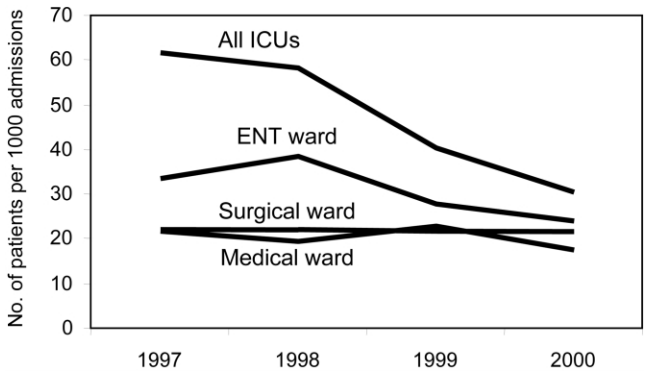

FIGURE 1. Evolution in the annual number of patients per 1,000 admissions in which clinical isolates yielded *Pseudomonas aeruginosa* in all intensive care units (ICUs) compared with medical, surgical, and ear-nose-throat (ENT) wards.

RESULTS

From 1998 to 2000, the annual incidence of ICU patients with clinical specimens positive for *P. aeruginosa* decreased by half (Table, Figure 1). During the same period, the annual incidences of such patients in the medical, surgical, and ear-nose-throat wards were also calculated and showed no significant change (Figure 1). None of these other wards were supplied with water by the same water networks as the ICU. The yearly number of specimens per patient, the distribution of anatomical sites from which *P. aeruginosa* was isolated, and the length of stay in the ICU did not change during the period 1998 to 2000 (Table). Results from the water system investigation showed that, despite the increase in temperature of the hot water, no significant decrease in the rate of faucet colonization was observed (Table) and there was no quantitative diminution of *P. aeruginosa* isolation from faucet swab specimens (data not shown). To understand this decrease in the incidence ICU patients colonized or infected with *P. aeruginosa,* comparison of the molecular epidemiology of *P. aeruginosa* isolates was performed between 1998 and 2000.

The total number of cases identified was 132 in 1998 and 65 in 2000. In 1998, there were 56 cases in group A, 39 cases in group B, and 37 cases in group C; in 2000, there were 17 cases in group A, 16 cases in group B, and 32 cases in group C. The incidence of cases in group A (ie, cases in which the isolate had a genotype identical to that of an isolate recovered from the water system) decreased significantly from 23.6 cases per 1,000 admissions in 1998 to 7.0 cases per 1,000 admissions in 2000 ($P < .01$). The incidence of cases in group B (ie, cases in which the isolate had a genotype identical to that of an isolate recovered from at least 1 other patient but not recovered from the faucets) decreased significantly from 16.5 cases per 1,000 admissions in 1998 to 6.5 cases per 1,000 admissions in 2000 ($P < .01$). However, the incidence of cases in group C (ie, cases in which the isolate had a unique genotype) remained nearly unchanged: 15.6 cases per 1,000 admissions in 1998, compared with 13.1 cases per 1,000 admissions in 2000 ($P = .64$) (Figure 2).

Regarding the cases in group A, the genotypes were first recovered from the faucets in 58 of 73 cases (44 of 56 in 1998 and 14 of 17 in 2000). The most prevalent genotype was isolated from both water distribution networks and was found in 39 cases (30 in 1998 and 9 in 2000). In the medical ICU network, 5 genotypes were isolated only from this network. They were responsible for 17 cases (13 in 1998 and 4 in 2000) in patients hospitalized in this ICU, and for only 3 cases (in 1998) in patients hospitalized in the other ICUs. In the other water network (that for the surgical, burn, and pediatric ICUs), 5 other genotypes were isolated, which were responsible for 12 cases in 1998 (and 0 cases in 2000) in patients hospitalized in these ICUs.

Among the 55 cases in group B, 6 epidemiological clusters (of 6, 3, 2, 2, 2, and 2 cases) were identified in 1998 and only 2 clusters (of 3 and 2 cases) in 2000. The 30 remaining cases (18 in 1998 and 12 in 2000) could not be grouped into clusters, since no overlapping period of hospitalization was recorded for the patients who harbored isolates with identical genotypes.

DISCUSSION

In several recent studies that have used molecular typing, the major reservoir of *P. aeruginosa* in an ICU in which there was not an epidemic was reported to be the endogenous flora

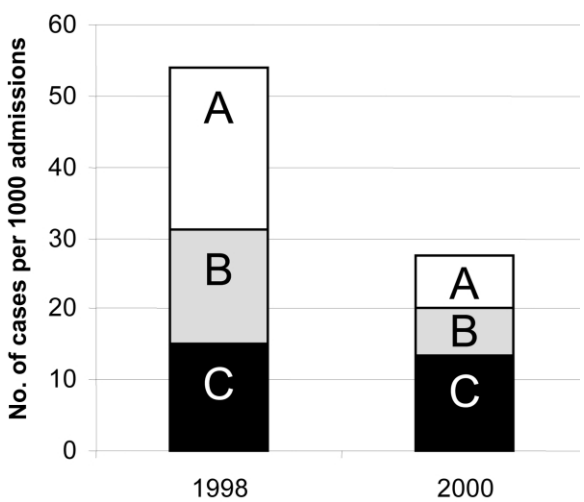

FIGURE 2. Evolution of the annual number of cases per 1,000 admissions in which clinical isolates yielded *Pseudomonas aeruginosa* among patients in the intensive care units. For case definitions, see Methods. Group A comprised cases in which the isolate had a genotype identical to that of an isolate recovered from a faucet. Group B comprised cases in which the isolate had a genotype identical to that of an isolate recovered from at least 1 other patient but not recovered from the faucets. Group C comprised cases in which the isolate had a unique genotype, suggesting that the source was probably endogenous.

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of the patients, suggesting that barrier precautions might not have a central role in the prevention of *P. aeruginosa* colonization or infection in a given patient.[3,7,8] The environment was identified as a nonnegligible source of *P. aeruginosa* when the aerator faucets and the sinks were highly contaminated[12,13] or during outbreaks.[4,5,14,15] Faucet water was mostly associated with *P. aeruginosa* infection when it was used for rinsing burned patients or medical devices after disinfection. However, studies have suggested that patients only rarely acquire the pathogen from this environmental source, other than during outbreaks.[16-18] Our results suggest that, before the implementation of infection control measures, an exogenous source (water or cross-infection) was possibly the ultimate source of *P. aeruginosa* in three-quarters of the cases and that the water system was likely to be the primary reservoir in half of the cases. These results are striking, because a relatively low proportion of swab specimens from ICU faucets (12%) revealed colonization, and water samples (not swab samples) failed to reveal the presence of *P. aeruginosa.*[10] The initial contamination of the faucets could have originated from retrograde colonization, but most of the *P. aeruginosa* genotypes (82%) were recovered from the faucets before they were recovered from patients, a finding that strongly suggested that the water system was the primary reservoir. Subsequent transmission of these strains to other patients via healthcare workers or other indirect transmission may also have occurred.

A limitation of the molecular epidemiology of this study is that it was based only on clinical specimens and that no routine bacteriological screening of the patients was performed during the study period. However, it has the advantage that specimen sampling between the 2 periods was comparable. Similarly, we may have underestimated the number of exogenous sources, because we did not perform extensive microbiological sampling of the environment other than in the plumbing system. However, the results observed after the implementation of infection control measures suggest that the decrease in the incidence of cases was the result of a decrease in the number of cases in which the *P. aeruginosa* strain originated from an exogenous source (ie, groups A and B), whereas the incidence of cases in which the strain had a unique genotype and an endogenous source (ie, group C) remained similar. These findings were sustained during the years 2001-2003 (data not shown). Because the infection-control measures that targeted the water supply failed to decrease the rate and degree of faucet colonization, the observed decrease in incidence was likely related to the other measures (mostly use of bottled water and hand disinfection). In addition, the distribution of the anatomical sites from which *P. aeruginosa* was cultivated did not change, supporting the finding that the observed effect was not the result of the introduction of bottled water for mouth care. The importance of compliance with hand disinfection has recently been emphasized.[19-21] It was shown that a sustained improvement in compliance with hand hygiene coincides with a reduction of nosocomial infections and transmission of methicillin-resistant *Staphylococcus* species.[21]

Intrinsic factors (such as virulence, adhesin production, or antibiotic resistance) might contribute to the ability of some strains to infect or colonize.[15] This hypothesis is supported by reports of outbreaks of *P. aeruginosa* infection that involved a single strain.[5,14,15] However, the role of intrinsic factors in our setting is unlikely, because 11 different strains were identified in both faucets and patients, and 6 other strains were recovered from several patients.[10]

Biofilm colonization of faucets with *P. aeruginosa* in the ICU is problematic, especially when a multidrug-resistant strain is involved.[5] In our setting, high water temperature and copper and silver ionization were not able to eradicate this colonization. All colonized faucets were changed, but recolonization occurred after several weeks (data not shown), probably because the biofilm was also present in the pipes. Despite a decrease in the number of cases after implementation of infection control measures, molecular typing of isolates suggests that a substantial proportion of cases (group A; Figure 2) may still ultimately have originated in the water distribution system. Final filtration, with microfilters at each faucet, might be considered to decrease further the number of cases, but studies are needed to assess the cost-effectiveness of this method.

In conclusion, we showed that the implementation of infection control measures can be effective in decreasing the incidence of *P. aeruginosa* colonization and infection in ICU patients when exogenous reservoirs contribute substantially to the rate of colonization and infection. Because *P. aeruginosa* is ubiquitous in the environment and colonizes up to 15% of hospitalized patients, control of the reservoirs is difficult. Therefore, efforts should primarily be focused on the reinforcement of infection control measures to limit the transmission of *P. aeruginosa.*

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Risk factors for *Pseudomonas aeruginosa* acquisition in intensive care units: a prospective multicentre study

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SUMMARY

Background: *Pseudomonas aeruginosa* is a major nosocomial pathogen in intensive care units (ICUs); however, endogenous versus exogenous origin of contamination remains unclear.

Aim: To identify individual and environmental ICU risk factors for *P. aeruginosa* acquisition.

Methods: A five-month prospective multicentric study was performed in ten French ICUs. Adult patients hospitalized in ICU for ≥24 h were included and screened for *P. aeruginosa* colonization on admission, weekly and before discharge. *P. aeruginosa* acquisition was

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defined by a subsequent colonization or infection if screening swabs on admission were negative. Water samples were obtained weekly on water taps of the ICUs. Data on patient characteristics, invasive devices exposure, antimicrobial therapy, *P. aeruginosa* water and patient colonization pressures, and ICU characteristics were collected. Hazard ratios (HRs) were estimated using multivariate Cox model.

Findings: Among the 1314 patients without *P. aeruginosa* on admission, 201 (15%) acquired *P. aeruginosa* during their ICU stay. Individual characteristics significantly associated with *P. aeruginosa* acquisition were history of previous *P. aeruginosa* infection or colonization, cumulative duration of mechanical ventilation and cumulative days of antibiotics not active against *P. aeruginosa*. Environmental risk factors for *P. aeruginosa* acquisition were cumulative daily ward 'nine equivalents of nursing manpower use score' (NEMS) [hazard ratio (HR): 1.47 for ≥30 points; 95% confidence interval (CI): 1.06–2.03] and contaminated tap water in patient's room (HR: 1.76; CI: 1.09–2.84).

Conclusion: Individual risk factors and environmental factors for which intervention is possible were identified for *P. aeruginosa* acquisition.

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Introduction

Pseudomonas aeruginosa is a ubiquitous micro-organism responsible for severe hospital-acquired infections.[1–3] Length of stay, severity of underlying disease and exposure to invasive procedures but also bacterial adherence and virulence factors, and antimicrobial drug resistance are associated with *P. aeruginosa* acquisition in ICUs.[4–6] Those risk factors were identified in single centre studies which most often focused on antimicrobial-resistant *P. aeruginosa* and rarely took into account the context of care delivery, i.e. colonization pressure or nurse staffing.[7] However, patient case-mix, ecology, organization, and practices vary across ICUs. Endogenous origin was regarded as the most relevant route of *P. aeruginosa* infection, but, during the last ten years, a significant proportion of *P. aeruginosa* isolates have been shown to stem from the ICU environment (moist reservoirs) and from cross-transmission.[8–11] Nseir *et al*. reported that the risk of multidrug-resistant *P. aeruginosa* acquisition was higher when the previous occupant of the room was colonized by such *P. aeruginosa*.[12] The relevance of exogenous reservoirs and the importance of cross-transmission were documented during outbreaks but their importance in non-epidemic situations remains controversial. A pilot study suggested that environmental factors might be associated with *P. aeruginosa* acquisition.[6] Unlike patient and pathogen characteristics, environmental factors such as nursing workload or contamination of water taps could be modified. A prospective cohort study in ten French ICUs, DYNAPYO (Dynamics of Acquisition of *Pseudomonas aeruginosa*), was performed to evaluate the respective contributions of individual and ICU environmental risk factors for *P. aeruginosa* acquisition.

Methods

Study design

A prospective five-month observational survey was performed from February to October 2009 in ten ICUs from eight French healthcare facilities including four medical, two surgical and four mixed (medical and surgical) ICUs. University hospitals of Besançon and Lyon included two ICUs each, university hospitals of Bordeaux, Garches, Montpellier and Paris, and general hospitals of Lens and Tourcoing included one ICU. These ICUs each had between nine and 20 beds and from 10 to 47 water taps. The average length of patient stay was eight to 16 days and ICUs admitted from 27 to 35 patients per month. Specific trained healthcare professionals were identified in each centre for the collection of data, which were entered on to a secured online electronic form. There was no policy to isolate patients with *P. aeruginosa* in each of the study ICUs, and changes such as to infection control initiative or antimicrobial stewardship were not implemented during the course of the study.

Exposure measurements

Every adult patient admitted for >24 h was included and had screening samples (oropharyngeal, rectal swab, and tracheobronchial aspirate) with 48 h of admission and thereafter once a week, and before discharge. The study was approved by the local ethics committees. Cetrimide agar was inoculated and identification of *P. aeruginosa* was performed after 24 and 48 h of aerobic incubation at 42 °C.

Water samples were performed weekly on water taps of the ICUs. Samples were taken on the morning before use, without prior disinfection; taps were opened and the first 250 mL flush of water collected immediately into a sterile flask with 5 mg sodium thiosulphate (pre-flush sample), the aerator was not removed but swabbed and the swab broken into the water sample; samples were processed by membrane filtration: 100 mL were filtered through a 0.45 mm pore size membrane filter; membranes were cultured aerobically on to cetrimide agar plates at 37 °C and examined for growth after 22 ± 4 and 44 ± 4 h; any colonies that grew on cetrimide agar were identified using the API 20NE identification system (bioMérieux, Marcy l'Etoile, France).

Patients found to be positive within the first 48 h of admission were considered to have imported *P. aeruginosa*. Acquisition was defined by a subsequent positive swab culture or *P. aeruginosa* infection during ICU stay if, on admission, screening swabs were negative and the patient was not infected yet by *P. aeruginosa*.

Demographic data were collected on admission, including: age, sex, immunodeficiency, primary diagnosis of infection, simplified acute physiology score within 24 h of admission (SAPS II), Charlson comorbidity index for chronic disease, history of previous hospitalization (<1 year before admission), previous antimicrobial therapy (<30 days), previous surgery (<30 days) and history of previous *P. aeruginosa* infection or colonization (yes/no).[13,14] During ICU stay, data regarding the use and duration of invasive devices and procedures were collected. Antibiotic treatment was recorded daily and classified (no antibiotic treatment; antibiotics normally non-active against *P. aeruginosa*; antibiotics normally active against *P. aeruginosa* including ureido- and carboxypenicillins, antipseudomonal cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, colimycin, fosfomycin).[6] If a patient received simultaneously both active and non-active antibiotics, the patient was considered to have been treated with active antibiotics.

The level of patient care was daily evaluated by the nursing staff using the 'nine equivalents of nursing manpower use score' (NEMS).[15] For each ward, the global daily average nursing workload (daily ward NEMS) was estimated by the sum of all the patients' daily NEMS divided by the number of patients.

The *P. aeruginosa* colonization status of the previous occupant of the bed and colonization of the bedroom water tap before *P. aeruginosa* acquisition (or discharge in case of no *P. aeruginosa* acquisition) was recorded. Daily indices of environmental pressure were calculated. Environmental exposure was assumed to be constant between two screenings. According to the organization of care by ICU staff and the localization of rooms on the ICU floor plan, patients in close proximity (same room or adjacent room) and water taps in close proximity (same room or usually used before entering the room) were defined for each patient. Two variables were then calculated: (i) the patient colonization pressure [cumulative number of positive patients in close proximity (colonized/infected patients, including patients with imported and acquired *P. aeruginosa*) from the admission to the previous day of *P. aeruginosa* acquisition/discharge, in patient-days]; (ii) the tap water colonization pressure (cumulative number of positive water taps in close proximity from the admission to the previous day of *P. aeruginosa* acquisition/discharge, in water tap-days).

Statistical analyses

Patients with imported *P. aeruginosa* were excluded from the risk factor analysis and only used to assess patient colonization pressure ($N = 180$). Only the first *P. aeruginosa* acquisition in the ICU during the study was considered. Hazard ratios for acquisition of *P. aeruginosa* were estimated for individual and environmental ICU factors using Cox regression model stratified on ward and taking into account left truncation. Factors concerning devices and procedures, antimicrobial therapy, patient and water colonization pressures and daily ward NEMS were introduced as time-updated factors. Because variables related to invasive devices and procedures were correlated, mechanical ventilation was included in the model as a surrogate variable for all other invasive procedures. Factors were selected in the final model following an unadjusted analysis ($P < 0.25$) and a backward-manual selection multivariable analysis ($P < 0.05$). Antimicrobial therapy, patient and water colonization pressure were systematically introduced into the model. Interactions between antimicrobial therapy and either patient colonization or water colonization pressures were tested ($P < 0.05$). Linearity of effects was checked; in cases of non-linearity, continuous variables were categorized according to the quantiles of the distribution. All analyses were performed using SAS software (version 9.1.3, SAS Institute, Cary, NC, USA).

Results

Among the 1314 patients without *P. aeruginosa* on admission, 201 (15%) acquired *P. aeruginosa* during their ICU stay. No *P. aeruginosa* outbreak was observed during the study. ICUs included 81–233 patients each (median: 123) and *P. aeruginosa* acquisition ranged from 11% to 21% according to the ICU. Incidence density of *P. aeruginosa* acquisition was 12.7 per 1000 days of hospitalization, ranging from 9.4 to 15.9 in ICUs. Median delay for *P. aeruginosa* acquisition was nine days [interquartile range (IQR): 6–15]. Main characteristics of patients are described according to their *P. aeruginosa* acquisition status in Table I.

Median cumulative patient colonization pressure was five patient-days (IQR: 2–14) for patients with *P. aeruginosa* acquisition and two patient-days (IQR: 0–6) for patients without *P. aeruginosa* acquisition ($P = 0.04$). Patients with *P. aeruginosa* acquisition were in a bed previously occupied by a positive patient in 23% of cases (22% for patients without acquisition; $P = 0.87$).

Water was contaminated in 17% of samples (2–62% according to the ICU; median: 7%). Median cumulative water tap colonization pressure was five positive water tap-days (IQR: 0–26) for patients with *P. aeruginosa* acquisition and zero (IQR: 0–6) for patients without acquisition ($P = 0.01$). In cases of *P. aeruginosa* acquisition, 32% of patients were previously exposed to a positive water tap in their bedroom (19% in case of no acquisition; $P < 0.01$).

Results of multivariate analyses are presented in Table II. Independent risk factors at individual level for *P. aeruginosa* acquisition were: older age, history of previous *P. aeruginosa* infection or colonization, longer cumulative duration of mechanical ventilation, and longer cumulative days of antibiotics non-active against *P. aeruginosa*. Among ICU environmental risk factors, positive tap water in patient's room and cumulative daily ward NEMS were independent risk factors.

Discussion

This prospective multicentre study evaluated the respective contributions of individual and ICU environmental risk factors for *P. aeruginosa* acquisition. In addition to individual risk factors, there were two significant environmental variables: previously contaminated tap water in the room and the cumulative ward level of nursing workload.

Selection biases were limited according to the study setting. The proportion of positive patients on screening swabs could have been underestimated because of an unknown, potentially suboptimal, sensitivity of the swab technique in detecting a small inoculum. However, this lower sensitivity was expected to be the same for all swabs and ICUs.[16] The overall prevalence of acquisition was 15% in our study that is in the range (from 6%

Table I
Characteristics of the patients according to their *Pseudomonas aeruginosa* status, DYNAPYO study (univariate analysis)

Characteristics	*P. aeruginosa* acquisition[a] ($N = 201$)	No *P. aeruginosa* acquisition[a] ($N = 1113$)	*P*-value
General characteristics			
Sex ratio (M/F)	1.91	1.35	0.27
Age (years)[b]	60.4 (18.4)	56.7(18.4)	0.02
Charlson comorbidity index on admission[b]	2.1 (2.3)	1.9 (2.2)	0.29
SAPS II score within the first 24 h[b]	50.9 (17.6)	42.7 (19.3)	0.03
Immunodeficiency on admission[c]	17 (9%)	127 (11%)	0.48
History of previous *P. aeruginosa* colonization or infection[c]	9 (5%)	18 (2%)	0.01
Hospitalization <1 year before admission[c]	95 (48%)	503 (46%)	0.51
Surgery <30 days before admission[c]	45 (23%)	238 (21%)	0.36
Antibiotics before admission[c]	74 (38%)	317 (30%)	0.34
Cumulated NEMS during ICU stay[d]	172 (110–308)	82 (26–205)	0.02
Exposure to invasive devices (duration in days)			
Duration of central vascular catheter[d]	5 (3–10)	0 (0–6)	<0.001
Duration of nasogastric tube[d]	5 (4–10)	1 (0–6)	<0.001
Duration of urinary catheter[d]	6 (4–11)	2 (0–7)	0.001
Duration of parenteral nutrition[d]	0 (0–1)	0 (0–0)	0.01
Duration of mechanical ventilation[d]	5 (4–10)	1 (0–6)	<0.001
Duration of non-invasive ventilation[d]	0 (0–0)	0 (0–0)	0.04
Bronchoscopic endoscopy[c]	30 (15%)	79 (7%)	0.15

DYNAPYO, Dynamics of Acquisition of *Pseudomonas aeruginosa* study; SAPS, simplified acute physiology score; NEMS, nine equivalents of nursing manpower use score; ICU, intensive care unit.
[a] Acquisition: at least one screening or diagnosis sample positive to *P. aeruginosa* during ICU stay. Characteristics during ICU stay were considered from admission to previous day of acquisition in cases of *P. aeruginosa* acquisition and from admission to previous day of discharge in cases of no acquisition.
[b] Mean (standard error).
[c] No. (%).
[d] Median (interquartile range).

to 32%) of reported estimations in ICU studies using similar screening method.[17–19] Water sampling at weekly intervals was preferred to sampling at times of patients' admission and discharge and to daily intervals as done in previously published studies and because of its feasibility.[6,9,11]

Hazard ratios (HRs) of individual risk factors for *P. aeruginosa* acquisition were stronger than of HRs of environmental factors: history of *P. aeruginosa* was strongest. In this latter case, *P. aeruginosa* acquisition might be a reappearance of an endogenous strain not identified on admission screening (but only strain typing could demonstrate that). However, by assessing the intrinsic endogenous risk and susceptibility for each patient to acquire *P. aeruginosa*, this factor is useful and simple to collect on admission.[16] The effect of the duration of the mechanical ventilation was previously described.[5–7,16,20–22] The third identified individual risk factor can be modified: the cumulative days of antibiotics not active against *P. aeruginosa*. Reducing susceptible intestinal flora

Table II
Risk factors for *Pseudomonas aeruginosa* acquisition[a] in DYNAPYO ICUs (multivariable Cox model)

Patient characteristics	HR	95% CI	*P*
Age (ref. ≤43 years)			
43–59	0.63	0.40–1.01	0.03
>59	1.06	0.72–1.56	
History of previous *P. aeruginosa* colonization or infection at admission	3.78	1.80–7.97	0.001
Cumulative duration of mechanical ventilation (for 10 days longer)	2.56	1.46–4.50	0.001
Cumulative days of antibiotics inactive against *P. aeruginosa* (ref. ≤1 day)	1.85	1.33–2.57	<0.001
Environmental (ICU) characteristics			
Cumulative daily ward NEMS (for 30 points higher)	1.47	1.06–2.03	0.02
Tap water of the room contaminated by *P. aeruginosa*	1.76	1.09–2.84	0.02

DYNAPYO, Dynamics of Acquisition of *Pseudomonas aeruginosa* study; ICU, intensive care unit; HR, hazard ratio; CI confidence interval; NEMS, nine equivalents of nursing manpower use score.
[a] Acquisition: at least one screening or diagnosis sample positive to *P. aeruginosa* during ICU stay. Characteristics during ICU stay were considered from admission to previous day of acquisition in cases of *P. aeruginosa* acquisition and from admission to previous day of discharge in cases of no acquisition.

would select naturally resistant *P. aeruginosa*. Patients with a history of long-course antimicrobial therapy, especially piperacillin, tazocillin, fluoroquinolones, and aminoglycosides, have been reported to be at greater risk of *P. aeruginosa* acquisition.[5,23–25]

Positive water in the patient's room was a risk factor for *P. aeruginosa* acquisition. Patients' contamination by a *P. aeruginosa* tap water strain has been estimated at 15–50% in the literature.[9,19,26–28] Petignat *et al.* demonstrated that *P. aeruginosa* colonization and infection in ICU patients decreased by eradicating *P. aeruginosa* in faucets and reinforcing standard precautions.[29] The efficacy of regular disinfection to control retrograde contamination has not been proven. However, water taps may be a reservoir of *P. aeruginosa* in ICUs. Water surveillance would assess patients' exposure to contaminated water. A definitive proof of the link between patient and environmental isolates of *P. aeruginosa* would be given by strain typing. Therefore according to our results, in cases of water contamination, decontamination actions and preventive measures would be a feasible preventive action. Although decontamination of a contaminated water tap has been widely studied, no review or guidelines on efficient decontamination strategy are available.[30–32] Promoting compliance with alcohol hand disinfection following hand hygiene in cases of *P. aeruginosa* water contamination should reduce *P. aeruginosa* transmission.

Cumulative daily ward NEMS assessed the cumulative global level of patients' care. A high workload in an ICU can decrease compliance to prevention measures and increase the risk of cross-transmission. To our knowledge, NEMS has never been used to assess risk for *P. aeruginosa* acquisition before; a previous 70-patient study used ICU-PRN (nurse workload system 'Projet de Recherche en Nursing en Réanimation') as explanatory variable for *P. aeruginosa* acquisition in ICU, but results were non-significant.[17] Increased nurse workload could result in underperforming in infection control measures (such as room cleaning or hand hygiene) which could favour both *P. aeruginosa* persistence in the environment and cross-transmission.[33] NEMS is a simple score that deserves to be used in ICU.

The cumulative number of positive patients in close proximity was not an independent risk factor for *P. aeruginosa* acquisition in multivariable analysis. In a previous single-centre study, the presence of positive patients in the ward on the previous day was independently associated with *P. aeruginosa* acquisition.[6] Analysis of the French national database of nosocomial infections surveillance in ICUs (REA-RAISIN) found that hospitalization in an ICU with elevated rate of *P. aeruginosa*-infected patients was significantly and independently linked with a pseudomonal nosocomial pneumonia, though the contamination of water taps and NEMS score were not assessed.[7] Cross-transmission has many variations according to the ward, and our study identified that colonization pressure was not the main environmental risk factor for *P. aeruginosa* acquisition.[4,18,34–40]

To conclude, a patient exposed to prolonged antibiotics inactive against *P. aeruginosa*, exposed to contaminated water in his room, and hospitalized in ICU with a high workload was more at risk for *P. aeruginosa* acquisition during his stay. These results suggest an exogenous, preventable acquisition of *P. aeruginosa* in ICU, which should stimulate research on policies of water decontamination and their impact on *P. aeruginosa* acquisition.

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Conflict of interest statement
None declared.

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The Role of Colonization in the Pathogenesis of Nosocomial Infections

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Symposium: Nosocomial Colonization

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The Role of Colonization in the Pathogenesis of Nosocomial Infections

Marc J.M. Bonten, MD, PhD; Robert A. Weinstein, MD

ABSTRACT

The close relationship between colonization and the development of nosocomial infections has been demonstrated. Patient-related factors, such as underlying illness for all major sites of infection and advanced age, and pathogen-related factors, such as the ability of bacteria to adhere to epithelial cells, play the major roles in the pathogenesis of colonization. However, exact mechanisms of colonization have not been elucidated, and modulation of bacterial adherence as a method of infection prevention remains experimental. Current methods of infection prevention, therefore, focus either on preventing growth of colonizing microorganisms or on preventing patient-pathogen contact. Topical antibiotics have been used as a method of colonization prevention. However, their effectiveness may be limited by increases in antibiotic resistance; moreover, the effects on patient outcome are controversial. Maintenance of the physiologic mucosal environment using nonantimicrobial agents seems a promising approach, but only a few studies demonstrating efficacy have been published. Prevention of colonization still must rely heavily on basic infection control measures to prevent contact between patient and pathogen (*Infect Control Hosp Epidemiol* 1996;17:193-200).

INTRODUCTION

Hospital-acquired infections have become increasingly complex over the past 20 years. The availability of sophisticated diagnostic and therapeutic modalities has led to higher survival rates for debilitated patients, creating a population at high risk of hospital-acquired infections. The frequent use of invasive devices in intensive care units (ICUs), especially central venous catheters and mechanical ventilators, is associated with further risk of nosocomial infections. Increasingly, broad-spectrum antimicrobial treatment of these infections has led to major problems with multiply antibiotic-resistant bacteria.

Preventing these infections requires an understanding of their pathogenesis. The occurrence of nosocomial infections most often involves a three-step process: first, colonization of a patient's mucosa or skin with a potential pathogen; second, access of the pathogen to a site where it may invade tissues, often in association with a foreign body such as an intravascular catheter or an endotracheal tube; and third, an imbalance among the pathogen's virulence factors and the host's defense factors, which eventually results in the infection. In essence, what colonizes our patients may infect them.

In this review, we will discuss the evidence for the importance, and the mechanisms, of the first step in the pathogenesis, colonization, and the possible ways to interfere in this process to prevent infection.

COLONIZATION WITH POTENTIAL PATHOGENS

The Respiratory Tract

Epidemiological observations. The development of respiratory tract colonization probably depends on the ability of the bacteria to adhere successfully to epithelial cells or mucus.[1,2] Although the exact mechanisms that facilitate adherence and colonization still

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TABLE 1
EXAMPLES OF FACTORS ASSOCIATED WITH RESPIRATORY TRACT COLONIZATION

Host Factors
Underlying illness
Cystic fibrosis
Chronic bronchitis
Alcohol abuse
Nicotine abuse
Diabetes mellitus
Malnutrition
Advanced age
Acute illness
Coma
Acidosis
Shock
Viral infection
Hypoxemia
Pathogen-specific factors
Adhesins
Mucins

are unclear, a number of epidemiologic observations have been made. For example, the development of ventilator-associated pneumonia (VAP) is related closely to preceding oropharyngeal colonization. More than 20 years ago, Johanson and coworkers demonstrated that 23% of colonized ICU patients developed nosocomial respiratory tract infections as compared to only 3% of noncolonized patients.[3] However, these data must be interpreted in light of the difficulty in differentiating colonization from infection in this patient population.

Recently introduced bronchoscopic specimen collection techniques, such as the use of protected-specimen brush (PSB) and bronchoalveolar lavage (BAL), probably have a higher specificity for differentiating colonization from infection in the lower respiratory tract.[4] Using these techniques, the relationship between colonization and infection of the respiratory tract has been reevaluated in mechanically ventilated ICU patients.[5,6] In one study, 11 of 64 patients developed 14 episodes of VAP; 17 (85%) of 20 bacterial species isolated in significant amounts from respiratory tract samples obtained by PSB or BAL had been isolated from tracheal aspirates prior to, or on the day of, diagnosis of pneumonia, and 10 (50%) had been isolated from the oropharynx.[5] In a second study, the proportion of pathogens colonizing the trachea and oropharynx prior to infection were 96% and 75%, respectively.[6] In that study, VAP was diagnosed in 37% of patients that acquired, and in only 8% of patients that did not acquire, colonization with gram-negative bacteria in the ICU.

Host factors associated with colonization. Many host factors have been associated with increased colonization of the respiratory tract (Table 1). Colonization itself seems to be a marker of underlying illness. For instance, oropharyngeal colonization with enteric gram-negative bacteria seldom occurs in healthy individuals.[3] In one study, introduction of a large inoculum of these bacteria into the mouths of healthy volunteers did not result in colonization.[7] However, with increasing illness or increasing age, oropharyngeal colonization with aerobic gram-negative bacteria occurs more frequently.[3,8] It has been hypothesized that, as the levels of salivary protease increase during illness, fibronectin is digested from the surface of epithelial cells.[9] As a result, the number of available binding sites for gram-negative bacteria increases. However, others failed to confirm this hypothesis.[10]

A change in acidity of salivary secretions also may play a role in bacterial adherence. Bacterial binding to tracheal cells was better under alkaline conditions (pH 7.2) than under more acidic conditions (pH 6.5).[11] Changes in acidity, theoretically, may affect the structure of bacterial and cell surfaces, formation and structure of adhesins and cell receptors, and the activity of protease and glycosidases.

Pathogen-specific factors. Adhesins are the most important pathogen-specific factors facilitating respiratory tract colonization. Pathogenic microorganisms contain hair-like filaments, known as pili or fimbriae, that attach specifically to carbohydrates on surface glycoproteins or glycolipids of epithelial cells.[12] Four groups of adhesins have been identified (Table 2). Adhesins belonging to the same group may be present on different species of bacteria, and, conversely, within a certain species different adhesins may be found.

Because not all bacteria that can adhere to epithelial cells have identifiable adhesins, either detection of fimbriae may not be sensitive enough or other nonfimbrial adhesins must be present.[12] For instance, mucins have been demonstrated to contain receptors for some bacteria and viruses that colonize the respiratory tract.[13] Although binding to mucins may facilitate mucociliary clearance, mucus also may protect microorganisms, such as *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, from host defense mechanisms.[14,15]

The bacterial source. Several sources of colonizing bacteria have been identified, some within the patient (endogenous) and others outside the patient (exogenous). The most important endogenous source for gram-negative bacteria is the gut. In

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TABLE 2
RELATION OF ADHERENCE MECHANISMS AND INFECTIONS

Type of Bacterial Fimbriae	Pathogen Exhibiting Fimbriae	Host-Receptor	Adherence Site	Infection
Mannose-susceptible Type I fimbriae	*Escherichia coli*	D-mannose	Tracheal epithelium	
Mannose-resistant P-fimbriae	*Pseudomonas aeruginosa*	Galactose	Urinary tract epithelium	Pyelonephritis
	Escherichia coli		Urinary tract epithelium	UTI
			Tracheal epithelium	Pneumonia
Mannose-resistant S-fimbriae	*Escherichia coli*	Sialylated glycocompounds	Blood vessels of the central nervous system	Meningitis
			Epithelial cells of the choroid plexus	
			Epithelial cells of ventricles	
	Haemophilus influenzae		Endothelial cells	Sepsis
			Tracheal epithelium	Pneumonia
Mannose-resistant Type 3 fimbriae	*Klebsiella* species	Unknown	Tracheal epithelium	Pneumonia
			Urinary tract epithelium	UTI
Type 1 C fimbriae	*Escherichia coli*	Unknown	Urinary tract epithelium	UTI
G-fimbriae	*Escherichia coli*	N-acetyl-D-glucosamine	Urinary tract epithelium	UTI
M-fimbriae	*Escherichia coli*	Galactose-N-acetyl-galactosamine	Urinary tract epithelium	UTI
Unknown	*Streptococcus pneumoniae*	N-acetylglucosamine	Tracheal epithelium	Pneumonia
			Oropharyngeal epithelium	
Unknown	*Mycoplasma pneumoniae*	Sialic acid	Tracheal epithelium	Pneumonia

Abbreviation: UTI, urinary tract infection.

healthy individuals, the aerobic colonic flora contains $>10^5$ *Escherichia coli* per gram of feces.[16] Moreover, some patients carry small numbers of *P aeruginosa* and *Enterobacter* species in their gut when admitted to the ICU.[17,18] Antibiotic use in the ICU may provide these bacteria with a selective growth advantage, allowing them to colonize other body sites.

Gastric colonization has been assumed to be important in the pathogenesis of colonization and infection of the respiratory tract.[19] In this hypothesis, bacteria that colonize the stomach subsequently colonize the oropharynx and trachea.[20] Under physiological conditions, gastric acidity (pH $\leq$4.0) prevents bacterial overgrowth of most bacteria. However, in critically ill patients, intragastric acidity may be reduced because of underlying illness, advanced age, or the administration of stress-ulcer prophylactic agents. In addition, administration of enteral feeding, which has a pH of approximately 6.0, has been associated with decreased intragastric acidity. However, the importance of this gastropulmonary route of colonization has been questioned.[5,6,21]

Contaminated medical devices, electric thermometers, or enteral feedings have been shown repeatedly to be exogenous sources for colonization and infection. Although strict hygienic measures should minimize transfer and colonization from these sources, animate exogenous sources, such as other patients in the ward and even healthcare workers, remain a risk for cross-colonization.[22-24] The relative contribution of each of these animate and inanimate sources of colonization has not been elucidated and probably differs among ICUs.

The Urinary Tract

Epidemiologic observations. The general belief that the ascending route of colonization is most important in the pathogenesis of urinary tract infections (UTIs) is based primarily on two epidemiologic observations. In the first place, these infections occur

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much more frequently in women, in whom the urethra is short and in direct contact with the moist environment of the vulvar and perirectal areas. Second, it has been shown that the pathogens causing UTI in women colonize the vagina and the periurethral area before infection occurs.[25]

Host factors associated with colonization. Normal urine has some important effects on bacterial growth. For instance, it possesses antibacterial activity for anaerobic and other fastidious bacteria, and high urea and low pH inhibit bacterial growth of *Enterobacteriaceae*.[26]

Hormonal changes probably influence bacterial colonization of the urinary tract. In fertile women, *Lactobacillus* species are the predominant microorganisms of the vaginal flora, and they maintain the normal acidic vaginal pH.[27] Estrogen deficiency is associated with decreased numbers of *Lactobacillus* species in the vagina.[28] As a result, mucosal pH is higher, colonization with enteric gram-negative bacteria occurs more frequently, and the incidence of UTIs is increased in postmenopausal women.[28] Urine of pregnant women also is more suitable for bacterial growth than urine from nonpregnant women.[29]

Pathogen-specific factors. E coli is the most important pathogen causing UTIs, and most infections are caused by only a few serogroups of this species.[30] Among different virulence factors that have been demonstrated, the ability to adhere to vaginal and uroepithelial cells probably is most important. As for pathogens colonizing the respiratory tract, adherence mostly is facilitated by fimbrial adhesins (Table 2). After bacterial entry into the lower urinary tract, type I adhesins probably facilitate colonization of bladder and lower urinary tract.[31] Subsequently, P-adhesins and nonfimbrial adhesins appear to facilitate infection of the pelvis and renal parenchyma.[32] With regard to nosocomial infections, it has been demonstrated that adherence to urinary catheters also is type I adhesin dependent.[33] The significance of bacterial adherence also has been demonstrated for other bacteria that occasionally cause UTI, such as *Proteus mirabilis*, *Staphylococcus saprophyticus*, and *Klebsiella* species.[34,35]

Nasal Colonization With Staphylococcus aureus

Nasal epithelial cells are an important site for colonization, multiplication, and dissemination of both methicillin-susceptible and -resistant *Staphylococcus aureus*. It has been demonstrated that other bacteria (*P aeruginosa*, other staphylococci, *Streptococcus pyogenes*, and diphtheroids) also have an ability to adhere to these cells.[36]

Adherence of *S aureus* to nasal epithelial cells is mediated by teichoic acid, a cell-wall component of staphylococci containing D-alanine and N-acetylglucosamine.[36] From the initial nasal source, the bacteria may disseminate to traumatized or disrupted skin and cause nosocomial wound infections. Attachment at these sites involves the interaction with proteins such as fibrinogen, fibronectin, laminin, thrombospondin, and, possibly, collagen IV.[37]

Intranasal carriage by patients has been associated with the development of staphylococcal infections in patients in hemodialysis and ICUs.[38] Casewell and Hill demonstrated that the incidence of surgical wound sepsis in nasal carriers of *S aureus* ranged from 5.6% to 16.5%, and 47% to 89% of the pathogens were similar to the strains colonizing the nose. The incidence of infection was 1.1% to 7.6% in noncolonized patients.[39]

PREVENTION OF COLONIZATION

Infection Control Measures

The institution of hygienic procedures is a cornerstone in all strategies to prevent colonization in hospitalized patients. Many studies have demonstrated the spread of bacteria from patient to patient via hands of healthcare workers, and the incidence of cross-colonization among patients has been reduced by enforcing handwashing between patient contacts.[40] The question of whether hands should be washed with antimicrobial soap, a general disinfectant, or just be rinsed with alcohol is raised frequently in hospitals. However, compliance with any handwashing guidelines is a problem. In one typical ICU, healthcare workers washed their hands after 41%, and physicians after only 28%, of patient contacts.[41]

In high-risk areas, even more precautions should be taken. High-risk areas are wards where patients are extremely susceptible to infection, such as hematology wards and neonatal ICUs; wards where many patients are heavily colonized with pathogenic bacteria, such as intensive care units; or wards where certain pathogenic microorganisms of epidemiologic concern are isolated frequently, such as outbreak wards. Under these circumstances, additional procedures are needed to prevent the spread of colonization. Specifically, in addition to handwashing, nonsterile examination gloves can be used to protect the healthcare worker from contamination with potentially infectious secretions, excretions, and antibiotic-resistant skin flora from patients. Sterile gloves should be used by healthcare workers to protect the patient against acquiring colonization during invasive procedures, such as suctioning intubated patients or touching open

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wounds.[42] Whether the use of cover gowns by personnel provides additional protection against patient colonization and infection remains to be established.[43]

Surveillance Cultures

The performance of surveillance cultures on a regular basis potentially may improve medical care for several reasons. Because there is such a close relationship between colonization and infection, knowledge of antimicrobial susceptibilities of colonizing bacteria may improve empiric antibiotic therapy. Moreover, epidemics or failures in infection control probably are detected earlier when colonization is monitored frequently. However, to the best of our knowledge, the effects of surveillance cultures never have been subjected to a controlled study, and surveillance cultures should not be recommended routinely. The optimal design for such a study would include a cost-benefit analysis, because a large proportion of colonized patients will not develop infection.

Modulation of the Colonizing Bacterial Flora

Modulation of the bacterial flora, as a matter of infection prevention, may be performed at present only by application of antimicrobial agents (applied systemically or topically) or by maintenance of the physiological mucosal environment.

Colonization resistance. In the human gut, the numbers of aerobic bacteria (predominantly *E coli*, streptococci, and enterococci) are far outnumbered by the anaerobic species. In an animal model, it has been demonstrated that an intact anaerobic flora protects the animal against overgrowth with enteric aerobic gram-negative bacteria, a phenomenon that has been called "colonization resistance."[16] This observation has been cited to explain why many patients receiving antimicrobial agents that are active against the anaerobic flora develop intestinal bacterial overgrowth with enteric gram-negative bacteria.

Systemic antibiotics. Systemic antibiotics have been used to prevent infection. They probably work by preventing the progression from colonization to infection more than by modulating colonization. Leukopenic patients often receive absorbable broad-spectrum antibiotics, such as fluoroquinolones, orally for both gut decontamination and systemic prophylaxis. The increasing prevalence of multiresistant microorganisms within hospitals justifies a rationale for restrictive use of these agents, and prophylaxis seems to be justified in leukopenic patients only.

Selective digestive decontamination. Selective decontamination of the digestive tract (SDD) has been studied extensively in ICU patients. SDD combines three potential preventive measures: the intestinal tract is decontaminated, from oropharynx to rectum, using topical, nonabsorbable antimicrobial agents; anaerobic bacteria are spared to maintain "colonization resistance"; and a systemic component (eg, a second- or third-generation cephalosporin) is added to prevent infections already incubating at the time of admission.[44] Despite approximately 50 clinical studies and five meta-analyses, the benefits of SDD in ICU patients remain unproven. Meta-analyses are hampered by lack of uniformity in the patient population, by use of many different antibiotic combinations, and by inadequate methodology to establish the diagnosis of the most important endpoint, VAP. The major concerns with regard to the routine use of SDD in ICUs result from the reported associations between the use of SDD and an increase in gram-positive colonization and infection,[45-47] the relation between SDD and selection of antibiotic-resistant bacteria,[48-50] and the lack of a reduction in mortality or duration of hospital stay.

Topical nasal decontamination. Many attempts have been made to eradicate nasal colonization by *S aureus*. The use of topical and systemic antibiotics either yielded poor results or was accompanied by selection of resistant strains.[38] The most favorable results are reported with the use of mupirocin, which is active against staphylococci, including MRSA, streptococci, and some gram-negative bacteria.[51,52] Mupirocin is not related structurally to other antibiotics and can be used only as a topical agent.

Mupirocin has been demonstrated to eradicate carriage of *S aureus* in healthcare workers[53] and of MRSA during outbreaks in Veterans' Administration nursing homes, ICUs, and neonatal nurseries.[38] Eradication of *S aureus* from the nares reduced the incidence of infections in hemodialysis patients[54] and in patients suffering from familial staphylococcal infections.[55] In thoracic surgery patients, the use of mupirocin nasal ointment was associated with a 0.5% rate of postoperative wound infections caused by *S aureus,* as compared to 3.5% in historical controls.[56]

However, any topical use of antibiotics is associated with concerns about emergence of resistance. Resistance to mupirocin has been reported, for instance, in dermatology wards where patients received prolonged treatment for infected skin lesions.[38] However, cross-resistance of mupirocin to clinically used antibiotics has not been demonstrated yet, probably because of its unique structure and mode of action. Nevertheless, prolonged and inappropriate use of mupirocin should be avoided to maintain its efficacy for eradicating MRSA.

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Maintaining the Physiological Mucosal Environment

Gastric acidity. Based on the hypothesis of the gastropulmonary route of colonization, several strategies have been developed to maintain intragastric acidity to prevent gastric colonization and the development of VAP.

Most studies focused on stress ulcer prophylaxis. Sucralfate, a cytoprotective agent that does not influence acidity, has been compared to antacids and H_2 antagonists. Although some trials reported significantly lower incidences of VAP in sucralfate-treated patients,[57-60] the results of the studies are contradictory.[6,61]

In addition, gastric colonization could be modulated by changing the administration of enteral feeding. For instance, enteral feeding could be administered intermittently, allowing a temporary decrease in intragastric pH to prevent bacterial overgrowth. Favorable results of this strategy were demonstrated by Lee and coworkers using a historical control group that had received continuous enteral feeding.[62] However, these benefits were not confirmed in two prospective studies.[63,64] In two other attempts, acidified enteral feedings[65] and intrajejunal administration of nutrition[66] were studied. Although both studies reported favorable effects, the small numbers of patients do not allow conclusions to be drawn.

Estrogen substitution. Recurrent UTIs in postmenopausal women, probably as a result of increased vaginal colonization with *Enterobacteriaceae*, have been prevented successfully by modulation of the vaginal flora with intravaginal estriol.[28] In a randomized, double-blind, placebo-controlled study, the use of topical estriol resulted in reappearance of lactobacilli in 61% of the patients. In addition, the mean vaginal pH declined from 5.5 to 3.8, the rate of vaginal colonization with *Enterobacteriaceae* fell from 67% to 31%, and the incidence of urinary tract infections in the estriol-treated patients was 0.5 episodes per patient-year, as compared to 5.9 episodes per patient-year in the control group ($P<.001$).[28]

Preventing Bacterial Adherence

Given the lack of knowledge about the mechanisms of bacterial adherence, it is clear that methods to prevent bacterial attachment are only in their infancy. The interaction between adhesins and cell receptors could be prevented theoretically either by soluble receptors that bind competitively to adhesins or, conversely, by soluble adhesins that competitively adhere to cell receptors. In another theoretical approach, antiadhesin or antireceptor antibodies may prevent the interaction between adhesin and cell receptor. Passive immunization with purified pili from developing enterotoxigenic strains of *E coli* in female pigs prevented their piglets from developing diarrhea,[67] and rats did not develop pyelonephritis after passive immunization with antisera produced with purified pili.[68]

Finally, adherence could be modified by altering bacterial surface proteins of either adhesins or cell receptors. This may be the mechanism by which aminoglycosides and ceftazidime, when used in subinhibitory concentrations, inhibited adherence of *P aeruginosa* and *E coli* to tracheal and buccal cells in vitro.[69]

CONCLUSIONS

Many data underscore the chronological relationship between colonization and the development of infection. Understanding the mechanisms of, and preventing, colonization—the first of the three steps leading to nosocomial infection—remains an important goal, especially for high-risk patients. However, there still is no ready answer to a seemingly simple question like "Why do hospitalized patients acquire colonization with enteric bacteria in their respiratory tract?" Moreover, in many cases, the exact source of these bacteria remains unknown.

The current knowledge about the initial step in the pathogenesis of colonization, adherence of the microorganism to the epithelial cells, is insufficient to develop adherence-targeted preventive strategies. Prevention of colonization, therefore, relies heavily on very basic infection control measures such as handwashing. Unfortunately, compliance with these measures often is poor. The development of alternative strategies, such as topical antimicrobial prophylaxis, is stymied by antibiotic resistance. Studies aimed at maintenance of the physiologic mucosal environment using nonantimicrobial agents may help avoid this pitfall.

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Conference on Reuse of Medical Devices

by Gina Pugliese, RN, MS
Medical News Editor

The Association for the Advancement of Medical Instrumentation is collaborating with a number of organizations to cosponsor a conference on "Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Healthcare Facilities" to be held April 9-10, 1996, in Washington DC. Cosponsoring organizations include the Health Industry Manufacturer Association, the Food and Drug Administration, American Medical Association, the Association for Professionals in Infection Control and Epidemiology, and the Association of Operating Room Nurses.

Conference topics will include reprocessing techniques, the perspective of technicians, an overview of standards and regulations, device design considerations, manufacturer responsibilities regarding reusable devices, and the future of reprocessing.

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Major article

Risk of infection following colonization with carbapenem-resistant Enterobactericeae: A systematic review

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Key Words:
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Background: Carbapenem-resistant Enterobacteriaceae (CRE) have emerged as important health care-associated pathogens. Colonization precedes infection but the risk of developing infection amongst those colonized with CRE is not clear.

Methods: We searched multiple databases for studies reporting rates of CRE-colonized patients subsequently developing infection.

Results: Ten studies fulfilled our inclusion criteria, including 1,806 patients used in our analysis. All studies were observational and conducted among adult inpatients. The cumulative rate of infection was 16.5% in our study. The most common site of infection was the lung, identified in half of patients, followed in decreasing frequency by urinary tract; primary bloodstream; and skin and soft tissue, including surgical sites. Colonization or infection by CRE prolonged stay and was associated with a 10% overall mortality in our analysis.

Conclusion: Our study results suggest an overall 16.5% risk of infection with CRE amongst patients colonized with CRE. Given the high mortality rate observed with CRE infection and the difficulty in treating these infections, research to investigate and develop strategies to eliminate the colonization state are needed.

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Since the early 1980s, carbapenems have been used with success as the last line of defense against multidrug-resistant gram-negative organisms. Widespread carbapenem-resistant Enterobacteriaceae (CRE) were unknown until the early 2000s.[1] During recent years, CRE have emerged as an important family of health care-associated pathogens worldwide, including in the United States.[2-5]

In January 2015, the Centers for Disease Control and Prevention revised the definition of CRE as organisms that are nonsusceptible to imipenem, meropenem, doripenem, or ertapenem or a documented isolate that possesses a carbapenemase.[6] A wide range of species in the Enterobacteriaceae family have been detected with carbapenemases, including *Klebsiella pneumoniae, Citrobacter freundii, Escherichia coli, Acinetobacter baumannii,* and *Enterobacter aerogenes*. *K pneumonia* carbapenemase and metallo-β–lactamase are the main mechanisms underlying resistance to carbapenems.[7]

Infections by CRE are considered a health care challenge because CRE isolates are usually extensively drug resistant and associated with high morbidity and mortality.[8,9] Colonization is considered to be a prerequisite for infection,[10] which suggests that prevention of CRE colonization is important in preventing the morbidity and mortality associated with these infections. However, the extent to which colonized patients develop infection with CRE is unclear. These data are important to guide decision making regarding infection-control interventions such as screening and contact precautions for colonized patients.

Therefore, we undertook a systematic review to understand the relationship between colonization with CRE and subsequent infection.

METHODS

During September 2014 and June 2015 we searched PubMed, Medline, Cochrane Library database, Cumulative Index to Nursing and Allied Health Literature, and Scielo databases from January 1,

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1991, the year before the first reported case of CRE, for relevant publications. No language restrictions were used. Key words used in the search, alone or in combination, were: *carbapenem-resistant Enterobacteriaceae*, Klebsiella pneumonia *carbapenemase*, *KPC*, *Verona integron-mediated metallo-beta-lactamase*, *CRE*, *carbapenem resistant*, *carbapenem nonsusceptible*, *carbapenemase*, *carbapenamase-producing*, *colonization*, *infection*, and *carriage*. Reference lists of studies included in this review were also searched for relevant articles.

Inclusion criteria

To be included in our systematic review, studies needed to be clinical trials or observational studies. Data had to be provided to calculate rates of infection in patients initially colonized. Studies that reported data on only colonization or infection or did not allow for determination of infections arising from previous colonization were not included, and studies that reported numbers of cultures or isolates, not patients colonized or infected, were excluded. Review articles, abstracts, and editorials were excluded.

Outcome measures

The primary outcome of interest was rate of CRE infection amongst those colonized with CRE.

Data extraction

Two investigators independently extracted data using a standard data collection form. Data extracted included study sample size, demographic characteristics of the study sample, number of patients colonized, site of colonization, number of subsequent infections, site of infection, length of hospital stay, and mortality rate. The two investigators reviewed studies that met the inclusion criteria. Disagreement amongst investigators was resolved by discussion and arriving at consensus.

Data synthesis

Descriptive statistics were used to determine the percentage of patients initially colonized with CRE who went on to develop clinical infection. The mean age of subjects with infection and length of stay were also calculated for the subset of studies for which these data were available. For the subset of studies for which mortality rates were reported, we calculated cumulative in-hospital mortality, which is reported as the proportion of patients with CRE infection or colonization who died with all patients infected or colonized with CRE as the denominator. Because of the heterogeneity in included studies, we did not perform a meta-analysis.

RESULTS

Study characteristics

Our search strategy yielded 1,709 reports, of which 178 were considered potentially relevant and abstracts were reviewed. Of these, the full text of 42 studies was retrieved and reviewed; 33 were ultimately excluded because data on the numbers of patients initially colonized who subsequently went on to develop infection were not reported. In fact, many were point prevalence reports of those colonized or infected and not consistent with the aim of our review (Fig 1). One additional study[11] was brought to the attention of the authors during the review process and was included in our analysis.

Ten studies fulfilled inclusion criteria; all were observational studies.[11-20] Characteristics of the studies meeting inclusion criteria are reported in Table 1. Among these 10 studies, 1,806 colonized patients were used in our analysis. For 2 studies,[13,14] only data on those patients with nosocomial acquisition of CRE were used for analysis because subsequent rates of infection were reported for only these patients. In a third instance,[15] the authors reported on rates of infection for those patients infected with *K pneumoniae* alone; thus, only this subset of patients was used in our analysis.

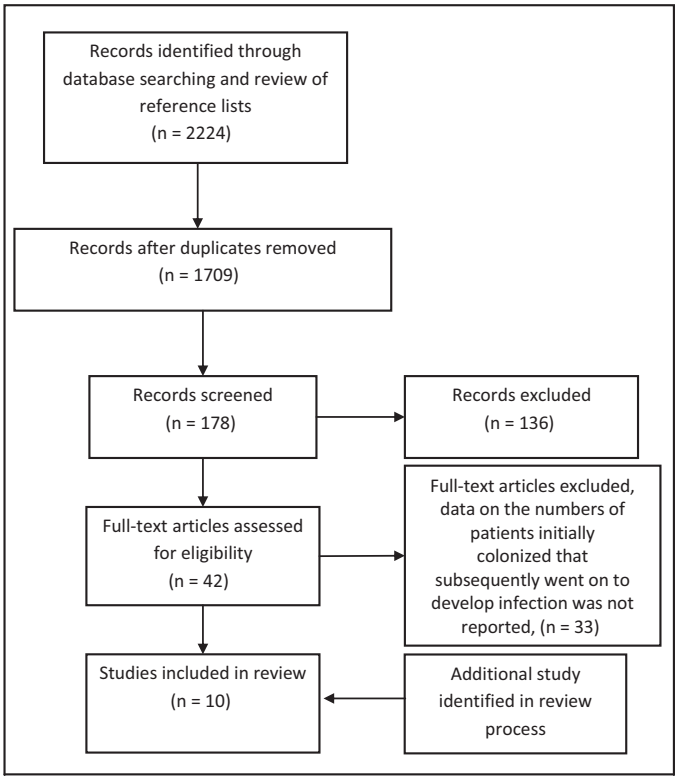

Fig 1. Flowchart depicting the selection process of studies included in the review.

