SCOTTISH HOSPITALS INQUIRY

Bundle of documents for Oral hearings commencing from 19 August 2024 in relation to the Queen Elizabeth University Hospital and the Royal Hospital for Children, Glasgow

Bundle 24 – Documents referred to in the Expert Report By Allan Bennett regarding Cryptococcus, and Supporting Documentation

Volume 4

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SCOTTISH HOSPITALS INQUIRY

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Emerging trends in the epidemiology of invasive mycoses in England and Wales (1990–9)

T. L. LAMAGNI[1]*, B. G. EVANS[1], M. SHIGEMATSU[1] AND E. M. JOHNSON[2]

[1] *HIV & STD Division, Communicable Disease Surveillance Centre, Public Health Laboratory Service, 61 Colindale Avenue, London NW9 5EQ, UK*
[2] *Mycology Reference Laboratory, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL, UK*

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SUMMARY

Invasive fungal infections are becoming an increasing public health problem owing to the growth in numbers of susceptible individuals. Despite this, the profile of mycoses remains low and there is no surveillance system specific to fungal infections currently existing in England and Wales. We analysed laboratory reports of deep-seated mycoses made to the Communicable Disease Surveillance Centre between 1990 and 1999 from England and Wales. A substantial rise in candidosis was seen during this period (6·76–13·70 reports per million population/year), particularly in the older age groups. Rates of cryptococcosis in males fluctuated over the decade but fell overall (1·05–0·66 per million population/year), whereas rates of female cases gradually rose up until 1998 (0·04–0·41 per million population/year). Reports of *Pneumocystis carinii* in men reduced substantially between 1990 and 1999 (2·77–0·42 per million population/year) but showed little change in women. Reports of aspergillosis fluctuated up until 1996, after which reports of male and female cases rose substantially (from 0·08 for both in 1996 to 1·92 and 1·69 per million population/year in 1999 for males and females respectively), largely accounted for by changes in reporting practice from one laboratory. Rates of invasive mycoses were generally higher in males than females, with overall male-to-female rate ratios of 1·32 (95% CI 1·25–1·40) for candidosis, 1·30 (95% CI 1·05–1·60) for aspergillosis, 3·99 (95% CI 2·93–5·53) for cryptococcosis and 4·36 (95% CI 3·47–5·53) for *Pneumocystis carinii*. The higher male than female rates of reports is likely to be a partial reflection of HIV epidemiology in England and Wales, although this does not fully explain the ratio in infants and older age groups. Lack of information on underlying predisposition prevents further identification of risk groups affected. Whilst substantial under-reporting of *Pneumocystis carinii* and *Cryptococcus* species was apparent, considerable numbers of superficial mycoses were mis-reported indicating a need for clarification of reporting guidelines. Efforts to enhance comprehensive laboratory reporting should be undertaken to maximize the utility of this approach for surveillance of deep-seated fungal infections.

INTRODUCTION

Fungal infections are becoming an increasingly important cause of morbidity and mortality in many countries [1–3]. Although some fungal species commonly exist as harmless colonizers of various bodily sites [4], they are also responsible for a vast array of clinical conditions, ranging from dermatological complaints to life-threatening systemic disease.

Invasive mycoses are a particular problem in hospital settings where distinct host and environ-

* Author for correspondence.

mental factors predispose patients to opportunistic infections. Fungal spores are commonly found as environmental contaminants, but their presence in hospitals is hazardous to certain patient groups. Hospital-acquired fungal infections may originate from endogenous flora in the case of yeast infections or are transmitted via healthcare workers and fomites or through the airborne spread of spores (conidia) [5]. Underlying diseases and treatments or procedures which predispose patients to invasive mycoses include those that impair the cellular immune response, the use of treatments that alter the balance of microflora, such as broad-spectrum antibiotics, invasive surgical procedures and the use of intravascular lines [6]. Invasive fungal infections are difficult to treat and can progress rapidly to death, case fatality rates of 19–65% for candidosis [7, 8] and 13–85% for aspergillosis [9, 10] have been reported. Invasive mycoses often pose the biggest threat to patients vulnerable to infection; aspergillosis is thought to be the leading cause of death following bone marrow transplantation [11].

The incidence of nosocomial fungal infection has increased over the last 20 years for a number of reasons relating to the emergence of new disease, increasing use of immunosuppressive treatments and invasive procedures, and to prolonged survival of highly susceptible patients [12–14]. The shift towards use of more intensive cytotoxic drug regimes, resulting in prolonged neutropenia, in patients with leukaemia has also led to longer risk periods in this group [15, 16]. Cancer patients are being treated with ever more aggressive chemotherapy regimens, leaving them susceptible to opportunistic infections. Organ transplantation is also becoming more common, necessitating the wider use of immunosuppressive therapy. Survival of preterm neonates is improving, creating a new group of highly susceptible patients. The onset of the HIV epidemic further added to this group of individuals with impaired immunity. These phenomena have served to increase the pool of individuals susceptible to opportunistic infections. The economic implications of nosocomial infection in England, fungal or otherwise, were estimated in a recent study to cost the NHS an additional £930 million each year [17].

Invasive mycoses pose a particular challenge to public health. Given that fungal organisms are common environmental contaminants, and colonizers of various body sites, prevention of infection is exceptionally difficult. For *Pneumocystis* and *Cryptococcus* species, and infections due to dimorphic fungi, disease can result from reactivation of infection acquired from the inhalation of airborne spores many years previously. Our ability to control these diseases is reliant on prevention of exposure and effective prophylactic treatment of vulnerable individuals, something that has been largely achieved for pneumocystis pneumonia but not for cryptococcosis.

Surveillance of mycoses in England and Wales is carried out primarily through systems monitoring hospital-acquired infections. Data are also available from routine laboratory reports of clinically significant fungal infections made to the PHLS Communicable Disease Surveillance Centre (CDSC). Laboratories throughout England and Wales are invited to report to CDSC all deep-seated fungal infections in which the organism has been isolated (or which have been diagnosed by antigen tests in the case of cryptococcosis) [18]. All reports of *Pneumocystis carinii* are accepted regardless of the method of detection. In this paper we summarize these reports in an attempt to review laboratory reporting of mycoses and, where appropriate, to describe the epidemiology of invasive fungal infection in England and Wales.

METHODS

Reports of fungal infections received by CDSC from microbiology laboratories in England and Wales between 1990 and 1999 were reviewed. Reports were made electronically through the CoSurv network or in paper format (CDR form 2 and computer-generated printouts). Ongoing checks were carried out by CDSC for possible duplication of reports.

A marker of the clinical significance of reported isolates was elicited by examination of the site of the specimen in combination with the organism reported. Specimen types were grouped according to site of infection (see Box). Isolation of a fungal species from the following site groupings was considered to indicate deep-seated infection: central nervous system; organs, tissue and tissue fluids; pulmonary (except for *Candida* spp.); blood (except for *Aspergillus* spp.); sputum (except for *Candida* spp.). Isolations of *Rhizomucor* and *Rhizopus* species from sites within the upper respiratory tract were also considered to reflect invasive disease. Isolations of *Candida* species from pulmonary sites or sputum were treated as being of indeterminate clinical significance as these were thought in many cases to be contaminants or harmless colonizers. Although possibly reflecting disseminated

Box 1. Specimen site groupings and hierarchy

1	Central nervous system
2	Blood
3	Organs, tissue and tissue fluids
4	Pulmonary
5	Sputum
6	Genitourinary
7	Gastrointestinal and anorectal
8	Eyes
9	Upper respiratory tract
10	Ears
11	Surgical devices
12	Subcutaneous
13	Cutaneous

infection, isolations of *Aspergillus* from blood cultures were similarly placed in this indeterminate category as this organism is very difficult to isolate from blood but is a common contaminant; therefore a positive culture is not proof of infection. All other isolations were considered to reflect superficial or subcutaneous infection. This included isolations of *Aspergillus* species from the ear, which may represent significant morbidity but do not represent deep-seated or invasive disease. Reports with isolations from more than one site were assigned according to the most invasive site (see Box for hierarchical ordering of sites).

Fungal infection reports were analysed by year of report, with further analyses by age, sex and region for *Aspergillus*, *Candida*, *Cryptococcus* and *Pneumocystis* species. Both standardized clinical descriptions and free-text clinical comments accompanying reports were examined for likely clinical significance and information on predispositions to infection. Reporting rates were calculated using mid-year resident population estimates for each corresponding year, age and gender grouping in each NHS regional office (Office for National Statistics: Population Estimates Unit, unpublished data). Regional population estimates were unavailable for 1990 and therefore substituted with 1991 population denominators. Rate ratios and exact confidence intervals were calculated using statistical software (StataCorp. 1999. *Stata Statistical Software: Release 6.0*. College Station, TX: Stata Corporation).

RESULTS

Overview of reports

A total of 11 702 fungal isolates were reported to CDSC between 1990 and 1999 from laboratories across England and Wales (Table 1). Reports were received from 267 PHLS and NHS laboratories, with 55% (6418/11 702) of all isolates being reported from PHLs. Seventy-three different fungal species were reported, 69 of which were fully identified. Isolations were made from 100 different specimen sites, with fungi isolated from more than one site in 6% (685/11 702) of reports.

Over half (59%; 6902) of all fungal isolates reported between 1990 and 1999 were of *Candida* species. A fifth (21%; 2510) of reports were of *Trichophyton* species, the next most commonly isolated species were *Aspergillus* (7%; 873), *Pneumocystis carinii* (6%; 668) and *Cryptococcus* (3%; 301).

Examination of specimen sites of fungal reports indicated a third (4012) to be superficial or subcutaneous infections and 6% (691) of indeterminate significance. Four percent (458) had missing information on site of isolation. The remaining 6541 reports appeared to indicate invasive infection. Reports of invasive mycoses from 46 different species were received, 43 of which were fully identified. Numbers of yearly reports of invasive mycoses by species are given in Table 2, excluding the following species for which less than 10 reports were received between 1990 and 1999 (number of reports): *Absidia corymbifera* (6); *Acremonium* sp. (3); *Blastoschizomyces capitatus* (1); *Cunninghamella bertholletiae* (1); *Exophiala dermatitidis* (2); *Fusarium* sp. (4); *Geotrichum* sp, (2); *Histoplasma* sp. (8); *Penicillium* sp. (5); *Phialophora richardsiae* (1); *Rhizomucor pusillus* (2); *Rhizopus sp*. (2); *Sporothrix schenckii* (1). Although not associated with invasive disease, five isolations from invasive sites of *Microsporum* sp. and two of *Trichophyton rubrum* were also reported.