All studies were conducted among adult inpatients, with 4[11,13,14,16] exclusively in an intensive care unit setting. One[17] included liver transplant recipients. One study[15] performed hospitalwide screening, whereas 4[12,18-20] performed screening on patients deemed to be at higher risk based on past acute- or long-term care admission, housing on a high-prevalence ward, or known contact with other patients infected or colonized with CRE. For the remaining studies, screening was performed at specified intervals for those patients admitted to an intensive care unit[11,13,14,16] (Table 1).

Seven studies examined carbapenem-resistant *K pneumoniae* exclusively,[12,14-19] 2 reported prevalence of carbapenem-resistant *A baumannii*,[11,13] and the remaining examined multiple CRE.[20] Seven reported site of infection[11-14,17,19,20] and 6 studies included data on length of stay amongst CRE cases,[12,14-17,20] whereas only 5 included mortality data.[12,14-17]

For those studies that reported data on gender,[11,14,17,18,20] men accounted for 33%-69.6% of patients colonized or infected with CRE. One study[18] found a predominance among women. Patient age ranged from 45-76 years across studies.[11,12,14,15,17,18,20] Four were conducted in the context of an outbreak.[15,17,18,20]

Risk of infection after colonization with CRE

Colonization was defined as gastrointestinal tract carriage determined by rectal swab in the majority of studies.[11,12,14,15,18-20] Five studies cultured specimens on MacConkey agar.[11,14,15,18-20]

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Table 1
Characteristics of studies included in the review

Study, year	Patient population	Outbreak setting	Screening frequency	No. of patients colonized by CRE	No. of patients who developed CRE infection	Colonization site	Infection site	Type of CRE	Mortality
Borer et al, 2012[12]	Adults, hospital-wide	No	Rectal cultures performed in patients admitted for nursing or other outside facility and on patients in high-risk units	464	42 (9.1%)	Rectal	Multiple	CRKP	18.75%, colonized 30%, infected
Cho et al, 2014[13]	Adults, ICU	No	On admission, weekly thereafter	530 with nosocomial acquisition included for analysis	111 (20.9%)	Nares	Multiple	CRAB	Not included
Debby et al, 2012[14]	Adults, ICU	No	Within 72 h of ICU admission, then twice weekly	48 with nosocomial acquisition included for analysis	20 (41.7%)	Rectal	Multiple	CRKP	17/48 (35.4%), patients with nosocomial acquisition
Wiener-Well et al, 2010[15]	Adults, hospital-wide	Yes	All hospitalized patients screened during 3 consecutive days	42, 16 with CRKP included for analysis	5 (31.3%)	Rectal, perianal, oral	Not included	CRKP	1/16 (6%) of all CRKP carriers
Papadimitriou-Olivgeris et al, 2013[16]	Adults, ICU	No	Upon ICU admission, days 4 and 7, and weekly thereafter	164	37 (22.6%)	Rectal	Not included	CRKP	41/127 (32%), colonized 22/37 (59.5%), infected
Lübbert et al, 2014[17]	Liver transplant patients	Yes	—	9	8 (89%)	Unclear	Multiple	CRKP	1/1 (100%), colonized 6 out of 8 (75%), infected (2 of these determined "noninfectious causes")
Pisney et al, 2014[18]	Adults, selected high-prevalence units	Yes	7 rounds of point prevalence screening on affected units	15	0 (0.0%)	Rectal	None	CRKP	Not included
Lowe et al, 2013[19]	Adults, hospital-wide	No	Known contact with CRE-infected patients were screened	9	4 (44.4%)	Rectal	Bloodstream, urine	CRKP	Not included
Schechner et al, 2012[20]	Adults, hospital-wide	Yes	All patients hospitalized in the past year and those with CRE-infected contact	502	38 (7.6%)	Rectal	Multiple	CRKP, *Escherichia coli*, *Enterobacter* spp, *Citrobacter freundii*	Not included
Latibeaudire, et al, 2015[11]	Adults, ICU	No	Upon ICU admission and weekly thereafter	49	34 (69%)	Rectal, tracheal	Multiple	CRAB	Not included

CRAB, carbapenemresistant *Acinetobacter baumannii*; *CRE*, carbapenem-resistant Enterobacteriaceae; *CRKP*, carbapenem resistant *Klebsiella pneumoniae; ICU*, intensive care unit.

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Alternative methods used included inoculation of brain-heart infusion broth[14,15] and use of CHROMagar *K pneumonia* carbapenemase Petri dishes (CHROMagar, Paris, France) for some or all specimens.[12,16,19] The study by Cho et al[13] was unique in using nasal swabs cultured on 5% sheep's blood agar plates. One study did not specify microbiologic techniques sufficiently to allow interpretation.[17]

Amongst the 1,806 initially colonized patients included in our analysis, 299 clinical infections were observed for a cumulative rate of 16.5%. Rates of infection in individual studies varied widely, from 0%[18]-89%,[17] although with the majority falling in the range of 7.6%-44.4% (Table 1).

Quantitative data regarding site of infection was reported for 223 previously colonized patients in the studies.[12-14,17,19,20] The most frequent clinical syndrome was pneumonia, identified in half of patients with CRE infection. Forty-four cases, or approximately 20%, of patients were diagnosed with urinary tract infection caused by CRE. In order of decreasing frequency, primary bloodstream infections, identified in 30 patients, and skin and soft tissue infections, including surgical site infection, identified in 16 patients, accounted for the remaining infections reported.

Risk of adverse outcomes following acquisition of CRE

There was no standardized method for reporting length of stay, so it is difficult to draw a meaningful conclusion regarding mean length of stay from currently available data. However, in studies that reported these data[12,14-17,20] colonization or infection with CRE significantly prolonged hospital stay, with 2 studies reporting hospitalization nearly 2-fold longer in those patients with CRE.[16,17]

The overall mortality rate was 10% for all patients infected or colonized. Amongst the 3 studies that reported mortality in the subset of patients with clinical infection separate from those colonized,[12,16,17] mortality rates ranged from 30%-75%.

DISCUSSION

Our results show that a substantial proportion of patients colonized the CRE go on to develop clinical infection with CRE. We summarized the results of the available literature and found an overall 16.5% risk of infection with CRE amongst patients colonized with CRE. Individual studies that have examined this question have found varying rates of infection, probably due to differences in type of organism, patient population, and clinical setting. Thus, the overall magnitude of risk was unclear. Our study provides an overall assessment of the risk of infection and has implications for infection preventionists and clinicians involved in the prevention and treatment of CRE.

Sites of screening and microbiologic methods varied across studies, contributing to the heterogeneity amongst studies. The most common site of screening for carriage was the gastrointestinal tract, specifically, rectal swabs. Wiener-Well et al[15] examined the sensitivity of 3 screening sites and found rectal swabs to be more sensitive that perianal or oral swabs. MacConkey agar was used most commonly for culturing CRE; however, a limited number of other methods were used in some studies. Future studies should compare the different types of selective media available to determine the best way to recover CRE from surveillance specimens. Studies also varied in the approach to screening for CRE; 9 of the 10 included studies reported screening method. Screening approach varied from hospitalwide point prevalence screening[15] to screening of those only with known contact with a CRE-infected patient.[19]

CRE infections are associated with considerable mortality with rates reported up to 50% in the literature.[9,21] Our review found similar rates, ranging from 30%-75% for those infected amongst the included studies. There are many contributors to this high rate of mortality, including longer length of stay,[12,14-17,20] poorer overall health status, site of infection, comorbid illnesses, and most importantly the limited antimicrobial options for treating these infections. Panresistant Enterobacteriaceae have been reported,[22,23] highlighting the challenges in treating these infections and underscoring the importance of antimicrobial discovery and development targeted to these organisms.

Given the heightened risk of mortality associated with CRE infection, >30% for patients in our review, our results emphasize the importance of identifying and eliminating the carrier state to prevent development of clinical infection with CRE. Studies have examined the role for oral decontamination therapy in decolonizing patients with CRE. In a modestly sized randomized controlled trial of 40 patients colonized the carbapenem-resistant *K pneumoniae*, the utility of treatment with combination of gentamicin and colistin in both oral, nonabsorbable solution and nasal gel delivery method was examined.[24] In the 20 patients randomized to treatment, the rate of rectal colonization at 2 weeks was significantly reduced, with 61.1% of patients decolonized in the treatment group versus 16.1% in the placebo arm. This difference persisted at 6 weeks, with 58.5% of patients remaining decolonized in the treatment arm versus 33.3% in the placebo arm. In a larger study,[25] 50 patients colonized with carbapenem-resistant *K pneumoniae* strains with demonstrated susceptibility to gentamicin and colistin were randomized to treatment with gentamicin, colistin, or combination therapy. Compared with the spontaneous eradication rate of 7% in their study, all 3 treatment arms demonstrated a significantly greater decolonization rate. The rate of decolonization with colistin was 50%, with gentamicin, 42%. Similar to the study by Saidel-Oades,[24] no significant adverse effects were noted. Interestingly, combination therapy yielded the lowest rate of decontamination, only 37.5%, although the differences amongst groups did not reach statistical significance. These promising results should be confirmed in larger studies and tested in both outbreak and endemic situations with close attention to analysis of gut microbiome changes as a result of the antibiotic agents.

Our study has several limitations. First, given the methodology of a systematic review, our results have the limitations of the included studies. Secondly, the studies were quite heterogeneous in their study methods and patient populations. We did develop a priori inclusion and exclusion criteria and did not include studies that did not clearly establish patients as colonized before developing clinical infection. Third, we were not able to study risk factors for development of clinical infection and mortality because of limited available data; this should be an area for future research. Fourth, the included studies used varying approaches to identifying cases of colonization and/or infection, potentially contributing to misclassification of colonization and infection. Similarly, many studies did not include data on those patients developing infection without preceding colonization, which precluded our ability to comment on the relative risk of infection in those patients with colonization. Limited by reported data, we were not able to comment on the time from colonization to the development of infection. Finally, publication bias is a concern.

CONCLUSIONS

We found that colonization with CRE poses a 16.5% risk of subsequent CRE infection. With a high rate of mortality associated with CRE infection, pending further research, eradication of colonization with CRE should be considered in select situations such as outbreaks. Future research should attempt to determine the utility of widespread routine surveillance for CRE amongst hospitalized patients as an infection control strategy as well as determine strategies of eradication of CRE colonization.

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Legionella in hospitals: a review

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Summary: Although epidemics of nosocomial Legionnaires' disease attract great attention, up to 30% of sporadic cases of hospital-acquired pneumonia are caused by legionellae. Legionellae are ubiquitous contaminants of potable water and can achieve high numbers in the hot-water systems of large buildings such as hospitals. They are present in the mains water supply in small numbers but are amplified considerably in the hospital's hot-water system. This is encouraged by water temperatures below 50°C, areas of stagnation and sludge formation, the presence of amoebae and other bacteria and the materials used in the piping. Formation of aerosols from contaminated water is a major mode of spread of legionellae, but there is evidence to suggest that aspiration is also a mode of entry.

Safe levels of legionellae in cooling towers have been defined, but not for hot-water systems. A combination of culture and antigen detection by immunofluorescence offer the best method for enumerating legionellae in environmental samples. Control involves a mixture of physical (heat, UV irradiation, sanitation) and chemical (hypochlorite, ozone) methods combined with good plumbing practice (e.g. arrangement of pumps and calorifiers, elimination of dead-legs). Adequate control can be costly and requires considerable attention to detail.

Keywords: Legionellae; nosocomial infection; hospital reservoirs.

Introduction

Since the initial description of an outbreak of pneumonia associated with an American Legion convention in Philadelphia in 1976[1] there has been great interest in legionellae in both the scientific and popular press. Although the initial outbreak was linked to a hotel, it is now clear that hospital-associated Legionnaires' disease, both epidemic[2,3] and sporadic[4,5] is not uncommon.

The infection generally presents as either a severe pneumonia with or without extrapulmonary manifestation or a milder 'flu-like' febrile illness (Pontiac fever),[6] but infection at other sites can occur. Such infections include pericarditis,[7] endocarditis[8] and cutaneous abscesses.[9]

In order for legionellae to produce disease, several factors must be considered. Firstly, because human-to-human transmission does not occur, there must be an environmental reservoir. Secondly there must be a mechanism for getting the bacterium from the reservoir to the site of

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infection and thirdly the organism must lodge in a susceptible host. In this review we will discuss the properties of legionellae, their reservoirs and modes of spread in hospitals and methods of detection and control of legionellae in the hospital environment.

The bacterium

There are currently 14 recognized serogroups of *Legionella pneumophila* and at least 29 other *Legionella* species (Table I) although there is still some disagreement over speciation. Not all of these bacteria have been associated with disease in man, and even within *L. pneumophila* serogroup 1 there appear to be subtypes of differing virulence.[10]

Legionellae are not thermophilic but are thermotolerant, i.e. they are able to survive at relatively high temperatures. For example, legionellae will multiply over the temperature range 20–43°C and can survive for varying periods at temperatures 40–60°C. At 50°C, 90% of the organisms are killed within 2 h. Legionellae can survive in water for over a year.[11] Legionellae are able to survive aerosolized in droplets 1–5 μm in diameter and there is evidence to suggest that virulent strains survive longer.[12] Legionellae appear to be able to adhere to substrata such as pipes, rubber, plastics and sediment and can thus persist even when there is constant flushing of a piped-water system. It is possible that they exist in biofilms with release of planktonic organisms. This may explain the discrepancy between the ability of disinfectants to kill legionellae in the test tube and that in piped water systems.

Finally, there is some evidence that legionellae are engulfed by amoebae and are able to survive and multiply inside them.[13] This might also be a mechanism prolonging survival of legionellae in water. It is also noteworthy that amoebae constantly generate superoxide,[14] thus the ability of

Table I. Legionella pneumophila *and* Legionella *spp.*

Legionella pneumophila (serogroups 1–14)*	*L. londiniensis*
*L. anisa**	*L. longbeachii**
L. birminghamensis	*L. maceachernii**
*L. bozemanii**	*L. micdadei**
L. cherrii	*L. nautarum*
L. cincinattiensis	*L. oakridgensis*
*L. dumoffii**	*L. parisiensis*
L. erythra	*L. quateriensis*
*L. feeleii**	*L. rubrilucens*
L. geestiae	*L. sainthelensi*
*L. gormanii**	*L. santicrucis*
*L. hackeliae**	*L. spiritensis*
L. israelensis	*L. steigwaltii*
L. jamestowniensis	*L. wadsworthii**
*L. jordanis**	*L. worsliensis*

* Associated with human disease.

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legionellae to survive in amoebae might be related to their ability to survive in a patient's alveolar macrophages.

Reservoirs

Legionellae are common contaminants of potable water. *Legionella pneumophila* has been isolated from drinking water from various parts of the world with a frequency ranging from 0 to 8·8% and isolation occurs more frequently in warm summer months.[15] It is probable that the small numbers of legionellae in the incoming water are amplified by the hot water systems in large buildings such as hospitals and hotels. For example, the water systems of over 50% of large establishments have been shown to contain *L. pneumophila*.[16] Large outbreaks of Legionnaires' disease have resulted from contamination of air conditioning and ventilation systems,[3,4,17] and legionellae can be found in large numbers in water in cooling towers. The other major site for colonization and multiplication of legionellae is the hot-water system, or other areas where water temperature is raised into the range 25–40°C. In many hospitals, hot water is produced by taking water through a number of heaters or calorifiers. The water then circulates throughout the hospital and returns to the calorifiers. The factors promoting the expansion and persistence of legionella populations have been described by others.[2,18] They include the arrangement of calorifiers, which should be in series rather than parallel, the presence of a dead space at the bottom of the calorifier which allows sludge and legionellae to persist, and maintenance of the hot-water output below 57°C. The presence of dead-legs in the hot-water system, e.g. associated with showers,[19–21] hosepipes or fire sprinkler systems, the use of spas or jacuzzis, and the addition of hot water to humidifiers or nebulizers[22] can all serve to amplify *Legionella* populations. We have even been able to find legionellae in the melt-water from ward ice-making machines. The pH, ionic composition and conductivity of the water and the nature and age of materials used to make pipes and valves also affect the growth and survival of legionellae. For example, some silicone-based rubbers enhance the growth of *L. pneumophila* by a factor of 10^5, whereas rubbers containing the accelerator thiuram inhibit growth.[23]

There has been considerable debate over the relative importance of the two major reservoirs, namely cooling towers and evaporative condensers and the hot-water system. It is clear that episodes of infection have arisen from contamination of water in cooling towers,[3,24] and the evidence linking such reservoirs and development of infection is strong. Indeed, in one outbreak of Legionnaires' disease in a retirement hotel in which *L. pneumophila* serogroup 1 was found in both the hot-water system and an evaporative condenser, it was demonstrated using air sampling and monoclonal antibody subtyping that the condenser was the source.[25] However, it has also been shown that Legionnaires' disease could occur in

areas where there was no possibility of aerosols from evaporative condensers.[20,26] Similarly outbreaks have been controlled by treatment of the water system alone even though a cooling tower was present.[27] It would appear that the two reservoirs are equally important.

Guidelines on acceptable levels of legionella contamination of cooling towers have been produced[28] but no such guidance is available for hot-water systems. In cooling towers *Legionella* counts of less than $10^3\,l^{-1}$ have been suggested as being acceptable, those above $10^5\,l^{-1}$ indicate that corrective action is required.

It has been demonstrated that there is a link between the extent of colonization of a hospital's hot-water system and the development of nosocomial Legionnaires' disease.[4,29] Thus, in an Ear Nose and Throat hospital where 67% of sites sampled contained legionellae, 30% of the cases of hospital-acquired pneumonia were due to legionellae. After chlorination of the system only 3·5% of sites were contaminated and none of the cases of hospital-acquired pneumonia were caused by legionellae. The patient group studied were resident on head and neck oncology wards and such patients are known to be highly susceptible to legionella infection. Nevertheless, it appears that once contamination of a hospital's hot-water system involves more than 30% of sites sampled, nosocomial Legionnaires' disease occurs.[19] Unfortunately, there is little information on the relationship between numbers of legionellae in the hot-water systems and development of infection. However, we were able to demonstrate that a few days prior to acquisition of pneumonia due to *L. pneumophila* serogroup 12 by a renal transplant recipient, the water from her hand basin hot tap contained 6×10^3 cfu l^{-1} of the same bacterium.[5]

Modes of spread

The most likely mode of spread is by aerosolized particles $< 5\,\mu m$ diameter containing legionellae. It is possible to infect guinea-pigs with aerosols containing as few as $3{\cdot}7 \times 10^4$ cfu *L. pneumophila* serogroup 1[30] and the bacteria can survive for up to 2 h in such aerosols.[31] Aerosols can be produced easily in evaporative condensers, cooling towers, jacuzzis, showers, nebulizers and in large buildings even by normal tap pressure. There has even been an outbreak of Pontiac fever due to *L. anisa* where an aerosol created by an artificial fountain was implicated.[32]

It has also been suggested that aspiration or direct inoculation of legionellae into the tracheo-bronchial tree via respiratory manipulation are important modes of transmission especially in head and neck surgery patients.[2,4]

Detection of legionellae in the environment

Perhaps the most difficult question to answer is should there be environmental testing and if so, how frequently? In general the indication

for environmental testing in hospitals is the presence of a cluster of cases of Legionnaires' disease or an increasing number of sporadic cases. Because legionellae are ubiquitous and because of the high cost, routine surveillance is not recommended.[33] Perhaps in the light of recent developments some re-appraisal of this position is necessary. Guidelines have been produced giving acceptable levels of legionellae in cooling towers, in order to comply with these it is thus necessary to measure levels actually present.

There does appear to be a relationship between the extent of environmental colonization and development of nosocomial Legionnaires' disease.[4] Methods for blanket control of *Legionella* in the hot-water system are expensive and not uniformly effective (see below) and we may now be reaching the point where it is more cost-effective to monitor environmental contamination than implement continuous control.

Finally, with patients increasingly taking recourse to the law, the high cost of hospital-acquired Legionnaires' disease might make regular testing more cost-effective. One author has suggested that hot water tanks and selected distal outlets be tested three to four times a year.[2] We would also suggest that the hot-water systems in areas where patients such as the immunosuppressed, those undergoing head and neck surgery and those with chronic pulmonary disease might warrant regular testing.

Detection of legionellae in the environment is now becoming simpler, cheaper and more accurate. The methods available for detecting legionellae in environmental samples fall into three categories: culture, antigen detection and genome detection.[2,6,15,20,34–36]

Quantitative culture usually involves concentration of water samples by centrifugation followed by incubation of the deposit on suitable selective media (e.g. buffered charcoal yeast extract agar with and without cysteine) at 37°C in a humid atmosphere for 7–10 days.[37] Colonies with the appropriate morphology are then serotyped with specific antisera. Although this is taken as the 'bench-mark' methodology it is rather slow and can be rendered imprecise by the presence of biocides and other bacteria that produce inhibitory compounds.[38] Another problem is the presence of non-culturable *L. pneumophila*.[15] These are bacteria that are visible by indirect or direct immunofluorescence but not recoverable on culture. It is possible that this is a form of 'heat shock' and it has been shown that if appropriate resuscitation procedures are employed the 'non-culturable' legionellae can be grown.[15]

Antigen detection is usually by immunofluorescence, which provides quantification. Although enzyme-linked immunosorbent assay (ELISA) systems have been devised,[36,39] they are not so readily rendered quantitative, since they will detect both bacterium-associated and soluble antigen. Immunofluorescence can be highly sensitive (94%) but is less specific (52%) compared with culture,[35] presumably because of the non-culturable legionellae.[15] Perhaps the use of vital dyes (e.g. that change colour when the bacterial respiratory chain is active) or antisera directed against *Legionella* heat-shock proteins might improve the specificity of such tests.

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Methods involving genome detection, e.g. DNA hybridization or polymerase chain reaction (PCR) are likely to suffer the same problems of quantification as ELISA, although PCR could determine viability if it were used to detect mRNA.

At present it would appear that a combination of culture and antigen detection offer the best method for quantification of legionellae in environmental waters.[35]

Control of legionellae in the hospital environment

Methods for control of legionellae in cooling towers and evaporative condensers are well-established and involve the use of biocides combined with good engineering practice.[40]

There are many methods, of varying efficacy, for controlling legionellae in hospital hot water systems. They fall into three categories: physical, chemical and good plumbing practice (Table II).

Physical methods

Raising the output temperature in the hot-water system, either intermittently or continually is a well-tried and successful method of elimination[2,27] and is often used in combination with hyperchlorination. Disadvantages of this method include: cost, risk of scalding and incomplete bacterial killing because of the temperature gradient between the calorifier and under-used peripheral outlets. Methods to circumvent this problem

Table II. *Some methods for control of legionellae in hot-water systems*

1. Physical		
(a) Heat	—Raising calorifier temperatures	
	—Instantaneous steam heat systems	
	—Self-regulating trace heat systems	
(b) UV irradiation		
(c) Sonication		
(d) Draining and flushing pipes with compressed air		
2. Chemical		
(a) To prevent scale formation		
(b) To maintain sediment in suspension		
(c) Biocides	—Sodium hypochlorite	
	—Ozone	
(d) Charcoal filters		
3. Good plumbing practice		
(a) Maintenance		
(b) Pumps and calorifiers in series not parallel		
(c) Elimination of dead-space in calorifiers		
(d) Removal of dead-legs		
(e) Regular flushing of outlets.		
(f) Use of Water Research Centre recommended components		

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include generation of hot-water at outlets via instantaneous steam heat[2] and the use of self-regulating trace heat elements that can maintain water in peripheral outlets at 50°C.[41]

Legionellae are readily killed by UV irradiation.[42] We have been able to demonstrate that fitting a UV irradiation device to a shower was able to maintain it free from legionellae following initial hyperchlorination (unpublished observation). Interestingly although this device did not eliminate other bacteria such as pseudomonads, no amoebae were found. On cessation of UV treatment amoebae and legionellae reappeared concurrently.

Chemical methods

These have been discussed extensively elsewhere.[6] Problems of their use include: inability to kill bacteria in biofilms, corrosion of pipework and generation of organic chlorine compounds. Ozonization of water presents another method of disinfection, and a comparison of various methods for elimination of legionellae has been produced.[2] Finally, charcoal filters have been shown to be of limited benefit in maintaining dental chair water systems free of legionellae.[43]

Good plumbing practice

Design and maintenance of hot-water systems are of prime importance in controlling legionellae in hospitals.[40] For example, elimination of dead spaces by avoiding the use of pumps and calorifiers in parallel, the use of devices to ensure complete water circulation in calorifiers and the elimination of dead legs are all of great importance. We have demonstrated that the fitting of automatic drain valves was ineffective in maintaining a reduction in the number of legionellae in shower water[21] but that regular flushing of the system was highly effective.

In conclusion, our understanding of the ecology, microbiology and pathogenesis of legionella infections has increased tremendously over the last decade. However, legionellae are still an important cause of hospital- and community-acquired pneumonia. For hospital-acquired Legionnaires' disease to develop, a series of events is required. Firstly, there must be a way for legionellae to gain access to the hospital's water system and it is likely that low numbers of legionellae gain access through the mains water supply. Next there must be an amplification step. Legionellae are thermotolerant, being able to survive at relatively high temperatures and they are able to multiply in lukewarm water both in sessile and planktonic forms. There must also be a way for the bacteria to gain access to their host's alveoli. This is usually by the aerosols that are readily produced in hospitals, but may occasionally be due to aspiration. Finally the aerosolized bacteria must lodge in a susceptible host. To prevent nosocomial Legionnaires' disease one or more of these four stages must be prevented. Efforts to decrease or prevent the amplification step are the most likely to be fruitful.

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Diagnostic methods Current best practices and guidelines for isolation of bacteria and fungi in infective endocarditis

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Advances in technology, especially echocardiography, have provided new tools for the better recognition and management of infective endocarditis (IE). Traditional blood cultures remain essential, however, not only for determining the etiological diagnosis of IE but also for antimicrobial susceptibility tests to guide therapy.

Within a decade of Ettlinger's first "hemoculture" using venous blood taken from a patient in Paris [1], use of blood cultures to diagnose bacterial endocarditis had become firmly established [2]. Before then, clinicians relied upon physical examination to detect the presence of "classic findings" such as subungual hemorrhages, Osler's nodes, or Roth spots to make a diagnosis of IE. These findings are not specific for IE, however, and in the absence of positive blood cultures they have limited diagnostic value today. Furthermore, these "classic findings" are seen less frequently in modern clinical practice than formerly, presumably because many cases are now diagnosed before the disease has been present long enough to give rise to these late findings [3]. Moreover, the incidence of IE due to viridans streptococci, one of the principal etiologic agents of subacute disease in which these findings would be more likely, is decreasing, while the incidence of disease due to organisms associated with acute infective endocarditis, especially *Staphylococcus aureus*, is increasing [4].

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Pathophysiology

Infective endocarditis is an endovascular infection which typically gives rise to a continuous bacteremia originating from the source of the infection, usually a vegetation on a heart valve. Early studies confirmed that the bacteremia of endocarditis is usually continuous, with ongoing shedding of organisms from the vegetation into the bloodstream and simultaneous clearance of bacteria by the reticuloendothelial system in the liver, spleen, and bone marrow [5]. Detection should therefore be relatively straightforward, and in practice the majority of blood cultures obtained from patients with IE are positive for the etiologic agent [6]. One early study demonstrated that two blood culture sets diagnosed 78% of cases, and that four blood culture sets diagnosed over 90% of patients with IE [7]. In a later series of 206 cases of bacterial endocarditis with positive cultures reported by Werner et al. [8], 95% of 789 blood cultures were positive for the etiologic agent. If all blood culture sets obtained were positive, especially if each set was drawn at least thirty minutes apart, the patient most likely had a source of infection that caused a continuous bacteremia, such as IE, rather than an intermittent bacteremia, such as an occult abscess.

Even in untreated patients with typical bacterial endocarditis the blood culture positivity rate is not 100%, for several reasons. Organisms in vegetations can be located on the surface or just beneath the outer fibrin layers of the vegetation, in close proximity to the bloodstream, or buried deep within the vegetation, therefore sequestered away from circulating blood [9]. Cessation of bacteremia and ''surface sterilization'' of the vegetation when the colonies are deeply buried has been described [10]. The inflammatory damage caused by the organisms continues beneath the surface, however, and valve ring abscesses and ruptured chordae are among the ensuing complications. This phenomenon explains why infected vegetations on valves removed at surgery should be ground up (for bacteria) or minced (for fungi) before culture—to minimize the occurrence of false-negative cultures.

The bacteremia in subacute IE is typically ''low-grade,'' with only 1 to 10 colony-forming units (CFU) per milliliter of venous blood [8]; therefore, an adequate volume of blood is needed for culture in order to maximize the chance of recovering organisms in the culture. Many years ago it was hypothesized that the recovery of microorganisms might be enhanced if arterial blood samples obtained before the blood had passed through the reticuloendothelial system of the liver, spleen, and marrow were cultured. Arterial blood does carry a somewhat higher count of bacteria [5], but it has now been concluded that there is no significant diagnostic benefit gained from using arterial versus venous blood for culture [5,11].

Role of microbiologic findings in diagnostic criteria

Blood culture results provide much useful diagnostic information that has immediate importance for management decisions. A positive result can

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provide the identity of the presumed etiologic agent, and the identity of the organism may point to the original source of bacteremia. For instance, isolation of viridans streptococci imply an oral or dental source whereas *Streptococcus bovis* points to possible colonic pathology such as a benign polyp or colonic malignancy as the source of bacteremia. This information is valuable because preventive interventions may decrease the risk of recurrent bacteremia and recurrent endocarditis. Endocarditis is itself a risk factor for recurrent endocarditis [12]. In addition, information that suggests a continuous bacteremia (i.e., 3 of 3 positive blood cultures collected at least 1 hour apart) provides clinical value. Earlier criteria for the diagnosis of IE (e.g., the Beth Israel criteria) [13] incorporated the finding of persistently positive blood cultures as a criterion for the diagnosis, but did not give any additional weight to the recovery of organisms that are typically associated with this disease. In 1994, the Duke Endocarditis Service published new diagnostic criteria which increased the sensitivity as well as specificity of clinical diagnosis of endocarditis [14]. These Duke criteria have been validated by many subsequent studies as being more sensitive than other published criteria and have also been shown to maintain a high negative predictive value [15].

The Duke criteria also give diagnostic weight to the recovery of certain species of microorganisms from the blood that are typically associated with IE. Identification of the organism may provide clues as to the origin of the infection. For example, recovery of *Enterococcus faecalis* from the blood of a patient in the community setting without an obvious primary focus of infection (such as a urinary tract infection) indicates a high prior probability of IE. If blood cultures had not been obtained, or were obtained and were negative, this valuable information would not be available to the clinician. More recently, proposed modifications to the Duke criteria have included bacteremia due to *S. aureus* regardless of whether the infection is nosocomially acquired or a removable source of infection is present [16]. A recent study reported that this organism is now the single most common cause of IE seen by the Duke Endocarditis Service [4].

A review of the individual value of each component of the Duke criteria for the diagnosis of IE shows that the major microbiological criteria had the highest relative importance [17]. In a study of the 67 pathologically proven cases, 53% of the ''definite'' cases became ''possible'' or ''rejected'' when the major microbiological criteria were subtracted.

The recovery of microorganisms from the blood in a patient with suspected IE does not, however, prove the diagnosis. The overall positivity rate for clinical blood cultures in the United States is ~10%, but 30% to 40% of these positives represent false-positive isolates. The most common cause of false-positive cultures is contamination arising from skin flora due to faulty skin preparation before venepuncture or from cultures that are obtained through contaminated indwelling vascular catheters. This issue becomes especially important when evaluating patients with suspected prosthetic valve

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endocarditis; here the leading etiologic species are coagulase-negative staphylococci, which are also the most frequent causes of false-positive blood cultures arising from skin flora. It is imperative, therefore, that skin is adequately prepared prior to obtaining blood cultures in these patients. Furthermore, blood for culture should not be drawn through indwelling vascular catheters, which may be contaminated. If false-positives are to be averted, the importance of meticulous techniques for obtaining peripheral venous blood cultures cannot be overemphasized.

The information obtained from the identification and susceptibility results of organisms isolated from the blood of patients with IE greatly aids the management of these patients. The identification of the organism can provide initial guidance with regard to appropriate antibiotic therapy, duration of therapy, and potential for outpatient therapy. The susceptibility profile of the isolate provides important information that assists the clinician in deciding the optimal therapeutic regimen, including the choice of single-agent versus combination therapy. For example, endocarditis caused by penicillin-susceptible (MIC ≤ 0.1 μg/mL) viridans streptococci may be treated with 2 weeks of combined penicillin and gentamicin while relatively resistant viridans strains (MIC $> 0.1 \leq 0.5$ μg/mL) require an additional 2 weeks of penicillin following the initial 2 weeks of combination therapy [18]. This information might also allow a physician to change a patient from an inappropriate antibiotic (chosen empirically for initial treatment) to an appropriate antibiotic. In a prospective epidemiological study of the clinical significance of positive blood cultures [19] such "corrections" of antibiotic therapy on the basis of susceptibility results were shown to decrease mortality.

Finally, identification of the etiologic species from blood cultures can provide important prognostic information about the potential for curative therapy without the need for adjunctive surgery. Patients infected with certain microorganisms are more likely to require surgical intervention. Prosthetic valve endocarditis due to gram-negative bacilli (other than the HACEK group), *S. aureus*, and yeasts are all associated with a high likelihood that surgery will be required [20]. In addition, native valve disease due to filamentous fungi is also a strong indication for surgical intervention [20].

Best practices for diagnosis of infective endocarditis due to bacteria

Because blood culture results are so critical to the diagnosis and management of patients with IE it is imperative that optimal procedures are utilized. The following guidelines define the authors' opinion of currently accepted "best practices" for evaluating patients with possible IE. The authors believe that use of these recommended practices will yield positive blood cultures in over 90% of cases of bacterial endocarditis and will optimize the yield of yeasts and filamentous fungi. If all cultures remain negative after application of these guidelines, the clinician must either conclude that the patient has blood culture-negative IE or consider an alternative diagnosis.

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Optimal antiseptic skin preparation

As mentioned previously, it is vitally important that adequate skin antisepsis be performed prior to venepuncture for drawing blood cultures to minimize contamination of the cultures with skin flora, which may confuse the diagnostic picture. To ensure proper collection of blood for culture it is helpful to have all of the necessary components, including skin preparation materials, venepuncture device, and bottles of culture media pre-packaged in a kit or procedural tray.

The recommended antiseptic skin preparation is liberal swabbing with 70% isopropyl alcohol applied in widening circles over a reasonably wide area of skin around the intended site for venepuncture and allowed to dry, followed by similar application of an iodophor or tincture of iodine over the same area [21]. It is important that adequate contact time with the skin be allowed for both the alcohol and the subsequent iodophor or tincture of iodine preparations, which should be allowed to dry completely by evaporation to maximize their antiseptic effect [21].

Whenever practicable, blood cultures should be obtained by way of fresh venepunctures, not through indwelling intravascular devices, as detailed in the guidelines provided by the American College of Physicians [22]. Blood cultures drawn through catheters are more likely to be contaminated [23].

Optimal timing of blood cultures

It has often been stated that the optimal time to obtain blood cultures on an individual patient is during the hour prior to the onset of chills or fever spikes [24]. Although this makes sense in theory, based on the presumed pathophysiology of bacteremia, it would clearly be difficult or impossible to accomplish from a practical standpoint. Who can predict within one hour when the next fever spike will occur? Clinical data that have been referenced to support this ''recommendation'' are unconvincing [25,26]. The continuous nature of most bacteremias associated with IE renders timing less important. The authors do not therefore recommend attempting to link the timing of blood culture draws to the timing of fever spikes.

In the patients who present with the syndrome of acute endocarditis caused by highly virulent pathogens such as *S. aureus*, any delay in treatment to obtain cultures spaced over more than 1 hour to ascertain whether a continuous bacteremia is present is neither warranted nor necessary. In this critical situation, two to three blood cultures should be obtained from separate venepunctures within 5 minutes of each other, prior to prompt administration of empiric antibiotic therapy.

If, on the other hand, the patient presents with the syndrome of subacute endocarditis, there is usually no need to initiate immediate empiric antibiotic therapy. For these infections it is more important to first attempt to establish a specific microbiological diagnosis. In this instance several blood culture sets should be obtained over a period of several hours to document

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continuous bacteremia, which may provide additional clinical information, especially if the echocardiogram is negative or equivocal.

Optimal number of blood cultures

What is the optimal number of separate blood cultures? The answer is debatable, but clearly one blood culture is inadequate [22]. A single blood culture, while better than none, suffers from at least three deficiencies: it does not maximize the chance of isolating the etiologic organism, it cannot demonstrate the presence of a continuous bacteremia, and it cannot distinguish between contamination and true bacteremia.

First, multiple cultures provide a higher volume of blood for culture; this is the single most important factor for successful recovery of microorganisms from the bloodstream of patients (Fig. 1).

Second, the rate of positivity increases (up to a point) as more cultures are obtained. For patients with culture-positive bactermia, it has been shown that two blood culture sets using adequate volumes of blood will isolate the etiologic organism in approximately 99% of cases, and that three sets will detect over 99% (Fig. 2). It is important to understand that these figures do not imply that only 1% of all patients with IE will have negative blood cultures. They also do not apply, for example, to patients receiving empirical antibiotics, cases of IE caused by yeasts or molds, or difficult-to-culture organisms such as *Bartonella* or *Coxiella*.

Third, multiple blood cultures are required to indicate that a bacteremia is continuous, as is often the case for intravascular infections such as IE. There is no standardized consensus definition of "continuous bacteremia." The

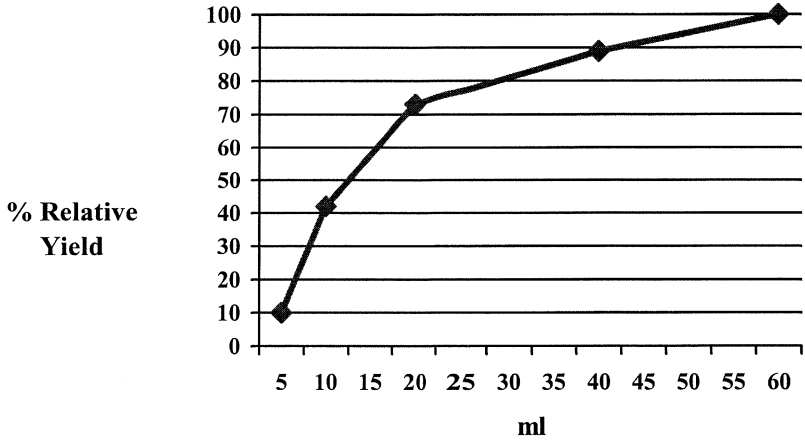

Fig. 1. Effect of volume on the relative yield of recovery of microorganisms from blood cultures. (*Data from* Refs. [44–46].)

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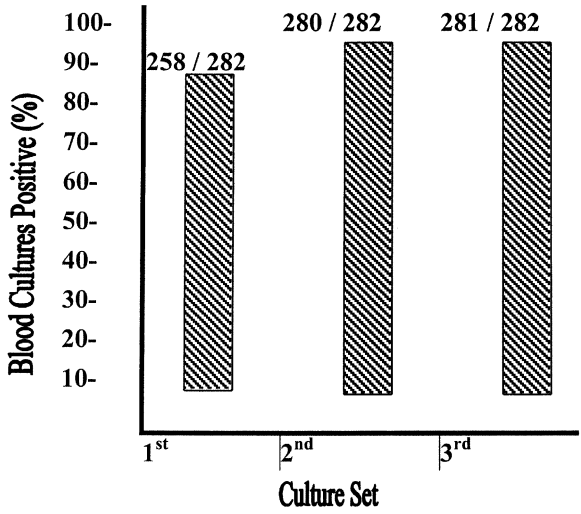

Fig. 2. Cumulative blood culture positivity rate from 282 patients with confirmed bacteremia or fungemia. (*Adapted from* Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 1983; 5(1):35–53, with permission.)

Duke criteria [14] suggest the following definition: two positive blood cultures for the same organism drawn 12 or more hours apart, or positive cultures for the same organism from all of three or a majority of four or more separate cultures, with the first and last being drawn at least one hour apart.

Finally, multiple blood cultures are also important to help determine whether a positive blood culture is due to contamination. It has been demonstrated that positives due to skin contaminants usually result in a single-positive blood culture set when multiple sets were obtained (Table 1).

In summary, because IE is associated with high mortality, and given the high value of recovery of the etiologic agent from blood, the authors strongly recommend that multiple separate blood cultures be obtained from all patients presenting with possible IE.

There is one important caveat. If the initial cultures from a patient suspected to have endocarditis are negative, it is intuitively attractive to consider ordering additional cultures. This is appropriate if the prior probability of IE remains high, but may well be inappropriate if the prior probability of IE is low or falling as the clinical evaluation progresses. Consistent with Bayesian theory, the lower the pre-test probability of IE, the higher the probability that any positive blood culture will be a false positive (which is fairly common in any case, due to contamination with skin flora). Clinicians and laboratorians should appreciate that in certain situations obtaining further routine blood cultures may be counter-productive, serving only to increase

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Table 1
Clinical significance of *Staphylococcus epidermidis* isolates from blood cultures

# of sets positive	# of sets obtained	% Significant	% Contaminate	% Indeterminate
1	1	0	97	3
1	2	2	95	3
2	2	60	3	37
1	3	0	100	0
2	3	75	0	25
3	3	100	0	0

Adapted from Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, Reller LB. The clinical significance of positive blood cultures in the 1990s. A prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of becteremia and fungemia in adults. Clin Infect Dis 1997;24:584–602, with permission.

rather than to decrease diagnostic uncertainty. In these situations, alternative diagnostic strategies should be undertaken [27].

Optimal volume of blood for culture

The authors recommend that 20 mL of blood be obtained for each two-bottle blood culture set. It is important to state that a ''set'' of blood cultures represents an independent venepuncture; regardless of how many bottles are inoculated from a single venepuncture, blood from a single venepuncture is defined as one set. Obtaining three blood culture sets therefore requires three separate venepunctures with 20 mL of blood obtained per set, for a total of 60 mL. These recommendations apply to adult patients only. In pediatric patients, 1 to 2 mL of blood per culture should be drawn for neonates, 2 to 3 mL for infants aged 1 month to 2 years, 3 to 5 mL for older children, and 10 to 20 mL for adolescents [28].

Optimal choice of blood culture media

There are several commercially available blood culture systems, each providing a choice of various blood culture media. No single blood culture system nor any single culture medium can detect all types of microorganisms that might be present in the bloodstream of patients with suspected IE. It is generally recommended that more than one type of blood culture medium be inoculated to increase the chance of recovery of certain microorganisms. Two-bottle blood culture sets often consist of one aerobic medium and one anaerobic medium. Since the incidence of anaerobic bacteremia is decreasing [29] and IE due to anaerobes is uncommon (<2%), it is not essential to obtain an anaerobic culture as part of every blood culture set that is drawn. Anaerobic cultures do have certain advantages beyond detection of strict anaerobes, however; they may enhance the recovery of certain facultative anaerobes such

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as streptococci, especially nutritionally variant streptococci (NVS). The authors therefore recommend that an anaerobic culture be included, although not with every blood culture set. If two separate blood cultures are drawn for evaluation of a patient with suspected acute IE, then one anaerobic bottle should be included in the total of four bottles inoculated from the two samples. If three separate blood cultures are obtained in the evaluation of a patient with suspected subacute infective endocarditis, then one or two anaerobic bottles should be included among the total of six bottles inoculated from the three samples. This recommendation places a premium on the recovery of the routine aerobic microorganisms that most commonly cause IE, but also optimizes the chances of recovering streptococci and other facultative anaerobes, and has the capacity to detect those rare cases of IE caused by strict anaerobes.

Frequently, patients who are undergoing investigation for possible IE have already been placed on empirical antibiotic therapy. This is the single most common reason for "culture-negative" IE [30]. To overcome the potential inhibitory activity of those antibiotics on bacterial growth it is desirable to utilize a blood culture medium that has been designed to counteract or neutralize the inhibitory effect of antibiotics. Examples of commercially available products that could be used in these situations are a BACTEC resin containing medium (BD Diagnostic Systems, Sparks, MD), or a BacT/Alert FA medium (bioMerieux, Durham, NC). The BACTEC medium contains resins that have been shown to neutralize many antibiotics that might be present in blood [31]. In addition, the resin granules provide a large solid surface area (like catheters and other foreign bodies), which may enhance the growth of certain species such as staphylococci and yeasts, thus optimizing recovery. The BacT/Alert FA medium contains activated charcoal to counter the potential inhibitory activity of antibiotics. Even these specialized media cannot always recover microorganisms from the blood of patients who have already received antibiotics, however; blood cultures should therefore be obtained from patients prior to the institution of empiric antibiotics whenever possible.

In earlier studies it was reported that another potential cause of blood culture-negative IE was the presence of the so-called nutritionally variant streptococci (NVS). These streptococcal species, since named *Abiotrophia defectiva*, *Abiotrophia adiacens*, and *Abiotrophia elegans*, require supplemental vitamin B6 or cysteine for growth. Today these do not need to be specially added to blood culture media because most commercially available media are already adequately supplemented, and in any case the high volume of human blood added to the culture media may provide these growth factors. If subcultures from blood culture bottles showing chains of gram-positive cocci do not grow on sheep blood agar with a tryptic soy base, presence of NVS should be suspected [32]. Competent clinical microbiology laboratories will perform additional tests to identify these organisms, which account for an estimated 5% to 6% of viridans streptococcal endocarditis cases [33].

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Optimal length of incubation for blood cultures

Modern commercially available automated blood culture systems demonstrate recovery of most clinically important pathogens from blood within 5 days, which is now the recommended duration of incubation for routine blood cultures. Previously it was argued that at least 2 to 3 weeks of incubation were required [34] for optimal recovery of the more fastidious organisms that may cause IE. The data supporting this recommendation were based on older manual blood culture systems and media and do not reflect the situation today. Even improvements in the media itself, while keeping to a manual system, have shown that the vast majority of fastidious organisms such as the HACEK group, Brucella spp, *Francisella tularensis*, and NVS can usually be recovered within 5 days [35,36]. With automated, instrumented blood culture systems some of these organisms may require longer incubation to reach the growth threshold required to trigger a positive signal. If the organisms were present in the original inoculum of blood, they will still be present in the bottles at 5 days, and therefore should have either multiplied enough by then to trigger a positive result or be detected by appropriate subcultures taken at 5 days. It is probably a better strategy to subculture negative bottles from patients with a significant clinical likelihood of IE onto enriched solid media after 5 days rather than select these bottles for extended incubation for 2 to 3 weeks. This approach, although previously recommended, has not been systematically evaluated using the improved automated blood culture systems available today.

Special considerations for the diagnosis of fungal infective endocarditis

Fungal endocarditis, once a rarity, is now being reported more often [37]. This increase may be attributed to "medical progress," because many of the risk factors for fungal endocarditis are associated with technologic advances such as long-term intravenous catheterization, prolonged use of antibiotics, dialysis, hyperalimentation, immunosuppression, and cardiovascular surgery, including implantation of prosthetic devices such as heart valves and pacemakers [37]. In addition, narcotic addiction is a major risk factor for the development of fungal endocarditis [38]. Fungal IE includes cases caused by yeasts and molds (filamentous fungi), with yeasts being more common.

Diagnosis of fungal IE presents a challenge for clinicians. When IE is suspected, the possibility of fungal etiology is seldom seriously considered until the routine blood culture results have been reported as negative. In a recent worldwide review of 270 cases of fungal endocarditis, this diagnosis was included in the differential diagnosis in only 48 cases (18%) at the time of presentation [38]. It is reasonable to assume that delayed diagnosis may contribute to the higher mortality rate associated with fungal endocarditis compared to subacute IE due to routine bacterial pathogens. The leading isolates causing fungal endocarditis are *Candida albicans*, non-albicans

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Candida spp, *Torulopsis glabrata*, and *Aspergillus* spp [37,38]. The following comments refer to special considerations for the diagnostic laboratory when this form of IE is suspected.

Blood cultures

Many of the same technical issues for diagnosis of bacterial endocarditis apply to the diagnosis of fungal endocarditis. Most routine manual and automated blood culture system media are able to support the growth of yeasts such as *Candida* spp. If routine blood cultures are negative but the diagnosis of IE remains likely, it may be useful to specifically request a "fungal blood culture" so media that optimally support the growth of yeasts are chosen. Overall, if optimal methods are utilized, the percentage of positive blood cultures from cases of endocarditis caused by *Candida* spp is 83% to 95% [39].

The best system for the recovery of filamentous fungi such as *Aspergillus* is the Isolator (Wampole Laboratories, Cranbury, NJ) lysis centrifugation tube. Even with this technique, the rate of recovery of filamentous fungi from the blood of patients with this form of IE is quite low, <30% of cases. It should be noted that the Isolator is also the best system for the recovery of some uncommon, fastidious organisms such as *Bartonella* that occasionally cause endocarditis.

Additional diagnostic tests

In a recent review of fungal endocarditis, it was shown that the overall sensitivity of pre-operative blood cultures for the diagnosis was only 45% [38]. The remainder of cases confirmed with a mycologic diagnosis were from cultures of the cardiac vegetation or from histologic examination of either the excised valve or embolus from a large artery. Emboli to large vessels are a characteristic feature of fungal endocarditis because the valvular vegetations with this disease are typically large and friable [40]. It is therefore important that any embolus removed surgically be cultured for fungi as well as bacteria, and also examined histologically for the presence of microorganisms. In addition, other sites that should be cultured as part of the work-up of a patient with suspected fungal endocarditis include any suspicious skin or vitreal lesions. A recent case report described the use of a direct DNA probe such as AccuProbe (GenProbe Inc., San Diego, CA) for the rapid diagnosis of *Histoplasma capsulatum* in an excised heart valve [40]. Because the sensitivity of methods using direct probes have not been shown to be equivalent or better than culture, however, they cannot yet replace culture-based methods for the diagnosis of this disease [41–43].

Serological testing for fungi can be useful in the diagnosis of deep-seated histoplasmosis or cryptococcosis [29]. Serology is seldom helpful for other species; to date there are inadequate data to justify the inclusion of serologic testing for the presence of antigens or antibodies to *Candida* or *Aspergillus*

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as part of the routine diagnostic work-up of a patient with suspected fungal endocarditis.

Summary of best practice guidelines for laboratory diagnosis of bacterial and fungal endocarditis

- Obtain blood cultures before starting antimicrobial therapy whenever possible.
- Always use strict aseptic technique and optimal skin preparation when collecting blood for culture.
- For suspected cases of acute IE obtain at least two, preferably three sets of blood cultures rapidly within 5 to 10 minutes of each other before starting empiric antibiotic therapy.
- For suspected cases of subacute IE draw three separate blood cultures, spaced 30 minutes to 1 hour apart. If these remain negative at 24 hours, draw two further separate cultures.
- The volume of each blood sample drawn should be 20 mL for adults; for infants and other children, draw the amount of blood outlined earlier in the chapter.
- Each separate blood culture should be divided for inoculation into two bottles.
- If all blood cultures remain negative at 5 days but IE remains likely on clinical grounds, subculture bottles onto chocolate agar plates if the bottles are not held beyond 5 days.
- Always culture any embolus or vegetation that has been surgically removed from a patient with suspected IE for both bacteria and fungi.
- If all of the above are negative, consider an alternative, mimicking diagnosis (e.g., antiphospholipid syndrome with systemic lupus erythematosus [Libman-Sacks]).

Conclusion

As the etiological character of IE changes, the microbiological tools used to confirm the diagnosis have also evolved. Here the authors have reviewed the current methods for optimal laboratory diagnosis of bacterial and fungal endocarditis using traditional growth-based technologies and offered good practice guidelines and recommendations. Newer techniques will be required to improve sensitivity of detection for known organisms and to identify emerging or as-yet unknown pathogens (see article by Lepidi et al. in this issue).

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Investigation of healthcare-acquired infections associated with *Pseudomonas aeruginosa* biofilms in taps in neonatal units in Northern Ireland

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SUMMARY

Background: In December 2011 and early 2012 four neonates died from *Pseudomonas aeruginosa* bacteraemia in hospitals in Northern Ireland.
Aim: To assess whether *P. aeruginosa* was associated with the neonatal unit taps and whether waterborne isolates were consistent with patient isolates.
Methods: Thirty taps and eight flow straighteners from the relevant hospitals were categorized and dismantled into 494 components and assessed for aerobic colony and *P. aeruginosa* counts using non-selective and selective agars. *P. aeruginosa* isolates were typed by variable number tandem repeat (VNTR) analysis. Selected tap components were subjected to epifluorescence and scanning electron microscopy to visualize biofilm.
Findings: The highest *P. aeruginosa* counts were from the flow straighteners, metal support collars and the tap bodies surrounding these two components. Complex flow straighteners had a significantly higher *P. aeruginosa* count than other types of flow straighteners ($P < 0.05$). Highest aerobic colony counts were associated with integrated mixers and solenoids ($P < 0.05$), but there was not a strong correlation ($r = 0.33$) between the aerobic colony counts and *P. aeruginosa* counts. Representative *P. aeruginosa* tap isolates from two hospital neonatal units had VNTR profiles consistent with strains from the tap water and infected neonates.
Conclusion: *P. aeruginosa* was predominantly found in biofilms in flow straighteners and associated components in the tap outlets and was a possible source of the infections observed. Healthcare providers should be aware that water outlets can be a source of *P. aeruginosa* contamination and should take steps to reduce such contamination, monitor it and have strategies to minimize risk to susceptible patients.
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Introduction

Water supplied via plumbing systems is not sterile and, as a consequence, plumbing materials can become contaminated with biofilm, which provides a niche for opportunistic pathogens.[1,2] Previous studies have demonstrated that pathogens

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including *Legionella pneumophila*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* may be present in biofilms in water systems and result in healthcare-associated infections.[3–10]

P. aeruginosa is commonly found in a wide range of moist, nutrient-limited environments and can colonize hospital and domestic water taps, sinks, drains, toilets and showers.[7,9,10] A number of plumbing materials, such as flexible hoses, have been identified as susceptible to such colonization.[11–13]

A number of colonizations and infections of neonates by *P. aeruginosa* occurred in neonatal critical care units of Northern Ireland hospitals in late 2011 and early 2012 with four patient deaths (http://www.rqia.org.uk/publications/rqia_reviews.cfm). At that time, swabs of the ends of taps and water samples were taken from taps within the units and were positive for *P. aeruginosa*. The water sample isolates were typed using variable number tandem repeat (VNTR) analysis, which indicated some commonality with the strains isolated from the babies (unpublished results). This study set out to determine whether *P. aeruginosa* was associated with particular tap components.

Following infection control measures taken during the incident, all taps and associated pipework were removed (e.g. thermostatic mixer valves, copper tubing, flexible hoses, plastic connectors and flow straighteners) from every neonatal unit in Northern Ireland, and replaced with new taps with reduced complexity (http://www.dhsspsni.gov.uk/hss-md-4-2012.pdf). This study set out to assess a representative number of these tap assemblies using microbiological culture and microscopy analysis for the presence of *P. aeruginosa* in biofilms that may have been present on the plumbing materials associated with the taps.

Methods

Transport and dismantling of taps

Several tap assemblies were chosen to represent the type and style of tap from neonatal units of seven hospitals in Northern Ireland and were removed and transported to Public Health England, Porton Down, Salisbury, where possible within 48 h of removal from the neonatal unit. The containers were held at 4 ± 2 °C prior to the taps being aseptically dismantled and divided into duplicate component parts for microbiology and microscopy, given a unique reference number, photographed and a description recorded. This dismantling process was carried out using manual hand tools to reduce any frictional heat build-up that may have reduced microbial viability. Both sensor (taps with an infra-red sensor that activated water flow) and non-sensor-operated taps shared common component parts in specific locations and for analysis components were categorized as follows: tap body, connectors, isolation valve, integrated thermostatic mixer valve and solenoid, mixer, flow straightener and solenoid; any water found in the transport bags was collected and analysed. Flow straighteners are the terminal component in most taps that produce a uniform flow of water without excessive splashing. The flow straightener components were further categorized into four subcomponents: the centrepiece, collar, filter and washer. Due to the highly varied designs of flow straighteners examined, each was categorized and details of the subjective complexity and material were also included in the analysis (Table I). Pipework used to connect the tap and solenoid valve was subdivided into copper or plastic flexible hose (Table I).