Aspergillosis

A total 873 *Aspergillus* isolates were reported between 1990 and 1999 to CDSC (Table 1). The clinical significance of 172 *Aspergillus* reports isolated from serum alone was questionable, so these were excluded from further analysis. A further 312 isolates were excluded on the basis of missing specimen information (53 reports) or because the sites from which they were isolated were not thought to represent invasive disease as follows: inner/outer ear (201), cutaneous/subcutaneous sites (38), upper respiratory tract (12), eyes (4), gastrointestinal/anorectal sites (2), genitourinary sites (1) and surgical device (1).

In total, 389 reports *Aspergillus* were thought to

Table 1. *Laboratory reports of all fungal isolates, by year of report (England and Wales: 1990–9)*

Species	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Absidia corymbifera		1	2		3	4		1	1		12
Acremonium spp.	1			1		5	3	1	3	2	16
Aspergillus spp.	44	48	63	39	42	31	17	166	207	216	873
Blastoschizomyces capitatus	1										1
Botrytis spp.				1							1
Candida spp.	356	328	361	399	485	644	636	1103	1279	1311	6902
Chrysosporium keratinophilum									1		1
Coccidioides immitis									1		1
Cryptococcus spp.	27	30	25	37	29	41	29	17	34	32	301
Cunningamella bertholletiae										1	1
Epidermophyton floccosum			5		2	11	2	2	8	1	31
Exophiala spp.	1			1	1	1				1	5
Fusarium spp.			1	4		5	3	3	6	9	31
Geotrichum spp.								2		2	4
Histoplasma spp.	3	2			1				2	1	9
Hyphozyma spp.									1		1
Malassezia spp.	2	3			8	48	5	7	3	2	78
Microsporum spp.			6	3	8	29	15	9	16	4	90
Mucor spp.			1	2				1			4
Penicillium spp.		1					1	2	3		7
Phialophora richardsiae	1										1
Pneumocystis carinii	78	152	87	26	50	58	61	53	54	49	668
Pseudallescheria boydii			1			1					2
Rhizomucor pusillus	1				1						2
Rhizopus spp.	1						4			2	7
Rhodotorula spp.	2		1	1	3	4	3	3	5	10	32
Saccharomyces spp.	2		1	2	1	1	2	4	3	24	40
Scopulariopsis spp.				2		14	8	3	11	7	45
Scytalidium dimidiatum									1		1
Sporothrix spp.							1		2	1	4
Trichophyton spp.			1		352	667	299	275	578	338	2510
Trichosporon spp.	4	2	1	2	2	5	1		3	1	21

Table 2. *Laboratory reports of invasive mycoses, by year of report (England and Wales: 1990–9)*

Species	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Aspergillus spp.	27	18	20	17	15	8	4	83	95	102	389
Candida spp.	344	317	349	387	463	536	552	683	804	722	5157
Cryptococcus spp.	27	30	25	36	25	41	25	15	27	28	279
Malassezia spp.	2	3			4		1	1	1	2	14
Pneumocystis carinii	78	152	85	26	48	56	57	35	39	19	595
Rhodotorula spp.	1		1	1	3	4	2	3	5	10	30
Saccharomyces spp.	2		1	2		1	2	4	3	5	20
Trichosporon spp.	3	2	1	2	2	1				1	12
Other species*	9	4	0	0	3	4	3	6	8	8	45

* Species with less than 10 reports received between 1990 and 1999.

represent invasive infection (Table 2). The majority specified *A. fumigatus* (338) as the causative organism, with a further 12 isolates identified as *A. flavus* and 6 each of *A. niger* and *A. terreus* (27 reports did not fully identify the organism).

The majority of *Aspergillus* isolates were from sputum (342) or pulmonary sites (32), indicative of lung disease. Other sites reported were CNS (5 cases) and organs, tissue and tissue fluids (10).

Two-thirds (265) of reports of aspergillosis contained standardized or free-text clinical information. Underlying diseases reported included: bronchiectasis (13 cases), neoplasms (12), chronic obstructive airway disease (9), leukaemia (8), Waldenström's macro-

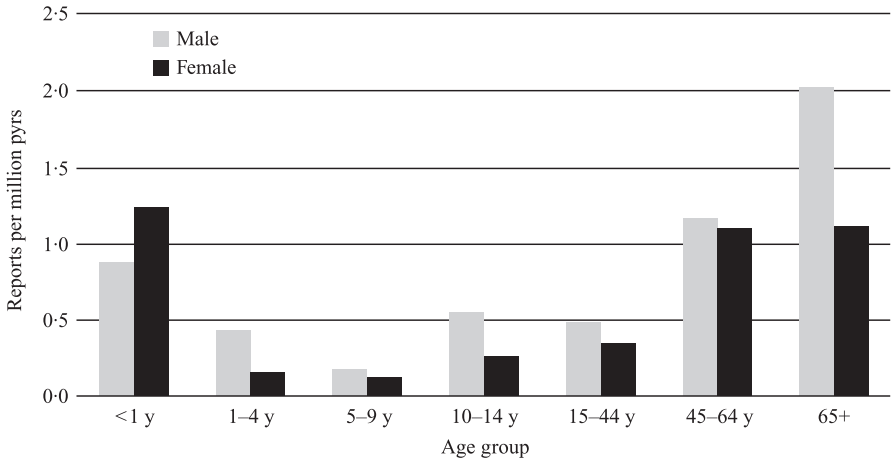

Fig. 1. Rates of aspergillosis laboratory reports, by age and sex (England and Wales: 1990–9).

Table 3. *Laboratory reports of aspergillosis per million population**, *by region* (*England and Wales: 1990–9*)

	Year of report										
Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Eastern											
Number	15	8	4	8	0	3	0	1	0	2	41
Rate	2·91	1·55	0·77	1·54	0·00	0·57	0·00	0·19	0·00	0·37	0·78
London											
Number	2	6	2	1	1	1	1	0	0	26	40
Rate	0·29	0·87	0·29	0·14	0·14	0·14	0·14	0·00	0·00	3·57	0·57
North West											
Number	0	1	1	1	0	0	1	77	93	68	242
Rate	0·00	0·15	0·15	0·15	0·00	0·00	0·15	11·89	14·36	10·51	3·74
Northern and Yorkshire											
Number	0	0	1	1	1	1	0	2	1	1	8
Rate	0·00	0·00	0·16	0·15	0·15	0·15	0·00	0·31	0·15	0·15	0·12
South East											
Number	0	0	0	1	9	2	1	2	1	2	18
Rate	0·00	0·00	0·00	0·12	1·07	0·24	0·12	0·23	0·12	0·23	0·21
South West											
Number	8	1	5	2	1	0	1	1	0	0	19
Rate	1·70	0·21	1·05	0·42	0·21	0·00	0·21	0·21	0·00	0·00	0·40
Trent											
Number	0	0	0	0	1	0	0	0	0	1	2
Rate	0·00	0·00	0·00	0·00	0·20	0·00	0·00	0·00	0·00	0·19	0·04
Wales											
Number	0	0	0	0	0	1	0	0	0	0	1
Rate	0·00	0·00	0·00	0·00	0·00	0·34	0·00	0·00	0·00	0·00	0·03
West Midlands											
Number	2	2	7	3	2	0	0	0	0	2	18
Rate	0·38	0·38	1·33	0·57	0·38	0·00	0·00	0·00	0·00	0·37	0·34
England and Wales											
Number	27	18	20	17	15	8	4	83	95	102	389
Rate	0·53	0·35	0·39	0·33	0·29	0·15	0·08	1·59	1·81	1·94	0·75

* Yearly regional population estimates used except for 1990 (1991 population denominator used).

globulinaemia (2), leucopenia (5), cystic fibrosis (5), sarcoidosis (2), HIV infection (2) and emphysema (1). In 40 cases the infection followed bone marrow or solid organ transplant and other invasive surgical procedures in 11 cases. Cytotoxic or steroid treatment was recorded for 7 cases.

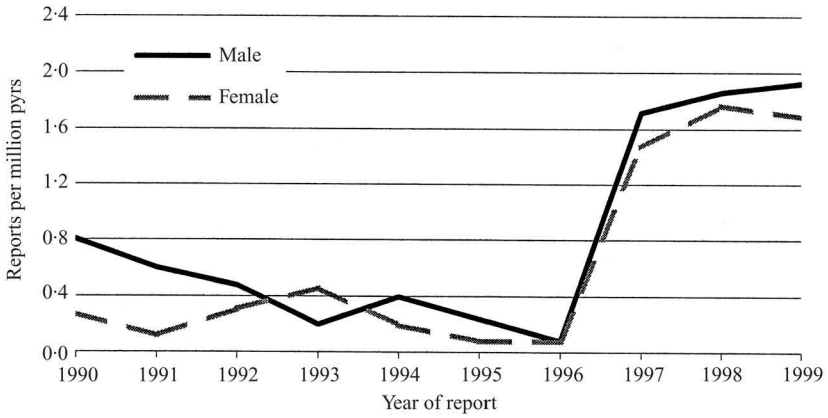

Fig. 2. Annual rates of aspergillosis laboratory reports, by sex (England and Wales: 1990–9).

Annual rates of aspergillosis by age group and sex are given in Figure 1. Age-specific rates of aspergillosis were highest for males in those 65 and over and for females in infants under 1 year. Rates were higher in males than females overall (rate ratio = 1·30, 95% CI 1·05–1·60), and in all age groups except in infants under 1 year. Only in the over 65 age group did this reach statistical significance (2·01 and 1·11 per million per year for males and females respectively; rate ratio = 1·82, 95% CI 1·25–2·65).

Regional yearly rates of all aspergillosis reported between 1990 and 1999 showed considerable variation, ranging from 0·03 (Wales) to 3·74 (North West) per million population per year (Table 3). Reports from the North West rose substantially in 1997, from 1 or 2 annual reports between 1990 and 1996 to 77 received in 1997, 93 in 1998 and 68 in 1999. Of the 280 aspergillosis reports received between 1997 and 1999, 169 originated from one laboratory.

Yearly changes in numbers of reports per million population are shown in Figure 2. Rates of reported aspergillosis in males and females remained between 0·08 and 0·80 per million between 1990 and 1996, after which reports rose to reach 1·92 and 1·69 per million respectively, mainly accounted for by the change in numbers of reports from one laboratory.