Microbiological analysis

Samples for microbiological assessment were placed in 10 mL of maximum recovery diluent together with 10 sterile glass beads (3 mm diameter, VWR International, Lutterworth, UK). The surface of each component was scraped with a sterile plastic loop (Sterilin, Newport, UK) and the suspension was then vortex-mixed (10 s) to remove biofilm and to suspend the micro-organisms. Each sample was then spiral-plated (Don Whitley, Shipley, UK) on to Plate Count Agar (Oxoid, Basingstoke, UK) and *Pseudomonas* selective agar (PCN, Oxoid, UK) for determination of aerobic colony count (ACC) and *P. aeruginosa* count respectively, and incubated at 30 °C (Plate Count Agar) or 37 °C (PCN) for 48 h.

Microscopy analysis for biofilms

Epifluorescent microscopy was carried out by staining each surface with propidium iodide (1 mg/mL) (VWR) for 1 min, rinsing off excess stain with sterile water and viewing each component part using a Nikon Labphot microscope with epifluorescence (50× water immersion lens).[14,15] The surface of each component was visualized and biofilm images recorded (Imagic Imaging Systems, Germany).

Where tap components were found to be culture-positive for *P. aeruginosa*, a selected number of these biofilm samples were also sent for scanning electron microscopy (SEM) using a Philips XL30 FEG scanning electron microscope.

Strain typing

P. aeruginosa isolates recovered from plumbing materials of two hospitals (up to a maximum of five isolates per tap) were typed using VNTR.[16]

Statistical analysis

Statistical analysis was performed using Stata 12.0 (2011; StataCorp, College Station, TX, USA). Colony counts below the detection limit were assigned an arbitrary value of the midpoint between zero and the detection limit, i.e. 10 colony-forming units (cfu) for *P. aeruginosa* and 100 cfu for aerobic colony count to allow inclusion in the statistical analysis. Tap components were categorized according to location within tap; type of tap (whether sensor or non-sensor); simple or complex flow straighteners; plastic or metal flow straighteners; flow straightener subcomponents; and the type of pipe between the solenoid and tap (copper or flexible hose) (Table I).

Three sets of statistical models were built: a logistic regression to indicate the difference in odds of colonization with *P. aeruginosa* between sensor and non-sensor taps; various single variable mixed-effects regression models to examine the unadjusted association between *P. aeruginosa* count and the various tap component categories; and a multiple linear mixed-effects regression to assess the adjusted significance of the differences in cfu counts between tap type and component categories and to assess any interaction between tap component and tap type. In the mixed-effects framework tap ID and

Table I
Summary statistics for tap component attributes indicating number in the sample and median *Pseudomonas aeruginosa* count for components with counts above detection limit

Sample location	All components		Components with *P. aeruginosa* cfu above detection limit		
	N	%	*N*	%	Median *Pseudomonas* cfu (IQR)
Tap component location					
Tap body	126	26	18	26	6340 (22,580)
Connectors	31	6	0	0	–
Isolation valve	30	6	0	0	–
Integrated mixer and solenoid	38	8	1	1	60 (0)
Mixer	98	20	2	3	20 (0)
Flow straightener	97	20	41	60	52,033 (816,820)
Solenoid	54	11	5	7	520 (23,380)
Water in tap sample bag	8	2	1	1	700,000 (0)
Tap type					
Sensor tap	395	80	63	90	23,400 (579,220)
Non-sensor tap	97	20	7	10	1440 (15,540)
Flow straightener components, sensor taps only					
Centrepiece	25	31	13	32	180,000 (898,200)
Collar	26	33	12	29	71,970 (1,308,020)
Filter	12	15	8	20	39,431 (307,280)
Washer	17	21	8	20	17,802 (43,300)
Simple flow straightener					
No	69	78	37	88	52,033 (895,600)
Yes	20	22	5	12	60,606 (58,806)
Flow straightener category					
A. Neoperl complex	70	72	37	90	52,033 (895,600)
B. Neoperl simple	10	10	4	10	31,203 (59,316)
C. Armitage Shanks	1	1	0	0	–
D. U/K	2	2	0	0	–
E. Plastic and metal filter	4	4	0	0	–
F. Metal Star	10	10	0	0	–
Metal flow straightener					
No	27	77	13	100	180,000 (898,200)
Yes	8	23	0	0	–
Connection between tap and solenoid					
Copper pipe	22	48	1	13	520 (0)
Flexi-hose	24	52	7	88	16,200 (573,720)

IQR, interquartile range.

hospital ID were modelled as random effects, and the tap components were modelled as fixed effects. ACC and *P. aeruginosa* counts follow an approximately log–normal distribution and were transformed to the log scale prior to analysis in the mixed effects regressions. For the logistic regression a tap was considered colonized with *P. aeruginosa* if it had at least one component with a count of *P. aeruginosa* above the detectable limit.

Results

Components for analysis

In all, 30 tap assemblies were received, three of which had a newly replaced flow straightener *in situ* as well as the original flow straightener (packaged separately). Five additional flow straighteners were also received without their associated tap assemblies. A total of 494 individual components or swab samples were generated from the taps and flow straighteners. Sensor taps (all of a single brand) accounted for 23/30 (73%) of taps, and four of the eight additional complex flow straighteners were also from the same brand of sensor taps.

Presence of P. aeruginosa and association with the aerobic colony count

P. aeruginosa was detected on 14% of the components (range of counts from 20 to 2.2×10^7 cfu per component) from different tap assemblies from different neonatal units (Table I). The correlation between the aerobic colony count and the *P. aeruginosa* count (Figure 1) for all 494 components was weak ($r = 0.33$). Many of the components that had a high aerobic colony count did not have detectable *P. aeruginosa*.

Tap type and the presence of P. aeruginosa

Sensor taps (Figure 2) had a significantly higher odds ($P < 0.05$) of having at least one component positive for

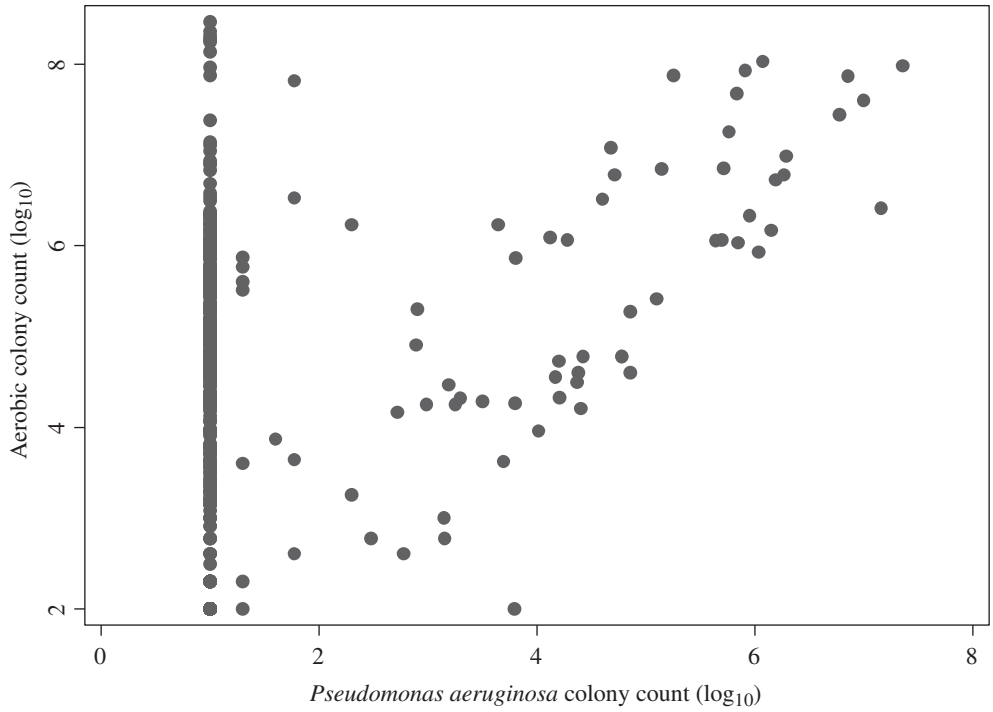

Figure 1. The relationship between *Pseudomonas aeruginosa* count (cfu per component) and aerobic colony count (cfu per component).

P. aeruginosa compared with non-sensor taps (Table II). Furthermore, non-sensor tap components yielded lower median counts of *P. aeruginosa* (1440 cfu) than sensor tap components (23,400 cfu) ($P < 0.05$) (Table I) (for those components with *P. aeruginosa* above the detection limit).

Influence of flow straightener type on P. aeruginosa counts

When both tap type and component type were considered jointly in the multiple regression model of log cfu counts, the flow straightener component in sensor taps was associated with significantly higher *P. aeruginosa* counts than the flow straightener component in non-sensor taps ($P < 0.05$ for interaction term). The expected *P. aeruginosa* count found in the non-sensor tap flow straighteners was 2% of the mean count found in the flow straighteners from sensor taps [95% confidence interval (CI): 0.3–14.1; $P < 0.01$]. Significant differences in cfu counts between tap types were not observed for the other tap components. When comparing *P. aeruginosa* counts within sensor-operated taps, the flow straightener was associated with more than 12 times the mean log cfu of the tap body component (95% CI: 6.07–25.50; adjusted model). This is the highest expected cfu of any component on a sensor tap (other components all have lower expected cfu than the tap body.)

Effect of flow straightener component on P. aeruginosa counts

Although flow straightener components represented 20% of all examined components, they accounted for 60% of components with detectable levels of *P. aeruginosa* (Table I). The single variable regression analyses of the flow straightener complexity, category and material indicated that, on average, complex flow straighteners had a higher expected *P. aeruginosa* count than a simple flow straightener: median of 1549 cfu and 3 cfu ($P < 0.05$), respectively. Complex flow straighteners were only present in sensor taps and so it is unclear whether this effect is related to the flow straightener design *per se*, or to another attribute of a sensor tap. The flow straightener categories C–F (Table I) did not have a significant association with raised *P. aeruginosa* colony counts.

P. aeruginosa colonization of flexible hose and copper pipe

The flexible hoses were associated with approximately three times higher *P. aeruginosa* count than copper pipe (95% CI: 0.91–9.58) but this was not statistically significant ($P = 0.07$), perhaps due to the low numbers of detectable counts and the low component numbers (Table I).

Comparison of component location for the aerobic colony count and P. aeruginosa count

The aerobic colony counts were significantly higher for the integrated mixer and solenoid than other components ($P < 0.05$), and were associated with higher counts than the tap body (95% CI: 18.77–269).

However, the flow straighteners were associated with higher *P. aeruginosa* counts than all other components, i.e. 7.81 times the *P. aeruginosa* count of the tap body (95% CI: 4.11–14.84). Both of these effects are highly statistically significant ($P < 0.01$). There was no significant difference ($P > 0.5$) in the presence of *P. aeruginosa* between the different flow straightener components including washers, filters, collars and centrepieces from sensor taps.

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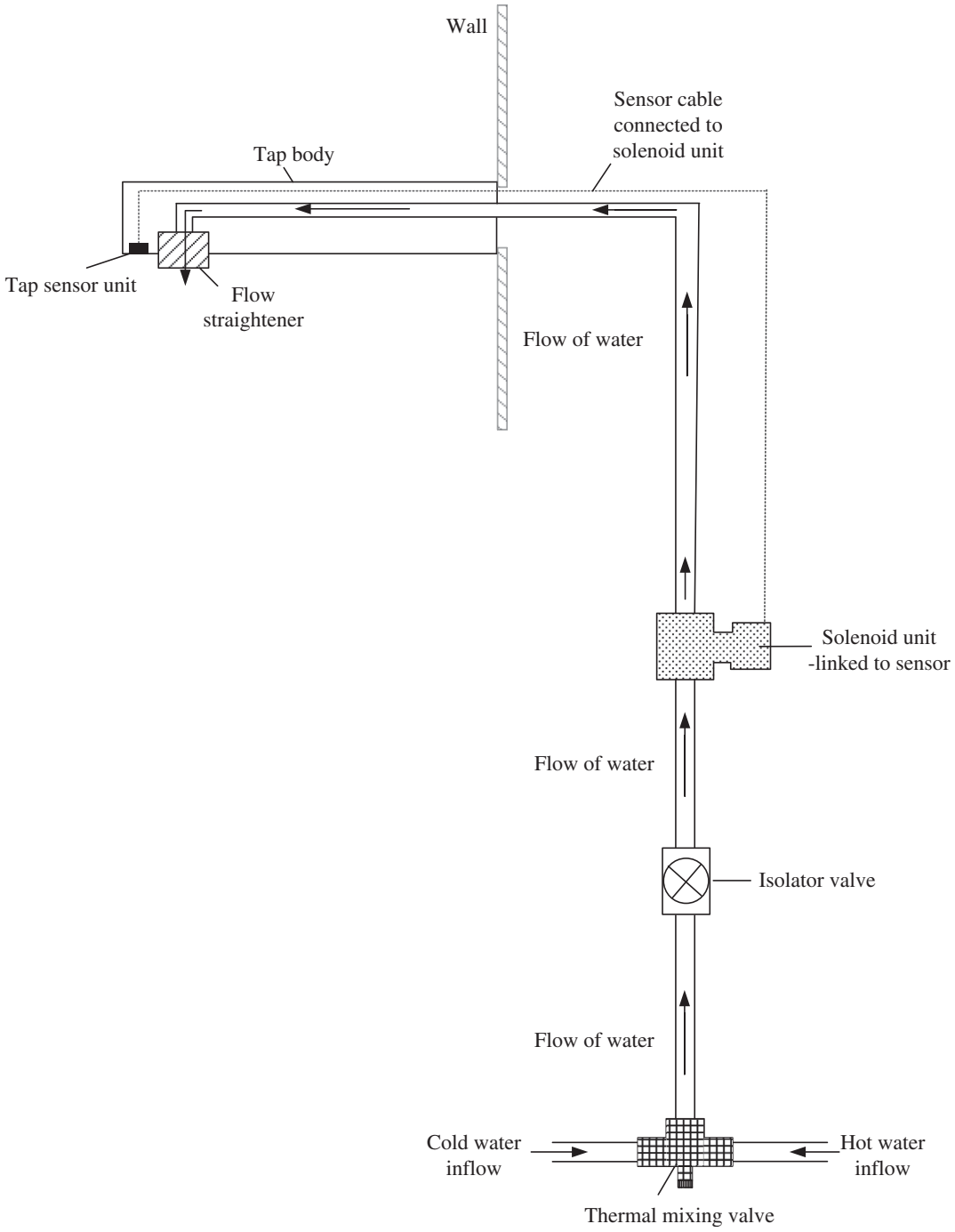

Figure 2. Schematic of water flow through the thermostatic mixer valve, isolator valve, solenoid unit and sensor operated tap.

Table II
Probability that different tap types will be colonized (above detection limits) with *P. aeruginosa* ($N = 35$)

Characteristic	Odds ratio compared to referent (95% CI)	*P*-value	Probability of *P. aeruginosa* above detection limit (95% CI)
Tap type			
Sensor tap	Referent		0.65 (0.44–0.82)
Non-sensor tap	0.11 (0.02–0.61)	0.012	0.17 (0.04–0.48)

CI, confidence interval.

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Results from the single variable regression models indicate that the highest aerobic colony counts are found on the integrated mixer and solenoid, which have on average 71 times the mean log cfu count of the reference (tap body) category (95% CI: 18.77–269). This finding contrasts with the results for *P. aeruginosa* from the adjusted model noted above which indicate that the highest *P. aeruginosa* counts are found on the flow straightener. In the unadjusted model the flow straightener is associated with 7.81 times the $\log_{10}$ *P. aeruginosa* cfu count of the tap body (95% CI: 4.11–14.84).

Typing of P. aeruginosa strains

P. aeruginosa isolates recovered from tap biofilm from three taps at one hospital had VNTR profiles that were indistinguishable from the strain found in both patients and tap water from that hospital (VNTR profile 12, 5, 1, 5, 2, 2, 8, 2, 9). Isolates from tap biofilms received from another hospital also had a VNTR profile that was indistinguishable from a patient strain (VNTR profile 8, 2, 5, 3, 4, 2, 7, 2, 10). In addition, strains from the second hospital's tap and water samples (VNTR profile 8, 3, 4, 5, 2, 3, 5, 2, 10) were also indistinguishable. However, these were distinct from the strain isolated from another patient (profile 11, 4, 5, 2, 2, 1, 8, 2, 12), although this strain had also been recovered from tap and tap water samples taken by the hospital at the time and sent to the reference laboratory for typing.

Microscopy analysis

Both epifluorescent microscopy and SEM detected the presence of microbial biofilms on a number of different components. Profuse biofilms were observed on sections of the plastic flow straighteners (internal, external curvatures, flat horizontal areas, mesh grids, and on the prongs) as well as on the internal and external areas of the metallic collars surrounding those flow straighteners (Figure 3).

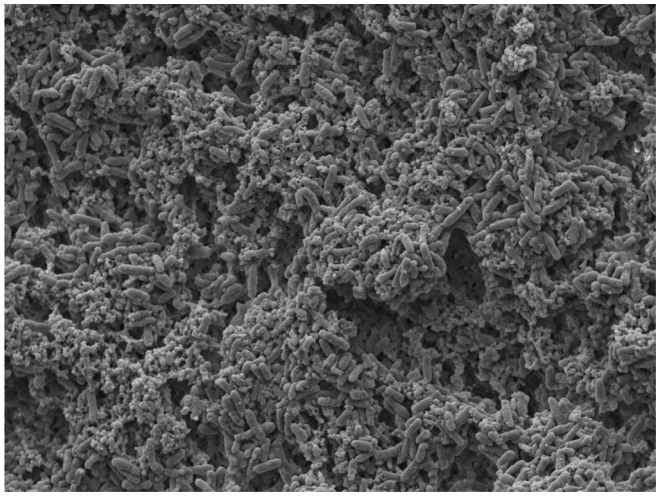

Figure 3. Scanning electron microscope image of biofilm on plastic complex flow straightener.

Discussion

P. aeruginosa can persist in hospital water systems for extended time periods and has resulted in a number of outbreaks.[1,7,9,17] Various medical devices and environmental reservoirs have been implicated in these outbreaks, including water, antiseptic solutions and lotions, endoscopy equipment, ventilators and mouth swabs.[16,18,19]

Following the incidents of *P. aeruginosa* in neonatal units in Northern Ireland hospitals, this study assessed the presence of *P. aeruginosa* and ACC in a range of different tap assemblies. Whereas only 14% of all components were positive for *P. aeruginosa*, some of them were heavily colonized with up to 2.2×10^7 cfu *P. aeruginosa* recovered.

ACC were recovered from 78% of components analysed (range: 200–2.9×10^8 cfu per sample) but there was little correlation between the aerobic colony count and the *P. aeruginosa* counts. ACC does not reliably predict the presence of *P. aeruginosa* and is not recommended in current Health Technical Memorandum addendum 04-01 on *P. aeruginosa* (https://www.gov.uk).

Whereas the highest ACC was associated with the integrated mixer and solenoid, the highest *P. aeruginosa* counts were associated with the flow straighteners, indicating that *P. aeruginosa* is predominantly associated with different tap components in comparison with the other aerobic microorganisms.

Previous studies have demonstrated the association of outbreaks with tap water in intensive care units where all the samples collected from the taps, water outlets and the water supply to the sinks grew >300 cfu/100 mL of a multidrug-resistant *P. aeruginosa* strain and the outbreak was controlled by replacing the sensor taps.[17]

Sensor taps are commonly used in healthcare facilities for staff handwashing and accounted for 79% of the tap assemblies that were received for analysis. These sensor taps had a greater probability of being colonized with *P. aeruginosa* than the non-sensor taps analysed, and our results show that the highest *P. aeruginosa* counts on sensor taps were associated with the flow straightener. However, although flow straighteners represented only 20% of tap components, they comprised 60% of all components with detectable levels of *P. aeruginosa* ($P < 0.05$). Detailed analysis of flow straighteners indicated that it was the complex flow straighteners (plastic ones) that had significantly higher counts of *P. aeruginosa* than more simple designs. In our observational study, complex flow straighteners were only found in sensor taps. This confounding makes it impossible to determine whether it was the design of the flow straightener or another attribute associated with a sensor tap that resulted in the presence of *P. aeruginosa*, e.g. lower water flow rates through the tap or different component materials, and therefore further work in this area is required in due course.

This would indicate that replacing the flow straightener or its component parts could still leave a *P. aeruginosa* biofilm on the adjacent tap surfaces.

There was no significant difference between the *P. aeruginosa* counts from flexible hoses versus copper pipes, although the sample size was small.

The typing data (VNTR) of the strains from two neonatal units linked the *P. aeruginosa* strains recovered from the biofilm on the tap surface and flow straightener, and/or the water from the tap and clinical samples isolated from the patients that died. This was most apparent in one of the hospitals,

where one strain clearly predominated among the patient, water and tap isolates, but not so clear for the samples from another neonatal unit in a different hospital, where a number of distinct strains were found among both patient and environmental isolates; some environmental strains were not represented at all among patient isolates. The strain of profile 8, 3, 4, 5, 2, 3, 5, 2, 10 of serotype 27, recovered from water and sink components, is quite frequently found in the environment globally and was not recovered from any patients from that hospital.[20,21]

Biofilms are an intrinsic way in which bacteria survive in water systems and were extensively observed on a range of flow straighteners and tap components using fluorescence microscopy and SEM. This current study of tap components from neonatal units in Northern Ireland indicated that *P. aeruginosa* heavily colonized specific tap components, i.e. the complex flow straighteners.

The tap outlets were supplied with water via a thermostatic mixer valve (TMV) which mixes the water from the hot and cold supply to deliver water at ~41 °C to prevent scalding during full body immersion. (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/144148/HTM_04-01_Part_B.pdf). However, TMVs are now frequently fitted to all outlets and as a consequence provide favourable temperature in the tap outlets at which *P. aeruginosa* are able to proliferate. The recently published Department of Health England Health Technical Memorandum 04-01 '*Pseudomonas aeruginosa* – advice for augmented care units' provides advice on what to do if there is *P. aeruginosa* contamination of the water system, including considering removal of flow straighteners, checking for underused outlets and flexible hoses and fitting of non-TMV mixing taps following a risk assessment (https://www.gov.uk/government/publications).

The results indicate that it was the more complex flow straighteners that were heavily contaminated and that these were only found in sensor taps (all of which were a single brand). Flow straighteners are present as the final component in the tap body to produce a non-splashing delivery of water. As the flow straightener and its associated components become colonized, the biofilm will shed micro-organisms into the water phase. With the flow straightener on the water–air interface, the ready supply of oxygen would facilitate *P. aeruginosa* to compete against facultative anaerobes in a biofilm – a factor that is not present in biofilms deeper inside the tap. This could be the reason why there was no link between ACC and *P. aeruginosa* on other tap components. Complex flow straighteners may also be fitted with a flow restrictor and have a large surface area:volume ratio, thus creating a moist, frequently stagnant environment in which the biofilm will grow.

Controlling biofilm could be inherently difficult as removal and replacement of the flow straightener and metal collar may still leave behind a *P. aeruginosa* biofilm on the tap body. Hence, the complete removal and replacement of the tap assembly may be a way in which hospitals can control the presence of *P. aeruginosa* in water samples from such outlets or by replacement of flow straighteners before significant biofilm can form or by physical removal of biofilm from the tap body when flow straighteners are replaced.[17]

Whereas the flow straightener components were contaminated with strains of *P. aeruginosa* indistinguishable from those isolated from patients, it is not possible to determine from the data whether the taps were the source of patient contamination, whether an external factor resulted in the contamination of both patients and taps, patient strains contaminated the taps or whether a mixture of these events occurred. As a consequence, hospitals should determine which patients are at risk in augmented units and undertake a review of not just the water system but also domestic environmental cleaning, infection control practices and related best practice assurances to assess possible tap contamination with patients' bacteria, to evaluate patient contact with water in particular wards and assess other control measures that may be required.

This study indicated that complex flow straighteners were implicated as an important area in the tap where *P. aeruginosa* accumulates. It may be prudent for manufacturers to redesign the tap outlet either to prevent contamination or to enable decontamination. When an increased incidence is identified, hospitals need to be aware that water outlets may be a possible source of *P. aeruginosa* and should take steps to assess the risks from water outlets and minimize risks to patients.

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None declared.

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Pseudomonas aeruginosa infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals

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***Pseudomonas aeruginosa* infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals**

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Running title: Transmission *P. aeruginosa* from water

Summary

Background*: Pseudomonas aeruginosa* is a common opportunistic pathogen and molecular typing in outbreaks has linked patient acquisition to contaminated hospital water systems.

Aim: To elucidate the role of *P. aeruginosa* transmission rates in non-outbreak augmented care setting in the UK.

Methods: Over a 16-week period, all water outlets in augmented care units of four hospitals were sampled for *P. aeruginosa* and clinical isolates were collected. Outlet and clinical *P. aeruginosa* isolates underwent whole genome sequencing (WGS), which with epidemiological data identified acquisition from water as definite (level 1), probable (level 2), possible (level 3), and no evidence (level 4).

Findings**:** Outlets were positive in each hospital on all three occasions, W (16%), X (2.5%), Y (0.9%) and Z (2%), and there were 51 persistently positive outlets in total. WGS identified likely transmission (at levels 1, 2 and 3) from outlets to patients in three hospitals for *P. aeruginosa* positive patients: W (63%), X (54.5%) and Z (26%). According to the criteria (intimate epidemiological link and no phylogenetic distance), approximately 5% of patients in the study 'definitely' acquired their *P. aeruginosa* from their water outlets in ICU. This study found extensive evidence of transmission from the outlet to the patients particularly in the newest hospital (W), which had the highest rate of positive outlets.

Conclusions: The overall findings suggest that water outlets are the most likely source of *P. aeruginosa* nosocomial infections in some settings, and that widespread introduction of control measures would have a substantial impact on infections.

Key Words:

P. aeruginosa, water-outlets, transmission, molecular-ecology, infections, augmented-care

Introduction

Pseudomonas aeruginosa is an important nosocomial pathogen in immunocompromised and critically ill patients and it is the fourth most common health care acquired infection (HCAI) pathogen in the USA [1] and the third most common cause of Gram-negative bacteraemia in hospitals in England, Wales and Northern Ireland [2].

Transmission of *P. aeruginosa* in the healthcare environment has been due to cross-contamination from a variety of environmental reservoirs [3-5]. Contaminated hospital water outlets have been implicated in outbreaks in neonatal and adult critical care units [3-9], including the high profile neonatal unit incident in Northern Ireland [8], where water outlets (last two metres) and associated fittings were identified as the source of infections that led to fatalities. Consequently, UK national guidance was published by the Department of Health for water sampling and control measures [10]. Whilst outbreaks of *P. aeruginosa* cross-infection in critical care units have been attributed to environmental sources, the role of such sources in sporadic (non-outbreak) pseudomonal infection situations has generally been poorly characterised in the UK [3].

Whole genome sequence typing (WGST) delivers a very high level of specificity and resolution compared to previously used typing methods such as pulsed-field gel electrophoresis (PFGE), and variable number tandem repeats (VNTR) which have been applied to *P. aeruginosa* outbreaks in critical care [4, 6, 8, 11]. WGST has been used to investigate transmission in outbreaks of *Staphylococcus aureus* [12] *Acinetobacter baumannii* [13] as well as *P. aeruginosa* [9, 14, 15] and previously used to demonstrate that transmission of waterborne isolates to burns patients most likely occurs during hydrotherapy, which is used in the management of burns patient's wounds [16].

To date there has been no comprehensive longitudinal multi-centre studies of the transmission dynamics of *P. aeruginosa* in UK critical care settings employing WGST. This study undertook a defined snapshot survey using WGST, simultaneously across four large UK hospitals, to investigate the burden

of *P. aeruginosa* infection or colonisation in adults (caused by transmission from water outlets) in the non-outbreak augmented care setting.

Methods

Study design and patients

Four NHS hospitals (anonymised to W, X, Y and Z) with diverse water sources, buildings, and plumbing infrastructures from a wide geographical area in England participated in this study (Table I). A total of 23 augmented care units (ACUs) across the 4 hospitals were studied, from which all 881 water outlets (774 taps and 107 showers) were sampled.

During a 16-week period (30th August - 6th December 2014) water sampling of outlets was undertaken at all sites, on three occasions (designated 'beginning', 'middle' and 'end') within a 10-day window. The ACUs of the hospitals were described as 'non-outbreak' with respect to *P. aeruginosa* as there was no observed increase in incidence of *P. aeruginosa* infections above the average number (from both clinical and environmental observations), and hence these units were not under enhanced surveillance.

All clinical isolates of *P. aeruginosa*, which were retained as part of routine care from patients satisfied the inclusion criteria as follows: an inpatient in one of the included wards for more than 48 hours, with no pre-existing *P. aeruginosa* colonisation/infection, and without an isolate with the same antibiogram and colony morphology having already been saved. The bed, ward number and nearest water outlet (make and model) when sample(s) were retained. No clinical details or patient identifiable data were recorded and we were unable to obtain ethical approval to screen all patients for *P. aeruginosa* carriage upon ICU admission.

Microbiological methods

Pre-flush samples were taken at each hospital as described previously [10]. Water (100 mL) samples were filtered (0.45µM membrane filter) and cultured on cysteine lactose electrolyte deficient agar (CLED) agar (Biomerieux)and on cetyl trimethylammonium bromide and nalidixic acid (CN) agar plate (Biomerieux) (48 hours incubation at 37°C). Oxidase positive colonies were identified using MALDI-TOF (Biomerieux).

Molecular methods and bioinformatics analysis

DNA extraction of *P. aeruginosa* isolates was performed by emulsifying (5µl) each bacterial colony in molecular grade water (100µl) and then heated (10 minutes at 95°C). DNA was isolated and cleaned by adding the supernatant to an equal volume of Ampure XP beads (Beckmann Coulter, High Wycombe, UK).

Whole genome sequencing was performed as described previously [16]. Reads were demultiplexed and then mapped against the *P. aeruginosa* PAO1 reference genome (NC_002516.2). Clusters were identified using a rapid genotyping method described previously [17]. To call variants, representative isolates from each cluster were mapped against *P. aeruginosa* reference genomes from RefSeq [18] and the NCTC 3000 project using bwa-mem (v0.7.15) [19]. The best reference was selected for each cluster based on greatest proportion of mapped reads. Variants were called using FreeBayes Variant calls and filtered for allele frequency and recombination. Phylogenetic trees were constructed for each cluster using RAxML (v8.2.10) [20]. In addition all isolates were MLST typed *in silico* using StringMLST (v0.5.1.1) [21, 22].

Definition of transmission events

The likelihood of transmission was assessed through a multifactorial approach taking into consideration i) the cluster defined dendrograms of environmental water and clinical isolates, ii) a measure of phylogenetic distance, and iii) the metadata relating to geographical locations when samples were taken.

- All clinical isolates within the same cluster as environmental isolates were analysed for potential transmissions by both phylogenetic distance and epidemiological link.
- Pairwise phylogenetic distances between clinical and environmental isolates were measured for each cluster with distances used to identify similarity to an environmental source.
- Metadata for clinical isolates defining ward and bed locations was analysed for potential epidemiological links to the sampling location of environmental isolates.

Four categories of transmission were defined; Level 1 (definite): 'Patient isolate genome indistinguishable from water outlet *P. aeruginosa* genome (phylogenetic distance of 0) with epidemiological link to at least ward level' (patient and outlet linked in time and place), Level 2 (probable): 'Patient isolate genome highly similar to an outlet genome (but with a phylogenetic distance of >0), and with an epidemiological link to outlet', Level 3 (possible) 'Patient isolate genome present in the same phylogenetic cluster as a *P. aeruginosa* water outlet genome but without an epidemiological link to outlet', and Level 4 (no evidence): clearly not a hospital water transmission (no hospital water isolates present in the same cluster as the patient isolates and no epidemiological link). The likely direction of transmission (and temporality) was then inferred based on the dates that the water and clinical isolates were collected.

Results

The overall proportion of outlets positive for *P. aeruginosa* (as a percentage of the total outlets sampled per hospital site) was 26.3%, 10.4%, 6.5% and 8.2% for hospitals W, X, Y & Z respectively. Some outlets (16% in hospital W) remained positive throughout the study (Table 1). The hospital built most recently (2010; with the newest plumbing system (hospital W)) had the highest rate of *P. aeruginosa* positive outlets, and the greatest number of clinical isolates. Hospital Y (the only hospital with a Copper Silver Ionisation (CSI) water treatment system for the prevention of Legionella infections), had the lowest positivity rates from the water outlets, and the fewest number of clinical isolates.

There were 120 clinical *P. aeruginosa* isolates collected from 78 eligible patients during the study (Supplementary Table II). The majority were from respiratory samples (50%), with the remainder from swabs (25.4%), urine (11.2%), blood cultures (6.7%), tissues (4.2%) and fluids (2.5%). Only one patient isolate was not included in the analysis due to inadequate sequencing depth.

Sequence diversity of *P. aeruginosa* isolates

A total of 552 (120 clinical and 432 water) *P. aeruginosa* isolates were analysed. One clinical isolate failed sequencing, and <10% of the water isolates could not be sequenced. We assigned the isolates to 17 clusters which were defined as clades containing at least one patient and one water outlet isolate i.e. a potential transmission. A total of 460 (83%) isolates belonged to a designated cluster, and clusters were consistent with the results of multi-locus sequence typing data reported by StringMLST (Supplementary Table III).

Analysis of the isolates for sequence diversity (Figure 1) demonstrated different clades associated with different hospitals. Hospital W had the largest number of positive water outlet isolates (181 from 231 sampled assets), but also the most defined clusters with the majority of the isolates belonging to one

of three large clusters (ST395, ST17 and ST179). The environmental water outlet isolates from the other hospitals were more diverse, with a smaller proportion of isolates contained in three small clusters (hospital X), one cluster (hospital Z), or with limited clustering (hospital Y).

Detection of linked environmental and clinical isolates

Although all clusters were analysed, further diversity analysis was performed on ST395, as it was present in each hospital (Supplementary Figure 2). Phylogenetic distance was lower within the same hospital when compared to different hospitals, suggesting each hospital was colonised by a local clone or clones. Phylogenetic distance of isolates from the same ward was similar to that of different wards, suggesting that transmission of *P. aeruginosa* to other wards only accounted for a minority of transmissions. Phylogenetic distance of isolates from the same outlet was markedly reduced compared to those from different outlets, suggesting the persistence of a single genotype within an outlet. We have chosen to represent phylogenetic distance data as a violin plot as it allows visualisation of the density distribution for the large number of datapoints from the pairwise comparison of isolates [23].

Clinical isolates (ranging from one to seven per patient) were collected from 78 patients across the four hospitals. The transmission category was determined for every clinical isolate, using the epidemiological information alongside phylogenetic distance. The highest transmission category was then recorded per patient (Supplementary Table IIII) and the positive water and patient sampling results for hospital W are presented (Supplementary Figure 3).

Direction of transmission inference

A summary of the patient acquisition/transmission events for each classification level, and each hospital, is shown in Supplementary Table II. Where multiple clinical isolates existed for a patient, all were investigated (to determine the particular classification levels of all the clinical isolates), and the highest classification level recorded in Supplementary Table III for that patient.

Using this approach, 5.1% of the patients were classified as level 1 ('definitely' acquired their *P. aeruginosa* isolate from hospital water outlets), 18% were level 2 ('probably'…), 24.3% are level 3 ('possibly'...), and 51.3% were level 4 (there is 'no evidence' that they have acquired their *P. aeruginosa* isolate from the hospital water outlets). A single clinical isolate from one of the patients could not be sequenced (1.3%). Consequently, considering the study as a whole, approximately 50% of patient's most likely acquired their *P. aeruginosa* isolate from the hospital water outlets.

Intra-hospital transmission events

The transmission rates varied across the hospitals involved in the study (Supplementary Table II) and were calculated per hospital by dividing those transmission events classified as level 1-3 by the total number of events and multiplying by 100. Hospital W had the highest transmission rate (63.3%), suggesting that nearly two-thirds of patients acquired their *P. aeruginosa* from the hospital water outlets whilst being nursed in augmented care. There were no patient acquisitions at hospital Y, and only a small number of clinical *P. aeruginosa* isolates were collected during the study period, despite a similar rate of sample collection.

Hospital W had four patients with a clinical *P. aeruginosa* isolate classified as level 1 ('definite'). For example, patient 28, was nursed close to an outlet which was positive on all three sampling events (30th August, 23rd October and 6th December 2014). Three clinical *P. aeruginosa* isolates were recovered from this patient (from sputum samples collected in early December) during the course of their treatment, and two of these clinical isolates were indistinguishable from the water isolates recovered from this outlet that was positive on all three sampling events. Interestingly, although classified as level 2 (owing to the phylogenetic distance being 0.002 and therefore greater than 0), this particular outlet had resulted in a previous patient (patient 3) also acquiring infection, with a swab and sputum sample positive on 8th September (water outlet first positive on 30th August). It is therefore assumed that both patients most likely acquired their *P. aeruginosa* from the identified hospital water outlet.

Although we only have evidence of 'definite' transmission from hospital W, there is evidence of probable (level 2), and possible (level 3) transmission for both hospitals X and Z. A probable transmission event in hospital X involved a patient who was nursed in a room with en-suite facilities including a handwash basin, and shower. *P. aeruginosa* was isolated from a blood culture collected from the patient on 21st October 2014 and is closely genetically related (phylogenetic distance of 0.003) to those collected from the handwash basin, and shower on both 3rd September and 27th October.

Hospital Z showed four probable transmission events, the temporality of which fit with the affected patients acquiring their *P. aeruginosa* from their nearest hospital water outlet. There was no evidence of water outlet to patient transmission at hospital Y.

Discussion

The majority of studies focusing on hospital water delivery and disposal have investigated distinct outbreaks of infection caused by *P. aeruginosa* often involving strains with an easily detectable phenotypic marker e.g. antibiotic resistance [9] . Nevertheless, such studies have established clear links between hospital water *P. aeruginosa* strains, and patient strains.

Many previous studies have been hampered by non-molecular typing methods with low discriminatory power, which overestimates transmission rates. Kerr *et al*. [3] concluded that because *P. aeruginosa* occurs in a wide range of hospital environments that investigations restricted to outbreaks are not sufficient to define directionality of transmission. This difficulty of interpretation was confirmed in a systematic review [24] which concluded that although water systems can act as a source of *P. aeruginosa* infection, the route of transmission was unclear, as was the directionality.

Strain typing studies using WGS are relatively uncommon and concentrate on outbreaks and did not examine water outlets as a source [7, 9, 15] with two others showing transmission from water outlets to a total of 5 patients [14, 16].

In order to understand the extent and dynamics of the colonisation of ICU tap water outlets a prospective longitudinal study is required. Only one such study has been published of 10 ICU wards in 8 separate hospitals in France performed in 2010 [25]. Over a 5-month period 233 taps were cultured for *P. aeruginosa* and patient colonisation was determined, as 17 patients were found to be colonised with a PFGE typed strain that matched a water isolate. The authors commented on "discrepancies" between units (e.g. 41/81 positive taps came from just 2 units) but did not investigate the reasons for the variation recommending that further studies on routes of transmission and control were needed.

Our study was a longitudinal, non-outbreak epidemiological study in four large hospitals housing 22 ICU wards with 881 tap outlets which were monitored. We looked for correlation between clinical disease associated isolates and water outlet isolates rather than asymptomatic colonisation, also using WGS in which we were able to assign directionality of transmission in some cases. We have shown 'definite' and 'probable' acquisition of *P. aeruginosa* in three of the four participating hospitals, affecting 19 of 78 (24.3%) patients. These definitions have been based on a measure of phylogenetic distance, epidemiological linkages and are further strengthened owing to the low diversity of water clones encountered (especially in hospital W), making patient contamination of the outlet unlikely. This is a highly conservative estimate, demonstrating that this is a significant potential problem in any ACU.

There is evidence of hospital-specific water associated clones of *P. aeruginosa*. MLST analysis of the WGS data shows that ST179 was found in hospital W only, and ST395 was predominantly associated with hospital W (although was also found in hospitals X, Y and Z) (Figure 1). We hypothesise that the low diversity of *P. aeruginosa* sequences found in hospital W was related to the age of the plumbing infrastructure (newest of the four). The majority (86%) of positive taps in hospital W were of one

model (compared to 38.5%, 64.2%, and 45% in hospitals X, Y and Z, respectively). A possible explanation for the presence of the dominant clone was that they were contaminated during manufacturer's wet integrity testing of the plumbing components prior to distribution[26]. ST395 has been widely reported in water systems and has been noted to carry a copper resistance gene which might aid survival in copper containing water distribution systems [27]. Interestingly the three large clusters from hospital W (ST395, ST17 and ST179) were also found in the other three hospital groups, suggesting that these represent common aquatic clones, despite considerable geographic separation (200 miles between hospital X and Z).

This is the first study to use patristic distances to investigate genetic relatedness of water and patient *P. aeruginosa* in the non-outbreak setting. The question of defining strain relatedness by differences in SNP carriage in WGS data is a vexed one. Attempts have been made to both define isolates as indistinguishable using a range of allowed SNP differences as well as proposing directions of transmission [28]. However, genome rearrangements can lead to overestimation of strain differences if SNP counts are considered in isolation. In the case of *Mycobacterium tuberculosis* (which has no horizontal gene transfer and a relatively slowly evolving genome) such numerical parameters would appear to be ideal and were proposed after sequencing sequential isolates from individuals and family clusters[28]. Their proposed 0-5 SNPs for indistinguishable isolates and >12 SNPs for distinct isolates was applied to a large 14 year outbreak of isoniazid resistant strains in North London [29]. They found that the majority of the 344 isolates differed by only 2 SNPs, but sequencing of 27 individual colonies cultured from a single patient specimen revealed up to 10 SNP differences between colonies. When applying the detailed epidemiological information to the data set it was clear that multiple transmission events had occurred without detectable SNP acquisition. This meant that even identical isolate pairs could not be deduced to have resulted from a direct transmission event without a supporting epidemiological link [29]. This experience has informed our criteria for assigning isolate

relatedness by including epidemiological information and using patristic distance rather than absolute SNP number differences.

There are some limitations of the study. Since the study was a short defined “snapshot” investigation of events over a defined time period, there was no follow up of the patient’s journey during their hospital admission. Consequently, the conservative definitions used to define the transmission events may have underestimated the true transmission rate. Patients were not screened for endogenous (asymptomatic) carriage on admission (owing to ethical considerations) which could be the route for colonisation and/or infection. Additionally, water sampling was performed on three occasions; therefore, it is possible that positives may have been missed in the gaps between samplings. Other wet sources (e.g. sink traps) or patient-to-patient transmissions were not investigated. As hospital Y had a CSI treatment system and copper ions are known to induce a persister state inducing a viable but non-culturable state thus rendering previously culturable bacteria unculturable on the same media [30, 31]. Pre-treatment with a copper chelator may have enabled more accurate viable counts of *P. aeruginosa* in Hospital Y.

The effect of CSI on bacteria other than Legionella in water distribution systems has been reported [32]. The CSI hot water samples showed no increase in species richness (defined by 16S rDNA sequencing) compared to the cold-water samples, whereas the non-CSI showed high diversity. The low rate of recovery of *P. aeruginosa* at hospital Y may be attributable to the CSI treatment, although other factors such as water temperature, plastic pipes and maintenance activity could also have contributed to this significant finding.

This was the largest prospective study that has demonstrated the importance of *P. aeruginosa* in hospital water, and evidence of onward transmission to patients. In hospital W (where outlets were identified as a likely source[33]), positive water samples have been drastically reduced across critical care in hospital W [34]. A series of holistic interventions (fitting new taps, filters on existing taps, improved tap cleaning, and appropriate disposal of patient waste water), resulted in a 50% reduction

in the number of *P. aeruginosa* clinical patient isolates over a year [34], and a 72% decrease in of *P. aeruginosa* acquisition when positive taps were removed and new ones fitted [35].

This study has shown that colonisation of ICU outlets acts as a persistent source of infection with *P. aeruginosa* and this varies in ACU depending on the level of contamination. There is substantial variation between different facilities and an urgent need to introduce control measures to reduce *P. aeruginosa* colonisation in ICU tap water.

Conclusion

In this longitudinal, non-outbreak study of four hospitals, very different levels and patterns of colonisation of water outlets was observed across all three sampling timeframes. Application of WGS to water and clinical isolates demonstrated convincing transmission (level 1) from water to patients for 5% of patients, and probable transmission (level 2) in a further 19% of the studied patients. Strains from water outlets caused a range of clinical infections, including a bacteraemia.

Hospital W had a strikingly high incidence of colonised outlets with a corresponding high incidence of patient isolates, supporting the transmission of *P. aeruginosa* from water outlets causing colonisation/infection in patients. This hospital also had a dominant clone (ST179) which exhibited low intra-isolate diversity, suggesting a common source. Hospital Y had the lowest incidence of colonised outlets which may have been due to the CSI treatment.

There was variability in the frequency of *P. aeruginosa* detected at different hospitals which correlated with the frequency of predicted transmissions. This suggests that in some settings water outlets are the most likely source of *P. aeruginosa* nosocomial infections for the patients in the study with widespread implications for control measures nationally and internationally

Conflict of Interest

None

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Table

Table I: The dates of construction, number of beds, and percentage of positive outlets per hospital

Hospital group	Details of the augmented care ward, including dates of construction	Number of beds	Number of outlets sampled during the study	Number of positive outlets (as a % of a sampled) per sampling perioc		
				Beginning	Middle	
W	4 wards. 1 hospital site. All 2010	100	231	57 (25%)	63 (28%)	
X	10 wards. 1 hospital site. Built 1974 (but revised 2002-2013)	165	400	47 (12%)	50 (12·5%)	
Y	5 wards across 2 hospital sites. All 1975	72	105	9 (9%)	5 (4·8%)	
Z	3 wards across 2 hospital sites. Two built 1978 (and revised 2009), the other built 2005	64	145	37 (25%)	19 (13%)	

Figures

Please see separate image files

Legends

Figure 1. Phylogenetic tree for all isolates generated using the rapid genotyping method. The five predominant clades are coloured according to their MLST clonal complex to aid interpretation (a clonal complex is defined as alleles exactly matching a sequence type or a single-locus variant of that sequence type). The annotation bars below indicate the hospital where that isolate was found and the type either water (blue) or patient (orange).

Supplementary Table II: The transmission events per Trust including the transmission events classified per patient, transmission rate, and clinical specimen types involved

Supplementary Table III: Table showing the multi-locus sequence typing data reported by StringMLST for all isolates for seven highly conserved housekeeping genes.

Where: *acsA* (acetyl-CoA synthetase), aroE (shikimate dehydrogenase), *guaA* (GMP synthase), *mutL* (DNA mismatch repair protein), *nuoD* (NADH-quinone oxidoreductase subunit C/D), *ppsA* (phosphoenolpyruvate synthase), *trpE* (anthranilate synthase component).

Supplementary Table IIII: Showing the categorisation of the transmission events per clinical isolate.

The table shows the clinical isolates, the ward and outlet that relate to each clinical isolate and the closest waters isolates (in terms of phylogenetic distances). This was then used to assign a category to that clinical/water isolate pair according to the definitions used in the paper:

Level 1 (definite): ‘clinical *P. aeruginosa* genome indistinguishable from water *P. aeruginosa* genome (phylogenetic distance of 0) with epidemiological link to at least ward level’, Level 2 (probable): ‘Patient genome most closely similar to an outlet genome (but with a phylogenetic distance of >0), and with an epidemiological link to outlet’, Level 3 (possible) ‘Patient genome present in the same phylogenetic cluster as a *P. aeruginosa* water genome but without an epidemiological link to outlet’, and Level 4 (no evidence): not clearly a hospital water transmission (no hospital waters present in the same cluster and no epidemiological link). ‘none’ refers to clinical isolates for which there were no related water isolates present in our sampling set.

Supplementary Figure 2. Violin plots showing phylogenetic distance between all pairs of isolates from the same hospital (left), the same ward (middle) and same outlet (right) for the ST395 cluster. Phylogenetic distance was calculated by the R package ‘ape’ [36] which are derived from the RAxML branch length themselves based on mutations per site.

Supplementary Figure 3. Map of four wards in hospital W, demonstrating association between water outlets and *P. aeruginosa* genetic clusters. The three water sampling periods (beginning, middle and end) are shown separately from top to bottom. *P. aeruginosa* isolates from water isolates (diamonds) and patients (circles) are coloured by their cluster type.

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Exogenous acquisition of *Pseudomonas aeruginosa* in intensive care units: a prospective multi-centre study (DYNAPYO study)

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SUMMARY

Background: *Pseudomonas aeruginosa* remains one of the most common nosocomial pathogens in intensive care units (ICUs). Although exogenous acquisition has been widely documented in outbreaks, its importance is unclear in non-epidemic situations.
Aim: To elucidate the role of exogenous origin of *P. aeruginosa* in ICU patients.
Methods: A chronological analysis of the acquisition of *P. aeruginosa* was performed using samples collected in 2009 in the DYNAPYO cohort study, during which patients and tap water were screened weekly. Molecular relatedness of *P. aeruginosa* isolates was investigated by pulsed-field gel electrophoresis. Exogenous acquisition was defined as

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Water taps
Pulsed-field gel electrophoresis

identification of a *P. aeruginosa* pulsotype previously isolated from another patient or tap water in the ICU.

Findings: The DYNAPYO cohort included 1808 patients (10,402 samples) and 233 water taps (4946 samples). Typing of 1515 isolates from 373 patients and 375 isolates from 81 tap water samples identified 296 pulsotypes. Analysis showed exogenous acquisition in 170 (45.6%) of 373 patients. The pulsotype identified had previously been isolated from another patient and from a tap water sample for 86 and 29 patients, respectively. The results differed according to the ICU.

Conclusion: Exogenous acquisition of *P. aeruginosa* could be prevented in half of patients. The overall findings of this survey support the need for studies on routes of transmission and risk assessment approach to better define how to control exogenous acquisition in ICUs.

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Introduction

Pseudomonas aeruginosa remains one of the most common hospital-acquired pathogens, and is endemic in many intensive care units (ICUs) [1,2]. Infections caused by *P. aeruginosa*, especially ventilator-associated pneumonia or bloodstream infections, are often severe and associated with considerable mortality in ICU patients; morbidity and mortality rates are even higher in cases of multi-drug resistance [3—6]. In ICUs, these infections are usually considered to be endogenous, arising from pre-existing colonization of patients. In addition, the roles of exogenous reservoirs and patient-to-patient transmission during outbreaks have been documented convincingly. However, the importance of exogenously acquired *P. aeruginosa* in non-epidemic situations remains uncertain [7,8]. Several epidemiological studies have indicated that colonization pressure seems to be a more relevant risk factor than exposure to antibiotics for the acquisition of *P. aeruginosa* [9—12].

A previous monocentric study showed a clear genetic temporal and spatial relationship between *P. aeruginosa* strains isolated from tap water samples and ICU inpatients [13]. Moreover, the first part of the multi-centre DYNAPYO (Dynamics of *Pseudomonas aeruginosa* Acquisition in ICU) study showed that exposure to contaminated tap water was a risk factor for *P. aeruginosa* colonization in ICU patients [14,15]. It is essential to better understand the true contribution of the exogenous acquisition of *P. aeruginosa* in ICUs to inform infection prevention and control strategies. As such, a chronological analysis was performed within DYNAPYO-participating ICUs to assess the respective contributions of *P. aeruginosa* exogenous acquisition by patient-to-patient transmission and from contaminated taps.

Methods

Study design and study population

The DYNAPYO study was a prospective 5-month observational survey performed in 2009 in 10 ICUs (four medical, two surgical, and four mixed medical and surgical ICUs) from eight French healthcare facilities: the university hospitals of Besançon and Lyon which included two ICUs; the university hospitals of Bordeaux, Garches, Montpellier and Paris; and the general hospitals of Lens and Tourcoing which included one ICU. These ICUs had nine to 20 beds with an average length of stay of 8—16 days (Table I).

The ICUs did not implement changes during the course of the study, such as infection control or antimicrobial stewardship initiatives. The ICUs had to follow French recommendations in terms of water use for care, and measures to prevent patient-to-patient transmission. Outlet taps were equipped with antibacterial filters in one ICU (ICU 1).

The DYNAPYO cohort included all adult patients admitted for more than 24 h during the study inclusion period. Demographic and epidemiological data (admission dates and discharge, room number) were collected prospectively. The type of tap (electronic or conventional) was recorded, and patients and tap water were monitored weekly for contamination with *P. aeruginosa* over the study period. Specific trained healthcare workers were identified in each centre for water sampling and for data collection on a secured online case report form. This study was approved by the local ethics committee, and the data underlying this study were restricted by the French data protection commission (Commission Nationale Informatique et Liberté, France).

Surveillance culture and microbiological analysis

During the data collection period, patients were screened on admission (within the first 48 h of ICU stay), then once per week, and finally on discharge or death. Screening samples were oropharyngeal swabs, rectal swabs and tracheobronchial aspirate (or sputum). Other clinical specimens were performed as clinically indicated (as appropriate).

Table I
Description of the intensive care units (ICUs)

	Type	Rooms (*N*)	Mean length of stay (days)
ICU 1[a]	Mixed	20	14
ICU 2	Mixed	16	12
ICU 3	Medical	12	10
ICU 4	Surgical	14	14
ICU 5	Medical	18	8
ICU 6	Medical	12	10
ICU 7	Mixed	18	11
ICU 8	Medical	15	11
ICU 9	Surgical	15	11
ICU 10	Mixed	15	16

[a] ICU with filtered taps.

Cold water samples were taken weekly from the 233 taps in the 10 ICUs (patients' rooms and other sites) for testing for *P. aeruginosa* (without colony count). Taps were turned on and the first 250 mL of water was collected immediately in a sterile flask with sodium thiosulphate. The aerator was swabbed and the swab broken into the water sample. Tap water samples were processed by membrane filtration. A volume of 100 mL was filtered through a 0.45-μm pore size membrane filter (Millipore Microfil, Molsheim, France). Swabs and filters were cultured on cetrimide-agar plates (Bio-Rad, Marnes-la-Coquette, France) at 37°C and examined for growth of colonies after 24 and 48 h. Any colony that grew on a cetrimide-agar plate was identified using the API20 NE identification system (bioMérieux, Marcy l'Etoile, France). All *P. aeruginosa* isolates were sent to the coordinating centre (Bordeaux) on semi-solid agar.

Genotyping

Molecular relatedness of *P. aeruginosa* isolates was investigated by pulsed-field gel electrophoresis (PFGE). Clonality of strains was investigated by PFGE with DraI digestion as described previously [16]. The banding patterns were analysed by scanning photographic negatives. GelCompar software was used for analysis of PFGE patterns (Applied Maths, Kortrijk, Belgium). Pulsotypes were defined according to international recommendations [17]. All patients' first isolates from each anatomic site were analysed. For each newly identified patient, we selected for comparison the isolates identified in tap water, the previous week and the following week.

Definitions

Exogenous acquisition was defined as colonization or infection by a strain of *P. aeruginosa* with a pulsotype previously isolated from another patient (i.e. patient-to-patient transmission) or from a tap water sample in the ICU. Patient-to-patient transmission was considered possible when a similar pulsotype was isolated in more than two patients hospitalized during an overlapping period without a similar pulsotype isolated from tap water. Exogenous origin from tap water was considered possible when a similar pulsotype was isolated in a patient and at least one ICU tap water sample prior to *P. aeruginosa* identification in the patient.

Results

Study population

Of the 1808 patients included in the DYNAPYO cohort, 206 were excluded because screening was not performed at admission. In total, 10,402 screening samples were taken and 427 patients were positive for *P. aeruginosa*; of these, 41 were found on entering the study. The average incidence of *P. aeruginosa* in the 10 ICUs was 12.7 per 1000 days of hospitalization (Table II).

Water samples

In total, 4946 water samples were obtained. Among the 233 taps screened, 81 (35%) were positive for *P. aeruginosa* at least once during the study, including 51 at the beginning of the study. The median duration of contamination was 5 weeks (range 1–13 weeks). The median duration of contamination differed between electronic and conventional taps (12.6 vs 8 weeks, respectively; $P=0.003$).

Genotyping and chronological epidemiological analysis

Typing of 1880 non-replicate isolates (1515 from 373 patients and 375 from 81 water samples) identified 296 pulsotypes. A total of 270 different pulsotypes were found in patients: 201 (74%) were sporadic, 52 were shared by patients, and 17 were shared by water and patient. Variations according to the ICU are shown in Table II.

The chronological epidemiological analysis showed exogenous acquisition in 170 (45.6%) patients out of the 373 patients for whom at least one isolate was available for typing, with variation according to the ICU (from 16.3% in ICU 7–85.7% in ICU 5; Table II). There was patient-to-patient transmission for 86 of the 170 patients (50.6%) and an exogenous origin from tap water for 29 other patients (17.1%). Moreover, it was not possible to draw conclusions for 55 patients from the two ICUs with the highest rates of positive tap water (ICU 5 and ICU 10) because pulsotypes were shared by many patients and tap water samples.

Discussion

To the authors' knowledge, the DYNAPYO study is the largest cohort study to assess the relative contribution of exogenous acquisition of *P. aeruginosa* in ICUs [14]. The present study showed an exogenous origin of *P. aeruginosa* in nearly half of the patients. Patient-to-patient transmission was more frequent than acquisition from tap water. At least half of the cases of colonization or infection by *P. aeruginosa* could be preventable. Furthermore, this study showed discrepancies in the rates of exogenous origin of *P. aeruginosa* according to the ICU, which could explain the differences in results in previous monocentric studies.

Patient-to-patient transmission occurs by carriage on the hands of healthcare workers or through contaminated medical equipment [18–20]. There is a considerable body of published literature for patient-to-patient transmission of multi-drug-resistant *P. aeruginosa* from outbreak reports [12,21,22]. The strict maintenance of infection control measures is essential to limit the spread of this bacteria. Infection control strategies to decrease the incidence of infection due to *P. aeruginosa* in ICUs mainly include bundle approaches involving general measures, disinfection and replacing reservoirs [23–25].

Previous studies found that clinical strains of *P. aeruginosa* were genetically related to the strains found in the patient's environment, such as in tap water, P-traps, sinks, handwashing stations, tap aerators or washbasin drains, but a causal link was controversial [25]. In a systematic review, seven monocentric studies were considered to provide plausible evidence of a link between tap water as a reservoir for *P. aeruginosa* and colonization/infection in patients in an endemic setting. In these studies, rates of exogenous acquisition of *P. aeruginosa* varied from 29% to 81% of patients, as in the different ICUs in the present study [13,19,26–30].

Table II
Data of surveillance, typing of isolates and chronological analysis

	ICU 1[a]	ICU 2	ICU 3	ICU 4	ICU 5	ICU 6	ICU 7	ICU 8	ICU 9	ICU 10	Total
Surveillance											
Patients included	203	135	152	138	295	190	200	123	204	168	1808
Patients colonized or infected	51	29	59	45	48	37	51	18	43	46	427
Patients colonized or infected at the beginning of the study	6	4	5	7	2	1	1	0	5	10	41
Incidence of patients colonized or infected (patients per 1000 days of hospitalization)	14.8	14.6	13.3	9.6	15.9	11.5	9.4	9.5	11.2	15.5	12.7
Tap water screened	45	15	28	31	25	15	29	10	15	20	233
Tap water positives	3	2	10	5	22	11	9	1	0	18	81
Tap water positives at the beginning of the study	5	3	9	5	19	0	2	0	0	8	51
Typing											
Patient isolates	69	90	223	155	166	72	227	82	100	331	1515
Water tap isolates	11	13	69	34	112	18	20	0	0	98	375
Patients with at least one isolate typed	34	26	54	44	42	27	49	18	38	41	373
Pulsotypes within patients	31	21	37	34	16	27	45	10	28	21	270
Sporadic pulsotypes	22	17	32	22	11	22	37	4	21	13	201
Shared by patients	8	2	4	9	0	4	8	6	7	4	52
Shared by water and patient	1	2	1	3	5	1	0	0	0	4	17
Pulsotypes in water taps	3	4	7	5	6	4	3	0	0	11	43
Sporadic pulsotypes	2	2	6	2	1	3	3	0	0	7	26
Chronological analysis											
Exogenous origin	17	10	23	23	36	6	8	8	10	29	170
Patient-to-patient transmission	14	6	4	20	1	5	8	8	10	10	86
Exogenous origin from water tap	3	3	19	3	/	1	0	0	0	/	29
Origin not concluded	/	1	/	/	35	/	/	/	/	19	55

ICU, intensive care unit.
[a] ICU with filtered taps.

In this study, there was wide heterogeneity in tap water contamination by *P. aeruginosa* among the ICUs. In common with other studies, the present study showed that *P. aeruginosa* may persist in tap water over prolonged periods, and that electronic taps are potential reservoirs of *P. aeruginosa* in ICUs [31–33]. Tap water could become positive for *P. aeruginosa* through contamination of the water supply or retrograde contamination (e.g. from splashing on to the tap when water is drawn, especially if the water flow impacts directly on the drain outlet, or if fluids are discarded inappropriately in handwash basins) [34,35].

Discrepancies observed between ICUs may be explained by various factors, including compliance with infection control measures, contamination load of the environment, and biological features of the pathogen (intrinsic fitness factors) [36]. Based on the study findings, the authors suggest monitoring tap water in ICUs with high rates of colonization or infection with *P. aeruginosa*, although there are no recommendations for systematic screening in search of *P. aeruginosa* in ICUs. Furthermore, these ICUs should consider eliminating work processes involving sinks in close proximity to patients, and favour the use of alcoholic hand disinfection in order to limit the spread of *P. aeruginosa* among patients. Some guidelines recommend sampling outlets in ICUs on a six-monthly basis and taking remedial action for outlets that are positive for *P. aeruginosa* [37].

This study had several strengths: the prospective multicentre design with a study population in accordance with most previous studies analysing *P. aeruginosa* colonization/infection in ICUs; the use of methods enabling a temporal relationship to be identified between water taps and identification of colonization/infection in patients; repeated sampling during the 5 months of the study; and the huge number of isolates typed by PFGE. However, some limitations should also be noted. First, isolates were selected for genotyping and this was performed from one colony of each positive culture; however, isolates were not available for some patients. So, the results may not accurately represent the whole epidemiology. A robust methodology would involve typing four to 10 different colonies from each culture. In a study of more than 1600 isolates of *P. aeruginosa*, the authors found that over 60% of the tap water samples were contaminated by *P. aeruginosa*, and 83% of the patient strains were classified as exogenous. They typed at least four colonies that were representative of the different morphological types of *P. aeruginosa* present on each culture plate [29]. Second, the limitation of water samples may have underestimated the number of exogenous sources, as extensive microbiological samples of the environment were not taken other than water taps and patients. The range of reservoirs in healthcare environments from which *P. aeruginosa* has been isolated is wide, including respiratory therapy equipment, ice makers, endoscopes and cleaning equipment [38,39].

In conclusion, this multi-centre study conducted in ICUs suggests that an exogenous origin of *P. aeruginosa* could be prevented in a substantial proportion of patients. Given the possible consequences of *P. aeruginosa* infection in ICUs, it is clear that strategies to prevent *P. aeruginosa* acquisition should become a key priority. There is a need for studies on routes of transmission and risk assessment approach to better define how to control exogenous acquisition in ICUs.

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Conflict of interest statement
None declared.

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SCOTTISH HOSPITALS INQUIRY

Review of the Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen Elizabeth University Hospital and Royal Hospital for Children, Glasgow

written by Professor PM Hawkey, Dr SG Agrawal and Dr LN Drumright

regarding Chapter 7

Expert Report prepared for the Scottish Hospitals Inquiry

Date: 10 June 2025

Mr. Sid Mookerjee, BSc, MSc, MPH, FRSPH

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1. Summary

1.1. This report has been prepared at the request of the Scottish Hospitals Inquiry and reviews Chapter 7 of the report of Professor Hawkey and colleagues, hereon referred to as the HAD report, on its methodology and conclusions from an epidemiological perspective.

1.2. This report does not address the *aspergillus* infections seen at QEUH and RHC. This subject is dealt with through a joint report by Dr. Sara Mumford and me, Sid Mookerjee.

1.3. The Inquiry has asked a series of questions in order to assist in this review.

2. Review of the HAD report

2.1. I am asked for my opinion on the validity of the principal proposition advanced that appears to be advanced at section 5.4.3 and section 6.1.2-6.1.3 that it would be possible to determine whether there was 'widespread contamination' of the hospital water system or 'inadequate or insufficient ventilation system' by considering whether there was an increased rate of infection in the patient population as a whole or in part'.

2.2. Dr Walker's expert report [1] and the Storrar and Rankin[2] HPS report , both evidence widespread contamination of the hospital water system. This contamination is noted to be owing to a wide range of gram-negative waterborne pathogens.

2.3. Dr Mumford's HAD review report [3] , sections 6.12 – 6.27 outline numerous points of evidence pointing to the widespread contamination of the hospital water system, with sections 5.13, 7.3, and 8.17 pertaining to the inadequacy of the ventilation system.

[1] Bundle 21, Volume 1, Document 5, page 180.
[2] Bundle 19, Document 21, page 174.
[3] Bundle 44, Volume 2 - Dr Mumford's Review of HAD Report 2025.

2.4. My 2024 supplementary report[4] noted the magnitude of the difference between the infection rate in wards 2A and post decant to 6A and the overall comparator units' rate over the period 2015-2022, specifically "*The IRR statistic shows that the year the Schiehallion opened – 2015, the magnitude of the difference between the 2A rate versus the comparator units' rate was 3 times. This rose to 18 times in 2018, at the point when patient admissions (among other actions) were restricted and the decant of patients to 6A (primarily) took place. At the time of the decant, the 6A rate was approx. 9 times that of comparator units', dipping to approx. 5, 7 and 8 times over 2019 – 2021, rising to 13 times in 2022 versus comparator units*".

2.5. Furthermore, the report evidences the association between the rate of water contamination and infection rate at the Schiehallion – "*the Pearson's correlation coefficient statistic compares two time series data points over a period of time and provides a figure indicative of how 'associated' these two time series are to each other. Recall that the Pearson's correlation coefficient in section 7.3 was 0.7, indicating a 'moderate – very strong' positive correlation between the trend in Schiehallion infection rates and the trend in water positivity, i.e. contamination. I recalculated the statistic, this time comparing the aggregated 2A and 6A rate against the water positivity figure for the period 2015 – 2019 (see paragraph 2.41 for an explanation of why I excluded the 2020 water positivity figure), with the resulting statistic = 0.6, interpreted as indicating a 'moderation – strong' association between infection rates and water positivity".*

2.6. There is clear evidence, borne out of analysing NHS GGC's own data on infections and activity, specifically that the rate of infections owing to environmental organisms was higher (for certain years) than that of comparator units across the UK, and correlates strongly with the trend in water contamination over 2015 – 2019, from which I surmise that long-term environmental contamination led to the increased rates of infections.

2.7. I am asked for my opinion on whether Paragraph [29] of the Executive Summary

[4] Bundle 21, Volume 1, page 71.

advances the proposition that amongst the paediatric haematology and oncology patients at the RHC and before that at Yorkhill the incidence of BSI Bacteraemia caused by 'environmentally relevant' microorganisms in a particular hospital or patient population is largely the same as the incidence of BSI Bacteraemia caused by microorganisms that are 'not environmentally relevant'.

2.8. In my 2024 Quantitative infection link report [5] as noted in paragraph 2.5 and 2.6 above, I evidenced the 'moderate-very strong' association based on the Pearson's correlation coefficient statistic, between rates of infections in the Schiehallion cohort and concurrent rates of water contamination. Furthermore, section 10.10 of the Quantitative report notes that the evidence presented satisfies all eight of the relevant Bradford Hill postulates, cementing the association between rates of infection caused by environmental organisms and rates of water contamination.

2.9. In my view as a clinical epidemiologist, trends in infections owing to environmental and non-environmentally relevant organisms are not mutually exclusive, that is the two can occur simultaneously, and the existence of one does not limit the other from being possible. In a clinical context, evidence of concurrent infections owing to non-environmentally relevant infections would necessitate a corresponding investigation to uncover the factors driving the cases. If infections from any source – environmental or otherwise – are high, it indicates a problem which ought to be dealt with to mitigate risk to patients. Furthermore, the differences in terms of habitat, pathogenic roles, and biochemical characteristics between environmental and non-environmental gram-negative bacteria make them unsuitable for comparison.

2.10. I am asked for my opinion on the relative merits of occupied bed days versus admission data and to set out my understanding of the consensus amongst medical epidemiologist about the challenges of comparing rates or incidence of bacterial blood stream infections in different groups of hospital patients in

[5] Bundle 21, Volume 1, page 3.

different hospitals and comment on the approach of the HAD Report to this issue.

2.11. The HAD report's use of rate of infections per 1000 bed days is in line with accepted scientific usage when adjusting incidence for activity over time.

2.12. The report makes comparisons between QEUH and other GCC hospitals, and Yorkhill – which is understandable as the first step in contextualising rates of infection, with the expectation, from an epidemiological viewpoint, to then extend the net wider and compare to other large tertiary hospital Trusts, which the HAD report does not do.

2.13. Benchmarking rates with other large hospitals across the UK allows for a) building a relevant frame of reference, i.e. where does the hospital rate in question sit within the context of other similar institutions in the country, b) peer comparisons, reducing biases which can creep into internal comparisons, i.e. structural issues which impact all local sites, c) allows for fair performance evaluation when comparing like patient cohorts across the country, and d) it is the internationally accepted gold standard utilised by UKHSA, ECDC, CDC and the WHO.

2.14. In my expert opinion, internal comparisons between RHC and Yorkhill does not adequately adjust for internal biases within an institution – QEUH, with and does not allow for a fair performance contextualisation of the rates of infection at the RHC. As noted in section 2.53 of my supplementary report [6] the comparator dataset used to contextualise the rates of infection in the Schiehallion cohort comprised of 140,689 admissions, 1232 gram-negative and fungal infections over a period of 8 years, providing a very high level of confidence in the statistic it delivers.

2.15. I've been asked for a formal comparison in tabular form of the categorisation of microorganisms into two classes within Table 1 and Table 2 (pages 60 to 61) with the categorisation of microorganisms in his report at 8.1.16[7], in the various

[6] Bundle 21, Volume 1, Pg 71.
[7] Bundle 21, Document 1, Page 25.

HPS reports from 2019[8] and the list of microorganisms used by Dr Kennedy in his reports based, according to his evidence, on the case definition for the 2018 Water Incident IMT[9].

2.16. Table 1 provides a comparison in tabular form of organisms included in each report noted in section 2.15.

2.17. Caveats – Quantitative report and CNR are environmental pathogen specific, with the HAD and Dr. Kennedy's reports including additional enteric pathogens, and the HPS reports including a much wider set of organisms such as gram-positive and non-environmental bacteria. Only pathogen names explicitly named in the HPS reports have been included in Table 1.

[8] The HPS Situational Assessment, Wards 2A/B, Royal Hospital for Children, NHS Greater Glasgow and Clyde, Appendix 4 (Bundle 7, Document 5, Page 205; The HPS SBAR: To support NHS GGC IMT Mycobacterium chelonae cases and the Incidence of gram-negative bacteraemia in the paediatric haemato-oncology, September 2019 (Bundle 3, Document 16, Page 127); the Draft HPS Review of NHS GG&C Infection Outbreaks in the Paediatric Haemato-oncology Data October 2019 (Bundle 7, Document 6, Page 219) and the final redacted HPS Review of NHS GG&C Infection Outbreaks in the Paediatric Haemato-oncology Data October 2019 - 29 November 2019 (Bundle 7, Document 7, Page 255).
[9] Bundle 6, Document 28, Page 121

Table 1: Tabular comparison of organisms included across the HAD, Quantitative Link, Case Note Review, Dr. Kennedy's and various HPS reports.

* ESBL – Extended spectrum beta-lactamase refers to an enzyme produced by certain bacteria and usually associated with enteric bacteria, i.e. those found in the bowel, and include a range of bacteria.

** Only pathogen names explicitly named in the HPS reports have been included in Table 1.

List of organisms	HAD report	Sid Mookerjee Quantitative report	Case Note Review	Dr. Kennedy report	HPS reports **
Abiotrophia species					Yes
Achromobacter denitrificans	Yes	Yes			
Achromobacter species	Yes	Yes	Yes	Yes	Yes
Achromobacter xylosoxidans				Yes	
Acinetobacter baumannii	Yes	Yes	Yes		
Acinetobacter baumannii complex	Yes	Yes	Yes		
Acinetobacter species	Yes				Yes
Acinetobacter lwofii				Yes	
Acinetobacter ursingii	Yes	Yes	Yes	Yes	
Actinomyces species					Yes
Aerococcus species					Yes
Aeromonas spp.					Yes
Aeromonas hydrophila/caviae	Yes	Yes	Yes		
Bacillus species					Yes
Bacteroides species					Yes
Bifidobacterium species					Yes
Brevibacterium species					Yes
Brevibacillus species					Yes
Brevundimonas species	Yes	Yes	Yes		Yes
Brevundimonas versicularis				Yes	
Burkholderia species					Yes
Burkholderia cepacia	Yes	Yes	Yes	Yes	

Burkholderia cepacia group	Yes	Yes			
Candida species					Yes
Capnocytophaga species					Yes
Cedecea lapagei				Yes	
Chryseobacterium species	Yes	Yes	Yes		Yes
Chryseomonas indologenes	Yes	Yes	Yes	Yes	
Citrobacter species					Yes
Citrobacter braakii	Yes	Yes	Yes		
Citrobacter freundii	Yes	Yes	Yes		
Citrobacter koseri	Yes	Yes	Yes		
Citrobacter youngae	Yes	Yes	Yes		
Clavibacter species					Yes
Clostridium species					Yes
Corynebacterium species					Yes
Cryptococcus neoformans	Yes				
Comamonas testosteroni				Yes	
Cupriavidus species					Yes
Cupriavidus pauculus	Yes	Yes	Yes		
Delftia acidovorans	Yes	Yes	Yes	Yes	Yes
Dermacoccus species					Yes
Dietzia species					Yes
Elizabethkingia meningoseptica	Yes	Yes	Yes	Yes	
Elizabethkingia miricola	Yes	Yes	Yes		
Elizabethkingia species	Yes	Yes	Yes		Yes
Enhydrobacter species					Yes
Enterobacter aerogenes	Yes				
Enterobacter species					Yes
Enterobacter cancerogenus	Yes	Yes			
Enterobacter cloacae	Yes	Yes	Yes	Yes	

Enterobacter cloacae complex	Yes	Yes	Yes		
Enterobacter cloacae ssp cloacae	Yes	Yes	Yes		
Enterobacter hormaechei	Yes	Yes	Yes		
Escherichia species					Yes
Fusobacterium species					Yes
Gemella species					Yes
Granulicatella species					Yes
Haemophilus species					Yes
Kingella species					Yes
Klebsiella oxytoca	Yes	Yes	Yes	Yes	
Klebsiella pneumoniae	Yes	Yes	Yes	Yes	Yes
Kocuria species					Yes
Lactobacillus species					Yes
Lactococcus species					Yes
Leclercia species					Yes
Leuconostoc species					Yes
Microbacterium species					Yes
Micrococcus species					Yes
Moraxella species					Yes
Morganella morganii				Yes	
Mycobacterium species					Yes
Neisseria species					Yes
Paenibacillus species					Yes
Pantoea species	Yes	Yes	Yes		
Pantoea agglomerans				Yes	
Paracoccus sp				Yes	Yes
Propionibacterium species					Yes
Proteus species					Yes
Proteus mirabilis	Yes				

Pseudomonas aeruginosa	Yes	Yes	Yes	Yes	
Pseudomonas chlororaphis				Yes	
Pseudomonas fluorescens				Yes	
Pseudomonas putida	Yes	Yes	Yes	Yes	
Pseudomonas stutzeri	Yes	Yes	Yes		
Pseudomonas oryzihabitans				Yes	
Pseudoxanthomonas Mexicana				Yes	
Raoultella species					Yes
Ralstonia picketii				Yes	
Raoultella planticola	Yes		Yes		
Rhodococcus species					Yes
Rhodotorula species					Yes
Rhizobium species					Yes
Rhizobium radiobacter	Yes	Yes		Yes	
Roseomonas species					Yes
Roseomonas mucosa	Yes	Yes	Yes		
Rothia species					Yes
Rothia mucilaginosa	Yes				
Salmonella species					Yes
Serratia species					Yes
Serratia fonticola				Yes	
Serratia liquefaciens	Yes	Yes	Yes		
Serratia marcescens	Yes	Yes	Yes	Yes	
Shewanella puterfaciens				Yes	
Sphingomonas paucimobilis	Yes	Yes	Yes		
Sphingomonas spp.				Yes	Yes
Staphylococcus species					Yes
Stenotrophomonas species					Yes
Stenotrophomonas maltophilia	Yes	Yes	Yes	Yes	

Streptococcus species					Yes
*ESBL**			Yes		
Herbaspirillum spp			Yes		
Candida albicans		Yes			
Candida fermentati		Yes			
Candida parapsilosis		Yes			
Candida tropicalis		Yes			
Mycobacterium chelonae			Yes		
Rhodotorula mucilaginosa		Yes			
Veillonella species					Yes

2.18. I am asked for my assessment of the methodology set out in section 7.2.2 to 7.2.6 and to compare the approach to that taken by me in my reports for the Inquiry. I am also asked for my views on approach taken to considering the potential for clusters and the factors considered in doing so, identify the source of the bed day data in Table 4 (page 66), and on the adjustment made by the HAD authors owing to "an uncharacteristically low number of bed days" at certain points.

2.19. On scrutiny, the following potential issues points can be noted.

1. There are inconsistencies in the dataset [10] used by the HAD authors. Dr Christine Peters' First Supplementary statement[11] details key inconsistencies, which for the sake of brevity I note in brief alongside additional points.

2. Adult patients designated by consultant are analysed corresponding to areas flagged as BMT, North and South, but data in the 'IPE admission hospital' does not always correspond to data in the 'IPE Admission ward'.

[10] IP Haem onc selected consultants from 2013 by sector. NHS GCC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.
[11] Dr. Christine Peters, First supplementary Witness Statement, 2025.

E.g. in the BMT tab, selecting 'QEUH' under column 'IPE admission hospital', line 2742 notes the 'IPE admission ward' as 'BOC Ward B8 - Haemopoietic Unit', which is a Beatson ward. Another example of this inconsistency is on line 548. Additional examples are provided in Dr. Peters' First Supplementary Statement.

3. Clustering based on presence/absence of whole genome sequencing is problematic considering the evidence regarding the lack of consistency in the range, volume and organisms tested pertaining to water sampling across the QEUH/RHC campus[12]. Furthermore, there is questionable levels of contemporaneous WGS testing to rule clusters in or out [13],[14],[15], calling into question the robustness of the clustering methodology adopted by the HAD authors.

4. The analysis underpinning Figures 1 – 4 includes environmental and non-environmental pathogens, including *Escherichia coli, Staphylococcus epidermidis, Coagulase negative staphylococcus* included alongside *Pseudomonas aeruginosa* (environmental bacteria). Owing to significant differences in habitat, pathogenic roles, and biochemical characteristics between environmental and non-environmental gram-negative bacteria, it is not epidemiological nor clinically sound to combine bacterial incidence in such a manner.

5. Highly specialised area such as BMT should have fewer bed days than non-BMT haemato-oncology, but here South sector has fewer bed days than BMT and the North [16] - 60930 adjusted bed days for BMT, 25417 bed days for the South and 48453 for the North. It would be useful to ask the authors their view on this.

[12] Bundle 21, Volume 1, Document 1, page 3.
[13] Bundle 8, Document 44, page 223.
[14] Bundle 8, Document 45, page 230.
[15] Bundle 8, document 46, page 239.
[16] Adult_Beddays spreadsheet, NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

6. As Dr. Mumford notes in her review [17] – "*The descriptors of the adult patients are confusing, so for instance the BMT group. In the supporting documentation[18] some of the patients included on the list are not patients who would require bone marrow transplantation according to the clinical details provided. For example, disorders of iron metabolism, hereditary spherocytosis and solid tumours. These patients do not have the same risk factors as those with haematological malignancies and are therefore not comparable*".

7. In relation the authors' point "an uncharacteristically low number of bed days" the South sector at the QUEH in September 2016, November 2019 and January 2023", they note adjusting some denominator data which appeared to them lower than the month-on-month average, which is in line with scientific methodology, albeit no reason is provided for the unusually low bed days figures at key points and further calls into question the robustness of the activity datasets used.

8. There are inconsistencies of note in the 'Adult Haem Onc 2013 – 31Aug23 – Matched GMC' spreadsheet [19]. Selecting 'QEUH' under 'Unit location group', there are 1483 rows under column 'Location' relates to SGH – Southern General Hospital. Dr. Peters' first supplementary statement details further examples.

9. On page 67, the authors note that the bacteraemia rate is higher than the Beatson and SGH GGC hospitals and write this off to increase to '*greater immune compromised status… indication of greater testing for bloodstream infection or other policy differences'*. It would be useful to ask the authors why they did not investigate this point further, by comparing the rate of infections over time to other large acute tertiary hospitals, a like to like

[17] Bundle 44, Volume 2, Document 15 – Dr Mumford's Review of HAD Report 2025.
[18] IP Haem onc selected consultants from 2013 by sector, NHS GCC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.
[19] Adult Haem Onc 2013 – 31Aug23 – Matched GMC.xlsx, NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

comparison, akin to undertaken in my Quantitative report [20].

10. Page 68 – 86 relates to adults. Adult and paediatric haemato-oncology patients differ significantly on points such as cancer aggressiveness, with paediatric cancers tending to be more aggressive, paediatric chemotherapy regimens being more intensive, often involving higher doses of certain chemotherapy agents, and differences in prognosis [21], [22], to note a few, I do not see how the adult focused analysis on these pages has any bearing on understanding the question as laid out in 'Direction 5' of the inquiry, which is concerned with rates of infections in the Schiehallion patient population and the role of environment.

2.20. I am asked for my assessment of the methodology set out in section 7.2.7 to 7.2.9. On scrutiny, the following additional potential issues points can be noted.

1. Figure 17, compares Yorkhill and QEUH rates of all bacteraemias, including gram-positive, gram-negative, environmental, non-environmental pathogens. In addition, scrutinising the paediatric data set provided 'RHC Haem onc adms from 2008 with monthly figures 2024-01-09' 23 the analysis combining patient groups who have haematological malignancies, the focus of this inquiry, alongside those with iron metabolism disorders, hereditary spherocytosis, sepsis, general vomiting and diarrhoea, and solid tumours, which is a very heterogenous mix of patients, therefore making the outcomes of the analysis unapplicable to understanding trends of environmentally relevant infections in the Schiehallion cohort.

2. Diluting the Schiehallion patient cohort which moved from Yorkhill to QEUH with additional patient groups, goes onto dilute the outcomes, which are not in keeping with the extensive reporting of infection rates in the Schiehallion

[20] Bundle 21, volume 1, document 1
[21] Stephen E Sallan, 'Myths and Lessons from the Adult/Pediatric Interface in Acute Lymphoblastic Leukemia' (2006) 2006 Hematology 128.
[22] 'Understanding the Differences Between Pediatric & Adult Cancers' (*Pediatric Cancer Research Foundation*, 27 March 2024) <https://pcrf-kids.org/2024/03/27/understanding-the-differences-between-pediatric-adult-cancers/> accessed 9 June 2025.
[23] RHC Haem onc adms from 2008 with monthly figures 2024-01-09, NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

unit at the RHC, with all reports [24] [25] [26] [27] [28] showing that the rates of bacteraemia with gram negative environmental organisms increased following the move of the Schiehallion Unit to RHC, peaking in 2017-18.

3. Internal comparisons within an institution of rates of infection over time are useful as an initial step to understand peaks in infection, but necessitates utilising the 'temporal mean', which is the arithmetic mean of the values and flag changes in infection trends by measuring the variance from the mean value at the minimum. The authors do not provide any statistical output to defend their view purported in paragraph one of section 7.2.8 - ' Incidence starts off at QEUH similar to that observed for the prior 10 years at Yorkhill, but then steadily declines from ~2018 onward'.

4. Analysing the bed days data used by the HAD report [29], hereby referred to as 'RHC BD 2025' specific to ward areas of 2A, 2B, 4B and 6A, and comparing it to the NHS GGC bed days dataset[30], hereby referred to as 'RHC BD 2024' relied on in my 2024 reports, I note stark differences in bed days assigned to wards 2A, 2B and 4B, with 6A bed days remaining fairly consistent, and a 10% decrease in the combined 2A, 2B, 4B and 6A bed days, 41206 in RHC BD 2024 versus 37720 in RHC BD 2025. These differences are set out in Table 2.

[24] Bundle 21, Volume 1, Document 1, page 3.
[25] HPS Report Water Contamination Summary of Incident and Findings - December 2018 Bundle 7, Document 2, page 32.
[26] Bundle 7, Document 7, page 250.
[27] Bundle 6, Document 36, page 795.
[28] Bundle 6, Document 38, page 975.
[29] RHC Haem onc adms from 2008 with monthly figures 09/01/2024, NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.
[30] RHC Haem Onc Admissions from 2015 to 2022, NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

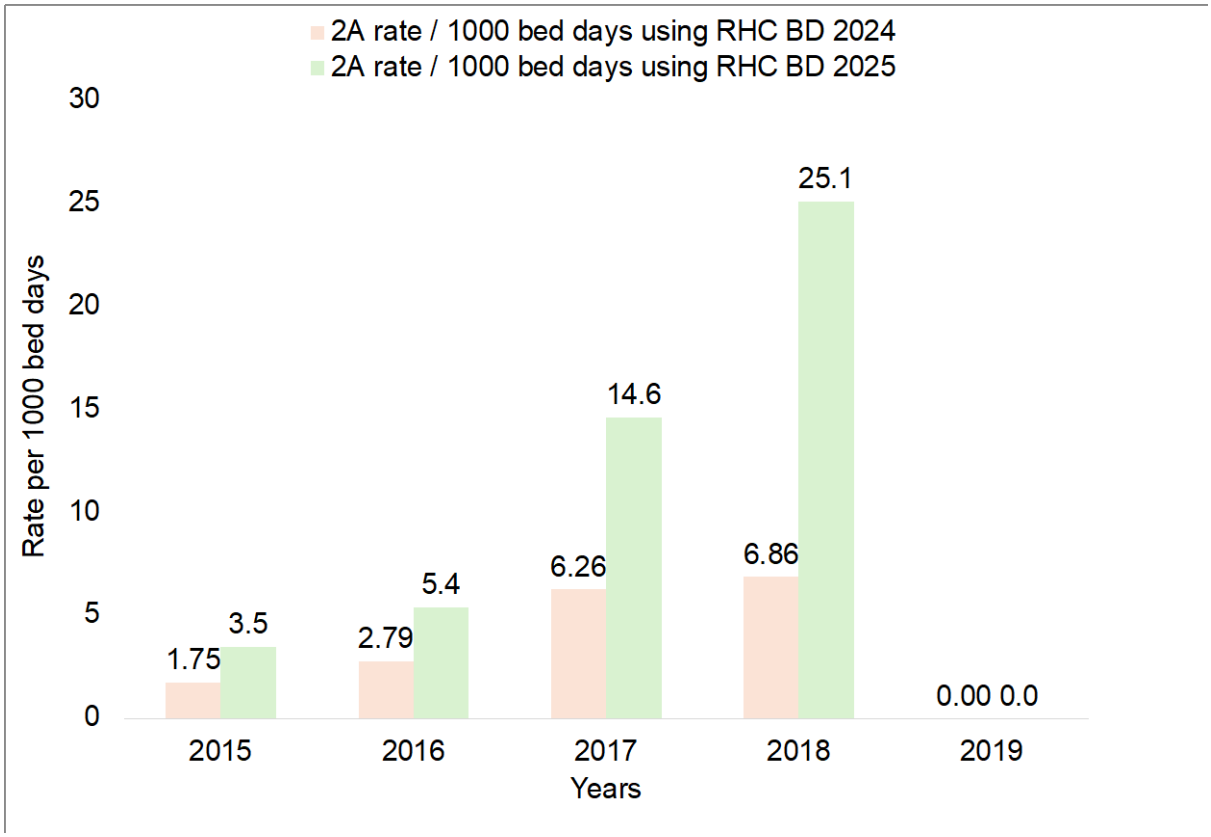
Table 2: Tabular comparison of bed days for wards 2A, 2B, 4B and 6A, RHC BD 2024 versus RHC BD 2025.

Dataset	Ward 4B	Ward 2A	Ward 2B	Ward 6A	Totals
RHC BD 2024	2039	25755	144	13268	41206
RHC BD 2025	855	11605	13271	12689	38420

Table 3: Tabular comparison of rate of infection for ward 2A, RHC BD 2024 versus RHC BD 2025.

Year	Ward 2A infections	Ward 2A, RHC BD 2024	Ward 2A, RHC BD 2025	2A rate / 1000 bed days using RHC BD 2024	2A rate / 1000 bed days using RHC BD 2025
2015	6	3434	1711	1.75	3.5
2016	18	6450	3334	2.79	5.4
2017	46	7344	3145	6.26	14.6
2018	29	4225	1155	6.86	25.1
2019	0	3	19	NA	0.0
2020	0	0		NA	NA
2021	0	0		NA	NA
2022	0	4299	2241	0.00	0.0

Figure 1: Comparison of rate of infections in 2A, RHC BD 2024 versus RHC BD 2025



5. If we are to recalculate the rates of infections in 2A using RHC BD 2025, for the period 2015 – 2022, as noted in Table 3 and illustrated in Figure 1, we see a doubling of the rate of infection for ward 2A over the period 2016 – 2017 and thereafter a tripling for the year 2018, as compared to the rates reported in my supplementary report [31] using RHC BD 2024. It would be valuable to understand why the two bed days datasets differ, and if the HAD authors were aware of the differences.

6. There are inconsistencies when comparing Dr. Drumright's [32] bacteraemia spreadsheet to the original bacteraemia spreadsheet provided by NHS GGC [33]. Table 4 provides a tabular comparison of ward areas which have

[31] Bundle 21, Volume 1, page 71.
[32] Paeds_micro_showing_deduplicated_and14_day_drop. NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.
[33] QEUH campus blood culture samples 1.1.15-31.12.22.xlsx - final version - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

environmental bacteria and fungal specimens linked to them. Note that Dr. Drumright's spreadsheet is missing environmental organisms from five RHC wards, equalling approximately 18 deduplicated environmental organisms. Would be useful to understand what the HAD authors think of this inconsistency and why it is so.

Table 4: Tabular comparison of ward areas which have environmental bacteria and fungal specimens linked to them.

RHC ward areas as they appear on spreadsheets	Dr. Drumright bacteraemia dataset	NHS GGC 2024 bacteraemia dataset
Clinical Decis. Unit RHC	Yes	Yes
Day unit ward 6A	Yes	Yes
ED emerg dept minor RHC	Yes	No
Neonatal ICU RHC	Yes	No
Ward 1C Day Care Un. RHC	No	Yes
Ward 1D paed critical care RHC	Yes	Yes
Ward 1E	No	Yes
Ward 2A	Yes	Yes
Ward 2B day care unit	Yes	Yes
Ward 2B day surgery	Yes	Yes
Ward 2C beds 1-20 RHC	Yes	Yes
Ward 2C beds 21-40 RHC	No	Yes
Ward 3A RHC	Yes	Yes
Ward 3B RHC	Yes	Yes
Ward 3C Renal DialUn RHC	No	Yes
Ward 3C Renal RHC	No	Yes
Ward 3C RHC	Yes	Yes
Ward 6A RHC	Yes	Yes
4BCH Kids BoneMarrTranRHC	Yes	Yes
Clinic 1 OP RHC	Yes	No

2.21. I am asked for my assessment of the methodology underpinning Figure 22 on page 113.

1. We understand as per Dr. Mumford's report [34] - "it would be expected that moving to a brand-new facility from a much older facility there would be a reduction in infections with environmental organisms. From Sid Mookerjee's work it is clear that this was the case for the Schiehallion Unit patients but the numbers of environmental infections rapidly increased to a peak in 2017 and a decline from 2018 due to multiple interventions including, but not limited to, point of use filters on taps, bottled water for washing and teeth cleaning, enhanced cleaning including silver hydrogen peroxide vapour (HPV) decontamination, mobile HEPA filters in patient's rooms and decant away from ward 2A/2B." The year-on-year fluctuations in the rate of infection per 1000 bed day at the RHC reflects this (blue line, Figure 22). Furthermore, it concurs with the Schiehallion overall rate trend graph, Figure 2, in my 2024 supplementary report [35]. Both figures chart an increase in the rate of infections from 2016, less than a year into the opening of the RHC, with the first peak in 2017, succeeded by a trough owing to the key mitigation interventions, particularly the decanting patients from 2A and 2B, to 4B and 6A. Both trends then chart a steep increase in the infection rate before a decline towards 2022. Lastly, the HPS Situational Assessment report [36], page 210 reiterates the rise in infections – "In the 2A/2B Group, the SPC chart shows a shift below the centreline for 17 months from January 2015 to May 2016, then a shift above the centreline from April 2017 to December 2017. The rate was also above the UCL, and therefore higher than expected". This is not in keeping with the authors' assertion that 'BSIs with environmentally relevant organisms are following the same pattern' as noted on page 112.

2. Using the data table underpinning Figure 22, provided by Dr. Drumright [37],

[34] Dr. Mumford HAD report review. 2025
[35] Bundle 21, Volume 1, document 3, page 86.
[36] Bundle 7, Document 5, page 210.
[37] Paeds_bsi_count_beddays_rates. NHS GGC - This document contains patient-identifiable data, therefore it has not been included in a Hearing Bundle.

Table 6 below details the cumulative sum of bed days, environmental bacteraemias, and a rate per 1000 bed days for three periods –

- Yorkhill 2005 to May 2015,
- RHC pre-decant June-2015 to September-2018
- RHC post-decant October-2018 to December-2022

Table 6: Yorkhill and RHC, pre and post decant figures

Indicator	Time period	Cumulative bed days	Cumulative environmental BSIs	BSI rate per 1000 bed days
Yorkhill	Jan 2005 to May 2015	68520	317	4.62
RHC pre-decant	June 2015 to Sept 2018	24363	122	5.00
RHC post-decant	Oct 2018 to Dec 2022	22555	60	2.66

3. Comparing the three periods' rates of infections, we note that the rate of infection at Yorkhill for the period 2005 up-until to move to RHC, rate of 4.62, and the pre-decant RHC rate, 5.00 are very similar, with a incidence rate ratio (IRR) of 0.92, evidence of their similarity. In contrast, the rate post-decant at the RHC, 2.66, and the pre-decant RHC rate, 5.00, are statistically significantly different, with an IRR of 1.88 (95% CI 1.38 – 2.56), evidence of the effect the specific infection mitigation, the decant from 2A, 2B to 4B and then 6A, had on the rate of infection at the RHC.

4. Both the above statistics – the lack of difference between the rate at Yorkhill to the pre-decant RHC rate, and the difference between the pre-and-post decant RHC rate is not in line with the assertions of the HAD authors that the rates follow the same pattern at Yorkhill and the RHC.

2.22. I am asked to consider section 7.1 and whether I can confirm the accuracy of

the reportage of *Pseudomonas aeruginosa* testing results on pages 57 to 58 and to produce a table from the water testing results for the QUEH already analysed setting the number of tests carried out at the QUEH for *Pseudomonas aeruginosa* and the number of positive results for *Pseudomonas aeruginosa* during the whole period of that data set with the results reported on a quarterly basis.

2.23. Table 7 details the *Pseudomonas. spp* positivity rate (%) by quarter for the period Aug-2015 to Jan-2021, taking into account water samples taken in the Schiehallion wards, utilising Dr. Chaput's water sampling data as described in my Quantitative Infection link report [38],alongside the number of positive *Pseudomonas. spp* samples.

2.24. Note as per Dr. Chaput's report [39], specifically Table 1, which states that any count of *Pseudomonas* bacterium found in water sampling from specialist high risk areas, such as the Schiehallion wards, is considered as per national guidance, to be out of spec.

2.25. Table 7 details the rate of *Pseudomonas. spp* results by quarter, for the Schiehallion wards, all of which are high risk areas, with rates of positivity of 2.6% in Q3 of 2015-16, 2.3% in Q1 of 2018-19, 1.6% in Q1 of 2019-20, all of which stand higher than those quoted in section 7.1 of the HAD report, i.e. 1.1% in high-risk areas.

2.26. Dr. Chaput's report, Table 3[40], notes the corrected *Pseudomonas* out of spec % for 2015 and 2016 as 7.95% and 4.87% respectively, albeit I understand this to be representative of the entire QEUH, some of which would have been in high-risk areas.

2.27. In keeping with the clinical opinion that any count in high risk areas, like the haematology-oncology units, is above the accepted threshold, the rates noted

[38] Bundle 21, Volume 1, page 3.
[39] Bundle 18, Volume 1, Document 2, Table 1.
[40] Bundle 18, Volume 1, Document 2, Table 3.

in 2.26 and 2.27 is clear evidence of high levels of contamination.

Table 7: *Pseudomonas. spp* results by quarter, for the Schiehallion wards.

Year-Quarter	Water samples taken (deduped, by quarter)	*Pseudomonas.spp* positive (deduped, by quarter)	*Pseudomonas.spp* positive (positivity rate % by quarter)
2015-16 Q2	2	0	0.0%
2015-16 Q3	78	2	2.6%
2015-16 Q4	47	0	0.0%
2016-17 Q1	0	0	NA
2016-17 Q2	0	0	NA
2016-17 Q3	0	0	NA
2016-17 Q4	56	0	0.0%
2017-18 Q1	0	0	NA
2017-18 Q2	94	0	0.0%
2017-18 Q3	46	0	0.0%
2017-18 Q4	737	4	0.5%
2018-19 Q1	174	4	2.3%
2018-19 Q2	80	0	0.0%
2018-19 Q3	167	0	0.0%
2018-19 Q4	454	4	0.9%
2019-20 Q1	614	10	1.6%
2019-20 Q2	453	5	1.1%
2019-20 Q3	288	3	1.0%
2019-20 Q4	353	3	0.8%
2021-22 Q1	361	0	0.0%
2021-22 Q2	427	0	0.0%
2021-22 Q3	328	1	0.3%
2021-22 Q4	7	0	0.0%

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Acute Lymphoblastic Leukemia in Adults and Children

Session Chair: Charles Linker, MD

Speakers: Stephen E. Sallan, MD; Nicola Gökbuget, MD; and Ching-Hon Pui, MD

Myths and Lessons from the Adult/Pediatric Interface in Acute Lymphoblastic Leukemia

Stephen E. Sallan

The development of effective therapy for children with acute lymphoblastic leukemia (ALL) is one of the great successes of clinical oncology, with long-term survival achieved in over 80% of patients. However, cure rates for adults with ALL remain relatively low, with only 40% of patients cured. With an age-unrestricted, biology-based approach, we anticipate a better understanding about why these outcome differences exist, and think that by extending successful pediatric clinical programs to include adult patients with ALL, we can directly compare uniformly treated adults and children in terms of response to therapy, toxicity and underlying biology.

"The child is the father of the man"…
William Wordsworth

Background

Within childhood acute lymphoblastic leukemia (ALL) populations, older children have had inferior outcomes[1] and within adult ALL populations, younger adults have had superior outcomes.[2,3] In recent reports, "overlapping" populations of older children and young adults have been compared for the likelihood of inducing a complete remission and long-term event-free survival (**Table 1**).[4-8]

None of the clinical trials listed in **Table 1** was designed to be compared to its age-overlapping pediatric or adult counterpart; thus, the comparisons were all retrospective. Although well-recognized age-related dissimilarities in the biology of ALL and event-free survival exist (**Tables 2** and **3**), the more favorable outcomes for older adolescents treated on pediatric regimens in **Table 1** cannot be explained by differences in patient characteristics. Nor would one expect other potential "adult vs. pediatric"

Table 1. Older child/young adult acute lymphoblastic leukemia (ALL) outcomes.

Country	Nat'l Trial	Age in Years	# Pts	CR (%)	5-Yr EFS (%)	Ref.
US:	CCG (P)	16-21	196	96	64*	4
	CALGB (A)		103	93	38*	
France:	FRALLE 93 (P)	15-20	77	94	67	5
	LALA94 (A)		100	83	41	
Holland:	DCOG (P)	15-18	47	98	69	6
	HVON (A)		44	91	34	
UK:	ALL97 (P)	15-17	61	98	65	7
	UKALLXII (A)		67	94	49	
Italy:	AIEOP (P)	14-18	150	94	80**	8
	Gimema (A)		95	89	71**	

Abbreviations: CR, complete remission; EFS, event-free survival; P, pediatric protocol; A, adult protocol
* 6-yr EFS
** 2-year overall survival

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differences (for example, co-morbidities associated with smoking, or host pharmacokinetic differences based on age) to play a substantial role in the findings summarized in **Table 1**.

There are clear differences between current adult and pediatric therapeutic approaches. For example, bone marrow transplantation in first remission is more common for adults (in part, because of the higher incidence of Philadelphia chromosome–positive ALL), and pediatric regimens generally include greater dose density of many chemotherapeutic agents (such as asparaginase, vincristine, corticosteroids and methotrexate).

What are some plausible explanations for these differences between pediatric and adult outcomes? In a thoughtful, provocative and prescient editorial, Dr. Charles Schiffer reflected on biological differences among the hosts and diseases, and also raised issues pertaining to clinical practice, such as the frequency of and familiarity with ALL protocols and care of such patients.[9] In addition to recognizing that ALL is the most common malignancy in children and relatively rare in adult oncology populations, he also noted that most children, but not most adults, with ALL were treated in the context of clinical trials, by experienced support teams, and that many adults were not treated at academic medical centers. The cultural differences between care of pediatric and adult ALL patients, described as "disparities in treating attitudes" by Boissel and co-workers,[5] was highlighted by Schiffer's colorful and accurate description of pediatricians' tight adherence to complex treatment protocols "…with a military precision on the basis of near-religious conviction about the necessity of maintaining prescribed dose and schedule come hell, high water, birthdays, Bastille Day, or Christmas." Although the necessity of such vigor has not been formally tested, the proof of the pudding is in the tasting.

Obstacles to Age-Unrestricted, Biology-based Treatment

Why have adults with ALL not received the same chemotherapeutic regimens as children?

There are two principal hurdles: 1) systems of care, and 2) regulatory impediments.

"Systems of care" and related differences

Referral patterns

Commonly held views suggest that pediatricians refer 15- to 20-year-old patients to pediatric academic medical centers where (a) > 90% of patients are < 15 years old, and b) > 90% of patients with ALL are enrolled on clinical trials.

Similarly, internists frequently refer 15- to 20-year-old patients to adult hospitals, mostly not academic medical centers, where a) > 90% of the patients are > 40 years old and b) most patients with ALL are not entered on clinical trials.[10]

Table 2. Acute lymphoblastic leukemia (ALL): incidence & biological differences.

	Children	Adults
Peak incidence	5 years	50 years
% of Leukemias	80-85%	15%
Chromosomes		
Ph+	3%	30%
MLL	1-2%	7%
TEL/AML1	20%	2%
Hyperdiploid	25%	5%
T-cell	10-15%	20-25%
Mature B	1-2%	3-5%

Table 3. Five-year event-free survival.

	Children	Adults
Pre-B	> 80%	30-40%
T-cell	75-80%	45-55%
Ph+	20-25%	< 10%
MLL	40-50%	20%
TEL/AML1	90%	N/A

Frequency of and familiarity with ALL

Although there might be as many adults with ALL as there are children with the disease[11] (**Figure 1**), the relative frequency of the disease is markedly higher in young children. ALL represents about 15% of all malignancies in 1-15 year olds, 5% in 15-19 year olds, and < 10% of malignancy in > 20 year olds.

Adherence to protocol and mitigating psychosocial factors

Although most of the drugs used for ALL today have existed for over four decades, little is known about their ideal antileukemia dose or the role of individual patient differ-

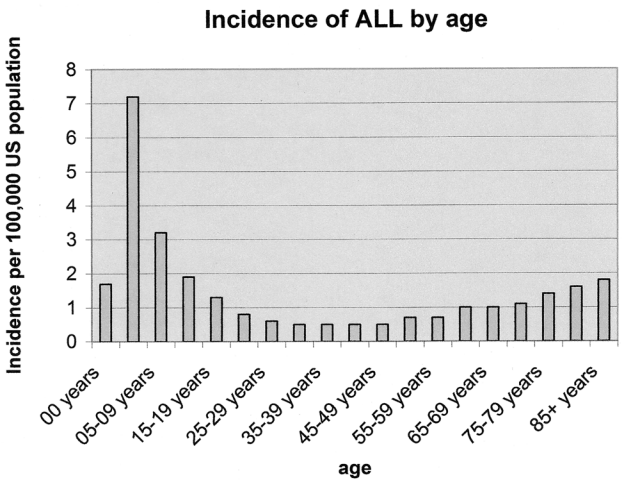

Figure 1. Incidence of acute lymphoblastic leukemia (ALL) by age, SEER 1992-1999.

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ences in drug metabolism. In pediatric practice, "pushing the dose" by using myelosuppression as the endpoint, assures delivery of the maximum dose for each child with each course of chemotherapy. Although it is difficult to assess whether such rigid adherence to protocols is necessary, Boissel and co-workers reported on one "therapeutic practice" measure. They determined the median number of days from the time of initial complete remission until the time of the first postremission chemotherapy: 2 days in the pediatric study and 7 days in the adult study; $P = 0.002$.[5]

Is this time-to-next-treatment measure a medical or psychological problem? Have the older patients fully recovered physiologically, or are there other mitigating features (e.g., "I need some time with my family") resulting in delays in therapy?

In the pediatric setting, in addition to the meticulous attention to detail by the pediatricians, a primary driver of subsequent therapy is the caretaker, usually the mother. ("What are we waiting for? The leukemia is growing back. Let's get going!")

Difficulties in comparisons

"Simple" apples-to-apples comparisons in a heterogenous disease such as ALL can be difficult, and occult differences in practices among internists and pediatricians might not be appreciated.

For example, **Table 1** demonstrates comparisons in the percentage of complete remissions between older children and adults. Not shown is the time to enter complete remission. All pediatric trials report complete remission at the end of approximately 1 month of multiagent chemotherapy. However, many adult trials report on the complete remission rates after 2 months of treatment (**Table 4**). Although the time to attain a complete remission was not of prognostic importance in at least one adult trial,[12] we found that in childhood ALL, the time to enter a complete remission had significant implications, and the difference between a complete remission in 1 month or 2 months was a matter of life or death for the majority of slow responders.[15]

Regulatory obstacles

No diseases begin or end at age 18 years. But current review systems of clinical trials (e.g., Institutional Review Boards) and funding sources (e.g., cooperative groups and pharmaceutical company-sponsored trials) frequently establish "biologically arbitrary" age limits for protocol eligibility and evaluation. Thus, diseases such as ALL, whose biology extends across a broad age spectrum, are usually approached as two diseases: childhood ALL (patients < 18 years old), and adult ALL (patients ≥ 18 years old). Such "age-restricted" criteria have played an integral role in our current understanding of the disease and its treatment. However, this approach, based on the single differentiating measure—patient age—has until recently hampered clinical research across a broader spectrum of the disease. Although likely a meaningful host variable, age, *per se*, is highly unlikely to trump leukemia biology or the impact of an individual treatment regimen. Overcoming such well-institutionalized barriers to research remains a formidable challenge.

Recently, the Dana-Farber Pediatric ALL Consortium, a group of nine collaborating pediatric centers with a successful record of ALL research and treatment,[16] joined together with nine adult institutions to form a Dana-Farber Combined Adult/Pediatric ALL Consortium.

In anticipation of a common approach to the over 18-year-old patients, we evaluated our oldest pediatric ALL population—those aged 15-18 years old.[17] Among 844 patients treated on two consecutive protocols (Dana-Farber 91-01 and 95-01) between 1991-2000, we found 51 (6%) were in the 15- to 18-year-old population. Statistically significant biological characteristics at the time of diagnosis included a higher proportion with T-cell ALL and a lower proportion with hyperdiploidy or the TEL/AML1 translocation compared to younger patients. Except for a lower incidence of complete remissions (1-14 year olds, 98% vs. 15-18 year olds, 94%; $P = 0.01$), there were no other statistically significant differences with regard to 5-year event-free survival (> 1-10 years old, 85%; > 10-15 years old, 77%; and >15-18 years old, 78%; $P = 0.10$). Patients 10 years old and older had more asparaginase-related pancreatitis and thrombosis, but there were no differences between the 10- to 14-year-old and 15- to 18-year-old populations. An updated analysis of the efficacy of our pediatric regimen for adult patients, 18-50 years old, appears as a 2006 American Society of Hematology abstract (DeAngelo et al).

We are now poised to investigate the biology of ALL and its treatment across a broad age spectrum, and to have common treatment regimens for common disease among patients ages 1-50 years old.

From our DFCI Combined Adult/Pediatric ALL Consortium, many myths have surfaced, and many lessons have been (and are being) learned.

Table 4. Time to complete remission in adult acute lymphoblastic leukemia (ALL) trials.

Study	% CR after One Cycle of Chemo	% CR after Two Cycles of Chemo
UKALLXII/ECOG[12]	51%	91%
CALGB 8811[3]	62%	86%
LALA-94[13]	72%	84%
Hyper-CVAD[2]	81%	92%
DFCI pilot[14]	82%	N/A

Myths and Lessons

Myth #1. It cannot be done!

You cannot have a common approach to therapy for children and adults. The disease biology, medical practices, regulations and cultures are incompatible with a uniform approach to clinical research.

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Lesson #1. It can be done!
(But it isn't easy.) Finding common ground requires time, communication and compromise. We are confident that our work within the DFCI Combined Adult/Pediatric ALL Consortium, as well as similar efforts by others who are striving toward the same goals—improved outcome for all, diminished toxicity and a better understanding of leukemia biology—will be rewarding and result in lives saved and quality years of life added.

Myth #2. Adults do not tolerate asparaginase.
It is generally assumed that adults are less tolerant to the acute and cumulative side effects of antileukemia drugs, and asparaginase, a drug used in all pediatric ALL regimens, is often cited as a prime example.

Lesson #2. Our initial adult protocol, a feasibility trial using one arm of our pediatric ALL regimen, includes 30 weeks of high-dose asparaginase therapy. Adults ages 18-50 years old and children ages 10-18 years old tolerate multiple weekly asparaginase doses (≥ 12,500 IU/m^2) with similar incidences of allergic reactions, pancreatitis and thrombosis.

Myth #3. Adults have a high rate of therapy-related deaths.
Some would argue against initiating combined adult/pediatric clinical trials because the remission mortality in children (now in the 1-2% range) is far less than in adults.

Lesson #3. In fact, the experience of Dutch[6] and British[7] investigators suggests that it is the practice of bone marrow transplantation in first remission for non-Philadelphia chromosome–positive ALL that accounts for the increased incidence of therapy-related deaths. Given the paucity of data supporting the superiority of bone marrow transplantation in first remission, many now agree that use of chemotherapy alone would result in both improved outcomes and in a lower incidence of therapy-related deaths. Our DFCI adult pilot protocol and mature pediatric protocols have ~1% incidence of therapy-related deaths.

Myth #4. Adults don't have asymptomatic central nervous system (CNS) leukemia at diagnosis.
And because they don't, the practice of routine sampling of CSF at the time of diagnosis in asymptomatic patients is variable among adult treatment regimens.

Lesson #4. ALL is always a systemic disease. About 2-3% of children and adults have symptomatic CNS disease at diagnosis and an additional 5-10% of asymptomatic children and 5% of adults have lymphoblasts in their CSF disease at time of diagnosis. Routine lumbar puncture at the time of diagnosis and prior to initiating systemic chemotherapy, as well as routine CNS treatment, should be part of ALL management for all patients. An argument against this practice, that one might inadvertently introduce blasts into the CSF, can be readily mitigated by the routine use of intrathecal chemotherapy at the time of the initial diagnostic lumbar puncture.

Myth #5. Who needs a parent or caregiver?
The patient and his or her physician are sufficient for optimal care and outcomes.

Lesson #5. Never underestimate the value of a caregiver! The best motivated patient and physician can have their well-intended treatments and outcomes enhanced by surrounding themselves with "a mother figure"; in fact, parental surrogates, committed family members and loved ones, as well as an extended supportive team of professionals (especially nurses), are likely to assure optimal adherence to protocol, emotional support, and (hopefully) better outcomes.

Conclusion

Retrospective analyses clearly demonstrate that current pediatric therapeutic regimens are more effective than adult regimens for 16- to 21-year-old patients. Current and future clinical trials will focus on "the ages of uncertainty": the 21- to 50-year-old population. Age-unrestricted, biology-based therapy should be the standard of all patients with ALL. Pediatricians and internists treating patients with ALL have much to learn from one another. Such efforts are likely to result in more cures and a higher quality of life among the survivors.

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SCOTTISH HOSPITALS INQUIRY

Review of the Expert Report for the Scottish Hospitals Inquiry on the evidence of risk of infection from the water and ventilation systems at the Queen Elizabeth University Hospital and Royal Hospital for Children, Glasgow

written by Professor PM Hawkey, Dr SG Agrawal and Dr LN Drumright

regarding *Aspergillus* infection

Expert Report prepared for the Scottish Hospitals Inquiry

Date: 4 June 2025

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1. Introduction

1.1. This report has been prepared at the request of the Scottish Hospitals Inquiry and reviews the report of Professor Hawkey and colleagues, hereon referred to as the HAD report, with respect to Chapters 6 and 8 from the different perspectives of a Microbiologist and Director of IPC, and a medical epidemiologist.

1.2. The Inquiry has asked a series of questions in order to assist in this review.

2. Review of the HAD report

2.1. The purpose of enhanced ventilation in hospital settings is to control the patient environment and to minimise the risk of hospital acquired infection. Ventilation with the correct number of air changes per hour helps to dilute and remove airborne contaminants such as viruses, bacteria and pollutants creating a healthier environment for patients and staff. The removal of airborne micro-organisms reduces the risk of cross infection between patients and healthcare workers. A filtered air supply will also mitigate the risk of infection owing to recognised airborne pathogens such as *Aspergillus*, *Cryptococcus* and tuberculosis. A constant supply of fresh air provides a more comfortable environment for the patient. Overall providing ventilation which addresses these factors can lead to better patient outcomes by reducing the risk of healthcare associated infections.