Candidosis

A total of 6902 laboratory reports of *Candida* species were received by CDSC between 1990 and 1999 (Table 1). Of these, 1745 did not meet our criteria for invasive disease and were excluded from further analysis. The majority (1108) of these exclusions were isolations from sites commonly colonized by *Candida* species or thought to reflect superficial or subcutaneous infection: gastrointestinal/anorectal sites (356), subcutaneous/cutaneous (307), ear (241), genitourinary sites (112), upper respiratory tract (50), surgical devices (16), eyes (15), pulmonary sites (11). Specimen information was missing from 118 reports. A further 519 reports were isolated from sputum specimens and therefore excluded as yeasts are a rare cause of pulmonary infection and the diagnosis is histological.

Reports of systemic candidosis formed over three-quarters (5157/6541) of all invasive mycoses reported to CDSC between 1990 and 1999 (Table 2). *C. albicans* was isolated in most (60%; 3104) of the candidosis reports, with substantial reports of *C. parapsilosis* (545) and *C.* (*Torulopsis*) *glabrata* (484) also being received. Other causative agents reported were *C. tropicalis* (195), *C. krusei* (78), *C. guillermondi* (33), *C. lusitaniae* (17), *C. famata* (22), *C. kefyr* (9), *C. inconspicua* (6), *C. lipolitica* (2) and one case each of *C. ciferrii*, *C. pelliculosa*, *C. norvegensis*, *C. humicola* and *C. rugosa*. Thirteen percent (657) were recorded as unnamed *Candida* species. The proportion of candidosis reports with *C. albicans* as the underlying agent showed little change between 1990 and 1999. Little variation was seen in the species distribution between males and females. Although *C. albicans* was the most commonly reported species across all age groups, distribution of other species varied across age groups. Nearly a quarter (23%; 172/757) of all paediatric (less than 15 years) candidosis cases were caused by *C. parapsilosis*, compared to only 9% (353/4144) of adult cases. Conversely, adult cases of candidosis showed higher proportions *C. glabrata*

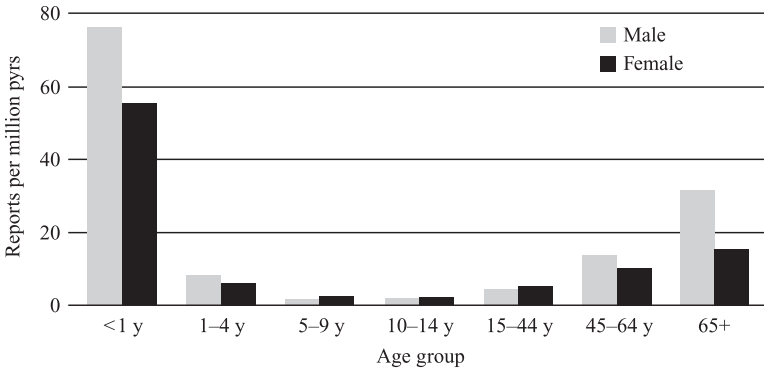

Fig. 3. Rates of candidosis laboratory reports, by age and sex (England and Wales: 1990–9).

Table 4. *Laboratory reports of candidosis per million population*, by region* (*England and Wales: 1990–9*)

	Year of report										
Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Eastern											
Number	14	18	20	25	32	44	36	32	39	58	318
Rate	2·72	3·50	3·86	4·81	6·13	8·37	6·80	6·00	7·25	10·70	6·05
London											
Number	92	79	78	80	76	84	87	107	127	77	887
Rate	13·35	11·47	11·30	11·54	10·91	11·99	12·30	15·02	17·67	10·57	12·64
North West											
Number	57	54	49	54	76	95	78	117	135	154	869
Rate	8·82	8·36	7·57	8·33	11·72	14·65	12·04	18·07	20·84	23·80	13·42
Northern and Yorkshire											
Number	35	47	34	63	56	75	53	74	92	65	594
Rate	5·45	7·32	5·28	9·76	8·66	11·60	8·20	11·46	14·23	10·06	9·20
South East											
Number	37	27	38	46	69	86	73	97	96	70	639
Rate	4·48	3·27	4·58	5·52	8·23	10·18	8·59	11·32	11·14	8·05	7·58
South West											
Number	33	30	35	24	38	52	74	58	57	56	457
Rate	6·99	6·36	7·37	5·03	7·92	10·77	15·28	11·90	11·63	11·35	9·50
Trent											
Number	38	25	39	29	51	28	34	49	63	79	435
Rate	7·55	4·97	7·71	5·71	10·01	5·48	6·64	9·55	12·27	15·35	8·54
Wales											
Number	12	11	21	31	24	30	55	47	84	65	380
Rate	4·15	3·80	7·25	10·67	8·24	10·29	18·83	16·06	28·64	22·13	13·04
West Midlands											
Number	26	26	35	35	41	42	62	102	111	98	578
Rate	4·94	4·94	6·63	6·62	7·74	7·91	11·66	19·17	20·82	18·37	10·91
England and Wales											
Number	344	317	349	387	463	536	552	683	804	722	5157
Rate	6·76	6·20	6·81	7·52	8·97	10·34	10·61	13·08	15·34	13·70	9·97

* Yearly regional population estimates used except for 1990 (1991 population denominator used).

(11%; 453/4144) than in paediatric candidosis (2%; 17/757).

The majority (98%; 5075) of patients with systemic candidosis had positive blood culture (candidaemia). Other sites from which *Candida* species were isolated include the CNS (42) and organs or tissue fluid (40).