2.2. At point 6.1, the authors of the HAD report conclude that the *"risk of infection arising from an "inadequate or insufficient" ventilation system would not be equal across all patient populations" and "would be most visible in these patients and one of the highest-risk populations is the group of patients with haematological, and other, cancers undergoing intensive chemotherapy and/or haematopoietic stem cell transplants (HSCT)".* Paragraph 6.1 of the HAD report relates to airborne diseases which the authors state are Aspergillosis, tuberculosis and viral infections. In principle we agree with this statement in that the most vulnerable patients; those with immunosuppression whether due to disease or treatment with corticosteroids, those in intensive care, cystic fibrosis patients, etc, would be most likely to acquire infection as a result of inadequate ventilation. However, as described in Dr Mumford and Ms

Dempster's earlier expert report[1] to the inquiry, the insufficient ventilation in ward 2A/2B contributed to the high levels of infection with environmental organisms, due to the failure to dilute and remove these, resulting in environmental contamination, and when looking at increases in infections this cannot be assumed to be restricted to typical airborne pathogens. As a conclusion to the preceding paragraphs in 6.1, this statement is, in our expert opinion, incomplete and fails to take into account the wider context of infection prevention mitigation which ventilation provides.

2.3. Aspergillosis is undoubtedly one of the potential consequences of inadequate ventilation in a healthcare facility. However, as described above it is not the only indicator. The epidemiology of Aspergillosis is multifactorial.

2.4. *Aspergillus spp* are ubiquitous in the environment and cause disease which often goes undiagnosed[2]. It is one of the most underdiagnosed infections in intensive care units[3]. An estimate of the burden of adult invasive aspergillosis[4] (IA) in the UK identified high risk populations as BMT patients, solid organ transplants, patients living with HIV, haematological malignancies, chronic obstructive pulmonary disease with emergency hospital admission, critical care patients, and patients with lung cancer. Critical care patients were identified as the largest at-risk group. Allergic bronchopulmonary aspergillosis complicates asthma and cystic fibrosis.

2.5. The estimated incidence of invasive aspergillosis is highest (8.1%) in allogenic bone marrow transplant patients, with AML patients (7.1%) and heart transplant patients (4.8%) also seeing high rates. Overall the numbers of infections annually in the UK range from 2901-2912 (excluding ITU patients), an overall rate of 4.59-4.61 per 100,000 population. The numbers of invasive aspergillosis cases in critical care patients is estimated to range from 387-1345, a rate of 0.61-2.13 per 100 000 population[5].

[1] Bundle 21, volume 1, paper 3, page 96.
[2] Pegorie M et al. Estimating the burden of invasive and serious fungal disease in the United Kingdom. Journal of Infection (2017) 74, 60-71.
[3] B Winters et al. Diagnostic Errors in the intensive care unit: a systemic review of autopsy studies. BMJ Qual Saf 2012;21:894–902.
[4] Pegorie M et al. Estimating the burden of invasive and serious fungal disease in the United Kingdom. Journal of Infection (2017) 74, 60-71.
[5] Pegorie M et al. Estimating the burden of invasive and serious fungal disease in the United Kingdom. Journal of Infection (2017) 74, 60-71.

2.6. In a study of invasive fungal infections in patients with haematological malignancy[6], 69% of invasive fungal infections occurred in patients with AML. Over half of all infections (346/538) were caused by moulds and 90% of mould infections were caused by *Aspergillus spp,* indicating that *Aspergillus spp* is the commonest invasive fungal pathogen in patients with haematological malignancies.

2.7. The function of enhanced ventilation is not only to filter the incoming air, but also to dilute any contaminants in the air, provide fresh air and environmental comfort. The failure to dilute contaminants in the air is, in our expert opinion, more likely to result in increased infection rates generally in immunocompromised patients than an observable increase in invasive aspergillus, only detected retrospectively with statistical analysis due to the small numbers of aspergillus infections seen in this group of patients. This is confounded further by the use of prophylactic anti-fungal drugs in patients perceived to be at risk of IA where ventilation is deemed to be inadequate. As a result, the observed incidence of invasive aspergillosis can be assumed to be an underestimate of the true risk of infection due to inadequate ventilation.

2.8. Aspergillus spores are ubiquitous in the environment. It is well recognised that some patients become colonised prior to their diagnosis and treatment for haematological malignancy. Stringent infection prevention and control and ventilation measures are put in place to prevent nosocomial (as opposed to endogenous) aspergillus infection in these patients. High quality enhanced ventilation with HEPA filtration of incoming air, high air changes of 12 air changes per hour (ach), and 6 Pa of positive pressure to the corridor are collectively required to minimise the risk of infection. In addition, prophylactic anti-fungal antimicrobials are used to minimise the risk of endogenous and nosocomial infection.

2.9. The upgraded ventilation on 4B provided HEPA filtration to the rooms only, 5.8-8.9 ach, and 6.6-13.9 Pa of positive pressure to the corridor. The changes provided an improved level of protection for the patients on 4B. 4C continued to have the same provision of ventilation as was standard across other wards, 2.8-3.3 ach, no HEPA filtration (mobile HEPA units in use), chilled beams and neutral to +1 Pa positive pressure[7] [8]. Although mobile HEPA units were used both in 4C for adult haematology

[6] Pagano L et al. The epidemiology of fungal infections in patients with haematological malignancies: the SEIFEM-2004 study. Haematologica 2006; 91:1068-1075.
[7] Bundle 21, Volume 1, document 8, page 611.
[8] Bundle 21, Volume 1, document 7, page 468.

oncology patients and in 2A / 6A for the paediatric haematology oncology patients, these have been found to have an insignificant effect on the incidence of IA in neutropenic haematology oncology patients[9]. Concentrations of airborne aspergillus spores even below 1 colony forming unit per cubic metre (cfu/m^3) have been shown to be sufficient to cause outbreaks in immunocompromised patients[10].

2.10. The risk of acquiring invasive aspergillosis in both adult and paediatric patients depends on their underlying disease and treatment (BMT, solid organ transplant, chemotherapy). In children, the highest risk is for those who undergo bone marrow transplant (BMT), with the greatest risk in those where the underlying disease is acute myeloblastic anaemia[11]. In adults, the highest risk is in those undergoing BMT. However, determining the comparative risk is complex. Children are thought to be more at risk, however the incidence of IA in both adults and children is increasing over time[12].

2.11. The methodology applied to case selection adopted in Chapter 8 of the HAD Report seems reasonable in the absence of clinical notes to confirm the working diagnosis and response to treatment which would normally be used by clinicians caring for a patient in making their judgement as to likelihood of infection.

2.12. Section 8.1.3 of the HAD report, Figure 24 provides details the incidence, i.e. case numbers only of *Aspergillus. spp* in the paediatric haemato-oncology population. A rate per 100,000 bed days or similarly appropriate denominator should have been calculated by the authors to account for any changes in activity over the period 2005 – 2022 and highlight peaks in incidence rate.

2.13. The definitions cited at 8.1 of the HAD report are consensus definitions agreed by the European Organisation for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group Education and Research Consortium (MSGERC)[13].

[9] Engelhart S et al. Impact of portable air filtration units on exposure of haematology-oncology patients to airborne *Aspergillus funigatus* spores under field conditions. Journal of Hospital Infection (2003) 54, 300-304.
[10] Vonberg R-P et al. Nosocomial aspergillosis in outbreak settings. Journal of Hospital Infection (2006) 63, 246-254.
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2.14. The definition of 'Proven' for *Aspergillus spp* involves recovery of the organism from a normally sterile site. In the guidance this specifically excludes broncho-alveolar lavage (BAL), paranasal or mastoid sinus cavity aspirate and urine. It could include a blood culture, however fungaemia due to *Aspergillus spp* is very rare and this is an unlikely source of a positive culture. Other sites where the organism might be recovered from include the cerebro-spinal fluid (CSF), heart, bones, soft tissues, kidney etc depending on the extent of the infection.

2.15. 'Probable' infection relies on host factors being present which confirm that he patient is immunocompromised, for example recent history of neutropenia, BMT at any time, haematological malignancy, corticosteroid use etc, combined with clinical features such as imaging evidence, plus mycological evidence, either culture for sites such as BAL or sinus aspiration OR antigen detection in plasma, serum, BAL or CSF. Beta D-glucan is not considered as mycological evidence here and the paediatric patients who had this as their only laboratory result on the data sheet provided did not have radiological evidence of fungal infection according to the imaging reports seen by us.

2.16. No blood cultures on the data set provided by NHS GGC grew *Aspergillus spp*. None of the cases identified in the Qualitative Report[14] were identified by blood cultures. PCR and aspergillus serology are done on blood samples and other clinical samples such as broncho-alveolar lavage.

2.17. The cases discussed in the earlier report from Dr Mumford and Ms Dempster[15] are identifiable from the data set provided. Incident Management Team (IMT) meetings were held for four cases of invasive aspergillosis.

2.17.1. IMT 05 August 2016[16] was held for two cases, one confirmed negative on admission screening and subsequently culture positive and PCR positive. The second case was positive on PCR one month later but was also known to have *candida tropicalis* infection. Both cases were identified as hospital acquired at the IMT. Issues with the pressure gradient, a torn ventilation duct, lack of HEPA filtration and ongoing construction on the QEUH campus were identified as risk factors. Actions from the IMT included environmental sampling, the introduction

[14] Bundle 21, volume 1, paper 3, page 96.
[15] Bundle 21, volume 1, document 3.
[16] Bundle 1, document 6, page 22.

of portable HEPA filters, anti-fungal prophylaxis to be given to at risk patients and enhanced cleaning.

2.17.2. IMT 07 March 2017[17] was held for an additional two cases and included an update on the patients from the previous IMT. The second case from the previous IMT was no longer classed as a case under EORTC classification. The other three patients are all classified as probable cases. The first of the two new cases was diagnosed on 23 Feb 2017 by PCR and the second diagnosed on 9 Feb 2017 by imaging only. No laboratory tests were done for the second case which is an additional case for 2017 not noted in the HAD report. At this IMT it was noted that the air quality conditions in the now closed Yorkhill Schiehallion unit were the same as those on 2A. The level of construction on the QEUH site was also noted. Actions from this meeting included giving antifungal prophylaxis to all patients, the wearing of masks when leaving the ward, air sampling and to undertake targeted water sampling. Concern was voiced that a new chemotherapy regime may be increasing the vulnerability of patients. A similar effect was being experienced in Lothian.

2.17.3. IMT 04/02/2019[18] was held for the ongoing cryptococcus cases. A patient was reported as *Aspergillus* PCR positive on CDU. Other investigations were normal (CT and ultrasound) and it was agreed that this patient was unlikely to be a case of aspergillosis. This patient was moved from 6A to CDU due to the mould issues identified.

2.17.4. IMT 01/08/2019[19] was held for the ongoing cryptococcus investigation. It was noted that air samples from patient's bathrooms on 6A taken on 15 July 2019 grew *Aspergillus spp*.

2.17.5. IMT 06/09/2019[20] was held for the ongoing gram-negative bacteraemia cases. It was noted that aspergillus had been isolated from air samples taken from the bathrooms on 6A. Mobile HEPA filtration was installed as a result.

[17] Bundle 1, document 9, page 35.
[18] Bundle 1, document 68, page 303.
[19] Bundle 1, document 75, page 334.
[20] Bundle 1, document 79, page 354.

2.18. In addition, Problem Assessment Group (PAG) meetings were held for further cases of aspergillus.

2.18.1. PAG 27/10/17[21] was held for a patient on 2A with *A. fumigatus* isolated from a broncho-alveolar lavage.

2.18.2. PAG 20/07/2018[22] was held for a case of aspergillosis on ward 2A classified as a probable infection.

2.19. It is unlikely that the decant of the ward from 2A/B to 6A and 4B would be an added risk factor for increased rates of invasive aspergillus infection. Patients would routinely leave the ward for investigations such as imaging so a move through the hospital would not be an unusual occurrence. For the BMT patients, they were moving to what was considered a safer environment on 4B so their risk would be expected to decrease as a result of the move. If surgical face masks were worn during the transfer, this would provide additional protection.

2.20. The paediatric cases from 2005 to 2015 and their location in Yorkhill by date and method of diagnosis are shown in the table below. One case on the data sheet was excluded as aged 19 on the date of diagnosis in 2014. The numbers of cases for 2010 differ from those in the HAD report in that the patient diagnosed on 30/12/2009 was tested again on 9/02/2010 during the same admission[23] having moved to ITU and therefore the second tests were classed as the same infection episode. This reduces the cases shown in Table 24 of the HAD report by one case in 2010 and one case in 2014.

[21] Bundle 2, document 25, page 66.
[22] Bundle 2, document 40, page 105.
[23] The HAD report case definition de-duplicated on the basis that 'where the same patient had multiple positive tests for the same admission episode, this was classified as one case'.

Table 4: Yorkhill *Aspergillus. spp* cases, 2005 – 2015

Location	Specimen type	Date collected	Investigation
Schiehallion YOR	Bronchio Alveolar Lavage	09/06/2005	ASP CULTURE
O.P.D. YOR	Sputum	16/10/2005	ASP CULTURE
Schiehallion YOR	Bronchio Alveolar Lavage	03/01/2008	ASP CULTURE
Schiehallion YOR	Bronchio Alveolar Lavage	27/11/2008	ASP CULTURE
Schiehallion YOR	Sputum	11/12/2008	ASP CULTURE
Schiehallion YOR	Sputum	02/04/2009	ASP CULTURE
Schiehallion YOR	Clotted Blood	30/12/2009	PCR
I.T.U. YOR	Clotted Blood	22/11/2010	PCR
I.T.U. YOR	Clotted Blood	03/02/2011	PCR
High Dependency Unit YOR	Bronchio Alveolar Lavage	30/03/2011	PCR
Schiehallion Ward YOR	Sputum	08/10/2012	ASP CULTURE
Schiehallion DCU YOR	Clotted Blood	16/04/2013	ASP SEROLOGY
ITU YOR	Clotted Blood	18/06/2013	PCR
Schiehallion Ward YOR	Clotted Blood	21/10/2013	ASP SEROLOGY
Schiehallion Ward YOR	Clotted Blood	18/12/2013	ASP SEROLOGY
Schiehallion Ward YOR	Clotted Blood	07/01/2014	ASP SEROLOGY
Schiehallion Ward YOR	Clotted Blood	29/12/2014	PCR

2.21. Consultant names are shown in the data set for *Aspergillus spp*.[24] All shown are consultants in paediatric haematology or paediatric oncology except Dr Claudio Giacomozzi (consultant in paediatric endocrinology).

2.22. The data set for *Aspergillus spp*. cases in the paediatric population does not contain any admission data. However, it does contain CHI numbers which allow cross referencing to the data set provided for paediatric haemato-oncology admissions[25]. The following details are noted:

[24] Complete Aspergillus Positives, Adults 2013-2023 & Paeds 2005 – 2022 provided by NHS GGC - This document contains datasets relied upon by the HAD Expert group, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.
[25] RHC Haem onc adms by episode Subtype.xlsx provided by NHS GGC - This document contains datasets relied upon by the HAD Expert group, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.

- The 2015 case was not admitted to Yorkhill at any time
- None of the 2018 cases were inpatients at the time of the move to 6A and subsequently diagnosed.
- One patient had been an inpatient during the decant from 2A to 6A in September 2018 but was not diagnosed until November 2019
- One 2019 case diagnosed on 1 Feb 2019 was moved to the CDU for part of their admission and was on the CDU at the time of diagnosis.
- One 2019 case was initially admitted to CDU on 21 Feb 2019 prior to being moved to 6A, however this was after the ward had moved back on 11 Feb 2019. The patient was on ward 6A when diagnosed on 27 Feb 2019.
- No other cases were involved in any of the ward moves.

2.23. From the data provided [26] [27] it can be derived that from 2008 to June 2015 at Yorkhill and June 2015 to 2022 at RHC, there were 53 paediatric haemato-oncology patients with positive tests for Aspergillus infection. A number of cases were excluded from further analysis, 2 excluded by their age (over 18 at the point of diagnosis), 4 excluded as they only appeared on the aspergillus positive spreadsheet and could not be cross referenced by CHI number on the admissions spreadsheet, and 5 excluded as they were only positive on a non-specific test (Beta D-glucan antigen). Of the remaining 43 patients, 8 had acute myeloblastic leukaemia (AML), 5 prior to the move to RHC and 3 post-move.

2.24. Based on the admission information, a total of 71 individual patients with AML had admissions to either Yorkhill (41) or RHC (30) during this period of time. By cross checking the CHI numbers the percentage of AML patients who were diagnosed with

[26] RHC Haem onc adms by episode Subtype.xlsx provided by NHS GGC - This document contains datasets relied upon by the HAD Expert group, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.
[27] Complete Aspergillus positives, Adults 2013-2023 & paeds 2005-2022.xlsx provided by NHS GGC - This document contains datasets relied upon by the HAD Expert group, and was provided to the Inquiry by NHS Greater Glasgow & Clyde on 18 February 2025. As it contains patient-identifiable data, it has not been included in a Hearing Bundle.

aspergillus at each hospital can be determined. A similar calculation was completed for patients with acute lymphoblastic leukaemia (ALL)

2.25. The expected rate of invasive aspergillus in paediatric AML patients is around 5.35% and 1.5% for ALL[28]. The table below shows the findings from the analysis of the data. It was not possible to determine with any confidence which patients had bone marrow transplant prior to their aspergillosis infection due to insufficient data.

Table 5: *Aspergillus. spp* cases by patient diagnosis

Diagnosis	Hospital	Number of cases	Number of Invasive Aspergillosis	Percentage of patients with IA
AML	**Yorkhill**	**41**	**5**	**12.1%**
	RHC	**30**	**3**	**10%**
ALL	**Yorkhill**	**227**	**6**	**2.6%**
	RHC	**174**	**18**	**10.3%**

2.26. Although the rates in each hospital are very similar for AML patients they are demonstrably higher than the expected rates. For ALL patients the rate of IA is much higher at RHC than that seen at Yorkhill and is higher than the AML rate. AML is accepted as having a higher risk for IA than ALL[29] [30].

2.27. A prospective survey of adults with haematological malignancies conducted from 2004 to 2009 [31] reported an overall IA incidence of 1.6 per 1,000 patient-days. Specifically, the incidence was 1.9 per 1,000 patient-days among acute myeloid leukaemia (AML) patients and 1.3 per 1,000 patient-days among acute lymphoblastic leukaemia (ALL) patients. It would be expected that a similar paediatric population would see a higher

[28] Crassard N et al. Invasive Aspergillosis in a paediatric haematology department: a 15-year review. Mycoses 51, 109-116
[29] Pagano L et al. The epidemiology of fungal infections in patients with haematological malignancies: the SEIFEM-2004 study. Haematologica 2006; 91:1068-1075
[30] Pegorie M et al. Estimating the burden of invasive and serious fungal disease in the United Kingdom. Journal of Infection (2017) 74, 60-71
[31] Nicolle M-C et al, 'Invasive Aspergillosis in Patients with Hematologic Malignancies: Incidence and Description of 127 Cases Enrolled in a Single Institution Prospective Survey from 2004 to 2009' (2011) 96 Haematologica 1685.

rate of IA. It should also be noted that this study did not include patients who underwent bone marrow transplant which is known to increase the risk of infection.

2.28. Using the data on *Aspergillus, spp* cases as per NHS GGC's 'COMPLETE ASPERGILLUS POSITIVES, ADULTS 2013-2023 & PAEDS 2005 deduplicated for paeds- 2022' spreadsheet and admission data – NHS GGC's 'RHC Haem onc adms from 2008 with monthly figures 2024-01-09', the rate of *Aspergillus.spp* infections for the period 2008 – 2022 has been calculated, in line with admission data ranging from 2008 – 2022.

2.29. Table 1 provides the *Aspergillus. spp* incidence for wards which had at least one case over the period 2008 – 2022 and had corresponding bed days data. Two wards which had one case each, Clinic 1 OP RHC and CritCar Un4 HDU 31-40 QEUH, did not have corresponding bed days data, and were therefore excluded from the analysis. Table 2 provides the corresponding bed days for the wards in Table 1. Table 3 details *Aspergillus spp* incidence rate by incidence wards for the period 2008 – 2022, alongside individual trend graphs to aid an understanding of peak rates and when they were recorded.

Table 1: *Aspergillus spp* incidence, Yorkhill and RHC wards over 2008 - 2022

Wards with *Aspergillus* cases + corresponding bed days	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
4BCH Kids BoneMarrTranRHC													1		
Clinical Decis. Unit RHC												1			
High Dependency Unit YOR				1											
ITU YOR			1	1		1									
Schiehall DaySur Wd2B RHC											1				1
Schiehallion YOR	3	2			1	2	2								
Schiehallion DCU YOR						1									
Ward 1D Paed CritCar RHC									2			1			
Ward 2A RHC								1	2	7	1				8
Ward 6A QEUH											1	5	2	2	
TOTALs	3	2	1	2	1	4	2	1	4	7	3	7	3	2	9

Table 2: Bed days, Yorkhill and RHC wards over 2008 - 2022

Bed days	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
4BCH Kids BoneMarrTranRHC		12				7	1				91	466	220	52	26
Clinical Decis. Unit RHC								9	115	931	764	499	983	1208	1170
High Dependency Unit YOR				37											
ITU YOR	204	7	35	118	8										
Schiehall DaySur Wd2B RHC								1775	3004	3975	3062				1455
Schiehallion YOR	3735	3410	2947	2263	3280	2199	2495	1065							
Schiehallion DCU YOR	3303	3363	3654	2855	3412	2946	3533	1553							
Ward 1D Paed CritCar RHC								67	172	334	37	10	48	192	11
Ward 2A RHC								1711	3334	3145	1155	19			2241
Ward 6A QEUH		2			9	10	5				1630	4277	2920	3504	358
TOTALs	7242	6794	6636	5273	6709	5162	6034	6180	6625	8385	6739	5271	4171	4956	5261

2.30 In Table 3, rates of *Aspergillus. spp* which exceed an incidence rate of 1.3 per 1000 bed-days (equivalent to patient-days) have been highlighted in red. Six of the ten wards at Yorkhill and RHC which saw at least one *Aspergillus. spp* infection and corresponding bed days data, have rates in excess of 1.3 per 1000 bed-days.

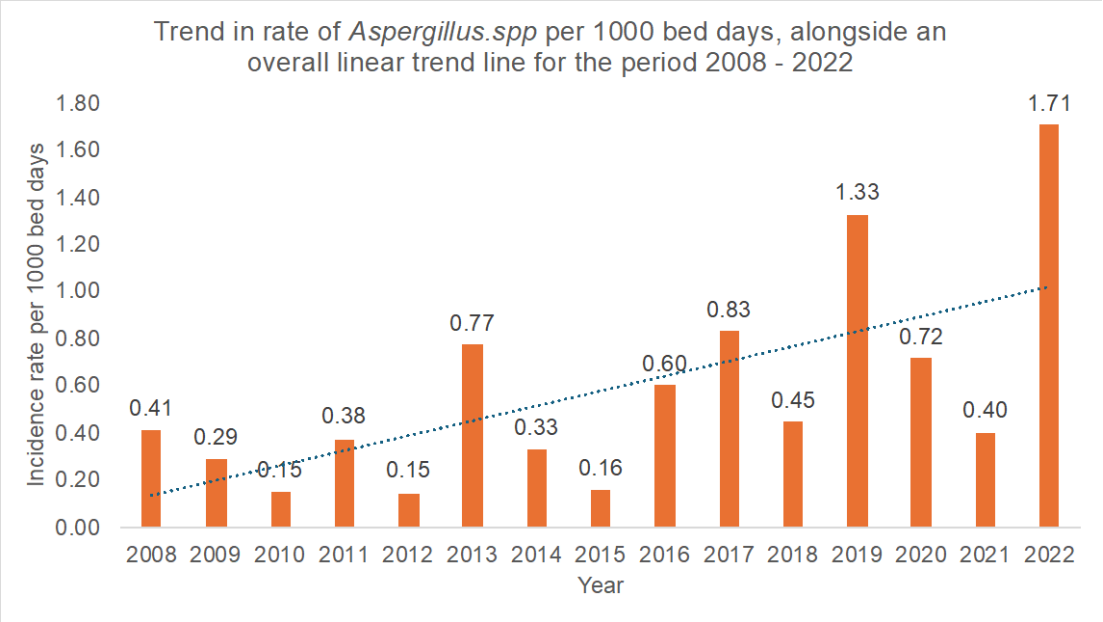
Table 3: *Aspergillus spp* rate of infections by Yorkhill and RHC wards, 2008 - 2022

	Aspergillus. spp incidence rate per 1000 bed days by year														
Wards with *Aspergillus* cases + corresponding BDs	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
4BCH Kids BoneMarrTranRHC	-	0.00	-	-	-	0.00	0.00	-	-	-	0.00	0.00	4.55	0.00	0.00
Clinical Decis. Unit RHC	-	-	-	-	-	-	-	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00
High Dependency Unit YOR	-	-	-	27.03	-	-	-	-	-	-	-	-	-	-	-
ITU YOR	0.00	0.00	28.57	8.47	0.00	-	-	-	-	-	-	-	-	-	-
Schiehall DaySur Wd2B RHC	-	-	-	-	-	-	-	0.00	0.00	0.00	0.33	-	-	-	0.69
Schiehallion YOR	0.80	0.59	0.00	0.00	0.30	0.91	0.80	0.00	-	-	-	-	-	-	-
Schiehallion DCU YOR	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	-	-	-	-	-	-	-
Ward 1D Paed CritCar RHC	-	-	-	-	-	-	-	0.00	11.63	0.00	0.00	100.00	0.00	0.00	0.00
Ward 2A RHC	-	-	-	-	-	-	-	0.58	0.60	2.23	0.87	0.00	-	-	3.57
Ward 6A QEUH	-	0.00	-	-	0.00	0.00	0.00	-	-	-	0.61	1.17	0.68	0.57	0.00
Annual incidence rates per 1000 bed days	0.41	0.29	0.15	0.38	0.15	0.77	0.33	0.16	0.60	0.83	0.45	1.33	0.72	0.40	1.71
Overall Yorkhill and RHC rates per 1000 bed days	0.342							0.756							

2.31 Utilising the overall *Aspergillus. spp* rate of infection at Yorkhill and RHC, seen in the bottom row of Table 3, 0.342 per 1000 bed days and 0.756 per 1000 bed days respectively, calculating the incidence rate ratio (IRR) and the corresponding 95% confidence intervals (CI), we get a IRR of 0.45 with a 95% CI (0.25-0.80), which notes that Yorkhill had an infection rate which was 55% lower as compared to the RHC, with the CIs not including 1, thereby making the difference statistically significant. In summary, the rate of *Aspergillus. spp* rate of infection at RHC was statistically significantly higher than the rate at Yorkhill.

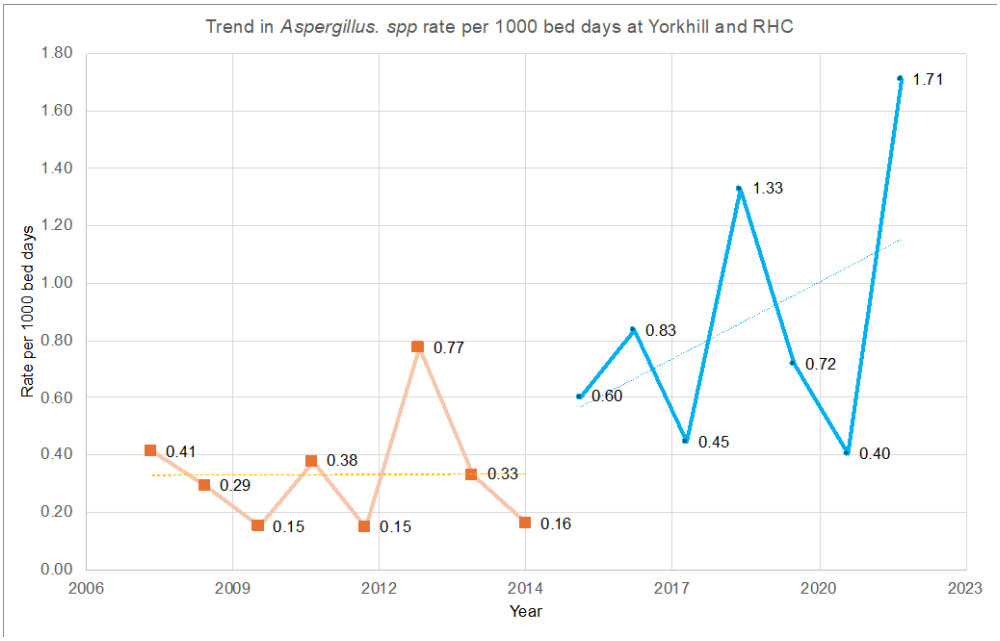
2.32 Figure 1 charts the annual incidence rates per 1000 bed days noted at the bottom of Table 3. We see an upward linear trend in infection rate from 2008 to 2022.

Figure 1: Trend in *Aspergillus spp* rate by year – paediatric haemato-oncology patients.



2.33 Figure 2 splits the trend chart into two separate scatter plots with individual trend lines, distinguishing between the trend in infection rate at Yorkhill, and thereafter at RHC, evidencing the difference in the rates of *Aspergillus* at Yorkhill and then at RHC – flatline trend at Yorkhill 2007 – 2014, followed by an upward linear trend for the period 2015 – 2022.

Figure 2: Yorkhill and RHC *Aspergillus spp* rates of infections with linear trend lines



2.34 In summary, the evidence discussed in sections 2.23 – 2.34 does not support the authors of the HAD report in their assertion that the there were 'low absolute numbers of cases' as whilst the numbers may appear relatively small, the rates are clearly higher than would be expected in a new facility such as RHC and overall significantly higher at RHC than Yorkhill.

Infectious Disorders • Research Paper

The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study

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Background and Objectives. The aim of this study was to evaluate the incidence and outcome of invasive fungal infections (IFI) in patients with hematologic malignancies.

Design and Methods. This was a retrospective cohort study of patients admitted between 1999 and 2003 to 18 hematology wards in Italy. Each participating center provided information on all patients with newly diagnosed hematologic malignancies admitted during the survery period and on all episodes of IFI experienced by these patients.

Results. The cohort was formed of 11,802 patients with hematologic malignacies: acute leukemia (myeloid 3012, lymphoid 1173), chronic leukemia (myeloid 596, lymphoid 1104), lymphoma (Hodgkin's 844, non-Hodgkin's 3457), or multiple myeloma (1616). There were 538 proven or probable IFI (4.6%); 373 (69%) occurred in patients with acute myeloid leukemia. Over half (346/538) were caused by molds (2.9%), in most cases *Aspergillus spp*. (310/346). The 192 yeast infections (1.6%) included 175 cases of candidemia. Overall and IFI-attributable mortality rates were 2% (209/11802) and 39% (209/538), respectively. The highest IFI-attributable mortality rates were associated with zygomycosis (64%) followed by fusariosis (53%), aspergillosis (42%), and candidemia (33%).

Interpretation and Conclusions. Patients with hematologic malignancies are currently at higher risk of IFI caused by molds than by yeasts, and the incidence of IFI is highest among patients with acute myeloid leukemia. *Aspergillus spp* are still the most common pathogens, followed by *Candida spp*. Other agents are rare. The attributable mortality rate for aspergillosis has dropped from 60-70% to approximately 40%. Candidemia-related mortality remains within the 30-40% range reported in literature although the incidence has decreased.

Key words: fungal infection, aspergillus, Candida, epidemiology, hematologic malignancies.

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The percentage of patients who develop invasive fungal infections (IFI) has increased dramatically in recent decades. Most of these infections occur in patients with hematologic malignancies.[1-3] This increase is attributed to host defense impairment due to intensive cytotoxic chemotherapies, hematopoietic stem cell transplantation, ablative radiation therapy, use of corticosteroids, cyclosporine, and new immunosuppressive agents.[3-8] *Candida spp.* have been the main cause of IFI, but recent autopsy and epidemiological findings indicate that an increasing number of infections are being caused by molds.[9-11] Most are attributed to *Aspergillus spp.*, and such infections have become a prime cause of death in patients with hematologic malignancies. During the last 20 years, other opportunistic fungal pathogens, such as *Fusarium spp.* and *Zygomycetes*, have also emorged[12-15] whereas infections caused by other fungi are still rare.[16-19] The true incidence of IFI among patients with hematologic malignancies remains obscure since data in the literature are based largely on reports from single institutions or analyses of selected subgroups of patients (e.g., those with acute leukemia or following stem cell transplantation).[7-8] The aim of the present study was to investigate current incidence and mortality rates for IFI in patients with hematologic malignancies in Italy.

Design and Methods

This retrospective cohort study was conducted in hematology wards of tertiary care centers or university hospitals located throughout Italy, between January 1999 and December 2003. Enrollment was limited to adult patients (aged over 16 years) with newly diagnosed acute myeloid or lymphoid leukemia (AML and ALL, respectively), chronic myeloid or lymphoid leukemia (CML and CLL, respectively), Hodgkin's or non-Hodgkin's lymphoma (HL and NHL, respectively), or multiple myeloma. Seven centers contributed data only on patients with acute leukemia. Patients with other types of hematologic malignancies (e.g., myelodysplastic syn-

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Table 1. Incidence of mold and yeast infections in patients with different types of hematologic malignancies.

HM	No. of patients	No. of IFI (incidence)	Molds		Yeasts	
			No. cases	Incidence %	No. cases	Incidence %
AML	3012	373 (12%)	239	7.9	134	4.4
ALL	1173	77 (6.5%)	51	4.3	26	2.2
CML	596	15 (2.5%)	14	2.3	1	0.2
CLL	1104	6 (0.5%)	5	0.4	1	0.1
NHL	3457	54 (1.6%)	30	0.9	24	0.7
HD	844	6 (0.7%)	3	0.35	3	0.35
MM	1616	7 (0.5%)	4	0.3	3	0.2
Total	11802	538 (4.6%)	346	2.9	192	1.6

HM: hematologic malignancies; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoid leukemia; NHL: non-Hodgkin's lymphoma; HD: Hodgkin's disease; MM: multiple myeloma; IFI: invasive fungal infection.

Table 2. Species distribution of invasive fungal infections in patients with hematologic malignancies.

Infections caused by	No. of cases (%)	Incidence %
Molds	**346 (100)**	**2.9**
Aspergillus spp.	310 (90)	2.6
Zygomycetes	14 (4)	0.1
Fusarium spp.	15 (4)	0.1
Others*	7 (2)	0.06
Yeasts	**192 (100)**	**1.6**
Candida spp.	175 (91)	1.5
Cryptococcus spp.	8 (4)	0.07
Trichosporon spp.	7 (4)	0.06
Others°	2 (1)	0.02

**Scedosporium spp. (n=3) , Acremonium spp. (n=2) , Cladosporium spp.(n=1) , Penicillium spp. (n=1); °Rhodotorula spp 1, Hansenula (n=1).*

dromes) or non-malignant hematologic disorders (hemolytic anemia; thrombocytopenias, aplastic anemia, etc) were excluded from this analysis, as were those patients undergoing autologous or allogeneic HSCT.

Each participating center completed a form which included information on all patients with newly diagnosed hematologic malignancies admitted during the survey period and on all episodes of IFI experienced by these patients. The consensus criteria proposed by the EORTC/ MSG were used to define IFI,[20] and analysis was restricted to infections classifiable as *proven* or *probable*. For each episode, additional information was requested: date of first positive identification, fungal species isolated, microbiological and histological findings (*in vivo* and *post mortem*), and outcome (assessed 30 days after diagnosis). Multiple episodes in the same patient were counted as separate infections unless they were caused by the same fungal agent. Overall and attributable mortality rates were estimated in the study population. Information was specifically requested on the antifungal prophylaxis protocols used in each center. Because the majority of the participating centers were members of the GIMEMA-Infection, their diagnostic work-ups were almost identical. These diagnostic works-ups include collection of blood cultures at the onset of fever, nasal, pharyngeal, and rectal swabs, serological tests for IFI, and computed tomography (CT) scans on the 4-7th day of fever. Additional examinations (e.g., abdominal sonography, sinus or brain CT, skin biopsy, bronchoalveolar lavage, fundus examination) were performed as indicated.[3,21]

Antifungal prophylaxis was administered in 13 centers. The use of itraconazole for this purpose increased (six departments in 1999, nine in 2000, and ten in 2002) with parallel reductions in fluconazole use. The therapeutic protocols used by the centers during the

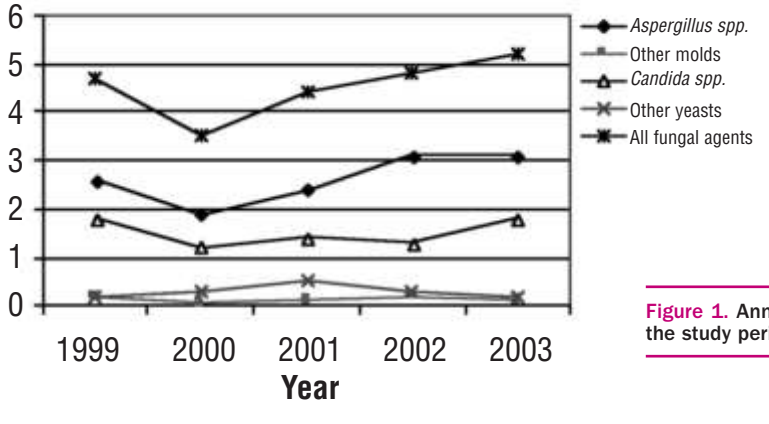

Figure 1. Annual incidence rates for IFI subgroups during the study period. RR 1.1 (95% CI 0.86-1.42; *p*= 0.452)

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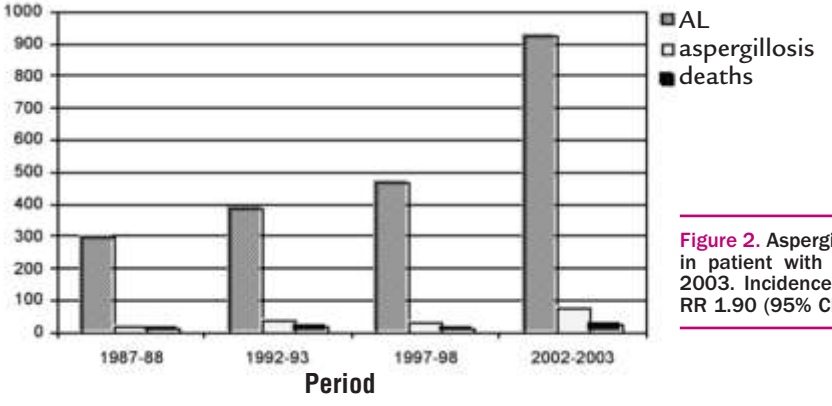

Figure 2. Aspergillosis and aspergillosis-related deaths in patient with acute leukemia between 1987 and 2003. Incidence: RR 0.81 (95% CI 0.50-1.30). AMR: RR 1.90 (95% CI 1.17-3.09).

Table 3. Molds infections in patients with different types of hematologic malignancies.

HM	*No. of patients*	*Aspergillus spp.*			*Zygomycetes*			*Fusarium spp.*		
		Cases (%)	*Deaths (AMR%)*	*Mortality (%)*	*Cases (%)*	*Deaths (AMR%)*	*Mortality (%)*	*Cases (%)*	*Deaths (AMR%)*	*Mortality (%)*
AML	3012	213 (69)	80 (38)	2.6	9 (64)	6 (66.7)	0.2	13 (87)	7 (47)	0.2
ALL	1173	44 (14)	19 (43)	1.6	4 (29)	2 (50)	0.08	1 (6.5)	1 (100)	0.08
CML	596	14 (4.5)	7 (50)	1.2	0	0	0	0	0	0
CLL	1104	5 (1.5)	4 (80)	0.4	0	0	0	0	0	0
NHL	3457	27 (9)	14 (52)	0.4	1 (7)	1 (100)	0.03	1 (6.5)	0	0
HD	844	3 (1)	2 (67)	0.2	0	0	0	0	0	0
MM	1616	4 (1)	3 (75)	0.2	0	0	0	0	0	0
Total	11802	310 (100)	129 (42)	1.1	14 (100)	9 (64)	0.07	15 (100)	8 (53)	0.07

HM: hematologic malignancy; AMR: attributable mortality rate; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoid leukemia; NHL: non-Hodgkin's lymphoma; HD: Hodgkin's disease; MM: multiple myeloma.

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study period were also similar and reflected current IDSA guidelines.[22,23] Six institutions had participated in a previous study on filamentous fungi infections in acute leukemia patients during the period 1987-1998,[3] and IFI incidence and mortality rates registered in these centers during the two study periods were thus compared.

Statistical methods

Associations between variables were evaluated using the χ^2 and Fisher's exact tests. Regression analysis with ANOVA was used to determine trends in incidence, while the Cochran-Armitage trend test was used to evaluate mortality trends. p values <0.05 were considered statistically significant. Statistical analyses were performed using SAS version 8.02.

Results

During the study period, 11,802 patients with newly diagnosed hematology malignancies were admitted to the 18 participating centers for treatment other than hematopoietic stem cell transplantation. Their underlying malignancies are reported in Table 1. Five hundred and thirty-eight proven or probable IFI were documented. The overall incidence of IFI, 4.6%, remained stable (RR=1.1; 95%CI=0.86-1.42; p=0.452), and no significant shifts were observed in the incidence of infections caused by individual pathogen subgroups (Figure 1). Molds were held responsible for 346/538 episodes (2.9%) and yeasts for 192 infections (1.6%) (Table 2). The incidence of IFI in the different subsets of patients ranged from 0.5% among patients with

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Table 4. Yeast infections in patients with different types of hematologic malignancies.

UHM	No. of patients	Candida spp.			Cryptococcus spp.			Trichosporon spp.		
		Cases (%)	Deaths (AMR%)	Mortality (%)	Cases (%)	Deaths (AMR%)	Mortality (%)	Cases (%)	Deaths (AMR%)	Mortality (%)
AML	3012	124 (71)	44 (35.5)	1.4	5 (62.5)	2 (40)	0.1	5 (71)	2 (40)	0.1
ALL	1173	22 (12.5)	8 (36)	0.7	2 (25)	1 (50)	0.08	2 (29)	0	0
CML	596	1 (0.5)	0	0	0	0	0	0	0	0
CLL	1104	1 (0.5)	0	0	0	0	0	0	0	0
NHL	3457	21 (12)	4 (19)	0.1	1 (12.5)	1 (100)	0.03	0	0	0
HD	844	3 (1.5)	0	0	0	0	0	0	0	0
MM	1616	3 (1.5)	1 (33)	0.05	0	0	0	0	0	0
Total	11802	175 (100)	57 (33)	0.5	8 (100)	4 (50)	0.04	7 (100)	2 (29)	0.02

HM: hematologic malignancy; AMR: attributable mortality rate; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoid leukemia; NHL: non-Hodgkin's lymphoma; HD: Hodgkin's disease; MM: multiple myeloma.

multiple myeloma to 12% in those with AML (Table 1). The overall mortality rate for fungal infections was 2% (209/11,802), and the IFI-attributable mortality rate (AMR) was 39% (209/538).

Molds infections

The majority of the mold infections (310/346; 90%) were caused by *Aspergillus spp.*, and these infections accounted for 58% of the IFI analyzed. Forty-one percent of the *Aspergillus* isolates were identified at the species level, and *A. fumigatus* was the main pathogen (68/310 episodes, 53%) (Table 3). Non-significant variations were observed in the annual incidence of IFI caused by the different *Aspergillus* species. An increasing trend was observed for *A. flavus* infections (5 cases in 1999 versus 14 cases in 2003), although this was also not statistically significant (RR 2.10, 95%CI 0.8-5.49; p=0.117). *Zygomycetes* and *Fusarium spp.* were responsible for 14 and 15 episodes (0.1%), respectively. The remaining six infections were due to *Scedosporium spp.*, *Acremonium spp.*, *Penicillium spp.*, or *Cladosporium spp.* Aspergillosis was most common in patients with acute leukemia (Table 3).

Almost half of these infections (43%; 111/259 evaluable cases) emerged during the first course of induction chemotherapy; 20 others (8%) occurred during consolidation chemotherapy in patients who were obviously in complete remission, and 73 (28%) emerged during a second or third course of induction chemotherapy for relapse of the hematopoietic malignancy. The remaining 55 patients (21%) were resistant to first-line chemotherapy and had received salvage therapy. The diagnosis of aspergillosis was classified as *probable* in 155 episodes (60%) and *proven* in the remaining 104 (40%). Only 22 of the proven diagnoses relied on autopsy findings.

Mortality rates ranged from about 2.6% in AML patients to 0.2% in those with multiple myeloma or Hodgkin's lymphoma. The AMR were 42% for aspergillosis, 64% for zygomycosis, and 53% for fusariosis (Table 3). Two of the three *Scedosporium* infections were fatal, as was one of the two infections caused by *Acremonium* and the single case due to *Cladosporium*. The patient suffering from a *Penicillium spp.* infection survived. The AMR for aspergillosis ranged from 38% in AML patients to 80% in those with CLL. An analysis of the relationship between the AMR for aspergillosis and the phase of the underlying malignancy (based on data for 259 assessable patients) revealed that 31 of the 112 deaths due to aspergillosis (28%) occurred during the induction cycle at the beginning of the treament of the hematologic malignancy. Over half of the patients who developed aspergillosis during a disease relapse (45/72; 63%) died, as opposed to only 25% (14/55) of those who were receiving salvage treatment and 20% (4/20) of patients whose infections occurred during complete remission.

Comparison of data for 1987 and 2003 on acute leukemia patients (available for six centers only) revealed increases in the absolute numbers of infections due to *Aspergillus spp.* and those caused by other molds although incidence rates remained stable for both types of infection (aspergillosis: RR 0.81, 95%CI

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0.50-1.30, *p*=0.38; other molds: RR 0.60, 95%CI 0.17-2.10). The AMR for aspergillosis dropped significantly (RR 1.90, 95%CI 1.17-3.09, *p*=0.019) from 60% (12 of 20 cases) in 1987 to 32% (24 of 76 cases) in 2003 (Figure 2), but there was no change in the rates for infections caused by other molds.

Yeasts infections

Most of the yeast infections were caused by *Candida spp.* (175 episodes, 33% of all IFI). The diagnosis was based on blood cultures in all cases. *Candida* non–albicans species were responsible for over half the episodes of candidemia (100/175; 57%) (Table 4). The remaining 17 yeast infections were caused by *Cryptococcus spp.* (8 cases), *Trichosporon spp.* (7 cases), and *Hansenula* and *Rhodotorula* (1 case each) (Table 2). Twelve of the 175 cases of candidemia (7%) were followed by chronic disseminated candidiasis. Eighty-three percent of the cases of candidiemia occurred in patients with acute leukemia (71% in AML, 12% in ALL). Overall, 3.5% (146/4185) of all patients with acute leukemia developed candidemia (Table 4). The incidence of *C. albicans* infections increased from 8% in 2001 to 19% in 2003 (RR 0.52; 95%CI 0.26-1.04; *p*=0.049).

The mortality rate was highest among AML patients (1.4%). There were no deaths due to candidemia among patients with CML, CLL, or HL. The AMR of patients with yeast infections was 33% for candidemia (57/175), 50% for cryptococcosis (4/8), and 29% for trichosporonosis (2/7). The patient who developed a *Hansenula* infection died while the one infected by *Rhodotorula* recovered from the infection. The AMR for candidemia ranged from 19% in NHL to 36% in ALL (Table 4). Species-specific AMR ranged from 6% for infections caused by *C. parapsilosis* to 54% for those due to *C. tropicalis.*

Discussion

In recent years numerous studies have been conducted to identify risk factors or prognostic factors for IFI, but only a few attempts have been made to assess the real incidence of these infections in patients with hematologic malignancies.[3-8] Data on the incidence of IFI in transplanted patients (one of the highest risk groups) are widely available, but they tell us very little about the situation in other patients being treated for hematologic malignancies.[7,8,24-26] The present study was not designed to establish an inventory of risk or prognostic factors or to evaluate the safety and efficacy of certain antifungal regimens. On the contrary, we terminated the investigation in 2003 to avoid a biased assessment of outcome related to the recent introduction of new and possibly more effective antifungal agents, such as voriconazole and caspofungin. Instead, our objectives were to determine the true incidence of IFI among patients with hematologic malignancies and to identity the predominant and emerging causes of these infections.

The reported incidence of proven or probable aspergillosis in patients with acute leukemias ranges from 6% to 12% in series that included both adults and children.[2-4,27,28] Our data confirm that molds were responsible for the majority of the IFI, and that aspergillosis is the most frequent complication in patients with hematologic malignancies. *A. fumigatus* is still the most frequently isolated species, while we did not observe the increase in infections due to *A. terreus* reported by other authors.[29]

Several studies have evaluated the incidence of candidemia, particularly in critical care settings.[30] Conversely there are far fewer reports on the frequency of candidemia among patients with hematologic malignancies.[21,30-34] Our data confirm that yeast infections are less common than mold infections in this population. *Candida spp.* were still the predominant yeast pathogens, but eight infections were caused by *Cryptococcus spp.*, which confirms our previous report on the possible occurrence in patients with hematologic malignancies of an infection typically associated with patients infected by human immunodeficiency virus.[19]

The influence of previous antifungal prophylaxis with fluconazole on the distribution of different *Candida spp.* has already been ascertained.[34,35] Patients who received fluconazole appeared more likely to acquire C. *non-albicans* infections than those who were not treated with it. As a result, *C. non-albicans spp.* became the leading cause of candidemia that developed during the administration of fluconazole prophylaxis. In our study, *C. non-albicans spp.* were responsible for 57% of all IFI episodes although the switch from fluconazole to itraconazole for prophylaxis seemed to be associated with a progressive increase in the percentage of bloodstream infections caused by *C. albicans.*

A previous study by the GIMEMA-Infection revealed that *Zygomycetes* were the second most frequent cause of mold infections in patients with hematologic malignancies.[14] During recent years, various studies have revealed apparent increases in the frequency of zygomycosis, particularly among patients who had received voriconazole for antifungal prophylaxis or treatment.[15,36] In our population, however, the incidence of *Zygomycetes* and *Fusarium* infections remained low; this finding might reflect our participating centers' limited use of voriconazole during the study period. On the other hand, in institutions where

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these infections are on the rise,[8,15] the increase might be due to other factors, such as more aggressive chemotherapy resulting in more severe immunosuppression that renders patients vulnerable to infections by these intrinsically less virulent fungi. As noted, there were no significant changes in the chemotherapy regimens used by the participating centers during the study period, which suggests that immunosuppression might be a major factor in the etiology of these rare fungal infections. It is rather difficult to establish the AMR for aspergillosis or other mold infections since the underlying hematologic malignancies represent an important determinant of outcome. A meta-analysis of 50 studies on aspergillosis revealed mortality rates of approximately 60% in acute leukemia and lymphoma patients and up to 90% in allogeneic stem cell recipients.[37]

About 2% of the patients with hematologic malignancies in our survey died as a result of their IFI, and 39% of the infections had fatal outcomes. The lowest AMR was observed in patients with trichosporonosis. Zygomycosis was associated with the highest mortality, i.e. 64%, which is consistent with the figure reported by Gleissner *et al*.[38] Interestingly, invasive aspergillosis caused by *A. fumigatus* carried a higher AMR than those recorded for *A. niger* and *A. terreus* infections. In the six participating centers with data collected 17 years ago, the incidence of aspergillosis among patients with acute leukemia appeared unchanged, but there was a significant reduction in the aspergillosis-AMR.[3] The AMR for infections caused by *Candida spp.* was 33%, which is consistent with figures published between 1993 and 1998 (34-39%).[21,30,31] Unfortunately, the mortality rate has not improved in spite of the availability of improved diagnostic tools and therapeutic alternatives that are safer than conventional amphotericin B.

Despite the limitations related to its retrospective nature, our study provides important information. The fact that we were able to collect relevant information on all patients admitted to the participating hematology departments during the study period is a very strong point of this survey. Our data confirm that, among non-transplanted patients, those with AML are at highest risk of developing an IFI. About 8% of our AML patients developed mold infections (mainly aspergillosis) whereas 4% had yeast infections. The reduction in aspergillosis-related mortality is a striking finding, which can probably be attributed to improved diagnostics combined with more appropriate treatment strategies since the reduction apparently predated the widespread use of new-generation antifungals. Indeed, the recent introduction into clinical practice of drugs such us voriconazole and caspofungin, which are effective against both *Aspergillus spp.* and *Candida spp.*,[38-40] and posaconazole, which also displays promising activity against *Zygomycetes*,[41] could conceivably lead to additional improvements in the prognosis of patients with hematologic malignancies.

We did not observe any increase in the frequency of infections caused by rarer fungal pathogens, so there appears to be no reason to include routine coverage for these obscure agents in standard antifungal regimens. Thus far, experience has justified decisions to initiate specific treatment only when serious suspicion of a particular infection has arisen. This may, however, change in the near future when the use of new forms of treatment for hematologic malignancies becomes more widespread, since new categories of patients will emerge who can be classified as *high risk*. Purine analogs, for example, which produce profound and prolonged immunosuppression, will extend the risk of IFI to other patients (e.g. those CLL) even in the absence of neutropenia or steroid therapy. The rate of fungal complications in NHL can also be expected to increase with the growing use of monoclonal antibodies, rituximab or alemtuzumab. Likewise, the continuous intensification of chemotherapeutic regimens with increasingly shorter intervals between treatment cycles could significantly increase the incidence of IFI in NHL patients.[4] When this occurs, it will be time for a new epidemiological survey, and the present data can be used for historical comparison.

All authors contributed equally to the manuscript.

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Review

Pediatric Invasive Aspergillosis

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Abstract: Invasive aspergillosis (IA) is a disease of increasing importance in pediatrics due to growth of the immunocompromised populations at risk and improvements in long-term survival for many of these groups. While general principles of diagnosis and therapy apply similarly across the age spectrum, there are unique considerations for clinicians who care for children and adolescents with IA. This review will highlight important differences in the epidemiology, clinical manifestations, diagnosis, and therapy of pediatric IA.

Keywords: aspergillosis; pediatric; antifungal

1. Introduction

Invasive aspergillosis (IA) is one of the most common and serious infectious complications occurring in immunocompromised children. The incidence of IA appears to be increasing in children as it has in adults [1–5]. Significant advances have occurred in the diagnosis and therapy of IA in the last 15 years, and more recent studies indicate that clinical outcomes have improved during this time period [6,7]. However, treatment success and long-term survival after IA diagnosis remain suboptimal.

The evidence that informs care for children with IA is largely extrapolated from adult studies or based on small non-comparative pediatric studies, predominantly including children with malignancy and/or hematopoietic stem cell transplant (HSCT) recipients. This review will describe the known differences in epidemiology, clinical manifestations, diagnosis and therapy of pediatric IA, and will highlight areas of opportunity for further research.

2. Epidemiology

2.1. Incidence and Vulnerable Populations

The population-based incidence of IA in children is unknown and likely varies across healthcare settings and internationally. According to data from the Kids' Inpatient Database, a representative dataset of inpatient hospitalizations in the United States, the annual incidence of IA was 437 cases/100,000 (0.4%) hospitalized immunocompromised children in the year 2000 [8]. Among the groups with highest incidence of IA are children severely immunocompromised due to allogeneic HSCT (annual incidence 4.5%), therapy for hematologic malignancies, especially acute myelogenous leukemia (AML; annual incidence 4%), and those with primary immunodeficiencies, particularly chronic granulomatous disease (CGD) (annual incidence 6.5%) [8]. Other populations susceptible to IA include children undergoing solid organ transplantation (SOT) (annual incidence 0.3%), autologous HSCT (annual incidence 0.3%), chemotherapy for solid tumor malignancy (annual incidence 0.1%), and those with advanced acquired immune deficiency syndrome or certain non-malignant hematologic disorders such as bone marrow failure syndromes or hemophagocytic lymphohistiocytosis [6,8–11].

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Additionally, IA has been reported in children with systemic lupus erythematosus receiving high dose corticosteroids and other intensive immunosuppression, and has also rarely been reported in preterm neonates [12,13]. The major predisposing risk factors for IA are similar in adults and children (Table 1), except that risk factors within pediatric SOT recipients are poorly characterized due to limited studies.

Table 1. Identified Risk Factors for Invasive Aspergillosis in Children.

Risk Factor	Comments
Shared by Multiple Groups	
Prolonged and severe neutropenia [6,10,14]	
High dose corticosteroids [6,10,14]	
Other immunosuppression [6,10,14]	
Specific to Children with Malignancy	
Acute myelogenous leukemia (*vs.* other malignancies) [11,15]	Majority of episodes are diagnosed during intensive phases of therapy [15]
Specific to HSCT [a] Recipients	
Allogeneic transplant (*vs.* autologous) [8,10,11,16]	
Unrelated donor (*vs.* related donor) [10,11,16]	
Cord blood transplant (*vs.* other stem cell source) [16]	
Graft versus host disease [8,10,11,16]	
Specific to SOT [b] Recipients	
Lung transplantation (*vs.* other organ transplants) [4,8,10,11]	Other risk factors related to SOT are poorly characterized in children
Specific to Primary Immunodeficiencies	
Chronic granulomatous disease [17]	Higher risk in lower quartiles of superoxide production [17]
Hyper-IgE syndrome [18]	Risk associated with pneumatoceles following bacterial pneumonias [18]
Other severe defects of phagocyte and/or cellular immunity	e.g. Severe combined immunodeficiency, Wiskott-Aldrich syndrome [6,10,19]

[a] Hematopoietic stem cell transplant; [b] Solid organ transplant.

2.2. Causative Species

The major causative species of IA are similar in children and adults, with multicenter studies reporting *Aspergillus fumigatus* as the most commonly identified species, responsible for 53% of cases in the largest pediatric series, followed by *A. flavus* [6,10,20]. Possibly due to differences in local epidemiology, some single centers have reported a predominance of *A. flavus* causing invasive disease in children [14,21,22]. This may also be due changes in clinical presentation of pediatric IA over time, since *A. flavus* is associated with primary cutaneous disease which was a common clinical presentation in early case series [23]. Other commonly reported species in children include *A. niger* and *A. terreus* [6,10,22].

2.3. Clinical Outcomes

Although estimates of mortality for children with IA are difficult to compare due to variable timing of outcome assessment, there have been apparent improvements in mortality over the past 15 years. A systematic review from 1995 to 1999 reported a 68% case-fatality rate for IA in patients under 20 years old [24]. Among 66 pediatric patients with cancer and IA diagnosed from 1962 to 1996, 85% died within one year following IA diagnosis [14]. In the largest multi-center study of children with IA, 52.5%

of patients diagnosed between 2000 and 2005 died during therapy [10]. More recent pediatric cohort studies that have transitioned to earlier outcome assessments at 12 weeks following diagnosis report lower mortality rates of approximately 30% [5,6,25]. Long term survival of children with IA at a single oncology center improved from 12.5% during 1986–2000 to 58% during 2001–2010 [7,26]. A similar improvement in mortality has been observed in adults with IA [20]. Although this improvement is encouraging, 30% short-term mortality is suboptimal, and IA is associated with other adverse outcomes including prolonged hospitalization, increased hospitalization costs, and interruption in therapy for malignancy [5,8,15].

Risk for poor outcomes among children with IA is driven primarily by host immune status and burden of infection. Children who developed IA in the context of allogeneic HSCT in one study had 6-fold greater odds of death during therapy, compared to those with other underlying conditions [10]. Among children with IA in the context of malignancy, a longer duration of neutropenia following IA diagnosis is associated with increased mortality [27]. Disseminated *Aspergillus* infection has been associated with increased mortality, and non-pulmonary primary sites, such as cutaneous disease, have been associated with better outcomes [14]. Surgical treatment, though not consistently reported across studies, has been associated with improved survival [10].

3. Clinical Manifestations and Diagnosis

3.1. Clinical Manifestations

Similarly to adults, the lungs are the most commonly affected organ in pediatric IA [3,6,10,11,14,20,21,26,28–31]. In a review of 139 pediatric IA cases, 80% of children had pulmonary disease, 14% cutaneous, 13% sinus, 8% cerebral, and 4% tracheobronchitis [10]. Although earlier pediatric studies suggested that cutaneous IA is more common in children than adults [21], more recent studies report lower rates of 14%–20%; this is thought to be due in part to the increasing use of central over peripheral venous catheters in pediatric oncology patients [3,10,14]. Cutaneous IA can be primary from direct inoculation or secondary due to hematogenous dissemination from another site [32]. Several case reports describe primary cutaneous IA in preterm neonates [32–34].

Disseminated infection, defined as disease occurring in $\geqslant$2 sites, has been reported in 10.5% to 38% of children with IA and can be due to hematogenous or contiguous spread [3,7,10,14,21,28]. *Aspergillus* can be locally invasive, with spread from the lung to the pleura, chest wall, and heart, or from the sinus to the central nervous system (CNS). Although no known studies have been performed, some authors suggest screening children with pulmonary IA for CNS involvement given the high mortality, late appearance of symptoms, and impact on treatment [35]. Uncommon sites of IA include the eye [20], head and neck including otitis media and mastoiditis [26], thyroid [36], and gastrointestinal including liver [3,21,31], heart including endocarditis [37] and pericarditis [26], and bone [14,38].

Although some patients with IA present with isolated fever, the clinical manifestations vary depending on which organ system is involved [14]. Fever is common across all presentations. Patients with pulmonary IA can present with cough, dyspnea, hemoptysis/hemorrhage, hypoxia, and pleuritic symptoms [14,28,29]. Cutaneous IA manifestations can be nonspecific and include erythematous plaques, papules, nodules, pustules, blisters, ecchymosis, and eschar formation, often at sites of prior trauma including intravenous catheter and tape sites [14,21,32]. Facial pain, nasal congestion and discharge, visual changes, and headache have been reported in patients with sinus involvement [11,14,39]. CNS IA can present with changes in mental status, visual disturbances, seizures, and focal neurologic deficits [14].

Atypical presentations without fever also occur [11]. It is also reported that up to 33% of patients with CGD who develop IA are asymptomatic and only 20% are febrile [30]. Patients with CGD, especially those infected with *A. nidulans,* can develop local extension to the pleura, chest wall, and vertebrae [30]. Unlike neutropenic patients who can suffer from extensive fungal angioinvasion with hematogenous dissemination, IA may cause more locally invasive disease in CGD patients

due to an ongoing and ineffective neutrophilic inflammatory response with persistent hyphae [30]. Patients with hyper-IgE syndrome can present with fungal superinfection of pneumatoceles resulting in aspergillomas and subsequent risk for invasion to the lung parenchyma or disseminated disease [30].

3.2. Diagnostic Methods

Although prompt IA diagnosis is important given the high morbidity and mortality associated with this infection, existing modalities, including microbiology, pathology, fungal biomarkers, and radiology, have limitations. With currently available diagnostic methods, many children with suspected invasive mold infections do not have an identified pathogen, and are treated empirically [40]. The median time from onset of symptoms to diagnosis was 11 days (range 0–69 days) in one cohort, reflecting challenges in establishing a diagnosis with currently available methods [14]. Establishment of an optimal diagnostic approach is limited by few pediatric IA diagnostic studies, and inconsistent application of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) classifications of proven and probable IA across different studies [41,42]. Further studies in high-risk pediatric patients are needed to establish the ideal timing, frequency, and combination of tests that result in earlier diagnosis.

3.2.1. Culture and Histopathology

Although culture from a sterile site and histopathologic evidence of tissue invasion remain the gold standard for diagnosis of IA, invasive procedures are needed to obtain samples and the diagnostic yield is low. In a pediatric cohort of 193 HSCT patients with fever, respiratory distress, or radiographic findings concerning for an invasive fungal infection (IFI), only 40% of the 101 bronchoalveolar lavages (BAL) performed were diagnostic, all for an infectious etiology, though 94% of the 19 lung biopsies performed yielded a diagnosis [43]. Furthermore, *Aspergillus* is rarely recovered from blood cultures [3,10,20]. Although the yield is low, positive cultures from affected sites are important because they identify the organism and provide antifungal susceptibilities in this era of emerging resistance even to first-line therapy [44]. Given the low yield of cultures and the morbidity and mortality of invasive procedures, the diagnosis of IA in many patients relies on indirect diagnostics including fungal markers and radiology.

3.2.2. Galactomannan Antigen

Galactomannan is a polysaccharide in the *Aspergillus* cell wall that is released during fungal growth and can be measured using an enzyme immunoassay. The limited studies evaluating the use of serum galactomannan in children are heterogeneous; some of the studies are single center, focus on those with malignancies and HSCT, use different definitions for IA, antifungal prophylaxis regimens, and galactomannan positivity cut-offs [31,45–52]. Serum galactomannan for prospective screening of high risk patients and diagnosing IA has been studied mostly in immunocompromised adults [45,53–56]. The sensitivity and specificity of serum galactomannan for the diagnosis of IA in children is similar to adults (Table 2) [55,57]. Although earlier studies reported higher false positivity rates in children than adults [58], more recently, the false positive rate in a pediatric cohort was 5.2% [47]. Preterm neonates have a false positive galactomannan rate as high as 83% [59]. The cause of the high false positive rates in this population are not known but could be secondary to cross-reactivity with *Bifidobacterium spp.* that is a normal part of the neonatal GI flora and present in formula milk [59–61].

Table 2. Fungal Biomarkers for Diagnosis of Invasive Aspergillosis.

Biomarker Attribute	Galactomannan Antigen (%, 95% CI)	1,3-β-D-glucan (%, 95% CI)
Performance in Serum		
Sensitivity—Adult [a] [55,62]	78% (70%–85%)	62% (48%–73%)
Sensitivity—Pediatric [b] [55]	84% (66%–93%)	Unknown
Specificity—Adult [a] [55,62]	85% (78%–91%)	91% (83%–95%)
Specificity—Pediatric [b] [55]	88% (60%–97%)	Unknown—likely less than adult
Causes of False Positive Result	Piperacillin-tazobactam, other beta lactam antibiotics, Plasmalyte, *Fusarium* spp., *Histoplasma capsulatum*, *Penicillium* spp. [45,60,63,64]	Ampicillin-sulbactam, piperacillin-tazobactam, intravenous immunoglobulin, albumin, certain hemodialysis filters, bacteremia [65–67]
Performance in BAL [c] Fluid		
Sensitivity—Adult [d] [68]	86%	n/a [f]
Sensitivity—Pediatric [e] [69]	78%	n/a
Specificity—Adult [d] [68]	91%	n/a
Specificity—Pediatric [e] [69]	92%	n/a

[a] Positivity cut-off ⩾0.5 optical density index (ODI); [b] Subanalysis of 7 pediatric studies within a meta-analysis; 5 studies used a positivity cut-off ⩾0.5 ODI, 1 a cut-off ⩾1.5 ODI, one did not report cut-off; [c] BAL; bronchoalveolar lavage; [d] Positivity cut-off ⩾0.8 ODI; [e] Positivity cut-off ⩾0.98 ODI; [f] Not applicable, test not performed on BAL fluid.

Although galactomannan increases with higher infectious burdens [70], it can have reduced sensitivity early in the infection and if the patient is receiving anti-mold therapy [70]. Lower galactomannan sensitivity and specificity has been reported in SOT patients [53]. Possibly due to the lack of angioinvasion, serum galactomannan has reduced sensitivity in patients with CGD [30,71]. In addition to screening and diagnosis, serum galactomannan has been studied as a predictor of clinical response and survival for patients with IA [72–75].

Galactomannan testing can also be performed on BAL fluid, cerebrospinal fluid (CSF), and urine. BAL galactomannan may be a helpful adjunct for the early diagnosis of IA though cut-off values for positivity have not been firmly established [69,76]. In a retrospective pediatric cohort of immunocompromised and immunocompetent patients, a BAL galactomannan cut-off of ⩾0.98 optical density index (ODI) resulted in a sensitivity of proven or probable IA of 78% and specificity of 92% [69]. This BAL cutoff when combined with a serum galactomannan of ⩾0.5 ODI yielded a sensitivity of 89% and specificity of 90% for proven or probable IA [69].

Few studies have assessed the utility of CSF galactomannan for the diagnosis of CNS IA and specific cut-off values for positivity have not been established. A recent study of pediatric and adult patients with CNS IA reported a CSF galactomannan sensitivity of 88% and specificity of 96% when using a cut-off of ⩾1 ODI [77]. Urine galactomannan has also been studied in children though is associated with high false positive rates [47].

3.2.3. Beta-D-Glucan

1,3-β-D-glucan (BDG) is a nonspecific cell wall component of many fungal pathogens including *Aspergillus*. A number of adult studies have assessed the performance of BDG in the diagnosis of IFI and specifically IA (Table 2) [62,78,79]. BDG for the diagnosis of IFI in children is limited to a few case reports and studies [46,67,80–82]. It has been difficult to establish an optimal positive cut-off for children because they have higher baseline levels than adults [83] and levels can be affected by colonization with *Candida* spp., among other causes of false positivity (Table 2) [84].

3.2.4. Molecular Diagnostics

Aspergillus polymerase chain reaction (PCR) diagnostics are promising for pediatric IA. In a meta-analysis, the sensitivity of the blood *Aspergillus* PCR ranged from 84%–88% and the specificity

from 75% to 76% [85]; the sensitivity of the BAL *Aspergillus* PCR ranged from 77% to 80% and specificity from 94% to 95% [85]. Fewer studies have assessed *Aspergillus* serum and BAL PCR for screening and diagnosis of IA in children [46,86–90]. Another emerging area is serum and BAL molecular diagnostics for identification of the *Aspergillus* species and the most common mutations that confer azole resistance [91,92]. *Aspergillus* PCR from the CSF [93], pleural fluid, fresh [94] and formalin-fixed paraffin-embedded tissue [95] has also been studied for the diagnosis of IA.

3.2.5. Emerging Diagnostics

Given the limitations of established diagnostic methods, further investigation has attempted to identify additional non-invasive methods to diagnose IA in early stages. Different fungal organisms causing pulmonary infection are associated with characteristic profiles of volatile secondary metabolites that can be detected in exhaled breath [96]. A recent study showed 94% sensitivity (95% CI, 81%–98%) and 93% specificity (95% CI, 79%–98%) for a combination of exhaled metabolites to identify proven or probable pulmonary IA in a cohort of adults with suspected pulmonary IFI [96]. The lateral-flow device (LFD) is another promising test. This point of care test detects *Aspergillus* glycoprotein antigen in both serum and BAL has been studied in adult cohorts [97,98]. While these novel diagnostics shows promise, they have not yet been evaluated in children.

3.2.6. Radiology

Plain film and computer tomography (CT) are used for the diagnosis of pulmonary IA, though radiographic findings overlap with those of other IFI. CT has higher sensitivity and specificity for early diagnosis of pulmonary IA [99,100]. Radiographic findings that are described as characteristic in adult pulmonary IA are less common in children. These include the 'halo sign' that occurs earlier in the course of infection, as well as cavitation and the 'air crescent' sign that occur upon immune reconstitution [10,101–103].

More commonly, pediatric patients with pulmonary IA have nodules, masses, peripheral infiltrates, consolidations, and pleural effusions [10,29,101,102,104]. In a retrospective review of pediatric patients with proven or probable pulmonary IA, the most common findings on CT or plain radiograph were nodules (59%) [10]. Nodules were more commonly reported in older compared to younger children (63% ⩾13 year-olds *vs.* 72%, 6–12 year-olds *vs.* 39%, 0–5 year-olds). Cavities were reported in 25% of patients and a minority of patients had the halo (11%) and air crescent (2%) signs. The reasons for the lower rates of cavitation and the halo and air crescent signs in children are unknown but differences in pathophysiology, immune response, and timing of imaging are possible explanations [10,101–103].

Radiographic findings of sinus and CNS IA are nonspecific. Common radiographic findings of sinus IA are mucosal thickening (67%), opacification (48%), and air fluid levels (24%) [10]. Radiographic findings of CNS IA include hypoattenuating lesions on CT and T2 hyperintense lesions on MRI, single or multiple ring enhancing lesions, or dural enhancement abutting sinus disease [105,106].

4. Treatment

During the last 15 years, multiple new antifungals have become available for treatment of IA, expanding options within three different antifungal classes. However, licensing of new antifungals for use in children lags behind adult approvals, and there are important age-specific pharmacokinetic differences. Table 3 summarizes the antifungals with activity against *Aspergillus*, their current pediatric licensing status and evidence and/or consensus guidelines supporting their use in children with IA. Off-label use of medications is common in pediatrics and agents may be commonly used for age groups and/or indications that lack regulatory approval [6,10,107].

Table 3. Antifungals for Treatment of Invasive Aspergillosis: Pediatric Licensing and Evidence Supporting Use in Children.

Class/Agent (Formulation—IV [a]/PO [b])	Ages Currently Licensed [c]	Pediatric Evidence	Clinical Guidelines
Polyenes			
Amphotericin B deoxycholate (IV)	All	First available agent	
Liposomal amphotericin B (IV)	⩾1 month	PK [d], safety [108]. Observational cohort study [109]	IDSA [d]: alternative primary therapy (A-I), salvage therapy (A-II). ECIL-4 [e]: 1st line (B-I), 2nd line (B-II)
Amphotericin B lipid complex (IV)	All	Observational cohort study [110]. Non-comparative trial (salvage therapy) [111]	IDSA: alternative primary therapy (A-I), salvage therapy (A-II). ECIL-4: 1st line (B-II), 2nd line (B-II)
Amphotericin B colloidal dispersion (IV)	All (no longer commercially available)	Non-comparative trial (salvage therapy) [112]	IDSA: alternative primary therapy (A-I), salvage therapy (A-II)
Triazoles			
Voriconazole (IV, PO)	⩾12 years [g]	PK, safety [113–117]. Non-comparative trial (salvage therapy) [118]. RCT[f] including children >12 years [119]	IDSA: primary therapy (A-I). ECIL-4: first line (A-I), second line for voriconazole-naïve patients (A-I)
Itraconazole (PO)	⩾18 years	PK, safety [120]	IDSA: salvage therapy (B-II). ECIL-4: 2nd line (no grade)
Posaconazole (IV, PO)	PO: ⩾13 years. IV: ⩾18 years	PK, safety [121–124]. Retrospective cohort study [125]	IDSA: salvage therapy (B-II). ECIL-4: 2nd line (no grade)
Isavuconazole (IV, PO)	⩾18 years		
Echinocandins			
Caspofungin (IV)	⩾3 months	PK, safety [126–129]. Retrospective cohort study [130]. Non-comparative trial (primary & salvage therapy) [131]	IDSA: salvage therapy (B-II). ECIL-4: 2nd line (A-II)
Micafungin (IV)	⩾4 months	PK, safety [132–135]. Non-comparative trial (salvage therapy) including pediatric patients [136]	IDSA: salvage therapy (B-II). ECIL-4: 2nd line (no grade)
Anidulafungin (IV)	⩾18 years	PK, safety [137]	

[a] IV; intravenous; [b] PO; oral; [c] Licensing status by United States Food and Drug Administration; not all drugs are licensed for primary therapy of invasive aspergillosis; voriconazole, isavuconazole, and amphotericin B deoxycholate are licensed for primary therapy; lipid amphotericin B formulations and caspofungin are licensed for salvage therapy in patients who are intolerant or unresponsive to other agents; posaconazole is licensed only for prophylaxis in the United States, but is licensed for salvage therapy by the European Medications Agency. Micafungin and anidulafungin are not licensed for IA but have *in vitro* activity similar to caspofungin.; [d] PK; pharmacokinetics; [d] IDSA; Infectious Diseases Society of America; combined adult and pediatric guidelines, evidence levels based on adult data [138]; [e] ECIL-4; Fourth European Conference on Infections in Leukemia; pediatric-specific guidelines [139]; [f] RCT; randomized controlled trial; [g] Voriconazole is approved by the European Medications Agency for children age 2–11 years.

4.1. Amphotericin B Formulations (Polyenes)

Amphotericin B exerts fungicidal activity by binding to ergosterol and altering permeability of the fungal cell membrane. It is active against *Aspergillus* species except for *Aspergillus terreus* [140]. Conventional amphotericin B deoxycholate was the first available antifungal with activity against *Aspergillus*, and was for many years the only therapeutic option for IA. Its disadvantages are nephrotoxicity and infusion-related adverse effects. Lipid formulations of amphotericin B are better tolerated with less nephrotoxicity [111,112]. Though they differ from one another and from the parent drug in pharmacokinetics, there is no available evidence showing differences in efficacy between any of the formulations [141]. Differences in tissue distribution should be considered when treating infections at specific sites, such as the CNS, because tissue distribution varies between formulations [142]. Lipid amphotericin B formulations remain options for treatment of patients who are intolerant or unresponsive to voriconazole [138,139]. Their potential advantages include lack of drug interactions and broader spectrum of antifungal activity, for patients in whom IA is not confirmed and other fungi are suspected.

4.2. Triazoles

Triazoles inhibit biosynthesis of ergosterol in the fungal cell membrane, and are fungicidal against *Aspergillus* [143]. Itraconazole was the first available triazole with activity against *Aspergillus*, but its use for treatment of IA in children is limited due to unreliable bioavailability [120]. Voriconazole became the first line antifungal for IA based on a clinical trial demonstrating superior efficacy and tolerability of voriconazole compared to amphotericin B deoxycholate among patients 12 years and older with definite or probable IA [119,138]. Survival at 12 weeks of therapy was 70.8% in the voriconazole group compared to 57.9% in the amphotericin B group, and severe drug-related adverse events were less common with voriconazole [119]. Although there are no comparative trials in children with IA under 12 years, voriconazole is recommended as primary therapy for pediatric IA based on pediatric safety data and a non-comparative compassionate use study demonstrating complete or partial response to voriconazole in 45% of children with IA and other IFIs who were refractory to or intolerant of conventional therapy [118,139].

Substantially higher doses of voriconazole are needed in young children (<12 years) to achieve adequate drug exposure [113–117]. This is due to linear voriconazole elimination kinetics in children over a wider range of doses compared to adults and adolescents, who have non-linear elimination kinetics at usual doses [113]. Non-linear kinetics result in large increases in exposure with small dose increases, and can be observed in younger children receiving higher voriconazole doses. Additionally, the oral formulation of voriconazole has lower bioavailability in children (44%–65%) compared to adults (>90%); this may be due to more extensive intestinal first pass metabolism in children [114,115,144].

There is substantial inter-patient variability in voriconazole exposure, partly due to polymorphisms in CYP2C19, and this variability may be greater in children than in adults [113–116,145]. Therapeutic drug monitoring (TDM) of voriconazole is recommended with a target trough range of 1.0–5.0 mg/L [139]. Correlation of treatment failure with trough levels <1.0 mg/L has been shown in young children as well as adolescents and adults [146–149]. A recent meta-analysis showed clear associations between therapeutic voriconazole levels and treatment success, and between supra-therapeutic levels and toxicity [150]. Voriconazole TDM has demonstrated benefits in children and adults, by reducing avoidable discontinuation of voriconazole and increasing likelihood of a successful response to therapy [149,151].

The most common adverse events associated with voriconazole include hepatic abnormalities, visual changes, and rash, often attributable to photosensitization [113,115,116,118,119]. Less common but serious toxicities include neurotoxicity (confusion and/or hallucination) and prolongation of the QT interval with associated arrhythmias [116,119,152]. Children receiving voriconazole prophylaxis have developed severe phototoxic skin reactions; the mechanism of this reaction is unknown [153,154].

Long term voriconazole exposure has been associated with acceleration of chronic phototoxicity and increased risk for skin cancers in immunocompromised patients [155]. Long term use of voriconazole has also been associated with elevated serum fluoride levels, with associated skeletal fluorosis and painful osteitis [156,157]. Voriconazole is an inhibitor of CYP3A4, CYP2C19 and CYP2C9 and is therefore subject to multiple drug interactions.

Newer triazole agents, posaconazole and isavuconazole, may serve a greater role for therapy of pediatric IA in the future, but their use is currently limited by a paucity of pediatric data. Although there are no published clinical trials comparing efficacy of posaconazole against voriconazole for IA, it is recommended as an option for salvage therapy in patients ⩾13 years of age on the basis of an externally controlled clinical trial in adults and adolescents with IA who were refractory to or intolerant of standard therapy [138,139,158]. It is available in oral suspension, oral tablet and IV formulations. Bioavailability of the oral suspension is unreliable and dependent on food intake [159]. This formulation has undergone limited evaluation in children, with observational studies characterizing pharmacokinetics, safety and efficacy in prophylaxis of invasive mycoses [122–124]. Preliminary results of a more extensive pediatric pharmacokinetic study indicate that target concentration attainment with the posaconazole oral suspension is poor, especially in young children [160]. The safety profile of posaconazole appears favorable, and further pharmacokinetic studies are in progress to establish optimal dosing of posaconazole in children, especially with the newer formulations, which are dosed differently than the suspension.

Isavuconazole, the most recently developed triazole, was found to be non-inferior to voriconazole for IA in adults [161]. Its potential advantages over voriconazole include broader spectrum of activity (including activity against mucormycosis), reliable pharmacokinetics, and a favorable safety profile [162]. However, there are currently no data to establish its use in children.

4.3. Echinocandins

Echinocandins inhibit synthesis of 1,3-β-D-glucan in the fungal cell wall and are fungistatic against *Aspergillus*. Caspofungin and micafungin are the best evaluated in children and have a favorable safety profile across multiple pediatric age groups [126,127,131–133]. Caspofungin and micafungin have been evaluated in non-comparative studies for treatment of patients with IA, including small numbers of children, mostly as salvage therapy for patients refractory to or intolerant of other agents [131,136]. Because of their fungistatic activity and lack of robust data for use as primary therapy, the echinocandins are recommended as second line therapy for IA in patients who are intolerant to or refractory to first line agents [138,139].

4.4. Combination Antifungal Therapy

Though not routinely recommended for primary IA therapy, use of combination antifungal therapy is common in both children and adults. Two recent multicenter pediatric cohort studies reported use of combination therapy in 46% and 54% of children with IA [6,10]. In one of these studies, children who received combination therapy were more likely to experience adverse events compared to those treated with monotherapy [6]. A recent clinical trial comparing voriconazole monotherapy to voriconazole and anidulafungin combination therapy for primary therapy of adults with IA showed a trend toward lower mortality with combination therapy (19.3% *vs.* 27.5% at 6 weeks; $p = 0.087$) but was underpowered for this primary endpoint [163]. The implications for management of children with IA are uncertain; recent European guidelines propose combination therapy as an option for pediatric IA, but with weak recommendations [139]. Combination therapy deserves further evaluation for safety and efficacy in children, given the major differences in antifungal pharmacology in children and adults.

4.5. Adjunctive Therapies

Colony stimulating factors and other therapies intended to reverse predisposing conditions and augment the immune response to *Aspergillus* are commonly given to children with IA [6,10,22].

As in adults, their specific contribution to clinical outcomes is uncertain, but an effect is biologically plausible due to the importance of immune reconstitution in recovery from IA. Adoptive transfer of donor-derived pathogen-specific T cells, a strategy used successfully against viral infections in HSCT recipients, is a promising option for IA therapy and is currently in early clinical development [164,165]. Many children with IA undergo surgery for disease control, with indications similar to those in adults; one pediatric study identified an association between surgical treatment and improved survival [10,22].

5. Conclusions

Important differences in diagnosis and treatment exist between adult and pediatric IA. Although there have been advances in the diagnosis, treatment, and outcomes of pediatric IA, challenges remain. Better characterization of IA in pediatric populations, such as those with SOT and primary immunodeficiencies, is needed. The greatest gains are likely to come from optimizing the diagnostic approach to IA, including incorporation of risk stratification, and establishing algorithms to determine which patients may be best evaluated through non-invasive tests, *versus* those that could benefit from invasive testing. Additional evaluation of combination antifungal therapy and pediatric studies of new antifungals will facilitate broader prophylaxis and treatment options in at-risk children. Novel treatment strategies such as immunotherapy may also play a role in the treatment of pediatric IA. Taken together, improved diagnostic and treatment modalities will enable earlier diagnosis and improve mortality for children with IA.

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Clinical Infectious Diseases

MAJOR ARTICLE

Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium

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(See the Editorial Commentary by Rogers on pages 1377–8.)

Background. Invasive fungal diseases (IFDs) remain important causes of morbidity and mortality. The consensus definitions of the Infectious Diseases Group of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group have been of immense value to researchers who conduct clinical trials of antifungals, assess diagnostic tests, and undertake epidemiologic studies. However, their utility has not extended beyond patients with cancer or recipients of stem cell or solid organ transplants. With newer diagnostic techniques available, it was clear that an update of these definitions was essential.

Methods. To achieve this, 10 working groups looked closely at imaging, laboratory diagnosis, and special populations at risk of IFD. A final version of the manuscript was agreed upon after the groups' findings were presented at a scientific symposium and after a 3-month period for public comment. There were several rounds of discussion before a final version of the manuscript was approved.

Results. There is no change in the classifications of "proven," "probable," and "possible" IFD, although the definition of "probable" has been expanded and the scope of the category "possible" has been diminished. The category of proven IFD can apply to any patient, regardless of whether the patient is immunocompromised. The probable and possible categories are proposed for immunocompromised patients only, except for endemic mycoses.

Conclusions. These updated definitions of IFDs should prove applicable in clinical, diagnostic, and epidemiologic research of a broader range of patients at high-risk.

Keywords. consensus; definitions; invasive fungal diseases; diagnosis; research.

The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions of invasive fungal diseases (IFDs) were last updated in 2008 [1]. These definitions achieved their original aim in fostering communication and enabling comparison of study findings among those engaged in research into IFD of patients with cancer and recipients of hematopoietic stem cell transplants (HSCTs) or solid organ transplants (SOTs) [2, 3]. Moreover, they have been adopted by regulatory agencies for evaluation of antifungals and have been used to evaluate diagnostic tests and to conduct epidemiologic studies [4–7]. Importantly, these definitions are specifically intended for these purposes only and not to direct or guide patient care.

The 2008 definitions had their shortcomings, including the facts that the definitions were unsuitable for patients with IFD in the setting of intensive care units (ICUs) or in pediatrics, data were insufficient to establish appropriate thresholds for detecting *Aspergillus* galactomannan (GM), and there was uncertainty about the role of (1,3)-beta-D glucan (BDG). Furthermore, nucleic acid amplification including polymerase chain reaction (PCR)–based tests were excluded because of lack of standardization and validation. Definitions for cryptococcosis and endemic mycoses also needed clarification, and there were no definitions for pneumocystosis.

PROCESS

Volunteers from the EORTC Infectious Diseases Group and the MSGERC were assigned according to their expertise to 10 working groups, each charged with appraising a particular topic (see list of contributors in the Notes section). The chairs of the EORTC and MSG (J. P. D. and P. G. P.) selected leaders for each working group, and S. C. served as executive secretary. After completing the first round of working group assignments, leaders presented each group's initial deliberations and recommendations at the 7th Trends in Medical Mycology Conference in Lisbon, Portugal, October 2015. A slide set was made available until 31 December 2015 online at www.e-materials.com/timm2015/invitation/Member and, on request, for public comment. After several iterations, the final draft of the manuscript was circulated to all members for their approval.

REVISIONS AND UPDATES

Special Populations

Pediatrics and patients in the ICU were considered as special populations. However, group 10 (IFD definitions in ICU patients) was unable to generate recommendations that preserved a level of certainty consistent with the existing definitions except for proven IFD (Table 1) and therefore undertook a separate initiative [8].

Pediatrics: Group 1

There was a clear need to establish pediatric-specific IFD definitions as the clinical and radiologic manifestations of IFD in children, that is, patients aged <18 years, may differ significantly from those in adults. Most importantly, the incidence of invasive candidiasis (IC) is higher in neonates than in other age groups [9–11]. The degree of prematurity, based on gestational age and birth weight, is a unique risk factor among neonates; hematogenous *Candida* meningoencephalitis affects premature infants disproportionately and has serious consequences including seizures, intraventricular hemorrhage, and developmental delay [12, 13]. With respect to IC due to non-*Candida albicans* species, *Candida glabrata* is the most common pathogen in adults, whereas *Candida parapsilosis* predominates in children and neonates [14]. Risk factors for invasive mold diseases include innate immunologic defects, with *Aspergillus nidulans* being associated with chronic granulomatous disease, while *Aspergillus fumigatus* is seen more often in other patient groups [15].

Neonates with IC often present with subtle clinical findings, and cultures are frequently sterile, including cerebrospinal fluid (CSF) samples from neonates with candidemia and concurrent *Candida* meningitis. Diagnosis is often inferred from insensitive

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Table 1. Criteria for Proven Invasive Fungal Disease

Fungus	Microscopic Analysis: Sterile Material	Culture: Sterile Material	Blood	Serology	Tissue Nucleic Acid Diagnosis
Molds[a]	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine	Blood culture that yields a mold[c] (eg, *Fusarium* species) in the context of a compatible infectious disease process	Not applicable	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
Yeasts[a]	Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells, for example, *Cryptococcus* species indicating encapsulated budding yeasts or *Candida* species showing pseudohyphae or true hyphae[d]	Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [<24 hours ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Blood culture that yields yeast (eg, *Cryptococcus* or *Candida* species) or yeast-like fungi (eg, *Trichosporon* species)	Cryptococcal antigen in cerebrospinal fluid or blood confirms cryptococcosis	Amplification of fungal DNA by PCR combined with DNA sequencing when yeasts are seen in formalin-fixed paraffin-embedded tissue
Pneumocystis	Detection of the organism microscopically in tissue, BAL fluid, expectorated sputum using conventional or immunofluorescence staining	Not applicable	Not applicable	Not applicable	Not applicable
Endemic mycoses	Histopathology or direct microscopy of specimens obtained from an affected site showing the distinctive form of the fungus	Recovery by culture of the fungus from specimens from an affected site	Blood culture that yields the fungus	Not applicable	Not applicable

Abbreviations: BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

[a]If culture is available, append the identification at the genus or species level from the culture results.

[b]Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Grocott-Gomori methenamine silver stain or periodic acid Schiff stain to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (eg, calcofluor or blankophor).

[c]Recovery of *Aspergillus* species from blood cultures rarely indicates endovascular disease and almost always represents contamination.

[d]*Trichosporon* and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae.

and nonspecific surrogate tests, such as increased C-reactive protein or thrombocytopenia, which has been shown to be a predictor of candidemia in infants [16, 17]. In neonates, a positive urine culture has a significance similar to that of a positive blood culture as an indicator of IC [16]. Radiographic findings are less specific in children than those reported in adults [18]. Chest computerized tomography (CT) scans in children with proven invasive pulmonary aspergillosis (IPA) commonly show nonspecific changes and not the halo sign, air crescent formation, or cavitation seen in adults [19].

There are also far fewer data to support the clinical use of nonculture-based fungal biomarkers in neonates and children [20], although the GM assay performs similarly in children and adults when used as an adjunctive tool to diagnose invasive aspergillosis (IA) [20, 21]. Likewise, there are few data regarding the use of BDG, *Candida* mannan antigen, and anti-mannan antibody biomarkers in pediatrics [22]. Recent data support the utility of BDG in CSF for the diagnosis and therapeutic monitoring of children with *Candida* meningoencephalitis [23], but the data are sparse regarding the utility of PCR assays and the T2Candida assay for diagnosis [24].

Diagnostic Tests and Imaging

In the previous definitions [1], indirect tests for diagnosing IFD were only included if there was sufficient evidence that they had been standardized and validated. Moreover, commercial tests were included only if criteria for interpretation had been provided. Hence, while tests for GM and BDG were incorporated, tests for detecting fungal nucleic acid were not [1]. Furthermore, there was no agreement about appropriate thresholds, so the manufacturers' analytical thresholds were adopted. The evidence for using GM to diagnose IA has grown considerably since then, and testing for BDG has been extended to a wide range of patients. With respect to *Aspergillus* PCR, the International Society of Human and Animal Mycology working group Fungal PCR Initiative (FPCRI; www.fpcri.eu) has made significant progress toward setting a standard for the technique after vigorous validation [25].

Imaging: Group 2

There is mounting evidence that the radiologic manifestations of invasive mold disease are more varied than previously recognized. The increased sensitivity of newer imaging techniques enables a greater number and depth of abnormalities to be seen in different anatomic regions. Recent data relating to the role of imaging in the diagnosis of IPA and pulmonary mucormycosis (PM) in adults suggest that a high-resolution CT scan (HRCT) is preferred to chest radiographs, magnetic resonance imaging (MRI), and positron emission tomography (PET), likely reflecting that HRCT is more sensitive than a chest

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radiograph, more widely available than MRI, and the experience with HRCT is much larger than with PET [26, 27]. Among patients with IPA, nodules or infiltrates with a halo sign remain useful among neutropenic patients but they are nonspecific for IPA in other groups [28]. Furthermore, the air crescent sign is a late and nonspecific sign. Among nonneutropenic patients, multiple pulmonary nodules and various nonspecific findings including bronchopneumonia, consolidation, cavitation, pleural effusions, ground glass opacities, tree-in-bud opacities, and atelectasis are found [29]. Overall, consolidation is the most frequent presentation of PM, followed by mass lesions, nodules, and cavitation [30]. Multiple nodules (more than10) and pleural effusions appear to be more frequent in PM than in IPA [31]. Moreover, the reverse halo sign is more specific for PM than IPA, although the differential diagnosis also includes other diseases including tuberculosis [32].

Aspergillus Galactomannan: Group 3

We evaluated *Aspergillus* galactomannan for both adults and children and specific patient groups and its utility and validity for different clinical specimens. We adopted different thresholds for different specimens rather than for different host groups [33–35] (Table 2). These differ from those recommended by the manufacturer of the GM assay (Platelia *Aspergillus* (Bio-Rad, CA), validated only for use in serum and bronchoalveolar lavage (BAL) fluid; however, detection of GM in plasma and CSF should support a diagnosis of IA [36, 37]. Exposure to mold-active antifungals compromises the utility of the GM test for IA [38] by reducing its sensitivity [39]. Therefore, caution should be exercised when GM is found to be absent from serum or plasma in patients receiving mold-active antifungals. There was consensus that similar GM thresholds are appropriate for adults and children.

BDG and T2Candida Assays: Group 4

The group considers detection of BDG to be suitable for diagnosing probable IFD in the appropriate clinical setting. This includes patients with hematologic malignancies with and without neutropenia, neutropenia following HSCT, and certain patients in the ICU who are at higher risk (>10%) for IC as a result of gastrointestinal surgery with recurrent anastomotic leaks, perforations of the upper gastrointestinal tract, or necrotizing pancreatitis when there is clinical suspicion of infection [40, 41]. A single threshold (>80 pg/mL) using the Fungitell test (Associates of Cape Cod, Falmouth, MA) is recommended; there is insufficient evidence to include assays produced by other manufacturers [42]. Confidence for true positive results increases with repeated positive tests and for values that greatly exceed the positivity threshold [43]. There may be variability in positive predictive value (PPV) and negative predictive value (NPV) based on patient population, but a single threshold is favored at this time. The group did not support the use of

Table 2. Probable Invasive Pulmonary Mold Diseases

Host factors
Recent history of neutropenia (<0.5 × 10^9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of invasive fungal disease
Hematologic malignancy[a]
Receipt of an allogeneic stem cell transplant
Receipt of a solid organ transplant
Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of ≥0.3 mg/kg corticosteroids for ≥3 weeks in the past 60 days
Treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days
Treatment with recognized B-cell immunosuppressants, such as Bruton's tyrosine kinase inhibitors, eg, ibrutinib
Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency)
Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids
Clinical features
Pulmonary aspergillosis
The presence of 1 of the following 4 patterns on CT:
Dense, well-circumscribed lesions(s) with or without a halo sign
Air crescent sign
Cavity
Wedge-shaped and segmental or lobar consolidation
Other pulmonary mold diseases
As for pulmonary aspergillosis but also including a reverse halo sign
Tracheobronchitis
Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
Sino-nasal diseases
Acute localized pain (including pain radiating to the eye)
Nasal ulcer with black eschar
Extension from the paranasal sinus across bony barriers, including into the orbit
Central nervous system infection
1 of the following 2 signs:
Focal lesions on imaging
Meningeal enhancement on magnetic resonance imaging or CT
Mycological evidence
Any mold, for example, *Aspergillus*, *Fusarium*, *Scedosporium* species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate
Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold
Tracheobronchitis
Aspergillus recovered by culture of BAL or bronchial brush
Microscopic detection of fungal elements in BAL or bronchial brush indicating a mold
Sino-nasal diseases
Mold recovered by culture of sinus aspirate samples
Microscopic detection of fungal elements in sinus aspirate samples indicating a mold
Aspergillosis only
Galactomannan antigen
Antigen detected in plasma, serum, BAL, or CSF
Any 1 of the following:
Single serum or plasma: ≥1.0
BAL fluid: ≥1.0
Single serum or plasma: ≥0.7 and BAL fluid ≥0.8

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Table 2. Continued

CSF: ≥1.0
Aspergillus PCR
Any 1 of the following:
Plasma, serum, or whole blood 2 or more consecutive PCR tests positive
BAL fluid 2 or more duplicate PCR tests positive
At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid
Aspergillus species recovered by culture from sputum, BAL, bronchial brush, or aspirate

Probable invasive fungal diseases (IFD) requires the presence of at least 1 host factor, a clinical feature and mycologic evidence and is proposed for immunocompromised patients only, whereas proven IFD can apply to any patient, regardless of whether the patient is immunocompromised. Probable IFD requires the presence of a host factor, a clinical feature, and mycologic evidence. Cases that meet the criteria for a host factor and a clinical feature but for which mycological evidence has not been found are considered possible IFD. (1,3)-beta-D glucan was not considered to provide mycological evidence of any invasive mold disease.

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; CT, computed tomography; PCR, polymerase chain reaction.

[a]Hematologic malignancy refers to active malignancy, in receipt of treatment for this malignancy, and those in remission in the recent past. These patients would comprise largely acute leukemias and lymphomas, as well as multiple myeloma, whereas patients with aplastic anemia represent a more heterogeneous group of individuals and are not included.

serum BDG to rule in patients for clinical trials or for defining IA or IC, as BDG detection is not specific for any one IFD. It was agreed that this test should only be used on serum samples, although the test has been used for CSF samples with some success to support a diagnosis of central nervous system (CNS) IFD in certain circumstances when other diagnostic tests are negative or inconclusive [44].

The T2Candida panel has been cleared by the US Food and Drug Administration for the detection of common *Candida* species from whole blood specimens. The test has a very high NPV but, as with all such tests, the PPV is variable and depends upon disease prevalence in a given patient population [45, 46]. The PPV increases from 62% among patients with sepsis, shock, or lengths of stay greater than 3–7 days in an ICU to 92% for bone marrow transplant recipients and patients with leukemia who are neutropenic but not receiving any antifungal prophylaxis. The test has been included as mycologic evidence to support a diagnosis of candidemia in selected clinical trials [47].

Aspergillus PCR: Group 5

In considering *Aspergillus* PCR, target species, patient populations, appropriate specimens for testing, technical issues, comparison with other biomarker assays, and unique attributes of PCR assays were reviewed. The data were sufficiently robust for performing *Aspergillus* PCR on serum, plasma, whole blood, and BAL fluid in adults. The group acknowledged that *Aspergillus* PCR data have been evaluated most extensively for adults with hematologic malignancies and HSCT. Systematic reviews of *Aspergillus* PCR methods on blood and BAL fluid conclude that PCR provides a robust diagnostic test for screening and confirming the diagnosis of *Aspergillus* infection [22, 48–53].

There are relatively few commercial PCR assays, and most investigators have developed methods in-house. As such, the FPCRI was established to develop criteria for *Aspergillus* PCR rather than a standardized method per se. Despite technologic variability, PCR performance was comparable with that for detecting GM and BDG [54]. Moreover, commercial PCR tests performed using methodology in line with the FPCRI recommendations provide a standardized approach that has been independently associated with improved performance. A unique feature of PCR is its ability to detect both genus and species of *Aspergillus*. PCR is also capable of identifying certain mutations associated with triazole resistance directly from clinical specimens [55–57].

Tissue Diagnosis: Group 6

Tissue diagnosis requires the presence of fungal elements in formalin-fixed paraffin-embedded tissue and signifies proven fungal disease but not the identity of the fungus involved. To achieve this, we recommend amplification of fungal DNA by PCR combined with DNA sequencing, but only when fungal elements are seen by histopathology. PCR would add value by allowing identification of the fungus to genus and possibly species levels. Because the technique used should be rigorously quality controlled, only laboratories with a proven record in performing DNA extraction from formalin-fixed tissue should undertake this. The identity of the fungus should be consistent with the histopathologic findings [58–60].

Other Disease Entities

Pneumocystosis: Group 7

The inclusion of *Pneumocystis jirovecii* pneumonia (PCP) diagnosis in the updated definitions was limited to patients not living with human immunodeficiency virus (HIV). Diagnosing PCP has been more difficult among these patients possibly due to a more focal pulmonary involvement, lower suspicion of disease, and lower sensitivity of traditional histologic and microscopy diagnostic tests [61]. As such, it is important to more fully define host factors for patients at increased risk for PCP. We selected receipt of therapeutic doses of corticosteroids for at least 2 weeks within the past 60 days; antineoplastic, antiinflammatory, or immunosuppressive treatment; and low CD4 lymphocyte counts due to a medical condition. This includes, but is not limited to, patients with primary immunodeficiencies, hematologic malignancies, SOTs, and allogeneic HSCT recipients [62, 63]. Clinical criteria in this population tend to be nonspecific and include cough, dyspnea, and hypoxemia. Radiographic abnormalities include bilateral ground-glass opacities and, less frequently, consolidation, small nodules, unilateral infiltrates, pleural effusions, and cystic lesions [61, 64, 65]. Amplification of *P. jirovecii* DNA by quantitative real-time PCR on BAL fluid, expectorated sputum, or oral wash specimens is preferred to qualitative PCR and is helpful to establish probable disease. However, further studies are needed

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to validate thresholds for positivity [66, 67]. Similarly, 2 or more serum BDG levels of ≥80 ng/L are useful for diagnosing probable disease in appropriate clinical context provided other IFDs have been excluded [68, 69].

Cryptococcosis: Group 8

A broader understanding of the natural history and host factors associated with cryptococcal disease warrants updating these definitions. We support the previous definitions of proven and probable cryptococcal disease in any host. However, we also recognize cryptococcal infection among individuals in high-risk host groups who have few, if any, symptoms and only a positive serum cryptococcal antigen test (asymptomatic cryptococcal antigenemia). This condition may be more common than symptomatic disease, and patients may develop clinical cryptococcal disease unless treated and so are now included in these definitions [70]. The term "disseminated cryptococcosis" as distinct from CNS cryptococcosis has been abandoned in favor of the terms "pulmonary," "CNS" and "other extrapulmonary sites." "Colonization" with *Cryptococcus* spp. is no longer included in the definitions as it is poorly understood and its natural history is unknown.

Identification to the species level for *Cryptococcus neoformans* and *Cryptococcus gattii* has become increasingly important based on reports that suggest different clinical presentations, outcomes, and responses to antifungal therapy between these 2 species [71, 72]. Verification of species that use CGB (L-canavanine, glycine, bromthymol blue) agar or matrix-assisted laser desorption ionization–time of flight mass spectrometry is recommended. Outcomes for HIV-associated cryptococcosis due to *C. neoformans* and *C. gattii* appear to be similar, and identification to the species level may be unnecessary [73, 74].

Endemic Mycoses: Group 9

The endemic mycoses are caused by environmental fungi that are usually restricted geographically and cause disease in immunocompetent and immunocompromised hosts. Causative agents include *Histoplasma capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*, *Blastomyces* species complex (eg, *B. dermatitidis*, *B. gilchristii*, *B. helicus*, *B. silverae*, and *B. parvus*), *Coccidioides immitis/Coccidioides posadasii*, *Paracoccidioides brasiliensis/Paracoccidioides lutzii, Sporothrix* species complex (*S. brasiliensis*, *S. schenckii sensu stricto*, *S. globosa*, and *S. luriei*), *Talaromyces* (formerly *Penicillium*) *marneffei*, and *Emergomyces* species (*E. pasteurianus*, *E. africanus*, *E. orientalis*, *E. canadensis*, and *E. europaeus*) [75–80]. Probable endemic mycoses are defined by evidence of environmental exposure to the fungus, a compatible clinical illness, and the presence of either *Histoplasma* or *Blastomyces* antigen in any body fluid or antibody to *Coccidioides* species in serum or CSF as recovery by culture and histopathologic evidence of infection is generally lacking. There are no approved serologic tests for *T. marneffei*,

Table 3. Other Probable Invasive Diseases

Candidiasis
Host factors
Recent history of neutropenia <0.5 × 10^9 neutrophils/L (<500 neutrophils/mm^3 for >10 days) temporally related to the onset of invasive fungal disease
Hematologic malignancy
Receipt of an allogeneic stem cell transplant
Solid organ transplant recipient
Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of ≥0.3 mg/kg corticosteroids for ≥3 weeks in the past 60 days
Treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days
Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, CARD9 deficiency, STAT-1 gain of function, or severe combined immunodeficiency)
Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids
Clinical features
At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks:
Small, target-like abscesses in liver or spleen (bull's-eye lesions) or in the brain, or, meningeal enhancement
Progressive retinal exudates or vitreal opacities on ophthalmologic examination
Mycological evidence
ß-D-glucan (Fungitell) ≥80 ng/L (pg/mL) detected in at least 2 consecutive serum samples provided that other etiologies have been excluded
Positive T2Candida[a]
Cryptococcosis
Host factors[b]
Human immunodeficiency virus infection
Solid organ or stem cell transplant recipient
Hematologic malignancy
Antibody deficiency (eg, common variable immunoglobulin deficiency)
Immunosuppressive therapy (including monoclonal antibodies)
End-stage liver or renal disease
Idiopathic CD4 lymphocytopenia
Clinical features
Meningeal inflammation
Radiological lesion consistent with cryptococcal disease
Mycological evidence
Recovery of *Cryptococcus* from a specimen obtained from any nonsterile site
Pneumocystosis[c]
Host factors
Low CD4 lymphocyte counts <200 cells/mm^3 (200 × 10^6 cells/L) for any reason
Exposure to medication (antineoplastic therapy, antiinflammatory, or immunosuppressive treatment) associated with T-cell dysfunction
Use of therapeutic doses of ≥0.3 mg/kg prednisone equivalent for ≥2 weeks in the past 60 days
Solid organ transplant
Clinical features
Any consistent radiographic features particularly bilateral ground glass opacities, consolidations, small nodules or unilateral infiltrates lobar infiltrate, nodular infiltrate with or without cavitation, multifocal infiltrates, miliary pattern [d]
Respiratory symptoms with cough, dyspnea, and hypoxemia accompanying radiographic abnormalities including consolidations, small nodules, unilateral infiltrates, pleural effusions, or cystic lesions on chest X-ray or computed tomography scan

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Table 3. Continued

Mycological evidence
ß-D-glucan (Fungitell) ≥80 ng/L (pg/mL) detection in ≥2 consecutive serum samples provided other etiologies have been excluded
Detection of *Pneumocystis jirovecii* DNA by quantitative real-time polymerase chain reaction in a respiratory tract specimen
Endemic mycoses
Host factors
Not applicable as these diseases affect both healthy and less healthy hosts
Clinical features
Evidence for geographical or occupational exposure (including remote) to the fungus and compatible clinical illness
Mycological evidence
Histoplasma or *Blastomyces* antigen in urine, serum, or body fluid
Antibody to *Coccidioides* in cerebrospinal fluid or 2-fold rise in 2 consecutive serum samples

Probable invasive fungal diseases (IFD)requires the presence of at least 1 host factor, a clinical feature and mycologic evidence and is proposed for immunocompromised patients only, whereas proven invasive fungal disease can apply to any patient, regardless of whether the patient is immunocompromised. Except for endemic mycoses, probable IFD requires the presence of a host factor, a clinical feature, and mycologic evidence, whereas cases that meet the criteria for a host factor and a clinical feature but for which mycological evidence has not been found are considered possible IFD.

[a]T2Candida is US Food and Drug Administration approved for the detection of *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* in blood.

[b]Cryptococcosis also occurs in phenotypically normal hosts.

[c]Definitions for human immunodeficiency virus–associated pneumocystosis are not included here.

[d]Bilateral, diffuse ground glass opacities with interstitial infiltrates are more common than other features such as consolidations, small nodules, thin-walled cavities, and unilateral infiltrates.

S. schenckii species complex, or *P. brasiliensis*. Exposure to 1 of these fungi is defined as a history of residence in an endemic area, no matter how remote, travel to an endemic area, or contact with fomites such as soil or vegetation that is derived from an endemic area.

Proven Invasive Fungal Disease

The revised definitions of proven IFD are shown in Table 1.

Probable Invasive Fungal Disease

Several changes were made to the definitions of probable IFD (Tables 2 and 3). For example, host factors were expanded to include inherited severe immunodeficiency and low CD4 lymphocyte counts. Radiographic features were expanded to include wedge-shaped and segmental or lobar consolidation and a reverse halo sign to indicate mold disease of the lower respiratory tract. Revised thresholds for GM now replace those of the manufacturer. *Aspergillus* PCR is now included, and there are mycologic criteria for non-HIV–associated pneumocystosis.

Possible Invasive Fungal Disease

While definitions of proven and probable disease have been shown to be reliable in research and attracted little controversy among the group, this cannot be said of the possible IFD category. There is much confusion about the difference between a host factor and a risk factor. As before, a host factor has been defined as a characteristic of individuals clearly predisposed to, and not simply at risk of, an IFD [1]. For example, while impaired gut wall integrity through surgery or illness may increase the risk of candidiasis, it was not considered specific enough to warrant inclusion as a host factor. Pulmonary abnormalities such as tree-in-bud opacities and interstitial abnormalities were excluded from the clinical features as they can be due to a wide range of pathologies in addition to IFD.

GENERAL POINTS

Throughout this process, we have emphasized the need to differentiate between definitions of IFD required for clinical research from those that influence clinical practice. In clinical practice, many would administer an antifungal agent to any patient at risk of IFD when fungi are detected by biomarkers in serum, plasma, whole blood, or relevant body site fluid without there being sufficient evidence to satisfy the consensus definitions of IFD. We also recognize that our definition of a host factor errs on the side of conservatism given the increasing use of drugs such as monoclonal antibodies for treating a variety of conditions.

Other controversial issues included distinguishing between the performance characteristics of tests for screening and confirmation, the impact of exposure to antifungal agents used for prophylaxis or treatment on imaging and diagnostic tests, and the use of biomarkers to monitor therapeutic outcome. We agree that further research will be required to evaluate the evidence for each of these assays. Finally, there was consensus that diagnostic strategies to determine the relative efficiency of an available test, alone or in combination with other diagnostic tests, should be considered further.

CONCLUSIONS

In summary, these revised definitions represent consensus expert opinion based on the best available evidence. As such, they will need to be reviewed regularly for their utility and relevance and, where possible, extended to other populations affected by IFDs. We acknowledge the limitations of these definitions, including the exclusion of certain cases of IFD. However, the reliance on host factors, clinical features, and mycologic evidence to define IFD in selected populations has proven its value for clinical trials, epidemiologic studies, and the evaluation of diagnostic tests.

Notes

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Diagnostic errors in the intensive care unit: a systematic review of autopsy studies

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ABSTRACT

Context: Misdiagnoses may be an underappreciated cause of preventable morbidity and mortality in the intensive care unit (ICU). Their prevalence, nature, and impact remain largely unknown.

Objectives: To determine whether potentially fatal ICU misdiagnoses would be more common than in the general inpatient population (~5%), and would involve more infections or vascular events.

Data sources: Systematic review of studies identified by electronic (MEDLINE, etc.) and manual searches (references in eligible articles) without language restriction (1966 through 2011).

Study selection and data abstraction: Observational studies examining autopsy-confirmed diagnostic errors in the adult ICU were included. Studies analysing misdiagnosis of one specific disease were excluded. Study results (autopsy rate, misdiagnosis prevalence, Goldman error class, diseases misdiagnosed) were abstracted and descriptive statistics calculated. We modelled the prevalence of Class I (potentially lethal) misdiagnoses as a non-linear function of the autopsy rate.

Results: Of 276 screened abstracts, 31 studies describing 5863 autopsies (median rate 43%) were analysed. The prevalence of misdiagnoses ranged from 5.5%–100% with 28% of autopsies reporting at least one misdiagnosis and 8% identifying a Class I diagnostic error. The projected prevalence of Class I misdiagnoses for a hypothetical autopsy rate of 100% was 6.3% (95% CI 4.0% to 7.5%). Vascular events and infections were the leading lethal misdiagnoses (41% each). The most common individual Class I misdiagnoses were PE, MI, pneumonia, and aspergillosis.

Conclusions: Our data suggest that as many as 40 500 adult patients in an ICU in USA may die with an ICU misdiagnoses annually. Despite this, diagnostic errors receive relatively little attention and research funding. Future studies should seek to prospectively measure the prevalence and impact of diagnostic errors and potential strategies to reduce them.

INTRODUCTION

Diagnostic errors are an underappreciated cause of preventable mortality and morbidity in hospitalised patients, accounting for an estimated 40 000–80 000 annual deaths in USA.[1] Patients in an intensive care unit (ICU) may be especially prone to suffer harm from diagnostic errors. They have limited reserve, often require fast diagnosis and treatment, are cared for by multiple clinicians and undergo frequent laboratory and imaging evaluations.[2] Harm may result from delay or failure to treat the correct underlying disease, complications of unnecessary diagnostic testing or treating a condition that is not actually present.[1]

Diagnostic errors are missed, wrong or delayed diagnoses that are detected by some subsequent definitive test or finding.[3] Autopsy-detected misdiagnoses are typically classified by the Goldman criteria and based on their clinical relevance and the potential that timely therapy would have changed the outcome. The Goldman criteria for autopsy confirmed discrepancies stratify misdiagnosis errors as major (Class I and II misdiagnosis) and minor (Class III and IV misdiagnosis). A Class I error is a major missed diagnosis with a potential adverse impact on survival (likely was the cause of death or contributed to the patient's death) and had that diagnosis been known would have changed management and outcome. A Class II error is a major missed diagnosis that did not have an impact on survival (was not the cause of death nor directly contributed to the patient's death). A Class III error is a minor missed diagnosis related to a terminal disease but not related to the cause of death, and Class IV errors are other missed minor discrepancies.[4]

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A 2003 systematic review of autopsy-confirmed misdiagnoses found that 9% were Class I errors and 15% were Class II errors.[5] After adjusting for declining autopsy rates over time, the authors estimated that 10% of all hospital deaths had a major diagnostic error and 5% of these were Class I errors. They also estimated that the Class I error prevalence in the ICU population was twice the overall prevalence in US hospitals.