Standardized clinical comments were available for 29% (1521) of candidosis cases (117 of cases less than

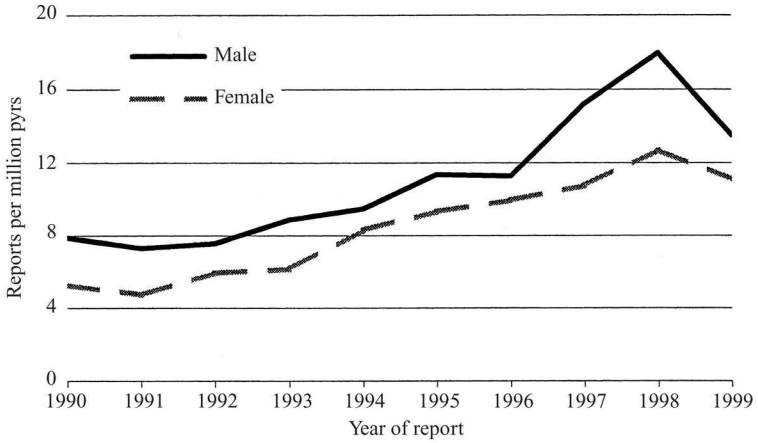

Fig. 4. Annual rates of candidosis laboratory reports, by sex (England and Wales: 1990–9).

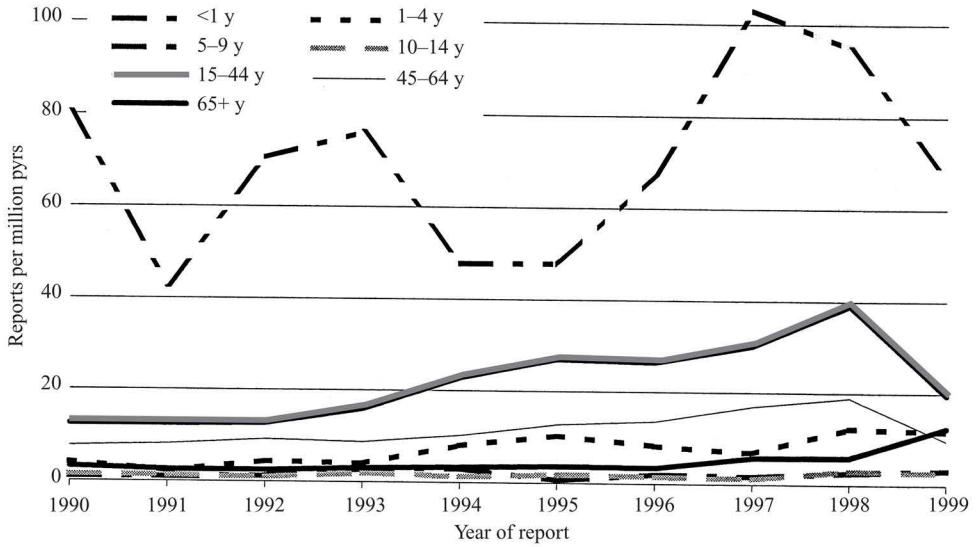

Fig. 5. Age-specific annual rates of candidosis laboratory reports (England and Wales: 1990–9).

1 year of age). Of these, a third (478) indicated that patients were immunocompromised (21% in under ones; 24/117), with use of intravenous catheters mentioned in 186 of these (12 of under ones). Use of intravenous catheters was mentioned in a further 918 cases (78 of the under ones). Additional free-text comments were recorded in 2401 reports (252 of under ones). Of these, 130 patients were noted as receiving total parenteral nutrition. Other predisposing factors noted included preterm birth (135 cases), pancreatitis (83), leukaemia (59), leucopenia (29), pancytopenia (2), neoplasms (49), cystic fibrosis (36), diabetes (31) and Whipple's disease (13). Four-hundred and eighty patients developed candidosis following invasive surgical procedures and a further 27 subsequent to solid organ or tissue transplant. Fifteen post burns cases were also reported.

Annual rates of candidosis reports by age and sex are shown in Figure 3. Highest rates were observed in infants under 1 year of age, 76·0 and 55·5 cases per million population per year for males and females respectively (rate ratio = 1·37, 95% CI 1·13–1·67). The majority of candidosis cases in infants occurred in those under 1 month old (58%; 260/446). Annual rates in age groups between 1 and 4 and 15 and 44 were less than 10 per million per year for males and females. Rates of reports were higher in males than females in most age bands, especially in those aged 65 plus, where rates in men were twice those in women (31·5 and 15·1 per million; rate ratio = 2·08, 95% CI 1·89–2·29).

Numbers of reports received from each region showed considerable variation (Table 4). The lowest incidence was observed in the Eastern region (6·05 per

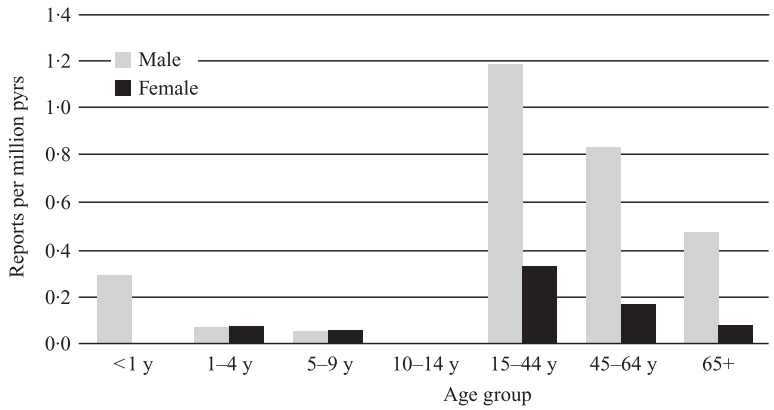

Fig. 6. Rates of cryptococcosis laboratory reports, by age and sex (England and Wales: 1990–9).

million population per year) and the highest in the North West (13·42 per million/year). All regions showed a general rise in candidosis reports between 1990 and 1999.