Nevertheless, the prevalence of diagnostic errors, the conditions ultimately diagnosed and the degree of harm from these errors are largely unknown. The methods to measure diagnostic errors are underdeveloped and most studies measuring patient safety ignore diagnostic errors.[1] In a recent study for example, diagnostic error was not a variable in examining temporal changes in patient harm over time.[6] As such, the problem of diagnostic errors remains largely invisible. The purpose of this study was to systematically estimate the prevalence and distribution of autopsy-confirmed diagnostic errors in the ICU population. We hypothesised that diagnostic errors would be more common in the ICU population than previously reported for the general hospital population. Additionally, based on a preliminary assessment of the literature, we hypothesised that most errors identified related to infections or vascular events.

METHODS

Search strategy

We conducted a systematic review of the published literature from 1966 through 2011 for observational studies that examined the prevalence of potentially lethal diagnostic errors in the ICU, as diagnosed by the reference standard of autopsy. Databases searched included PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature, and the Cochrane Database of Systematic Reviews using the Johns Hopkins Welch Medical Library online electronic resources. There were no language limitations. The full search strategies (see online appendix) were designed by librarians from the Johns Hopkins Welch Medical Library (KR, VG) together with clinical investigators (BW, DNT). All of the clinical investigators that served as reviewers have expertise in critical care or evaluation of diagnostic errors. Two reviewers (JC and SGK) independently screened each abstract and all studies considered eligible by either reviewer were passed on to two reviewers (JC and BW) for a full article review to determine inclusion in the final analysis. Disagreements between these reviewers were resolved by group consensus. Bibliographies from retrieved articles were screened for other relevant studies; none were identified. We did not attempt to contact authors or identify meeting abstracts, unpublished studies, or other grey literature.

Study selection

We included studies if they provided autopsy-confirmed misdiagnosis data on adult patients who died while in an intensive care setting. Studies without original patient data were excluded. We did not set standards for autopsy performance or for conduct and reporting of individual studies (eg, prospective vs retrospective). Two types of studies were defined: (1) general misdiagnosis studies (reporting on all misdiagnoses or all misdiagnoses of a minimum severity),[7–37] and (2) disease-specific studies (reporting only on misdiagnoses of particular conditions, such as pulmonary embolus).[38–46] We excluded disease-specific studies from our analysis. A flowchart of our study selection is in figure 1.

Analysis

Four authors (JC, BW, AN, SMG) reviewed the general misdiagnosis studies and abstracted the data for pooled analysis. These data included number of deaths, autopsies and errors, error class, underlying diagnoses, country and ICU type. We did not assign a quality rating. Errors were defined by the Goldman Classification scheme. Class I are missed major diagnoses with potential adverse impact on survival and that would have changed management. Class II is a missed major diagnosis that would not have impacted survival and that

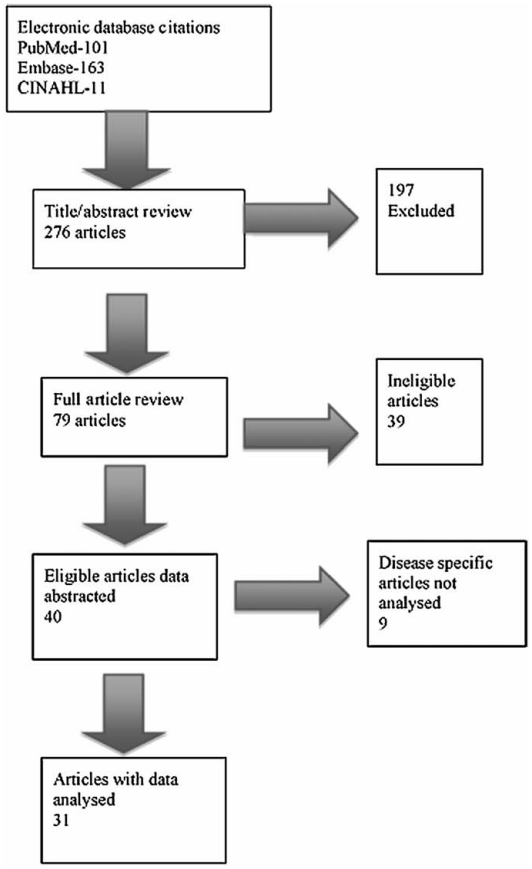

Figure 1 This figure shows the study selection flowchart.

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would not have changed therapy. Class III and Class IV are minor errors related to the terminal disease but not death and completely unrelated to either, respectively. The majority of studies reported Class I[7–16 18–32 34–37] and II[7 9 10 12 13 15 16 18–32 34–36] error data, and less reported Class III[9 10 12 13 15 16 18–20 22 26 30 31] and IV[9 12 13 16 18 20 23] error data.

We defined four broad disease categories to assess the distribution of types of misdiagnoses. These categories included: (1) vascular (myocardial infarction, pulmonary embolus, stroke, any organ infarct, any vessel rupture or dissection, and any haemorrhage), (2) infectious (bacterial, aspergillosis, other fungal, viral, and tuberculosis), (3) mechanical pathophysiological states (cardiac tamponade, bowel obstruction, perforated viscus, congestive heart failure, and cardiac valve failure), and (4) neoplastic/other. Some studies treated pneumonia and aspergillosis separately from other infections.[10 14 19 20 25 34] We aggregated these results during the analytic phase.

Data were tabulated using Microsoft Excel 2008 for Mac (Microsoft Corp., Redmond, Washington, USA). Descriptive statistics were calculated using Stata V.11 (Stata Corp., College Station). A p value <0.05 was considered statistically significant. A logistic regression model was performed to create a hypothetical prediction of the prevalence of misdiagnoses at higher autopsy rates to predict what the actual error rate might be if every patient who died in an ICU always had an autopsy. A random intercept logistic regression model was fit to the class I misdiagnosis rate (number of class I errors vs number of autopsies performed) as a non-linear function of the per cent of autopsies performed (natural spline with two degrees of freedom). A likelihood ratio test was performed with R V.2.11.1 (R Foundation for Statistical Computing, Vienna, Austria) to determine if the non-linear function was justified over a linear fit. The random intercept was included to account for natural variation across studies as is standard in meta-analysis. We controlled for size of study (number of autopsies) and the study time period in this model.

This report was prepared according to Meta-analysis of Observational Studies in epidemiology (MOOSE) guidelines for systematic reviews of observational studies.[47]

RESULTS

Of 276 abstracts screened, 78 were advanced to full article review (κ for concordance was 0.97; SE =0.11). We identified 41 studies and subsequently excluded one by group consensus because the unit studied could not be clearly identified as an ICU. Of the remaining 40 studies, 30 reported general misdiagnosis (28 retrospective) representing a total of 5863 autopsies and were included in our analysis, and nine were disease-specific misdiagnosis and excluded from our analysis (tables 1 and 2). The study periods ranged from 11 months to 300 months and included a wide range of ICU types. Autopsy rates ranged from 6% to 100% (weighted mean 41%, median 43%).

The prevalence of autopsy-detected misdiagnoses ranged from 5.5% to 100%. In aggregate, 28% (n=1632/5863) of autopsies reported at least one misdiagnosis (figure 2). Of the 31 studies, 27 reported class I error data, 25 class II, 13 class III, and 7 class IV error data. Class I diagnostic errors were identified in 8% (n=343/4514); Class II errors in 15% (n=637/4403); Class III errors in 15% (n=264/1711); and Class IV errors in 21% (n=175/816) of autopsies reporting (figure 2). Class I misdiagnoses were less prevalent at higher autopsy rates (figure 3) although total misdiagnoses were not (data not shown). The predicted prevalence of potentially lethal misdiagnoses, when extrapolated to a hypothetical 'perfect' 100% autopsy rate was 6.3% (95% CI=4.0% to 7.5%). Our model estimates the Class I error prevalence as a function of autopsy rate; the model result (6.3%) differs from the measured prevalence (8%), which is simply the average misdiagnosis prevalence from all autopsies reporting Class I errors. A likelihood ratio test was used to determine if the non-linear function was justified over a linear fit (p=0.021). In addition, a likelihood ratio test was used to determine if the autopsy rate (based on natural spline fit) provided any information for the class I misdiagnosis rate (p=0.002). After accounting for the autopsy rate, the estimated SD of the log odds of a class 1 misdiagnosis across studies was 0.58 (SE: 0.11), translating to study to study variation accounting for roughly 10% of the total variation.

Final diagnoses were provided in 25 of 31 studies for Class I misdiagnoses and in 16 of 31 studies for Class II misdiagnoses (figure 4 and box 1). Among all Class I errors, vascular events (41%) and infections (41%) were the most common diagnosis, followed by mechanical pathophysiological states (10%), and neoplasms and other conditions (9%). For all Class II errors, vascular events (47%) were the most common diagnosis, followed by infections (29%), neoplasms and other conditions (21%), and mechanical obstructive pathophysiological conditions (4%). Within these broad categories, pulmonary embolus, myocardial infarction, pneumonia, and aspergillosis were the most common diagnoses in the class I errors. Cumulatively, they accounted for about one-third of all potentially lethal diagnostic errors (box 1).

DISCUSSION

In this study, 28% of autopsied ICU patients had at least one misdiagnosis. Of the autopsies reporting misdiagnosis error classifications, 8% were Class I (major and

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Table 1 Included studies of intensive care unit (ICU) misdiagnoses in general

Author	Year	Country	Type of study	Length of study period (months)	ICU type	Total # of deaths	No of autopsies used for analysis
Berlot[7]	1999	Italy	Retrospective	36	Mixed	346	159
Blosser[8]	1998	USA	Retrospective	12	Med/ coronary	132	41
Combes[9]	2004	France	Prospective	36	Med/surg	315	167
Dimopolous[10]	2004	Belgium	Retrospective	12	Med/surg	489	222
Duke[11]	1999	Australia	Retrospective	24	Not given	238	Not given
Fernandez-Segoviano[12]	1988	Spain	Prospective	30	Mixed	Not given	100
Fish[13]	2000	USA	Retrospective	72	Burn	94	88
Gerain[14]	1990	Belgium	Retrospective	11	Oncology	48	34
Gut[15]	1999	Brazil	Retrospective	36	Med/surg	152	30
Kallinen[16]	2008	Finland	Retrospective	72	Burn	74	71
Koch[17]	2008	Germany	Retrospective	120	Medical	1205	1205
Magret[18]	2006	Spain	Retrospective	46	Mixed	525	80
Maris[19]	2007	Belgium	Retrospective	24	Med/surg	786	289
Mort[20]	1999	USA	Retrospective	72	Surg	560	149
Nadrous[21]	2003	USA	Retrospective	24	Multiple	1597	455
Ong[22]	2002	USA	Retrospective	24	Trauma/burn	158	153
Palazon-Sanchez[23]	1999	Spain	Retrospective	12	Med/surg	67	24
Papadakis[24]	1991	USA	Retrospective	24	Medical	401	172
Pastores[25]	2007	USA	Retrospective	69	Med/surg	658	86
Perkins[26]	2003	Great Britain	Retrospective	42	Med/surg	636	38
Podbregar[27]	2001	Slovenia	Retrospective	24	Medical	270	126
Podbregar[28]	2011	Slovenia	Retrospective	20	Medical	373	170
Roosen[29]	2000	Belgium	Retrospective	24	Medical	108	100
Saad[30]	2007	Brazil	Retrospective	24	Coronary	Unclear	161
Sharma[31]	2005	India	Retrospective	36	Trauma/burn	249	163
Silfvast[32]	2003	Finland	Retrospective	48	Mixed	388	346
Simon[33]	2001	Hungary	Retrospective	12	Medical	163	110
Tai[34]	2001	USA	Retrospective	24	Medical	401	91
Tejerina	2010	Spain	Retrospective	300	Mixed	2857	866
Twigg[36]	2001	Great Britain	Retrospective	36	Med/surg	252	97
Yalamarthi[37]	1998	Great Britain	Retrospective	24	Not given	233	70

ICU, Intensive Care Unit.

potentially lethal), and 15% were Class II (major but not lethal) despite presumably aggressive diagnostic assessment in the critical care environment. Given the 540 000 deaths annually during or immediately following an ICU stay,[48] and extrapolating to a 100% autopsy rate, our findings suggest that 34 000 (95% CI =22 600 to 40 500) ICU patients in the US may die as a result of a Class I error annually, assuming that the error was the cause of death. This predicted number is comparable to the estimated deaths from central line-associated bloodstream infections in the ICU[49] and from breast cancer[50] in the US, and does not consider misdiagnosis-related morbidity among intensive care patients. These results also suggest that despite the dropping autopsy rate over time, autopsy remains a crucial asset especially in ICU patients.

We proved our hypothesis that infections and vascular events would culminate a large majority of the diagnostic errors. Vascular events and infections were the leading potentially lethal misdiagnoses accounting for almost 82% of potentially lethal Class I errors and almost 76% of the serious but not lethal Class II errors. Neoplastic disease was the third most common overall misdiagnosis category but was rarely deemed lethal. Mechanical pathophysiological states were uncommon. Importantly, pulmonary embolus, myocardial infarction, pneumonia, and aspergillosis accounted for over one-third of all Class I misdiagnoses. These four particular diagnostic errors may be useful as outcome measure for designing early interventions to reduce diagnostic errors as a whole.

Our results add new information to the field of diagnostic error research. Shojania *et al*[5] conducted a systematic review of autopsy-detected diagnostic error prevalence rates over time, but they did not describe specific misdiagnoses in the ICU or elsewhere. We included the seven ICU studies from their review in our analysis to offer refined estimates of the prevalence of

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Table 2 Condition/disease specific misdiagnosis studies not analysed

Author	Year	Country	Type of study	Study period (mos)	ICU type	Total no of deaths	No of autopsies analysed	No of missed diagnoses	Disease or diagnosis
deHemptinne[38]	2009	Belgium	Retrospective	36	Med/surg	169	64	15	ARDS only
Du[39]	1996	China	Retrospective	492	Surg	Not given	3447	80	Fungal only
Hasani[40]	2005	Iran	Prospective	12	Mixed	Not given	43	2	Meningitis only
Janson[41]	1976	Germany	Retrospective	24	Surg	100	100	48	CXR misDx only
Petersen[43]	1999	Denmark	Prospective	12	Multiple	659	141	70	Pneumonia only
Pinheiro[44]	2007	Brazil	Retrospective	96	Not given	592	22	7	ARDS only
Al-Saidi[42]	2002	Canada	Retrospective	67	Med/surg	59	28	10	Bone marrow transplant only
Tejerina[45]	2009	Spain	Retrospective	150	Not given	1633	253	111	VAP only
Widimsky[46]	1985	Czech-Slovakia	Retrospective	120	Coronary	Not given	31	12	PE only

ARDS, Acute Respiratory Distress syndrome; CXR, chest X-ray; ICU, Intensive Care Unit; misDx, misdiagnosis; mos, months; PE, pulmonary embolus; VAP, ventilator-associated pneumonia.

misdiagnoses in the ICU by error class and autopsy rate. Shojania *et al* projected that, despite a reduction of 33% in the rate of class I errors over 4 decades, a US hospital would likely continue to observe a class I error at a rate of 4.1%–6.7% across all patients. While a few of the studies in Shojania's review included ICU patients in addition to general ward and emergency department patients, our results suggest that the rate of class I errors are 16%–48% higher in the ICU patient population then the general hospital patient population as a whole. Methodologically, we found that autopsy rates of 30%–40% or higher are likely to produce fairly accurate estimates of the overall prevalence of potentially lethal misdiagnoses in the ICU population, despite potential biases inherent in autopsy case selection.[51]

Observed diagnostic error frequencies were highest in studies with the lowest autopsy rates. Clinical selection of the most complex cases to undergo autopsy is believed to contribute to this effect.[51] To counter this effect, we have modelled the measured error frequency as a function of autopsy rate, controlled for study size and time and extrapolated out to conditions of 100% autopsy. Nevertheless, it is possible that other factors may contribute to the difference seen in studies with low autopsy rates such as a more detailed and rigorous approach to autopsy and error finding. Additionally, the level of diagnostic certainty as to the cause of death is likely to influence the autopsy rate. Podbregar *et al*[27] attempted to measure this influence finding that diagnostic certainty significantly influenced the decision to pursue an autopsy though there were no differences in the class I error rate. As a consequence, our extrapolated estimate for the total error frequency likely represents a conservative, minimum estimate of the true frequency as aggregated from the present data.

Although we could not measure morbidity associated with misdiagnosis in survivors, the consequences and costs are likely substantial. ICU-based studies have reported complications of misdiagnosis, such as re-operation,[52] and increased length of stay.[53] The most frequently reported non-lethal long-term consequences of misdiagnosis are neurological. Preventable or treatable neurological complications of critical illness such as stroke may go unrecognised with devastating consequences.[54–56] Robust estimates of the morbid consequences of diagnostic errors will likely require prospective studies.

Risk predictors for misdiagnosis in the ICU at the patient, organisational or provider level are incompletely defined and there is sparse data on root causes of misdiagnosis in the ICU. Off-hours occurrence appears to be a risk factor for at least some types of ICU misdiagnosis.[53] Patient demographics and institutional factors have been cited as possible influences.[5 57 58] There is

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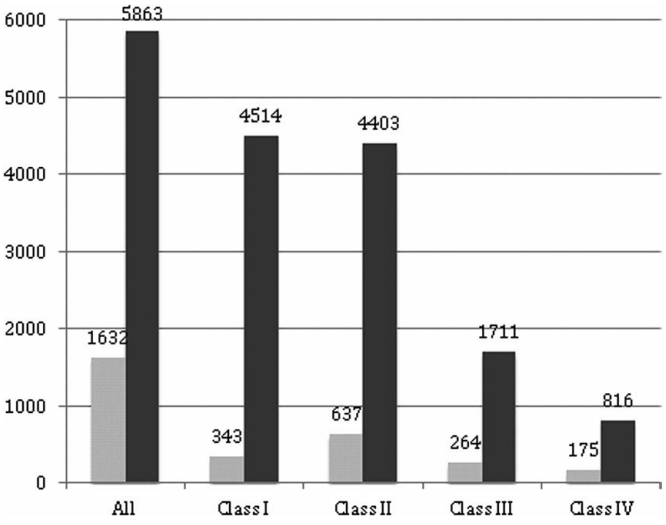

Figure 2 This figure shows the proportion of all autopsies that identified a misdiagnosis, and the proportion of autopsies that identified a misdiagnosis stratified by the Goldman criterion (Class I, II, III, and IV). The grey bars represent identified misdiagnosis and the black bars represent all autopsies reported for total and class. Of the 30 studies included in this analysis, 26 reported class I error data, 24 class II, 13 class III, and 7 class IV error data. Thus, the sample sizes by Goldman classification varied. Stratified by class, 8% of autopsies detected a Class I error, 15% detected a Class II error, 15% detected a Class III error, and 21% detected a Class IV error.

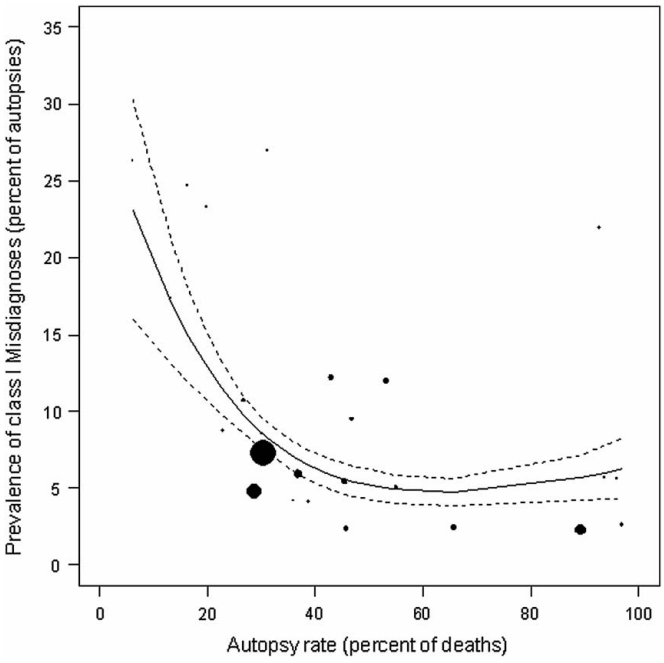

Figure 3 This figure illustrates the prevalence of Class I misdiagnoses (as a per cent of autopsies) and the autopsy rate. Each point on the figure represents data from one study. The plotting symbols are proportional to the number of autopsies performed in each study (with larger plotting symbols indicating larger number of autopsies). The solid line represents the logistic regression models estimate for reported values. The dotted lines represent the 95% CIs. The curve was generated using a natural spline with two degrees of freedom.

evidence that the patient's clinical presentation such as greater co-morbidity or illness complexity increases the risk of misdiagnosis.[52 53 56 59] The diagnostic process is complex[60] and errors may occur at any stage during the process, often at multiple stages.[61] Some misdiagnoses may be directly linked to limited sensitivity and specificity of individual tests in the critical care setting,[62] but remediable causes are most often associated with failures in information processing. Cognitive errors are also a major contributor to misdiagnoses and a recent review by Graber *et al*[63] identified a large number of possible approaches (some tested and some not) that may help reduce cognitive errors and hence, misdiagnoses.

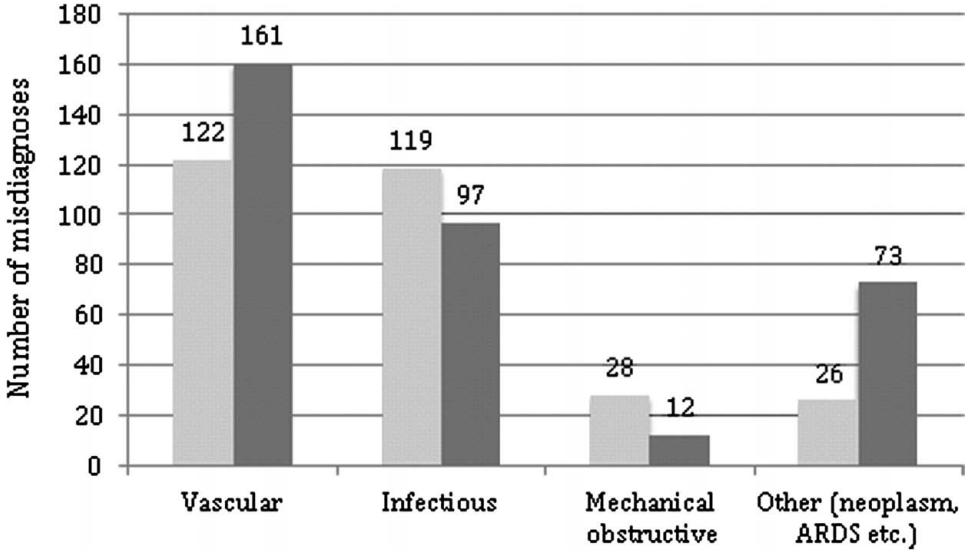

Figure 4 This figure shows the most common misdiagnoses by broad disease categories for Class I and II errors. These categories are vascular (myocardial infarction, pulmonary embolus, stroke, any organ infarct, any vessel rupture or dissection, and any haemorrhage), infectious (bacterial, aspergillosis, other fungal, viral and tuberculosis), mechanical pathophysiological states (cardiac tamponade, bowel obstruction, perforated viscus, congestive heart failure and cardiac valve failure), and neoplasm and other (includes acute respiratory distress syndrome (ARDS)). The number of Class I (black bar) and Class II (grey bar) errors are compared for each diagnostic category. The data for Class I errors represents 24 of the 30 studies in the analysis and data for Class II errors represents 16 of 30 studies.

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Box 1 Breakdown of misdiagnoses by final diagnosis reported

Vascular events
  Haemorrhage
Vascular rupture
Aortic aneurysm/dissection (11, 1)
Other (2, 1)
Abdominal bleed (21, 41)
Other (5, 12)
  Thrombosis
Pulmonary embolus (37, 64)
Other (1, 0)
  Ischemia
Myocardial infarction (31, 33)
Bowel infarction (13, 10)
Stroke (1, 0)
Infections
Mechanical obstructive pathophysiology
  Cardiac tamponade (10, 0)
  Perforated viscus not designated peritonitis (9, 4)
  Bowel obstruction (1, 4)
  Tension pneumothorax (1, 0)
  Congestive heart failure (2, 2)
  Cardiac valvular lesion (not endocarditis) (5, 2)
Other
  Malignancy (6, 47)
  Pulmonary oedema (not acute respiratory distress syndrome) (2, 2)
  Acute respiratory distress syndrome (3, 1)
  Pancreatitis (4, 1)
  Cirrhosis (0, 1)
  Airway obstruction (2, 0)
  Other (9, 22)

Values in parentheses are the actual total number of class I and class II identified in studies reporting this data.

A major impediment to better understanding of diagnostic errors is the declining autopsy rate.[5] The reasons for this are multi-factorial and likely include medical culture and societal attitude shifts. One alternative that might improve the rate of autopsy is the 'virtual autopsy' using sophisticated radiological techniques as opposed to dissection. Wichmann *et al*,[64] recently reported a comparison of 'virtual autopsy' to traditional autopsy in ICU patients suggesting that it compares favourably and had a higher acceptance rate in terms of per cent of deaths referred to post-mortem analysis. With further refinement this approach may offer a way to continue to obtain the post-mortem information required.

Our systematic review has significant policy implications. Diagnostic errors have not been the focus of patient safety campaigns or systematic studies.[1 65] A 2003 report of 93 Agency for Health Research and Quality (AHRQ)-funded safety projects found only one focused on misdiagnosis.[65] A recent Special Emphasis Notice has sought proposals for these errors in ambulatory and emergency department settings,[66] but it has not been linked to a funded request for applications.

Our review has limitations. We did not search the grey literature nor did we follow-up with authors, pursue secondary references or consult with experts and publication bias could, in theory, have inflated our measured prevalence. Virtually all studies were retrospective in nature and there is potential for selection bias in those autopsied though Podbregar *et al*[27] found that while diagnostic certainty did influence the rate of autopsy, it did not influence the rate of class I errors. Unfortunately, the reliability of determining whether an error occurred and its severity could not be determined to any meaningful extent in the reviewed studies. Thus our estimate of the class I (lethal) diagnostic error frequency may either be an over- or under-estimate of the true frequency. Debate continues on the extent to which this is a potential problem.[11] There was some heterogeneity in the study populations included in our review and the measured misdiagnosis prevalence varied even at high autopsy rates, so misdiagnosis prevalence might not be evenly distributed. We did not assign a quality rating to the studies analysed based on autopsy confirmed diagnosis being a 'gold standard'. Autopsy, as a 'gold standard', is potentially flawed as the decision to perform an autopsy or the results of the autopsy may be influenced by the degree of involvement of clinicians in the judgement of diagnostic errors, the degree to which pathologists knew the clinical history and working diagnoses at the time that they performed autopsy and the format/context in which requests for autopsy were transmitted. The original study authors may have misclassified the types of diagnostic errors, resulting in inaccurate estimates of lethality or preventability and disease frequency estimates might be distorted. Nevertheless, given the consistency with which studies reported missed infections and vascular events as a common source of lethal diagnostic errors, these are likely important.

In conclusion, this systematic review and analysis suggests that between 22 600–40 500 ICU patients die each year in the US with and potentially from a diagnostic error and many more suffer a clinically relevant diagnostic error. These data suggest that diagnostic errors are a significant cause of preventable harm in hospitalised patients. To this point, diagnostic errors have received relatively little attention and research funding, leaving the methods to measure them immature; this must change. Future studies should seek to prospectively measure the prevalence and direct impact of diagnostic errors, including those that are non-lethal, and potential strategies to reduce them.

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Impact of portable air filtration units on exposure of haematology–oncology patients to airborne *Aspergillus fumigatus* spores under field conditions

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Aspergillus fumigatus; Air sampling; Invasive aspergillosis; Haematology–oncology; Portable air filtration units

Summary We undertook a one-year study to investigate the impact of the NSA model 7100A/B portable air filtration unit on exposure of haematology–oncology patients to airborne *Aspergillus fumigatus* spores under field conditions. Weekly measurements for airborne *A. fumigatus* were conducted in indoor and outdoor air, and surveillance for invasive aspergillosis was based on a combination of ward liaison, targeted chart review and consultation with the medical staff. The mean indoor *A. fumigatus* counts (8.1 cfu/m^3; range, $<$0.8 to 42 cfu/m^3) reflected the fungal load of outdoor air (9.4 cfu/m^3; range, $<$0.8 to 50 cfu/m^3), and were reduced by only about one third in rooms with portable air filtration units (5.3 cfu/m^3; range, $<$0.8 to 41 cfu/m^3). During the study period, a total of five cases (incidence density, 0.8 per 1000 patient-days) of invasive aspergillosis (one proven case, four suspected cases; case fatality rate 40%) were recorded. None of these five patients was allocated to a room with portable air filtration unit, however, the difference between incidence densities in rooms with and without portable air filtration units was non-significant (Fisher's exact test, $P = 0.33$). Due to the noise level and thermal discomfort, patient compliance with the air filtration units was poor. We conclude that under field conditions this air filtration unit cannot be recommended for prevention of invasive aspergillosis in neutropenic haematology–oncology patients.
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Introduction

Invasive aspergillosis plays an important role in the morbidity and mortality of immunosuppressed patients suffering from neutropenia.[1–4] Preventive measures are important in the control of invasive aspergillosis because diagnosis is difficult and the outcome of treatment is poor,[5] and good air quality in critical areas in hospitals is necessary to minimize the incidence of invasive aspergillosis.[6–10] Passweg et al.[11] showed that the use of high-efficiency particulate air (HEPA) filtration and/or laminar

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air-flow systems to prevent infections decreased transplant-related mortality and increased survival after allogeneic bone marrow transplantation for leukaemia. However, in older hospital buildings the installation of central air supply systems is sometimes not possible due to limited space. Rutala et al.[12] demonstrated that portable air filtration units can rapidly reduce levels of airborne particles, and there are some indications that such devices can also be beneficial as an interim solution to reduce indoor aspergillus levels.[13–19] To date, there exist no randomized controlled trials on the clinical efficacy of portable air filtration units. Table I gives an overview of studies addressing the impact of different portable air filtration units on fungal exposure of patients at risk of acquiring invasive aspergillosis. A direct comparison of these studies is difficult due to the different methods used. Most studies show a significant reduction of airborne fungal exposure and recommend these devices as an interim solution, e.g. in older hospital buildings where the installation of central air supply systems is not possible due to the limited space, or as an additional measure during construction or renovation works.[13–19] However, the clinical value of these devices is not supported by strong evidence. The purpose of our study was to investigate the impact of a specific (low-priced) type of portable air filtration unit on exposure of haematology–oncology patients to airborne *Aspergillus fumigatus* spores under field conditions.

Table I Studies addressing the impact of portable air filtration units on fungal exposure of patients at risk of acquiring invasive aspergillosis

Author	Setting	Trade name (manufacturer)	Filter specification	Air-flow per hour (m^3/h)	Air-changes per hour (1/h)	Reduction of fungal air counts[a]		
						Filtered air (mean cfu/m^3)	Unfiltered air (mean cfu/m^3)	Level of significance
Rhame et al. (1984)[18]	BMT ward	Dexon model 685 (Dexon Inc., Minneapolis, MN, USA)	HEPA	1020	20–30	8–33	82–123	-
Opal et al. (1986)[16]	Military medical center during construction works	Cleanaire (Air Techniques, Baltimore, MD, USA)	HEPA	-	-	0.008 ± 0.003 (*Aspergillus* spp.)	1.7 ± 0.2 (*Aspergillus* spp.)	$P = 0.025$
Perraud et al. (1992)[17]	Haematology ward	Airvar (Dianorel, Lyon, France)	HEPA	350–1000	17–18	0–4	2–24	$P < 0.05$
Loo et al. (1996)[14]	Haematology ward	Elixair 600 (Ufox, Finland)	HEPA type	350–700	17–18	0–2	0–11	$P < 0.05$
		Cloud 9 Sterile-Air (Mason Engineering and Designing Corp, Wooddale, IL, USA)	HEPA	-	12–22	-	-	-
Richardson et al. (2000)[19]	Haematology ward	Envair (Envair Ltd Rossendale, Lancashire, UK)	-	-	-	5–11	99–121	$P = 0.02$
Mahieu et al. (2000)[15]	Neonatal ICU during renovation works	MedicClean Air Forte (Willebroek, Belgium)	HEPA	60–250	3	73 (*Aspergillus* spp.)	252 (*Aspergillus* spp.)	$P = 0.004$

BMT = bone marrow transplantation; HEPA = high-efficiency particulate air filter; ICU = intensive care unit.
[a] Total fungal counts unless stated otherwise.

Materials and methods

Setting

The Department of Internal Medicine I at the University Hospital Bonn includes an 18-bed inpatient unit for adult haematology–oncology patients (predominantly patients with acute leukaemia, multiple myeloma, and malignant non-Hodgkin's lymphoma), which is located on the first floor of a separate building surrounded by a forested area. All patient rooms are naturally ventilated (windows, doors) because the installation of a ventilation system with HEPA filtration is not possible because of the limited space in the building. However, patients are instructed to keep the windows closed during periods of neutropenia, and to wear masks when leaving their room. Moreover, regular policies for aspergillus prevention included disinfection of horizontal surfaces including the floor on a daily basis, prohibiting potted plants and flower arrangements within the

whole unit, avoidance of showering during neutropenia, and use of sterile water for any other applications. Specific policies exist for construction or renovation activities, however, no such activities took place during this study. Patients with an expected duration of profound neutropenia of >10 days received antifungal prophylaxis with itraconazole.

Portable air filtration units

We tested three units of the NSA model 7100A/B Environmental Air System (National Safety Associates Limited, Memphis, TN, USA). After a pre-filter panel, the units contain a tri-media-filter (for gases/odours) and a HEPA-type filter that should be replaced at a minimum of every six months and two years, respectively, according to the manufactures' instructions. The NSA unit has a 95% filtering capacity for particles >0.3 μm, and a flow (at its 'high' 'low' settings, respectively) of 168 resp. 112 m^3/h at a noise level of 57 resp. 55 dB(A). Three patient rooms (mean room volume, 38.0 m^3; single rooms, 29.2 m^3; double rooms, 46.9 m^3) were equipped with one unit each. The patients in these rooms were advised to keep the windows closed as much as possible, and to run the NSA unit at the 'high' setting. However, due to the high noise level, the units mostly were put to the 'low' setting, or sometimes even stopped during the night. Questioning patients and staff was the only means of determining how long the units were in operation and how long the windows were left open.

Air sampling and laboratory analysis

Air sampling was conducted using the Sartorius MD8 Air Sampler (Sartorius Ltd, Epsom, Surrey, UK) at a flow of 5.0 m^3/h and an 80 mm diameter gelatine membrane filter (pore size 3 μm; Sartorius, Goettingen, Germany). During a period of one year (May 1996 to April 1997), weekly sampling was undertaken at three locations; (1) outdoors, inside patient rooms (2) without, and (3) with, portable air filtration units. All sampling locations were examined in triplicate for 5 min per sample (total air volume, 1250 L). The filters were incubated at 37°C on Sabouraud dextrose agar for a total of 72 h. *A. fumigatus* was identified by light microscopy.

Surveillance for invasive aspergillosis and statistical analysis

During the study period, all cases of suspected or proven invasive aspergillosis were recorded. Invasive aspergillosis was defined according to previously published criteria.[20] As a case-finding method, a combination of ward liaison, targeted chart review and consultation with the medical staff was employed. Infection rates were calculated per 1000 patient-days. Fisher's exact test (version 6; Epi Info, CDC, Atlanta, GA, USA) was used to compare observed with expected proportions. A *P*-value of less than 0.05 was considered statistically significant.

Results

Air sampling

Overall, the mean indoor *A. fumigatus* counts (8.1 cfu/m^3; range, <0.8 to 42 cfu/m^3) were slightly lower compared with outdoor samples (9.4 cfu/m^3; range, <0.8 to 50 cfu/m^3), and were further reduced by 35% in rooms with portable air filtration units (5.3 cfu/m^3; range, <0.8 to 41 cfu/m^3). Only 11.5% of samples from rooms with portable air filtration units exceeded a value of 10 cfu/m^3. The corresponding proportion in unfiltered indoor air, as well as in outdoor air was 25.0%, however, the difference failed to reach statistical significance ($P = 0.076$). Values of <1 cfu/m^3 were obtained in 19.2% and 13.5% of samples from rooms with, and without, portable air filtration units, respectively, whereas the proportion in outdoor air was only 7.6%; however, the differences also failed to reach statistical significance ($P = 0.085$). We found no marked seasonal variation of indoor or outdoor *A. fumigatus* counts.

Surveillance data

During the study period, five cases of invasive aspergillosis (one proven, four suspected) were recorded in patients with acute leukaemia, two (40%) of the patients died. The overall incidence density for (suspected or proven) invasive aspergillosis was 0.8 per 1000 patient-days. None of these five patients had been allocated to a room with portable air filtration unit, however, the difference between incidence densities in rooms with and without portable air filtration unit (i.e. five infections per 4800 versus none per 1200 patient-days) was non-significant ($P = 0.33$).

Peak counts and aspergillosis cases

Counts of >30 cfu/m^3 ('peaks') were detected in 1/52 (1.9%) samples from rooms with air filtration,

2/52 (3.8%) samples from rooms without air filtration, and 6/52 (11.5%) outdoor samples. Seven of these nine peaks were not related to cases of invasive aspergillosis. However, in two cases of suspected invasive aspergillosis (dates of onset: 20 and 23 August 1996), which had both received chemotherapy for acute myeloid and acute lymphocytic leukaemia, respectively, during the first week of August an association in time did occur. In the week before chemotherapy (29th July), we detected concentrations of 31 cfu/m^3 (outdoor) resp. 16 cfu/m^3 (indoor) and the week after chemotherapy (12 August) there was an indoor peak of 42 cfu/m^3; all other counts during this time period (i.e. about six weeks before onset of the two cases of invasive aspergillosis) were <10 cfu/m^3. The remaining three cases of invasive aspergillosis did not show any correlation with peaks.

Discussion

Our study shows that under field conditions the NSA air filtration unit Model 7100A/B reduced the indoor *A. fumigatus* counts by about one third compared with rooms without air filtration unit. However, this degree of reduction does not seem to be of clinical relevance for patients at high risk, as the air filtration units were not able completely to remove *A. fumigatus* spores from indoor air. It is not known whether there is a 'safe' level of exposure, however, in order to prevent exogeneous aspergillus infections in neutropenic patients, their environment should be as free as possible from aspergillus spores, i.e. considerably below 1.0 cfu/m^3.[7,13,21–22] From the clinical point of view, our surveillance data did not allow a conclusive assessment of the efficacy of the air filtration units. It may be that a study of a greater number of rooms equipped with such units might reveal a small but statistically significant effect. In addition, the use of antifungal prophylaxis with itraconazole in patients with prolonged severe neutropenia may have interfered with the effect of the environmental intervention.

There are several limitations that might explain why the NSA air filtration unit failed to reduce *A. fumigatus* spores further. First, the 95% filtering capacity does not meet the criterion of portable HEPA filtration units, which is defined as a removal of at least 99.97% of particles >0.3 μm in diameter.[12] We are not sure, however, if this is the most important point as the NSA air filtration unit has been shown effectively to reduce particles of this range[12] and Miller-Leiden et al.[23] found that air filters containing efficient, but non-HEPA filter media were as effective as air filters containing HEPA filter media. Second, the flow of the air filtration units was relatively low, reaching a room air exchange rate in the patient rooms of between 2.4 to 3.8 per hour at the low (3.6 to 5.8 per hour at the high) setting. The CDC recommend at least 12 room air changes per hour in order to minimize fungal spore counts.[13] Third, the patient's compliance with the air filtration units was low due to the noise level and heat generated by the units, particularly at high outdoor temperatures when patients transiently opened the windows.

We conclude that the specific air filtration unit investigated in this study cannot be recommended for prevention of invasive aspergillosis in neutropenic haematology–oncology patients. Further studies on this topic should be conducted using air filtration units with higher filtering capacity, higher airflow, lower noise level, and ideally with the air intake from outside the patient's room.

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REVIEW

Nosocomial aspergillosis in outbreak settings

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KEYWORDS
Aspergillus outbreaks; Aspergillus; Environmental air sampling/culturing; Immunocompromised hosts; Outbreak investigations

Summary Nosocomial aspergillosis represents a serious threat for severely immunocompromised patients and numerous outbreaks of invasive aspergillosis have been described. This systematic review summarizes characteristics and mortality rates of infected patients, distribution of *Aspergillus* spp. in clinical specimens, concentrations of aspergillus spores in volumetric air samples, and outbreak sources. A web-based register of nosocomial epidemics (outbreak database), PubMed and reference lists of relevant articles were searched systematically for descriptions of aspergillus outbreaks in hospital settings. Fifty-three studies with a total of 458 patients were included. In 356 patients, the lower respiratory tract was the primary site of aspergillus infection. Species identified most often were *Aspergillus fumigatus* (154 patients) and *Aspergillus flavus* (101 patients). Haematological malignancies were the predominant underlying diseases (299 individuals). The overall fatality rate in these 299 patients (57.6%) was significantly greater than that in patients without severe immunodeficiency (39.4% of 38 individuals). Construction or demolition work was often (49.1%) considered to be the probable or possible source of the outbreak. Even concentrations of *Aspergillus* spp. below 1 colony-forming unit/m^3 were sufficient to cause infection in high-risk patients. Virtually all outbreaks of nosocomial aspergillosis are attributed to airborne sources, usually construction. Even small concentrations of spores have been associated with outbreaks, mainly due to *A. fumigatus* or *A. flavus*. Patients at risk should not be exposed to aspergilli.
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Introduction

Aspergillus spores are ubiquitous in their distribution, and inhalation of spores is believed to be the usual route of transmission. In most healthy individuals, spores are removed by functional innate defence mechanisms such as monocyte-derived and resident macrophages.[1] Unfortunately, in severely immunocompromised hosts, such as patients suffering from haematological malignancies[2–6] or solid organ transplant patients,[7–11] invasive aspergillosis (IA) may represent a serious complication in the course of disease. The incidence of IA is increased in these high-risk patients[12] with an overall case-fatality rate of one-half to two-thirds.[13] IA is difficult to treat and multi-variate analysis has revealed it to be an independent risk factor for mortality in critically ill patients.[14]

To prevent hospital-acquired aspergillus infections, high-risk patients are usually placed in protective isolation rooms in which positive air pressure is maintained compared with surrounding areas.[15] These special rooms are provided with high-efficiency particulate air (HEPA) filters and an air flow of at least 12 air changes/h because HEPA filtration significantly reduces the concentration of fungal spores[16] and the incidence of IA.[17] In addition, horizontal laminar air flow (LAF) is provided in some facilities which drives contaminants out through the ducts.[18] However, additional protection due to LAF remains a matter of debate and the use of LAF is not explicitly recommended by the Centers for Disease Control and Prevention (CDC), the Infectious Disease Society of America (IDSA) or the American Society of Blood and Marrow Transplantation (ASBMT) for the care of haematopoietic stem cell transplant recipients.[15,19] Despite such guidelines for the care of highly susceptible patients and maximum protective efforts, nosocomial outbreaks of IA do occur.[19,20] This systematic review was carried out to summarize the data from all nosocomial aspergillus outbreak reports published to date.

Methods

Collection of data

The outbreak database (www.outbreak-database.com), a web-based register of nosocomial epidemics, was searched for outbreaks due to any type of *Aspergillus* spp.[21] Furthermore, a PubMed search (1 January 1966—15 August 2005) was performed to identify additional aspergillus outbreaks by using the term 'outbreak' in combination with 'aspergillus' or 'aspergillosis'. References were subsequently screened for additional descriptions of aspergillus epidemics in hospital settings.

Extraction of data

The following data were obtained: (1) number of patients involved in the outbreak; (2) number of patients that died due to aspergillus infection; (3) underlying diseases of affected patients; (4) primary site of mycosis; (5) distribution of *Aspergillus* spp. in clinical specimens; (6) concentration of airborne mould spores in diverse hospital areas; (7) genotyping of fungal isolates to confirm an epidemiological relationship; (8) source of the outbreak.

Evaluation of data

Outbreak data were evaluated by both authors independently. Patients were grouped depending upon underlying diseases into the following classes: (1) haematology and bone marrow transplantation; (2) solid organ transplantation; (3) other immunodeficiency; (4) no known severe immunodeficiency. If a relevant immunodeficiency could not be excluded but definite classification of underlying disease was not possible either, patients were grouped as 'other immunodeficiency'. Mortality was calculated for all groups and then compared. Statistically significant differences in mortality rates in diverse patient groups were determined using Chi-squared test (Epi-Info software, Version 3.3.2). Outbreak sources were considered 'probable' if stated so by the authors of the outbreak. Sources were considered 'possible' if there were circumstances that may have been the source of the outbreak but no exact link could be made with infection.

Results

A total of 53 outbreaks and 458 affected patients were included in this review. Comprising a total of 299 individuals (65.3%), haematological malignancies were the predominant underlying disease. In all but one outbreak, air was the route of fungal spore transmission and the major site of primary infection (356 patients) was the lower respiratory tract.[22] Surgical site infections and superficial skin infections were observed far less frequently (24 patients each). Interpatient spread was only described on one occasion.[23] Species identified most often from clinical samples were *Aspergillus fumigatus* (154 patients) and *Aspergillus flavus* (101 patients). Species differentiation was not

Table I Characteristics of patients and causative *Aspergillus* spp. in nosocomial outbreaks

Author (year, country)	Patient group (*N* patients)	Patients (*N* fatal)	Primary site of infection (*N*)	Clinical *Aspergillus* spp. isolates (*N*)
Gage *et al.* (1970, USA)[53,54]	T-SURG (4)	4 (3)	Endocarditis (4)	*fumigatus* (3); *glaucus* (1)
Burton *et al.* (1972, USA)[55]	RTX (4)	4 (0)	LRTI (4)	*fumigatus* (4)
Rose (1972, USA)[39]	HEMA (?); others (?)	Total: 23 (total: 12)	LRTI (23)	*fumigatus* (≥12)
Aisner *et al.* (1976, USA)[40]	HEMA (8)	8 (≥3)	LRTI (7); sinusitis (1)	Unknown (8)
Kyriakides *et al.* (1976, USA)[56]	RTX (3)	3 (1)	LRTI (3)	*fumigatus* (3)
Arnow *et al.* (1978, USA)[57]	RTX (3)	3 (1)	LRTI (2)	*fumigatus* (3)
Mahoney *et al.* (1979, USA)[58]	HEMA (5)	5 (3)	Sinusitis (3); LRTI (2)	*fumigatus* (1); unknown (4)
Lentino *et al.* (1982, USA)[49]	RTX (7); HEMA (3)	Total: 10 (total: 4)	LRTI (10)	Unknown (10)
Sarubbi *et al.* (1982, USA)[59]	HEMA (?); others (?)	Total: 22 (total: 1)	LRTI (1)	*flavus* (22)
Gustafson *et al.* (1983, USA)[60]	RTX (9)	9 (7)	LRTI (8); epidural abscess (1)	*fumigatus* (3); unknown (6)
Gerson *et al.* (1984, USA)[61–63]	HEMA (15)	15 (?)	LRTI (15)	Unknown (15)
Grossman *et al.* (1985, USA)[64]	HEMA (6)	6 (0)	Skin infection (6)	*flavus* (3); *fumigatus* (2); *niger* (1)
Krasinski *et al.* (1985, USA)[65]	Neonates (1)	1 (1)	Skin infection (1)	Unknown (1)
Rotstein *et al.* (1985, USA)[66,67]	HEMA (10)	10 (10)	LRTI (9); sinusitis (1)	*fumigatus* (7); *flavus* (3)
Opal *et al.* (1986, USA)[68]	HEMA (7); steroids (3); ONCO (1)	7 (7) 3 (3) 1 (1)	LRTI (11)	*flavus* (4); *fumigatus* (1); *niger* (1); unknown (5)
Allo *et al.* (1987, USA)[69]	HEMA (9)	9 (2)	Skin infection (9)	*flavus* (8); unknown (1)
Perraud *et al.* (1987, France)[70,71]	HEMA (22)	22 (18)	LRTI (22)	*fumigatus* (22)
Ruutu (1987, Finland) [72,73]	HEMA (8)	8 (8)	LRTI (8)	*fumigatus* (8)
Sherertz *et al.* (1987, USA)[3]	HEMA (14)	14 (13)	LRTI (14)	*fumigatus* (?); *flavus* (?)
Weems *et al.* (1987, USA)[74]	HEMA (3)	3 (3)	LRTI (3)	Unknown (3)
Harvey *et al.* (1988, UK)[75]	ICU patients low risk (2); high risk (2)	2 (2) 2 (2)	Endocarditis (3)	*fumigatus* (≥3)
Barnes and Rogers (1989, UK)[18]	HEMA (6)	6 (6)	LRTI (6)	Unknown (6)
Hara *et al.* (1989, USA)[76]	HEMA (1); steroids (1)	1 (1) 1 (1)	LRTI (1); sinusitis (1)	*terreus* (2); *fumigatus* (1)
Hopkins *et al.* (1989, USA)[77]	RTX (3); HEMA (2); ONCO (1)	3 (1) 2 (0) 1 (1)	LRTI (6)	*fumigatus* (6)
Mehta *et al.* (1990, India)[78]	T-SURG (4)	4 (4)	Endocarditis (4)	*fumigatus* (1); unknown (3)
Weber *et al.* (1990, USA)[79]	HEMA (18)	18 (18)	LRTI (17)	*fumigatus* (1); unknown (3)
Arnow *et al.* (1991, USA)[44]	HEMA (12); LiTX (3)	12 (?) 3 (?)	LRTI (15)	*flavus* (9); *fumigatus* (6)
Humphreys *et al.* (1991, UK)[48]	ICU patients low risk (3); steroids (3)	3 (0) 3 (2)	LRTI (≥4)	*fumigatus* (6); *flavus* (1)
Loosveld *et al.* (1992, The Netherlands)[80]	HEMA (5); ONCO (1)	5 (3) 1 (0)	LRTI (6)	*fumigatus* (6)
Pla *et al.* (1992, Spain)[81]	LiTX (2)	2 (2)	SSI (2)	*fumigatus* (2)

Table I (*Continued*)

Author (year, country)	Patient group (*N* patients)	Patients (*N* fatal)	Primary site of infection (*N*)	Clinical *Aspergillus* spp. isolates (*N*)
Richet *et al*. (1992, USA)[50]	T-SURG (6)	6 (0)	SSI (6)	*fumigatus* (6)
Flynn *et al*. (1993, USA)[82]	HEMA (3); ONCO (1)	3 (3) 1 (1)	LRTI (4)	*terreus* (4)
Tritz and Woods (1993, USA)[4]	HEMA (4)	4 (4)	LRTI (4)	*terreus* (4); *fumigatus* (1)
Buffington *et al*. (1994, USA)[24]	HEMA (7)	7 (6)	LRTI (7)	*fumigatus* (3); *flavus* (2); unknown (2)
Iwen *et al*. (1994, USA)[83]	HEMA (5)	5 (5)	LRTI (5)	*fumigatus* (2); *flavus* (1)
Tang *et al*. (1994, UK)[25]	RTX (2)	2 (1)	LRTI (2)	*fumigatus* (2)
Bryce *et al*. (1996, Canada)[41]	Surgery (?); burn unit (?)	Total: 4 (?)	Skin infections (4)	Unknown (4)
Leenders *et al*. (1996, The Netherlands)[26,84]	HEMA (5)	5 (?)	LRTI (2); sinusitis (1); eye infection (1); mastoiditis (1)	*fumigatus* (3); *flavus* (2)
Loo *et al*. (1996, Canada)[85]	HEMA (36)	36 (17)	LRTI (34); sinusitis (2)	*flavus* (10); *fumigatus* (6); *niger* (1)
Singer *et al*. (1998, Germany)[22]	Neonates (4)	4 (3)	Skin infection (4)	*fumigatus* (3); *flavus* (1)
Tabbara and al Jabarti (1998, Saudi Arabia)[86]	Cataract surgery (5)	5 (?)	Eye infection (5)	*fumigatus* (5)
Gaspar *et al*. (1999, Spain)[87]	HEMA (11)	11 (1)	LRTI (11)	Unknown (5)
Mellado *et al*. (2000, Spain)[27]	Steroids (8); other lung disease (2)	Total: 10 (total: 6)	LRTI (10)	*fumigatus* (10)
Thio *et al*. (2000, USA)[28]	HEMA (21)	21 (6)	LRTI (21)	*flavus* (≥5); unknown (16)
Burwen *et al*. (2001, USA)[29]	HEMA (6)	6 (?)	LRTI (6)	*flavus* (6)
Lai (2001, USA)[88]	HEMA (3)	3 (2)	LRTI (3)	*flavus* (2); unknown (1)
Oren *et al*. (2001, Israel)[89]	HEMA (31)	31 (0)	LRTI (31)	Unknown (≥15)
Hahn *et al*. (2002, USA)[90]	HEMA (10)	10 (8)	LRTI (10)	*flavus* (≥7); *niger* (≥2)
Pegues *et al*. (2002, USA)[23]	LiTX (3)	3 (≥2)	SSI (2); LRTI (1)	*fumigatus* (3); *flavus* (1); *oryzae* (1)
Lutz *et al*. (2003, USA)[30]	Surgery (3); RTX (1); T-SURG (2)	3 (1) 1 (0) 2 (1)	SSI (6)	*fumigatus* (4); *flavus* (2)
Myoken *et al*. (2003, Japan)[31]	HEMA (3)	3 (?)	Stomatitis (3)	*flavus* (3)
Panackal *et al*. (2003, USA)[32]	RTX (4)	4 (4)	LRTI (3)	*fumigatus* (4)
Heinemann *et al*. (2004, Belgium)[33,91,92]	T-SURG (9)	9 (2)	SSI (9)	*flavus* (9)

?, exact number unknown; T-SURG, thoracic surgery; RTX, renal transplantation; HEMA, haematology and bone marrow transplantation; ONCO, non-haematologic malignancy; ICU, intensive care unit; LiTX, liver transplantation; LRTI, lower respiratory tract infection; SSI, surgical site infection.

performed in 108 clinical isolates. Genotyping of clinical and environmental isolates was performed in 11 studies[23–33] and revealed similar or identical band patterns of at least some isolates in all but four of those investigations.[27,29,30,32] Detailed data concerning patients' characteristics and causative *Aspergillus* spp. in each outbreak are summarized in Table I.

Table II shows mortality rates in various groups of patients. In 49 patients, the distribution of

Table II Number of patients with different underlying diseases and associated mortality

Underlying disease	No. of patients	Mortality (%)
Haematologic malignancy	299	57.6
Solid organ transplantation		55.9
Renal transplantation	36	
Liver transplantation	8	
Other immunocompromised patients		52.3
High-dose steroid therapy	15	
Neonates	5	
Other malignancy	4	
Chronic lung disease	2	
ICU patients ('high risk')	2	
No exact classification possible	49	
Patients without severe immunodeficiency		39.4
Thoracic surgery	25	
Cataract surgery	5	
ICU patients ('low risk')	5	
Other surgical patients	3	
Total	458	55.0

ICU, intensive care unit.

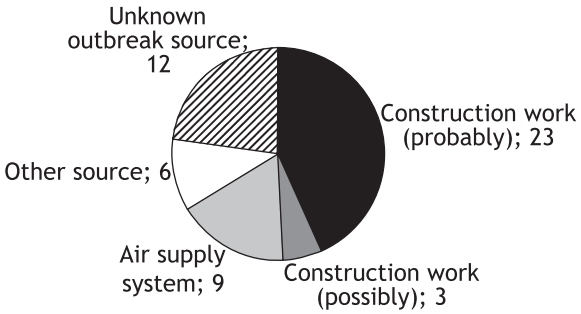

Figure 1 Distribution of sources of nosocomial aspergillus outbreaks.

underlying diseases was not described in detail but all of those diseases did show a relevant immunodeficiency. Therefore, these patients were grouped as described in the Methods section. Mortality was greatest (57.6%) among patients suffering from haematological malignancies. The mortality in this group was significantly higher compared with the mortality of patients without any known immunodeficiency (39.4%; $P < 0.05$).