Annual reporting rates for candidosis showed a rising trend between 1990 and 1998, from 6·76 to 15·34 per million population, with a drop in 1999 to 13·70 per million. Rates of both male and female candidosis reports rose during this period (Fig. 4). Rates of male cases outnumbered female across the entire period (rate ratio = 1·32, 95% CI 1·25–1·40), incidence in males ranging from 13% higher (1996) to 54% higher (1991) than females. The rise in candidosis reports was seen in most age groups (Fig. 5) aside from infants less than 1 year of age, with rates in all age other groups at least doubling between 1990 and 1998.

Cryptococcosis

Between 1990 and 1999, 279 reports of invasive cryptococcal infection were received (Table 2), 263 of which were fully identified as *C. neoformans* and one as *C. albidus* infection.

Half of reports (83/173) came with accompanying clinical comments indicating patients to be immunocompromised. Underlying HIV infection was known in 57 cases. Other reported underlying predisposing factors included hepatitis C infection (2 cases) and diabetes (1 case).

Age and sex-specific incidence of cryptococcosis reports are shown in Figure 6. Rates were low in children and highest in 15–44 year olds. Substantially higher rates were reported for men than women (rate ratio = 3·99, 95% CI 2·93–5·53 overall), from 4 times greater in 15–44 year olds (rate ratio = 3·57, 95% CI 2·45–5·31), to 5 (rate ratio = 4·86, 95% CI 2·43–10·78) and 6 times (rate ratio = 5·85, 95% CI 1·89–24·05) greater in those aged 45–64 and 65 plus, respectively.

Regional reporting rates of cryptococcosis are given in Table 5. Highest rates were observed in London (1·97 per million population per year). Outside London, annual rates ranged from 0·20 in Trent to 0·46 per million in the South West.

Annual laboratory reports of cryptococcal infection were substantially higher in males than females between 1990 and 1995 (Fig. 7). Rates of male cryptococcosis fell substantially between 1995 and 1997, from 1·38 to 0·31 per million although showing a slight rise subsequently to 0·66 per million in 1999. Rates in women increased between 1990 and 1998, from 0·04 to 0·41 per million, although falling in 1999 to 0·11 per million.

Pneumocystis carinii

P. carinii infection was the second most commonly reported deep mycosis, 595 reports were received between 1990 and 1999 (Table 2). Over half (55%; 327) of the infections were detected through sputum specimens, most of the others (44%; 261) were diagnosed from other pulmonary specimens, 6 were detected from blood samples and 1 from bone marrow.

Of the 595 *P. carinii* reports, 409 had accompanying clinical information. One hundred and eighty-seven (46%) were described as immunocompromised. Underlying HIV infection was present in 181 patients. Other conditions associated with immunosuppression mentioned include organ/tissue transplant (11 cases), lymphoma (3), leukaemia (2), Wegener's granulomatosis (2), cystic fibrosis (1), severe combined immune deficiency (1) and systemic lupus erythematosus (1).

Table 5. *Laboratory reports of cryptococcosis per million population*, by region (England and Wales: 1990–9)*

Region	Year of report										
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Eastern											
Number	2	1	2	0	2	1	4	1	1	1	15
Rate	0·39	0·19	0·39	0·00	0·38	0·19	0·76	0·19	0·19	0·18	0·29
London											
Number	16	19	14	14	14	21	10	8	14	8	138
Rate	2·32	2·76	2·03	2·02	2·01	3·00	1·41	1·12	1·95	1·10	1·97
North West											
Number	2	3	0	4	1	3	2	0	2	3	20
Rate	0·31	0·46	0·00	0·62	0·15	0·46	0·31	0·00	0·31	0·46	0·31
Northern and Yorkshire											
Number	0	4	0	1	0	2	2	0	2	3	14
Rate	0·00	0·62	0·00	0·15	0·00	0·31	0·31	0·00	.031	0·46	0·22
South East											
Number	4	3	3	7	3	7	0	2	3	2	34
Rate	0·48	0·36	0·36	0·84	0·36	0·83	0·00	0·23	0·35	0·23	0·40
South West											
Number	1	0	2	7	3	4	1	0	2	2	22
Rate	0·21	0·00	0·42	1·47	0·63	0·83	0·21	0·00	0·41	0·41	0·46
Trent											
Number	0	0	2	2	0	1	2	1	0	2	10
Rate	0·00	0·00	0·40	0·39	0·00	0·20	0·39	0·19	0·00	0·39	0·20
Wales											
Number	1	0	0	1	0	0	1	1	0	2	6
Rate	0·35	0·00	0·00	0·34	0·00	0·00	0·34	0·34	0·00	0·68	0·21
West Midlands											
Number	1	0	2	0	2	2	3	2	3	5	20
Rate	0·19	0·00	0·38	0·00	0·38	0·38	0·56	0·38	0·56	0·94	0·38
England and Wales											
Number	27	30	25	36	25	41	25	15	27	28	279
Rate	0·53	0·59	0·49	0·70	0·48	0·79	0·48	0·29	0·51	0·53	0·54

* Yearly regional population estimates used except for 1990 (1991 population denominator used).