In 24 outbreaks, volumetric air samples in the hospital and outdoors were taken during the environmental investigation (Table III). The concentration of airborne fungi in patient care areas

Table III Concentration of airborne aspergillus spores (colony-forming units/m^3)

Study	Site of renovation	Operating theatres	Wards, stairs, elevators	Rooms (no HEPA)	Rooms (HEPA)	Outdoor samples
Arnow *et al.*[57]	>235.0		>235.0			
Lentino *et al.*[49]		0.0	0.0	0.0		
Rotstein *et al.*[66]			0.0–5.0	0.0–5.0		3.0
Opal *et al.*[68]	5.9		1.7		<0.1	0.1
Allo *et al.*[69]		0.0–400.0				
Perraud *et al.*[70,71]				6.0		
Sherertz *et al.*[3]			0.2	<0.1	<0.1	1.0
Harvey *et al.*[75]		0.0	0.0	0.0		0.0
Barnes and Rogers[18]			133.0			12.0
Arnow *et al.*[44]			1.1–>90			
Humphreys[48]			0.0			
Richet *et al.*[50]		0.0	0.0			
Flynn *et al.*[82]			>71.0			
Buffington *et al.*[24]	Took volumetric air samples but did not describe exact results of environmental investigation					
Leenders *et al.*[26,84]			0.0–<0.1		0.0–<0.1	0.9–1.3
Loo *et al.*[85]			6.8			
Singer *et al.*[22]				0.0		
Gaspar *et al.*[87]	48.0		5.0–35.0			
Thio *et al.*[28]			0.8–18.0			
Lai[88]			0.0–5.9		0.0	
Oren *et al.*[89]	15.0		15.0		0.2	
Hahn *et al.*[90]			<4.0–>150.0			
Pegues *et al.*[23]			1.1–106.8			
Heinemann *et al.*[33,91,92]			<5.0–115.0			

HEPA, high-efficiency particulate air.

during outbreak investigations ranged from 0 to more than 100 spores/m^3.

As shown in Figure 1, construction work or renovation activities within the hospital or in surrounding areas were most commonly (49.1%) considered to be the probable or possible source of the nosocomial aspergillus outbreak, followed by a contaminated or defective air supply system (17%). In 12 of the 53 outbreaks, the source remained unknown or was not described.

Discussion

Today, construction in or around hospitals is a never-ending phenomenon. This review suggests that construction, renovation, demolition and excavation activities are the main causes of nosocomial aspergillus outbreaks. This is plausible because renovation and demolition work have been shown to increase the amount of airborne fungal spores dramatically,[34] and in consequence increase the risk for aspergillus infection in susceptible patients.[35] Routes of aspergillus transmission in nosocomial outbreaks other than airborne spores are rarely described.[22] Although aspergilli have also been detected in water supply systems, there is no evidence that nosocomial outbreaks derive from the plumbing system.[36,37]

No *Aspergillus* spp. may be disregarded with respect to severity of infection. Instead, any *Aspergillus* spp. in air samples from special care areas should raise concern of invasive infection, although certain *Aspergillus* spp. are more commonly involved in nosocomial outbreaks than others. This may, in part, be explained by an increased pathogenicity of this species, but it may also reflect the natural distribution of aspergilli in the environment.[38]

Environmental investigations are usually initiated in order to assess the quality of air and to identify potential sources of the epidemic. In many studies, the burden of fungal spores in air has been evaluated by gravity sedimentation methods alone (e.g. open Petri dish method).[39–41] However, this method is not volumetric, cannot be calibrated, and hence provides qualitative rather than quantitative results. In preference, air samples should be drawn by impacting particles from an air stream on to agar surfaces after centrifugal acceleration (e.g. Anderson sampler). This technique is more sensitive, allows determination of aspergillus spores in a standardized volume, and is recommended for calculation of the actual density of fungal contamination.[3,42] To date, the minimal airborne concentration of aspergillus spores necessary to cause infection in patients with significant immunodeficiency remains unknown.[43] Even concentrations of airborne aspergillus spores below 1 colony-forming unit (cfu)/m^3 have been shown to be sufficient to cause outbreaks in immunocompromised patients.[3,44] In contrast, the rate of nosocomial IA did not increase in other studies when a median spore count of 0 cfu/m^3 was maintained or high-efficiency filtration face masks were used for neutropenic patients outside patient rooms during the construction period.[45,46]

In accordance with guidelines published by the Healthcare Infection Control Practices Advisory Committee of the CDC, the Association for Professionals in Infection Control and Epidemiology, the IDSA and the ASBMT,[19,20,47] the authors' recommend application of the following measures for nosocomial IA prevention:

(1) avoid non-emergent admissions during heavy construction periods;
(2) if possible, locate high-risk patients as far as possible from areas of demolition or construction;
(3) seal off patient care areas with adequate and impermeable barriers, and keep doors and windows closed;
(4) verify that HEPA air filtration is sufficient and proper air exchange rates are maintained. Check possible plugging or leakage of air filters. Ensure that air pressure relationships are adequate compared with adjunct rooms. Aim for positive pressure in patient rooms and for negative pressure in in-house construction areas;
(5) provide treatment in the patient's room if possible. Transport via an alternate route, schedule transportation during periods with minimal construction activity, minimize waiting times outside, and use appropriate face masks for susceptible patients if it is necessary for them to leave their rooms and pass through potentially contaminated areas;
(6) wet-clean wards thoroughly without raising dust;
(7) surveillance of infections in patients that are at increased risk for IA should be performed.

There are limitations in this approach to determine the minimal infectious dose of aspergillus spores by outbreak analysis. First, environmental investigation starts some time after an increased incidence of nosocomial aspergillosis is recognized, but the concentration of environmental airborne fungal spores varies constantly, e.g. due to climate changes or phases of spores' dissemination from construction work.[48] As such, a single peak of increased spore concentrations at the time of

infection may not be detected. Spores may have settled down in the meantime, and can be detected by environmental swabs while volumetric air samples remain free of fungi.[49,50] Second, after an outbreak has occurred, air sampling should be conducted using large volumes (>1000 L) to increase the likelihood of detecting a low level of spores;[28] however, that was not the case in many outbreak reports. Third, standardized criteria for definite, probable or possible IA were not used in all the studies included in this review. In 2002, international definitions for IA in immunocompromised patients were proposed, but data of earlier outbreak investigations may be influenced by different criteria for IA determination.[51] Finally, despite wide diversity within each species of aspergillus,[52] genotyping was seldom performed to ascertain a common source of infection.[23–33] Even if indistinguishable strains are found, it is also noteworthy that nosocomial aspergillosis is one of the few settings where nosocomial outbreaks can, and probably usually, occur due to a variety of unrelated strains. Thus, findings of similar strains in the nosocomial outbreak setting may also represent inadequate sensitivity of typing methods.

Prospective approaches where the actual airborne fungal concentration is determined in small time intervals in patient care areas during hospital construction periods could be helpful to identify an increase of airborne mould concentrations at an early stage before cases of nosocomial aspergillosis are observed. Detection of airborne aspergilli in high-risk patient care areas should always draw attention to the potential risk of a serious infection. Based on the data available at present, it is concluded that airborne mould spores at any concentration may represent a threat for severely immunocompromised patients.

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Invasive mold infections in iatrogenically immunocompromised children: An eight-yr review

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Abstract: IMI are emerging as an important cause of mortality and morbidity among the growing number of immunocompromised children. A retrospective chart review was performed in all patients with a proven diagnosis of IMI over an eight-yr period (1997–2004) at The Hospital for Sick Children, Toronto, Canada to document the incidence, clinical spectrum, microbiology, treatment, and outcome of pediatric IMI. Twenty-eight patients developed IMI over the study period (10 cancer, 12 HCT, and six SOT patients). IMI occurred in 0.51%, 2.2% and 3.2% after a median time of 118, 60 and 71 days, among cancer, HCT and SOT recipients, respectively. *Aspergillus* spp. infection was diagnosed most commonly (23 patients) and the most common site of infection was the lung (21 patients). Patients at increased risk included those with acute myelogenous leukemia, allogeneic unrelated HCT recipients, graft-versus-host disease, and lung transplant recipients. The mortality after one yr was 60% among cancer patients, 58% among HCT patients, and 16% among SOT patients.

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Key words: invasive mold infection – transplantation – cancer – immunocompromised host

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Children diagnosed with cancer or undergoing HCT or SOT are at risk of opportunistic infections including IMI. The incidence of IMI has been increasing over the past decade, commensurate with the rise in immunocompromised patients (1, 2) and filamentous fungi (molds) now represent the major cause of death due to fungi among immunocompromised patients receiving fluconazole prophylaxis (3). IMI is associated with a high mortality (1, 4, 5), and excess health care costs of approximately US$40 000 per case (2).

Shifts have been observed in the epidemiology of fungal disease with the widespread use of chemoprophylaxis regimens in high risk groups. Infections due to *Candida albicans* have decreased due to the use of fluconazole prophylaxis, while the rate of resistant filamentous fungi has increased (1). The use of itraconazole and voriconazole prophylaxis in some centers has been associated with reduced rates of aspergillosis, but increased rates of zygomycosis (6, 7). In this context, current data on the epidemiology, management, and outcome of IMI in pediatric populations are needed. We, therefore, undertook a detailed descriptive study of the epidemiology, clinical features, prophylaxis, treatment practices, and outcome of IMI in children managed at our institution over an eight-yr period (1997–2004).

Abbreviations: ALL, acute lymphoblastic leukemia; AMB, amphotericin B; AML, acute myeloblastic leukemia; CF, cystic fibrosis; CI, confidence interval; CMV, cytomegalovirus; CNS, central nervous system; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; ICU, intensive care unit; IMI, invasive mold infection; NS, not significant; OR, odds ratio; SOT, solid organ transplantation.

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Methods

A retrospective chart review of all cases of IMI at our institution between January 1, 1997 and December 31, 2004 (eight-yr period) was conducted. The study was approved by the research ethics board at our institution. This is a quaternary level institution with large oncology, HCT and SOT services, including kidney, liver, heart, lung, small bowel, and multivisceral transplantation.

Cases were identified through a review of microbiology records. Only cases of proven and probable IMI were included. Proven IMI was defined as culture of the mold from biopsy or autopsy tissues, with or without histopathologic evidence of tissue invasion. Probable IMI was defined as culture of the mold from respiratory tract secretions when signs and symptoms consistent with pneumonia were present. Cases of possible IMI, including CF patients with culture-positive respiratory specimens who had not received lung transplantation, were not included in this series. Both patients diagnosed with IMI during life and those diagnosed at necropsy were included. Patient records were reviewed and data was extracted relating to patient demographics, diagnosis, microbiologic confirmation, treatment, and outcome.

For the purpose of quantifying risk factors for IMI, appropriate control groups were selected from available hospital databases. Limited data were available concerning the diagnosis of GVHD in HCT patients, so a smaller subset (77 patients treated between 2003 and 2004) was used to examine whether GVHD was a risk factor for IMI. Data were descriptively summarized with comparative statistics performed where appropriate (statistical software: SPSS 15.0, SPSS Inc., Chicago, IL, USA).

Results

Twenty-eight patients with IMI were identified (10 cancer, 12 HCT, and six SOT) at our institution between January 1997 and December 2004. During this same period, an additional 1966 patients were diagnosed with cancer (393 ALL, 97 AML, 333 lymphoma, 673 solid tumor, and 470 CNS malignancy), 528 patients underwent HCT (178 allogeneic related, 143 allogeneic unrelated, and 207 autologous), and 187 patients underwent SOT (100 heart, 58 kidney, 15 liver, 10 lung, two small bowel, and two multivisceral), but were not diagnosed with IMI. The median age of patients diagnosed with an IMI was nine yr (range two months to 17 yr), and 46% were female. The underlying diagnosis and the type of transplantation are shown in Table 1.

Incidence of IMI

As summarized in Table 1, the incidence of infection (expressed as the proportion of patients in each category who developed mold infection) ranged from 0.51 (cancer patients) to 3.2% (SOT patients). Microbiological diagnosis was made at a median of 60 days after the date of diagnosis (cancer patients) or the date of transplantation (HCT or SOT patients), as shown in Table 1, although late presentation (beyond six months) was common (32% of cases).

Clinical presentation

Table 2 shows the clinical symptoms according to the site of mold infection. In this regard, the predominant symptoms and signs were referable to the lungs and sinuses. In five cases, fever was absent, including one case of *Aspergillus* pneumonia in a heart transplant patient, and four cases of extra-pulmonary disease at presentation (two cases of sacral ulcer, one thumb lesion, and one nasal septum lesion). In nine cases, the diagnosis of IMI was made more than six months after transplantation or cancer chemotherapy, including eight cases of *Aspergillus* pneumonia and one case of *Alternaria* pneumonia, and sinusitis. Infection at all sites was associated with a high mortality (Table 2): 50% of patients with pneumonia, 40% of those with sinusitis, and two of three patients with soft tissue infection died, both of whom had evidence of disseminated disease (pneumonia and osteomyelitis, respectively).

Risk factors

Table 1 summarizes the risk factors for mold infection in this cohort. Cancer patients with a diagnosis of AML were more likely to develop mold infection (OR = 45; 95% CI 11–180; $p < 0.0001$) than patients with other cancer types. Among HCT patients, those undergoing allogeneic unrelated transplantation were more likely to develop mold infection than allogeneic related or autologous transplant recipients (OR = 8.1; 95% CI 2.2–30; $p = 0.0009$). In addition, GVHD was associated with mold infection (OR = 9.9; 95% CI 2.0–50; $p = 0.002$) among allogeneic transplant recipients. Among SOT patients, lung transplant recipients were more likely to develop mold infection than liver, heart, or kidney transplant recipients (OR = 18; 95% CI 3.1–99; $p = 0.0043$). This difference remained statistically significant (OR = 12, 95% CI 2.1–67; $p = 0.012$) even after the exclusion of kidney transplant recipients, who are known to be at lower risk of IMI. All three lung transplant patients with mold infection had an underlying diagnosis of CF, and were colonized with *Aspergillus* prior to transplantation. No association was observed between the CMV serostatus (recipient or donor) and mold infection among HCT patients ($p = 0.56$, NS) or SOT patients ($p = 0.57$, NS). Neutropenia was present at the time of diagnosis in all cancer patients, in

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Table 1. Description of cohort of 28 patients who developed mold infection by category of immunocompromise

	Cancer (n = 10)	HCT (n = 12)	SOT (n = 6)	Overall (%)
Underlying diagnosis and type of transplant where applicable (n)				
	AML (7) ALL (2) Medulloblastoma (1)	Allogeneic unrelated (9) AML (3) SCID (2) ALL (1) Hurler's syndrome (1) JMML (1) NHL (1) Allogeneic related (2) AML (1) Aplastic anemia (1) Autologous (1) ALL (1)	Lung (3) CF (3) Heart (2) HLHS (1) Cardiomyopathy (1) Liver (1) Cirrhosis due to CF (1)	
Incidence (%)				
Proportion developing a mold infection (95% CI)	0.51 (0.28–0.93)*	2.2 (1.6–2.9)	3.2 (1.5–6.8)	
Timing of infection (days)				
Time to microbiological diagnosis†, median (range)	118 (15–616)	60 (11–386)	71 (17–1382)	60 (11–1382)
Type of mold				
Aspergillus spp.				
A. fumigatus	1	8	5	14 (50)
A. flavus	6	2		8 (29)
A. terreus		1		1 (4)
Zygomycete	1		1	2 (7)
Fusarium oxysporum		1		1 (4)
Alternaria spp.	2			2 (7)
Proven or probable disease				
Proven	9	7	4	20 (71)
Probable	1	5	2	8 (29)
Site of infection				
Lung	6‡	9	5	20 (71)
Sinuses	3	2		5 (18)
Soft tissue§	2¶	1	1	4 (14)
Risk factors				
High risk category	AML; OR = 45 (11–180)**	1. Allogeneic unrelated; OR = 8.1 (2.2–30)†† 2. GVHD; OR = 9.9 (2.0–50)‡‡	Lung transplant; OR = 18 (3.1–99)§§	
Outcome (%)				
One-yr mortality (95% CI)	60 (45–75)	58 (44–73)	16 (1.5–32)	50 (41–59)¶¶

HCT, hematopoietic cell transplantation; SOT, solid organ transplantation; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NHL, non-Hodgkin's lymphoma; SCID, severe combined immunodeficiency; JMML, juvenile myelomonocytic leukemia; CF, cystic fibrosis; HLHS, hypoplastic left heart syndrome; GVHD, graft-versus-host disease; OR, odds ratio; CI, confidence interval.

*$p = 0.0001$ for difference (vs. HCT), $p < 0.0001$ (vs. SOT).

†From the date of cancer diagnosis (cancer patients) or date of transplantation (HCT and SOT patients).

‡One patient had liver and spleen (biopsy-proven) in addition to lung (radiographic) lesions.

§Soft tissue lesions: hand, thumb, sacrum, and nasal septum.

¶One patient had both lung (radiographic) and soft tissue-hand (biopsy-proven) lesions.

**$p < 0.0001$; control group consisted of 1966 patients with a diagnosis of cancer, without mold infection, between 1997 and 2004.

††$p = 0.0009$; control group consisted of 528 patients who underwent HCT transplantation, without mold infection, between 1997 and 2004.

‡‡Among allogeneic HCT recipients only, $p = 0.002$; control group consisted of 77 patients who underwent allogeneic HCT transplantation, without mold infection, between 2003 and 2004.

§§$p = 0.0043$; control group consisted of 187 patients who underwent solid organ transplantation, without mold infection, between 1997 and 2004.

¶¶Fourteen patients (50%) died in the first year following cancer diagnosis or transplant, and one patient died 385 days after HCT, for an overall mortality of 54% in this series.

5/12 = 42% of HCT patients, and in none of the SOT patients (overall 15/28 = 54%).

Species of mold

Table 1 describes the type of mold isolated and the site of infection. *Aspergillus fumigatus* was the most common pathogen, followed by other *Aspergillus* species. Table 2 documents the clinical presentation according to causative pathogen. The dominant syndrome was pneumonia caused by *Aspergillus* species. The mortality was 57%, 38% and 100% among patients infected

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Table 2. Clinical and microbiological features of pediatric mold infection, according to site of infection

	Lung/sputum (n = 20)	Sinus (n = 5)	Soft tissue (n = 3)	Overall (%)
Signs and symptoms, n (%)				
Fever	19 (95)	5 (100)	0	24 (86)
Cough or respiratory distress	12 (60)	1 (20)	0	13 (46)
Sinus pain or headache	0	2 (40)	0	2 (7)
Soft tissue swelling or ulceration	1 (5)*	0	3 (100)	3 (11)
Mortality	11 (55)	2 (40)	2 (66)	15 (54)
Fungal pathogen, n (%)				
Aspergillus spp.				
A. fumigatus	13	1	0	14 (50)
A. flavus	3	3	2	8 (29)
A. terreus	1	0	0	1 (4)
Zygomycete	1	0	1	2 (7)
Fusarium oxysporum	1	0	0	1 (4)
Alternaria spp.	0	2	0	2 (7)

*One patient had both pulmonary lesions and an ulcer of the hand, which was culture positive for *Aspergillus flavus* on biopsy.

with *A. fumigatus*, *A. flavus*, and *A. terreus*, respectively. The mortality was 50% among the small number of patients infected with zygomycetes and *Alternaria* spp. and the single patient with fusariosis died.

Antifungal chemoprophylaxis

Fifteen patients (54%) had received antifungal prophylaxis prior to developing IMI, including fluconazole (13 patients) and itraconazole (two patients). Both patients receiving itraconazole were lung transplant recipients with an underlying diagnosis of CF who were colonized with *Aspergillus* spp. prior to transplantation.

Antifungal treatment

In 19 cases (68%), antifungal treatment was commenced empirically at a median of six days (range 1–51 days) prior to definitive microbiologic diagnosis, due to clinical suspicion of IMI. Empiric regimens consisted of AMB in 16 cases and caspofungin in three cases. In a further seven cases, treatment was started in response to laboratory diagnosis, on the same day or up to four days later. In two cases, no antifungal therapy was administered prior to death and diagnosis of IMI was made at autopsy. Table 3 shows the duration of the various antifungal regimens used, as well as the mortality among patients with IMI despite antifungal therapy. Most patients received AMB alone, while nine patients (32%) received combination antifungal therapy.

Outcome

Figure 1 shows the mortality during the first year after diagnosis (cancer patients) or transplantation (HCT and SCT patients) for the 28 IMI patients. In all cases, the cause of death was attributed to the fungal infection. The one-yr mortality is given in Table 1. Fifteen patients with mold infection were admitted to the ICU, for a median duration of 13 days (range 2–82 days). Of these patients, 12 died and the remaining three patients recovered with critical supportive care including mechanical ventilation after a period ranging from seven to 71 days in ICU.

Table 3. Antifungal therapy

	No. patients (%)	Median duration (days, range)	Mortality (%)
No antifungal therapy*	2 (7)	0	2 (100)
AMB monotherapy	17 (61)	32 (3–777)	10 (59)
AMB + caspofungin	4 (14)	62 (31–100)	1 (25)
AMB + caspofungin + voriconazole	4 (14)	225 (118–289)	1 (25)
AMB + caspofungin + itraconazole	1 (3)	30	1 (100)

AMB, amphotericin B.
*Two patients were diagnosed with IMI at autopsy, having never received antifungal therapy.

Discussion

In an era of expanding antifungal armamentarium and increasing incidence of mold infections (1), we report a comparatively large and detailed study of immunocompromised children with IMI from a single center. Noteworthy features of this series include the broad range of immunocompromised children (cancer, HCT, and SOT patients), the spectrum of pathogens (all invasive molds, in contrast to several previous studies that examined invasive aspergillosis) and the stringent inclusion criteria (only proven or probable cases).

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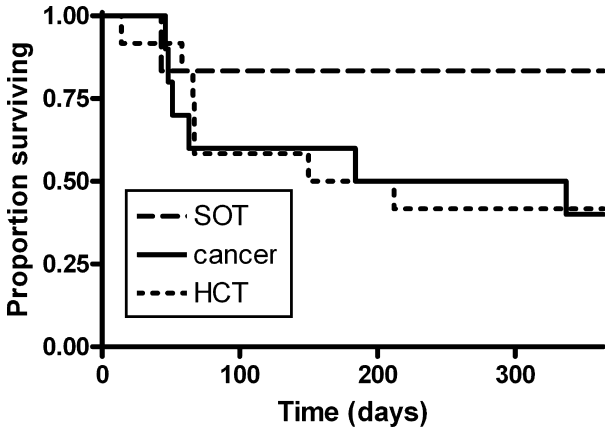

Fig. 1. Kaplan–Meier survival curves for 28 patients diagnosed with invasive mold infection (IMI), according to category of immunocompromise [cancer, solid organ transplant (SOT) or hematopoietic cell transplant (HCT)], beginning from time of cancer diagnosis or transplantation. The difference in survival between categories was not statistically significant (p = 0.32).

The incidence of IMI varies between categories of iatrogenic immunocompromised individuals. Cancer patients in our series were significantly less likely to develop IMI (0.51%) than HCT (2.2%) and SOT (3.2%) patients. Similarly, the reported incidence of invasive aspergillosis among children with malignancy in the USA (0.5%) was lower than the incidence in pediatric HCT patients (3.4%) (2). In a prospective multicenter USA study, the cumulative incidence of IMI 12 months after transplantation was highest among HCT recipients (3.3%) and varied within SOT patients from 2.4 (lung transplant patients) to 0.1% (kidney transplant patients) (8). Variable rates of IMI have been reported in retrospective analyses: pediatric HCT recipients, 4.79% (9) and 5% (10); adult HCT recipients, 12% (1) to 15% (4); adult lung transplant patients 1.6% (11).

Unlike previous studies (1), we did not find evidence of an increase in mold infections over time. In a similar study from our institution conducted between 1979 and 1988, 39 cases of invasive aspergillosis were documented over a 10-yr period (3.9 cases/yr) (12), while the rate of Aspergillus infection during our study period was 3.1 cases/yr. Given that the number of immunocompromised patients has increased over time, the incidence of IMI (as a proportion of patients at risk) appears, in fact, to have decreased. Of note, patients have been managed in a new hospital building since 1988; therefore, the apparent higher rate of aspergillosis in the previous study may be attributable to environmental factors associated with the older building.

The timing of IMI in relation to cancer diagnosis or transplantation (HCT and SOT patients) was similar in our series (median 60 days) and other studies (1, 4). Late presentation (more than six months after onset of immunocompromised state) occurred in nearly a third of cases, when the index of suspicion for fungal infection may be lower. The lung was the most common site of infection, followed by the sinuses, as has been observed in other series (11, 13, 14). This is of relevance to the investigation of patients with suspected aspergillosis: patients with normal CT scans of their sinuses and chest have a low likelihood of aspergillosis, thereby potentially influencing choice of empiric antifungal therapy.

Clinical features reflected the site of infection: fever and cough or respiratory distress occurred in most patients with lung involvement; fever and sinus pain or headache predominated in patients with sinus involvement. Atypical presentations including IMI without fever were observed, predominantly among patients with extrapulmonary disease.

Aspergillus species predominate as the causative agent of IMI (1, 4). *Aspergillus fumigatus* was most common in our and other series (1, 4). The occurrence of a case of *A. terreus* is concerning, given the resistance of this pathogen to polyene antifungals such as AMB (15), widely used for empiric therapy at our institution. The fatal case of *Fusarium oxysporum* infection in our series highlights the poor outcome associated with this unusual but emerging fungal pathogen (5). The two cases of *Zygomycete* infection in our series may reflect the increasing incidence of this group of molds (16), difficult to distinguish from Aspergillus on clinical grounds but noteworthy for their reduced susceptibility to azole antifungals (16).

Our retrospective series confirms previously identified risk factors for IMI. Among cancer patients, IMI occurs more often in AML patients than in ALL or other malignancies (2, 13). We confirmed a strong association between a diagnosis of AML and IMI by comparing cancer patients with IMI in our series to a set of 1966 cancer patients without IMI at our institution over the same period (OR = 45; 95% CI 11–180; p < 0.0001). Among HCT patients, allogeneic transplant recipients have higher rates of IMI than autologous transplant recipients (2, 10), and we found a significant association (p = 0.0009) between allogeneic HCT and IMI. Among allogeneic HCT recipients, GVHD is a recognized risk factor for IMI (10). In one USA series, the risk of IMI was 42% in patients with grade III or

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IV acute GVHD, 14% in patients with grade I or II acute GVHD, and 6% among patients without GVHD (4). Likewise, in our series, GVHD was associated with IMI (OR 9.9; 95% CI 2.0–50; p = 0.002). Compared with other SOT recipients, the rate of IMI was higher among lung transplant recipients in our series, as in previous reports. In one series, the cumulative incidence of invasive aspergillosis one yr after SOT was 2.4% (lung) vs. 0.8% (heart), 0.3% (liver) and 0.1% (kidney) (8). Similarly, in a retrospective series of SOT patients, the attack rate of invasive aspergillosis was 12.8% (lung) compared with 0.7% (liver), 0.4% (kidney), and 0.4% (heart) (11). At our institution, during the study period, three of 13 lung transplant patients developed Aspergillus infection, compared with 10 of 187 non-lung SOT patients (OR 18; 95% CI 3.1–99; p = 0.0043). Other studies have cited neutropenia as a risk factor for IMI (5), which was common in our patient series. We did not detect an association between CMV serostatus and risk of IMI, although CMV infection has been shown to predispose to IMI in other studies (4). These risk factors (AML, allogeneic HCT, and GVHD) collectively point to aggressive iatrogenic immunosuppression as the underlying reason for susceptibility to IMI. Increased rates of IMI, particularly aspergillosis, in lung transplant patients can be explained by the exposure of the allograft to the external environment (in contrast to kidney, heart, and liver transplants) and high pretransplant rates of Aspergillus colonization or infection in the CF population, which constitutes a large proportion of lung transplant patients.

Although 13 patients in our series received fluconazole prophylaxis, few received chemoprophylaxis with activity against filamentous fungi. Two lung transplant patients received itraconazole, yet still developed pulmonary aspergillosis. The American Society of Transplantation and American Society of Transplant Surgeons recommend prophylaxis with voriconazole, itraconazole or echinocandins with or without nebulized AMB for a duration of 4–6 months (17) for lung transplant recipients, based on evidence from case series (11, 18–20) and one non-randomized retrospective study (21). The use of antifungal prophylaxis has been recently reviewed for cancer, HCT, and SOT patients (22–24). Of note, the triazole antifungal posaconazole, with broad spectrum activity against filamentous fungi has recently been approved for prophylaxis in allogeneic HCT recipients with GVHD and in patients with acute myelogenous leukemia undergoing induction chemotherapy based on two large, prospective randomized controlled trials (25, 26).

Variability in the antifungal regimen and duration of therapy in our series reflect a lack of data on the optimal treatment for mold infections, as highlighted by a recent systematic review (27). Similar to other reports (13, 28), the most commonly prescribed antifungal for invasive fungal infection at our institution was AMB. Caspofungin (usually in combination with other antifungal agents) has been used to treat IMI in pediatric patients with variable efficacy (29–32), and was the second most commonly used antifungal in our series. Voriconazole, which demonstrated superior efficacy against invasive aspergillosis compared with AMB in a randomized controlled trial (33), has been used as salvage therapy in immunocompromised children in previous series (26, 34) and was the third most commonly used antifungal in our cohort. There is considerable interest in, though little clinical data at present (3) to support the use of combination antifungal therapy, which was used in 25% of patients in our series. Mortality appeared lower in patients receiving dual or triple antifungal therapy, relative to AMB alone, although difference did not achieve statistical significance.

Six of 28 (21%) patients in our series were diagnosed with invasive fungal infection at autopsy, and two of these patients had received no antifungal therapy during life. Likewise, in previous autopsy series, fungal infections feature prominently among clinically unsuspected causes of death (35–38). Among patients with hematologic malignancies undergoing autopsy at a tertiary center in the USA, 31% had invasive fungal infection, and 75% of these cases were not diagnosed ante-mortem (39).

Mortality after IMI is high, as demonstrated in our and other series. Fifteen of 28 children (54%) with IMI died, similar to the mortality in a multicenter retrospective review of pediatric invasive aspergillosis of 52.5% (2). Among cancer patients, mortality one yr after diagnosis was 60%, similar to the 79% mortality in patients with hematologic malignancy and fusariosis (40). Among HCT recipients, the one-yr mortality of 58% observed in our series is comparable with the rate observed in one prospective study (54–85%) (8). Trials of antifungal therapy have reported somewhat lower mortality rates, as in a randomized controlled trial that showed death because of Aspergillus in 29% of the group receiving voriconazole vs. 42% of those receiving AMB (33). Mortality in reports involving only

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pediatric patients varies from 18% (13) to 88% (10), with small number of patients in these series. A multi-center study of children with invasive aspergillosis in the USA documented an in-hospital mortality of 18% and an overall mortality of 44% (2).

In summary, IMI is an unusual but often fatal complication among immunocompromised children. Aspergillus species predominate, and risk factors include acute myelogenous leukemia, allogeneic unrelated HCT, GVHD, and lung transplantation. The optimal therapy has not been defined, and mortality remains high despite antifungal treatment.

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Original article

Invasive aspergillosis in a paediatric haematology department: a 15-year review

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Summary

Invasive aspergillosis (IA) is an increasingly common and often fatal fungal infection in children with haematological disorders. To describe the epidemiology, diagnosis, treatment and outcome of IA in children, retrospective review of the medical records of proven and probable IA between January 1986 and December 2000 was used. Twenty-four patients with IA were identified (10 proven and 14 probable) with a median age of 8.5 years. The incidence of IA was particularly high in acute myeloblastic leukaemia (5.35%) and leukaemia relapse (4%). Twenty-two patients presented with lung involvement. Broncho-alveolar lavage led to a diagnosis in 11 cases, but diagnosis was difficult and repeated invasive explorations were required. Antifungal therapy mainly consisted of amphotericin B. Eight patients underwent open-thorax surgery without any complication. Nine patients (37.5%) were cured of IA and three are still alive. The mortality was 87.5%. Three patients died of massive haemoptysis, including two before neutropenia recovery. Four patients presented with IA recurrence and three were cured again. Despite significant progress having been made in the treatment and diagnosis of IA, it is still a devastating complication in children with haematological disorders. New antifungal therapies and strategies are promising, but objective data are still lacking.

Key words: Invasive aspergillosis, haematology, cancer, children, lung.

Introduction

Aspergillus is a ubiquitous and saprophytic mould. Its airborne conidia are extremely common in the environment, including air within hospitals, and can be responsible for patient contamination. Since the first description in 1953[1] invasive aspergillosis (IA) has become an increasing concern. IA is now the second most common cause of fungal infection after candidosis,[2,3] and it is the foremost cause of death owing to fungal infection.[4–6] Diagnosis of IA is often difficult. Despite therapy, the prognosis is considered very poor, especially in agranulocytic patients.[7]

To describe the characteristics of IA among children with haematological diseases and immunodeficiency syndromes before the advent of new drugs, such as voriconazole or caspofungin, we reviewed the medical records of proven and probable IA in children over a 15-year period.

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Materials and methods

Patient population

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Debrousse Hospital, Lyon, France is a tertiary referral centre. This single-centre retrospective study was performed from January 1986 to December 2000 on a large population with malignant and non-malignant haematological diseases and immunodeficiency syndromes. During this period, we performed 379 allogeneic HSCT. Patients were treated according to the European Organization for Research and Treatment of Cancer (EORTC), the French Society for Pediatric Oncology (SFOP) and the French Society of Bone Marrow Transplantation (SFGM) protocols. Patients undergoing HSCT and those with prolonged neutropenia were cared for in a laminar air flow (LAF) room. No patient received primary prophylactic therapy against *Aspergillus*. On the other hand, secondary chemoprophylaxis against *Aspergillus* [intravenous amphotericin B (AmB) or itraconazole] was systematically initiated after a first history of IA for all new neutropenic period or HSCT. Empirical broad-spectrum antibiotic therapy was immediately started for febrile neutropenic patients after an evaluation, including a chest radiograph. Intravenous AmB desoxycholate (0.5 mg kg^{-1} day^{-1}) was started after 72 h of persistent fever or sooner in the case of a pulmonary manifestation or an abnormal chest X ray.

Identification and case definition

Cases of IA were identified by review of the infection surveillance department data, pathology reports and microbiological reports. Patients were included if they had proven or probable IA as defined by the EORTC criteria.[8] Proven IA requires histopathology or cytopathology showing *Aspergillus* hyphal invasion, or a positive culture from a radiologically abnormal site that is normally sterile. Probable infection is defined by microbiological criteria in a patient with typical host factors for IA, such as current neutropenia, fever refractory to antibiotics, graft vs. host disease (GVHD), prolonged use of corticosteroid, fever and either neutropenia in the previous 60 days, or the use of immunosuppressive agents or previous fungal infection. Microbiological criteria include sputum or bronchoalveolar lavage (BAL) culture positive for *Aspergillus*, or positive *Aspergillus* antigen in two consecutive blood samples. Clinical criteria include nodular opacities on computerised tomography (CT) scan. Possible IA is defined as having the host factors for IA but lacking the clinical or microbiological features. Possible IA cases were excluded from our study. Medical records of the identified cases were reviewed retrospectively for patient's demographics, underlying diseases, diagnostic procedures, extent of the infection, treatment and outcome.

Microbiology

A direct examination of the samples [BAL, peritoneal liquid, pus, tracheal aspiration, cerebrospinal fluid (CSF)] and biopsies (lung, pericardium) was performed after Giemsa and Grocott staining. The specimens were inoculated onto Sabouraud-chloramphenicol media, and the species were identified by their morphology. Until 1997, Latex agglutination (Pastorex® *Aspergillus*; BioRad, Marnes-la-Coquette, France) was used for galactomannane antigen detection in samples of BAL, CSF and serum. After 1997, sandwich enzyme-linked immunosorbent assay (Platelia® *Aspergillus*; BioRad) was used for antigen detection. If IA was suspected, antigen assay was performed, but it was not routinely done.

Results

Patient demographics and underlying conditions

Twenty-four cases of IA were identified (10 proven and 14 probable): 12 males and 12 females with a median age of 8 years at IA diagnosis (Table 1). The median age at the time of the underlying disease diagnosis ranged from 0 to 16 years (median, 7 years). The interval between the onset of the underlying disease and the diagnosis of IA was 1.5 to 132 months (median, 8.5 months). Underlying diseases are reported in Table 2. Among the 13 patients treated for acute myeloblastic leukaemia (AML) and acute lymphoblastic leukaemia (ALL), six (46%) were in relapse. Twenty-two patients (92%) had experienced neutropenia (neutrophil count <0.5 G l^{-1}) for 13–277 days (median, 101 days). Among those 22 patients, 11 (50%) were still neutropenic at the time of IA diagnosis. For the others, the time between neutropenia recovery and onset of IA was 1–480 days (median, 40 days). Twenty-one patients (87.5%) had received chemotherapy and 10 (41.6%) had been treated with allogeneic HSCT before IA. Prior to IA, antifungal therapy was administered to seven children (29%): four patients with persistent undocumented fever and three patients with *Candida albicans* infection. All patients were treated with AmB or its lipidic formulations. Ten patients (42%) were treated with corticosteroid at the time of diagnosis of IA with a median dose of 1 mg kg^{-1}.

For nine patients (38%), a concomitant infection was diagnosed. One patient presented with a systemic

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Table 1 Clinical features of patients with invasive aspergillosis

Patient	Age (years)	Underlying disease	Proven/probable	Presenting site	Isolates	Mycological diagnosis procedure	Antifungal therapy	Surgery	Other therapy	Outcome	Survival after diagnosis (days)
1	9	AML	Probable	Lung	Lung	BAL	AmB	No	Leucocyte transfusion	Died of IA	0
2	0.7	SCID	Probable	Lung	Lung	BAL	AmB, 5FC	No	No	Died of MFC	3
3	3.3	AML	Proven	Lung	Lung	BAL	AmB, 5FC	Lobectomy	Leucocyte transfusion, i.v. miconazole	Died of IA	25
4	7.1	AML	Probable	Lung	Lung	BAL	AmB	No	No	Cured from IA	2035
5	7.8	ALL	Probable	Lung	Lung	BAL	AmB	No	No	Died of IA	4
6	13.2	ALL relapse	Proven	Lung	Lung	Lung biopsy, BAL	AmB, itraco	Lung lesion resection	No	Cured from IA	320
7	0.75	FLH	Proven	Lung, pericardium	Lung, pericardium	Pericardium biopsy	AmB, 5FC	Lobectomy	No	Cured from IA	Alive
8	14	CGD	Proven	Lung, chest wall, ribs, vertebra, endocardium	Lung, chest wall	BAL, chest wall pus culture	AmB, AmB lip, itraco	Lung lesion resection, abscess drainage	Gamma IFN, leucocyte transfusion, voriconazole	Died of IA	290
9	9	ALL relapse	Proven	Lung	Lung	Lung biopsy	AmB, itraco	Lung lesion resection	No	Cured from IA	100
10	14.4	MDS	Probable	Lung	–	Positive antigen in blood	AmB	No	No	Died of IA	10
11	4.75	Hurler disease	Probable	Lung	Lung	Tracheal aspiration	AmB, itraco	No	No	Died of MFC	23
12	8.25	ALL	Probable	Lung	Lung	BAL	AmB, itraco	No	No	Cured from IA	65
13	5.1	Fanconi anaemia	Probable	Lung	Lung	BAL	AmB, itraco	No	No	Died of MFC	48
14	14.3	JMML	Probable	Lung, brain	Lung	BAL	AmB, AmB lip, itraco	No	No	Died of IA	17
15	5.1	ALL relapse	Proven	Mastoid, meninges, bone	Mastoid	Mastoid pus culture	AmB, AmB lip, itraco	Mastoid debridement	Leucocyte transfusion	Cured from IA	120
16	17.25	AML relapse	Probable	Lung	Lung	Tracheal aspiration	AmB	No	No	Died of MFC	2
17	13.8	ALL	Proven	Lung, peritoneum, meninges	Lung, peritoneum, meninges	Tracheal aspiration, PL culture, antigen in PL, antigen in CSF	AmB, itraco	No	Intraperitoneal infusion of AmB and 5FC, intrathechal infusion of AmB	Died of IA	5
18	3.75	SCID	Probable	Lung	Lung	BAL	AmB, itraco	No	No	Died of MFC	90
19	2.4	ALL relapse	Probable	Middle ear	Middle ear	Middle ear pus culture	AmB, AmB lip, itraco	No	No	Cured from IA	53

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Table 1 (*Continued*)

Patient	Age (years)	Underlying disease	Proven/probable	Presenting site	Isolates	Mycological diagnosis procedure	Antifungal therapy	Surgery	Other therapy	Outcome	Survival after diagnosis (days)
20	8	AML	Proven	Lung	Lung	Lobectomy	AmB, itraco	Lobectomy and lung lesion resection	No	Cured from IA	Alive
21	14	AML relapse	Proven	Lung	Lung	Lobectomy	AmB, itraco	Lobectomy	No	Died of IA	160
22	7.9	Aplastic anaemia	Proven	Lung	Lung	Lobectomy	AmB, itraco	Lobectomy	Leucocyte transfusion	Cured from IA	Alive
23	11.7	SCID	Probable	Lung	Lung	Sputum culture	AmB lip, itraco	No	No	Died of IA	45
24	12	Fanconi anaemia	Probable	Lung	–	Positive antigen in blood	AmB lip	No	No	Died of MFC	7

AML, acute myeloblastic leukaemia; SCID, severe combined immunodeficiency; ALL, acute lymphoblastic leukaemia; FLH, familial lymphohistiocytosis; CGD, chronic granulomatous disease; MDS, myelodysplasia; JMML, juvenile myelomonocytic leukaemia; BAL, bronchoalveolar lavage; PL, peritoneal liquid; CSF, cerebrospinal fluid; AmB, amphotericin B; 5FC, flucytosine; itraco, itraconazole; AmB lip, lipidic formulation of amphotericin B; IA, invasive aspergillosis; i.v., intravenous; IFN, interferon; MFC, multifactorial cause.

adenovirus infection. Four patients presented with sepsis involving *Alcaligenes dentrificans* ($n = 1$), *Staphylococcus haemolyticus* ($n = 1$), *C. albicans* ($n = 1$) and *Capnocytophaga* ($n = 1$). For four patients, microbiological investigations on BAL samples revealed (besides *Aspergillus*) the presence of *P. aeruginosa* ($n = 1$), *Herpes simplex virus 1* ($n = 1$), *Coronavirus* ($n = 1$) and *C. albicans* ($n = 1$). At the onset of IA, cytomegalovirus (CMV) serology was positive in 10 patients (42%), but only two had experienced severe infection during previous immunosuppression period.

Mycological studies

Aspergillus species was isolated from 22 patients: *Aspergillus fumigatus* (18 cases), *Aspergillus nidulans* (three cases), *Aspergillus flavus* (one case) and *Aspergillus terreus* (one case). Two species were isolated in BAL culture (*A. fumigatus* and *A. nidulans*) from only one patient (patient 18).

Clinical, radiological and biological findings

The lung was the most commonly involved organ (22 cases). Nineteen patients (80%) had a single localisation involving lung in 18 cases and middle ear in one case. For the others, infection was multifocal. Fever was observed in 22 patients (90%). Median time between the first symptom and diagnosis confirmation was 12 days (0–230 days).

Lung involvement

In eight cases (36%), chest radiographs showed the typical nodules or air crescent sign of invasive pulmonary aspergillosis (IPA). A CT scan was performed in 15 patients, of which 11 (74%) displayed evocative aspects with nodules, halo sign or air crescent sign.

Among the 11 diagnoses of IA based on BAL examination, seven (64%) were made on the first BAL, two (18%) on the second BAL and two on the third BAL. In five cases, diagnosis was made on lung specimens from surgical biopsy or lobectomy. Among those five cases, surgery was the first diagnostic procedure for two patients. For two others, surgery was performed after a negative culture for *Aspergillus* on BAL samples. For the fifth case, surgery was performed after a negative BAL and a negative culture from a needle biopsy specimen.

Other IA localisation included the central nervous system (three), bone (two), mastoid (one), middle ear (one), peritoneum (one), pericardium (one), endocardium (one) and chest wall (one).

No autopsy was performed in our study. No blood culture was positive for *Aspergillus.* The C-reactive

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Table 2 Underlying diseases, IA incidence in our study compared with previous paediatric studies

Underlying diseases (number of patients)	IA incidence	IA incidence for Abbasi *et al.* [12] (%)	IA incidence for Zaoutis *et al.* [26] (%)	IA incidence for Groll *et al.* [27] (%)
AML (112)	6/112 (5.35%)	4	3.7	27.77
Total ALL (464)	7/464 (1.5%)	1	0.6	2.06
ALL relapse (101)	4/101 (4%)			8.57
SCID (ND)	3/ND			
Fanconi anaemia (ND)	2/ND			
Aplastic anaemia (30)	1/30 (3.3%)	4.6	1.4	
CGD (ND)	1/ND	7	6.5	
JMML (ND)	1/ND			
Hurler disease after HSCT (ND)	1/ND			
FLH (ND)	1/ND			
Myelodysplasia (ND)	1/ND			

IA, invasive aspergillosis; AML, acute myeloblastic leukaemia; ALL, acute lymphoblastic leukaemia; SCID, severe combined immunodeficiency; ND, not determined; CGD, chronic granulomatous disease; JMML, juvenile myelomonocytic leukaemia; HSCT, haematopoietic stem cell transplantation; FLH, familial lymphohistiocytosis.

protein assay was assessed in 23 patients and ranged from 13 to 440 mg l^{-1} (median, 158 mg l^{-1}).

Therapy and outcome

Twenty-two patients (92%) received intravenous AmB desoxycholate over a period of 2–131 days (median, 26 days). Six patients were treated with a lipidic formulation of AmB as Ambisome® (Gilead Sciences, Foster City, CA, USA) (one patient) and Abelcet® (Zeneus Pharma, UK) (five patients) for 3–43 days (median, 19 days). Fifteen patients (58%) received itraconazole for 3–949 days (median, 38 days), and three patients were treated with flucytosine for 2–60 days (median, 33 days). Fourteen patients (58%) received G-CSF. Other treatments are summarised in Table 1. Reversible renal function impairment occurred in: five patients on AmB desoxycholate; one patient on itraconazole; and two patients on Abelcet®. No patient required dialysis.

Surgery

Eight patients underwent thoracic surgery (thoracotomy): one resection of a unilateral lesion, one resection of bilateral lesions (two left and one right), four lobectomies (including one patient who underwent a controlateral lesion resection at a later time). In two surgeries, a total resection was impossible. There were no severe surgical complications.

Outcome

Nine patients (37.5%) were considered cured of IA. Among those nine patients, three patients (patients 7, 20 and 22) are still alive at follow-up of 15, 9 and 7 years on April 2007 and six died from relapses or complications of the underlying disease with a median survival of 110 days after IA (53 days to 5.5 years). Nine patients died of IA (fatal IA outcome). For the six other patients, the cause of death was multifactorial. Median survival for patients with fatal IA outcome was 17 days (0–290 days). Median survival for patients with multifactorial cause of death was 15 days (0–90 days). For patients with fatal IA outcome, causes of death were: massive haemoptysis (three patients); pulmonary failure (three patients); pulmonary failure and intracranial hypertension with cerebral herniation (one patient), multiple organ failure related to IA (two patients). Hence, after the diagnosis of IA, 21 patients (87.5%) died with a median survival of 45 days (0–5.5 years), and to date, only three patients are alive.

Four children suffered IA recurrence. Recurrences occurred during a neutropenic period for patient 6, and after an allogeneic HSCT for patients 20 and 21. For patient 22, IPA relapsed at the same location despite normal granulocyte count. It is remarkable that among the four patients with IPA recurrence, three had favourable outcomes with two still alive and well.

Discussion

Gerson *et al.* [9] demonstrated that neutropenia is a major risk factor for IA in adult patients. Twenty-two of our patients (91.6%) presented with at least one neutropenic period before IA, all of which were subsequent to cytotoxic chemotherapy, except for one patient with aplastic anaemia. However, only 50% of our patients were neutropenic at the time of IA

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diagnosis. This could be the result of two factors. First, some IPA diagnoses were probably delayed owing to the unavailability of a CT scan in the hospital. Therefore, CT scan was delayed until after haematological recovery and LAF room discharge. Second, our study included a high number of cases of congenital immunodeficiency and allogeneic HSCT. These cases involve severe, extensive and global immunodeficiency beyond the quantitative decreased level of neutrophil granulocyte. Only two children did not experience neutropenia: patient 2 with adenosine deaminase deficiency severe combined immunodeficiency (SCID) and patient 8 with chronic granulomatous disease.

The underlying diseases and respective incidence of children with IA are reported in Table 2. Our data are compared with the main published paediatric series. In our study, the incidence of IA in children with AML was high (5.35%) despite the use of the LAF room for the duration of all neutropenic periods. Only one patient with aplastic anaemia developed IA (incidence: 3.3%). In adult series, the incidence of IA in aplastic anaemia ranges from 5% to 10%.[10,11] The underlying conditions displayed paediatric specificities: three patients with SCID (patient 2 with adenosine deaminase deficiency; patient 18 with purine nucleoside phosphorylase deficiency; and patient 23 with HLA class II antigen deficiency); two patients with Fanconi anaemia; and one patient with Hurler disease that developed IA after HSCT. One patient presented with a familial lymphohistiocytosis.

Invasive aspergillosis and a disseminated form of IA were probably underestimated in our study for three reasons. First, autopsies were rarely performed in our institution. In the study of Abbasi *et al.* [12], autopsy was performed in 68% of the cases. Among the 26 cases of IPA, autopsies allowed the diagnosis in 13. Among the 24 children with premortem diagnosis of unifocal involvement, 15 were found to have multifocal infection at postmortem. Second, systematic empirical antifungal therapy may have cured some patients before diagnosis. Third, because of ethical considerations, we did not pursue diagnostic exploration in palliative-care patients. Autopsies are needed to be performed more systematically in our institution in order to better estimate the incidence and extent of *Aspergillus* infection.

Infection was multifocal in 20% of cases, involving two, three or four organs. Lung was the most commonly involved organ with 22 patients (92%) having unifocal or multifocal lung infection. IPA was particularly frequent among our patients (70% for Abbasi *et al.* [12] and 41% for Walmsley *et al.* [13]). This high frequency of lung involvement in our study, compared with previous studies, is probably related to systematic BAL whenever a lung infection was suspected. Massive haemoptysis is a major threat during IPA.[14] Three of our patients (1, 5, 21), i.e. one-third of the death imputed to IA, died suddenly in this dramatic and uncontrollable situation. The role of granulocytes in this complication has been emphasised.[15] In most cases, haemoptysis occurs within 7 days after the neutrophil count exceeds 0.5 G l^{-1}. Caillot *et al.* recommend an early surgical resection of a threatening lesion detected on repeated chest CT scans prior to neutropenia recovery.[16] However, it is noteworthy that only patient 5 presented with haemoptysis after neutropenia recovery. Both patients 1 and 21 were still neutropenic at the time of haemoptysis.

As demonstrated in previous studies,[16,17] CT scan is more effective in establishing a diagnosis than chest radiograph, demonstrating an evocative aspect in 74% vs. 36% in our study. BAL sensitivity is above 60% in adult reports[18] and 55% in our series. No incident was reported during endoscopy and BAL. Based on BAL, trans-thoracic biopsy or surgery findings, diagnosis was made on the first investigation in nine patients; on the second investigation in four; and on the third investigation in three. IPA diagnosis is difficult and explorations should be repeated.

The absence of sinus and skin aspergillosis is remarkable in our study. Skin aspergillosis has been reported to be more common in children than in adults. Abbasi *et al.* [12] reported 13 children (20% of their patients) with skin involvement. Walmsley *et al.* described 16 cutaneous aspergillosis (41% of their patients), and a review of the literature revealed that 67% of skin involvement cases occur in children.[13] Arm boards, commonly used in children in order to prevent movement at the peripheral catheter site, are implicated.[13] The systematic use of central venous catheter avoids the use of peripheral lines. The absence of any bandages or arm board may explain the absence of any skin infection in our population.

Twenty-three *Aspergillus* species were isolated from 22 patients. As reported in other reviews,[10] *A. fumigatus* is the most common species responsible for IA in our study (78% of cases). It is noteworthy that in the two North American paediatric studies (Memphis, TN, USA[12] and Toronto, ON, Canada[13]), epidemiology was different: *A. flavus* was the most frequently identified species (55% and 65%, respectively), as *A. fumigatus* was identified only in 29% and 15% of cases, respectively.

Until the end of our study, only AmB and its lipidic formulations, itraconazole and flucytosine, were

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routinely used as antifungal therapy in our institution. All patients received AmB (desoxycholate or lipidic formulation) as the 'gold standard' antifungal therapy.[19] New drugs, such as voriconazole and caspofungin, have recently become available and should improve the prognosis.[20–23]

Eight patients underwent open chest surgery without any complication. Among them, five experienced a favourable outcome. Our results suggest an added benefit of surgical resection, and are in accordance with the data already published.[24] All surgeries were performed after haematological recovery in order to prevent relapse and dissemination during future immunosuppression periods. Earlier surgery may improve survival by reducing the risk of fatal haemoptysis.[16]

The prognosis of IA remains dismal.[25] IA is considered a major death risk factor in children with malignancies with relative risk (RR) estimated at 13.5 by Zaoutis *et al.* [26]. In our study, the overall mortality was 87.7%. This result is comparable with other paediatric studies: 85% for Abbasi *et al.* [12] in 1999; 74% for Walmsley *et al.* [13] in 1993, in which 41% were skin aspergillosis, explaining a better prognosis; and, 70% for Groll *et al.* [27], in which definition criteria were more permissive than ours.

In our study, the cure rate of IA was at least 37.5% with a median follow-up of at least 6 years. Thirty-seven per cent to 62% of the deaths were directly related to IA with a median survival of 17 days (0–312 days). These results are comparable with adult series.[7,28] In addition, among the four patients developing IA recurrence, three were cured and two of them are long-term survivors.

Conclusion

Our study demonstrates that IA is a frequent and deadly complication in severely immunocompromised children, in particular for children treated for AML. Despite progress in diagnostic procedures and therapeutics, mortality remains high. Future studies and research should focus on the identification of patients at risk, an effective prophylactic treatment, and earlier diagnosis. Newly available drugs may improve prognosis, but controlled clinical trials are still required.

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Dear Colleagues

Further Update on Standards on Healthcare Associated Infections

In February 2023, DL (2023)06 was issued informing Health Boards that the previous healthcare associated infection (HCAI) standards and antibiotic use indicators originally due to expire in 2022, were extended to 2024. Since this letter, an objective to review the HCAI standards and indicators was included in the current two-year HCAI Strategy 2023-2025.

Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) Scotland were commissioned and in their response, outlined a range of options based on the analysis of the current targets and trends, as well as stakeholder engagement.

The Cabinet Secretary for Health and Social Care considered the recommendations and has approved that the standard **should be no increase in the incidence (number of cases) of *Clostridioides difficile* infection (CDI), *Escherichia coli* bacteraemia (ECB), and *Staphylococcus aureus* bacteraemia (SAB) by March 2026 from the 2023/2024 baseline.**

ARHAI Scotland will provide Boards with the 2023/2024 baseline number of CDI, ECB and SAB cases to enable local monitoring.

The national HCAI surveillance programme in Scotland is under review with the recommendations due to Scottish Government by November 2025 (a deliverable within the HCAI Strategy). The surveillance review will consider new standards once the direction of the national mandatory HCAI surveillance programme has been set. Further communications on the surveillance programme will be shared at a later date.

From:
Interim Chief Nursing Officer,
Anne Armstrong

27 March 2025

DL (2025) 05

Addresses

For action

NHS Scotland Chairs,
NHS Scotland Chief Executives,
Chief Officers Health and Social Care Partnerships
Local Authorities
HR Directors,
Medical Directors,
Nurse Directors,
Primary Care Leads,
Directors of Pharmacy,
Directors of Public Health,
Directors of Dentistry,
Optometric Advisors,
All Independent Contractors (Dental, Pharmacy, General Practice and Optometry),
Infection Control Managers
Infection Control Doctors
Infection Control Nurses

Enquiries

Scottish Government
Directorate for Chief Nursing Officer
Email: cno@gov.scot

We recognise the hard work that Health Boards have undertaken to work towards these targets in challenging circumstances and we expect Health Boards to maintain standards and continue to prioritise reductions in CDI, ECB and SAB.

Finally, the UK AMR National Action Plan (NAP) sets out renewed UK-wide antibiotic use targets – namely the ambition to, by 2029, reduce total antibiotic use in human populations by 5% and to achieve 70% of total use of antibiotics from the Access category across the human healthcare system. Both targets are set against a 2019/20 baseline. The Scottish Government is currently working with the Scottish Antimicrobial Prescribing Group on a set of supplementary antimicrobial prescribing targets to support the high level NAP goals across both primary and secondary healthcare settings. Further details on these supplementary targets in a further communication in due course.

If you have any questions about the content of this publication, please contact the HCAI/Antimicrobial Resistance Team at HAI-AMR_Policy_Unit@gov.scot.

Yours sincerely,

ANNE ARMSTRONG
INTERIM CHIEF NURSING OFFICER

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OCID: ocds-r6ebe6-0000789140
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Deadline Time:
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Abstract: Scottish Government and NHSScotland are exploring options for a single national Infection Prevention Control eSurveillance solution that will ensure consistent application across all of NHSScotland.
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