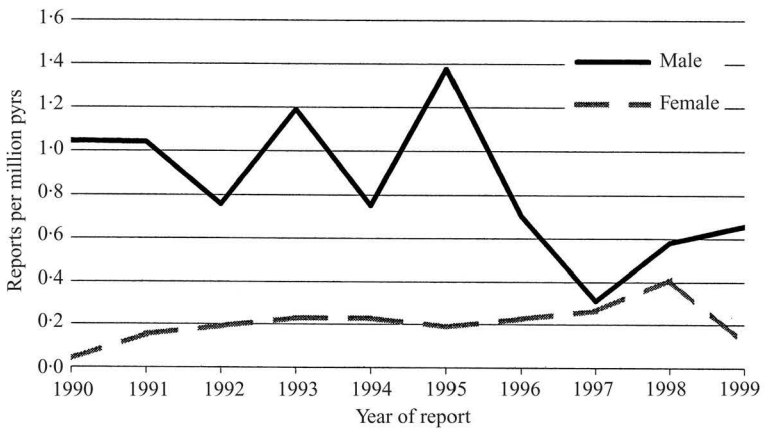

Fig. 7. Annual rates of cryptococcosis laboratory reports, by sex (England and Wales: 1990–9).

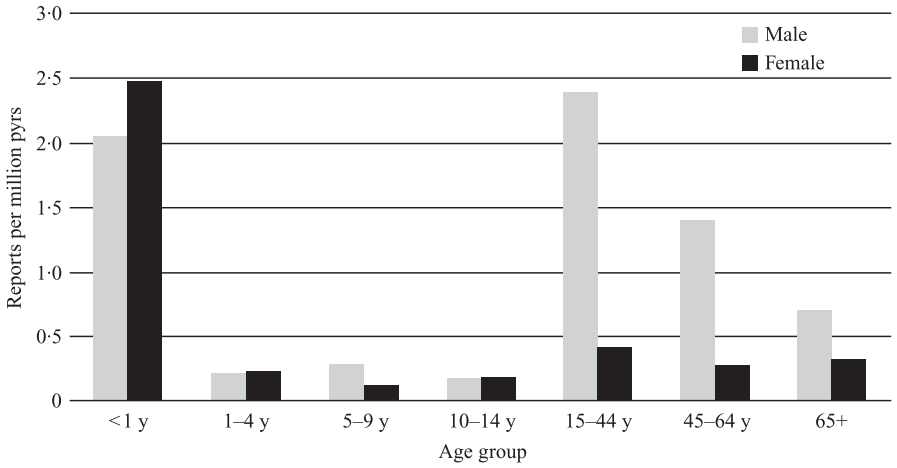

Fig. 8. Rates of *Pneumocystis carinii* laboratory reports, by age and sex (England and Wales: 1990–9).

Table 6. *Laboratory reports of* Pneumocystis carinii *per million population*, by region* (*England and Wales: 1990–9*)

	Year of report										
Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	1990–9
Eastern											
Number	4	6	7	3	3	7	3	3	7	4	47
Rate	0·78	1·17	1·35	0·58	0·57	1·33	0·57	0·56	1·30	0·74	0·89
London											
Number	54	91	41	3	3	0	5	3	1	0	201
Rate	7·84	13·21	5·94	0·43	0·43	0·00	0·71	0·42	0·14	0·00	2·86
North West											
Number	1	0	2	0	1	1	0	0	4	0	9
Rate	0·15	0·00	0·31	0·00	0·15	0·15	0·00	0·00	0·62	0·00	0·14
Northern and Yorkshire											
Number	0	27	10	4	0	3	5	0	2	2	53
Rate	0·00	4·20	1·55	0·62	0·00	0·46	0·77	0·00	0·31	0·31	0·82
South East											
Number	6	2	1	3	12	10	11	1	2	0	48
Rate	0·73	0·24	0·12	0·36	1·43	1·18	1·29	0·12	0·23	0·00	0·57
South West											
Number	2	7	10	3	19	18	15	4	10	3	91
Rate	0·42	1·48	2·11	0·63	3·96	3·73	3·10	0·82	2·04	0·61	1·89
Trent											
Number	10	17	12	9	9	14	13	20	9	6	119
Rate	1·99	3·38	2·37	1·77	1·77	2·74	2·54	3·90	1·75	1·17	2·34
Wales											
Number	0	2	0	0	0	3	5	3	1	2	16
Rate	0·00	0·69	0·00	0·00	0·00	1·03	1·71	1·02	0·34	0·68	0·55
West Midlands											
Number	1	0	2	1	1	0	0	1	3	2	11
Rate	0·19	0·00	0·38	0·19	0·19	0·00	0·00	0·19	0·56	0·38	0·21
England and Wales											
Number	78	152	85	26	48	56	57	35	39	19	595
Rate	1·53	2·97	1·66	0·51	0·93	1·08	1·10	0·67	0·74	0·36	1·15

* Yearly regional population estimates used except for 1990 (1991 population denominator used).

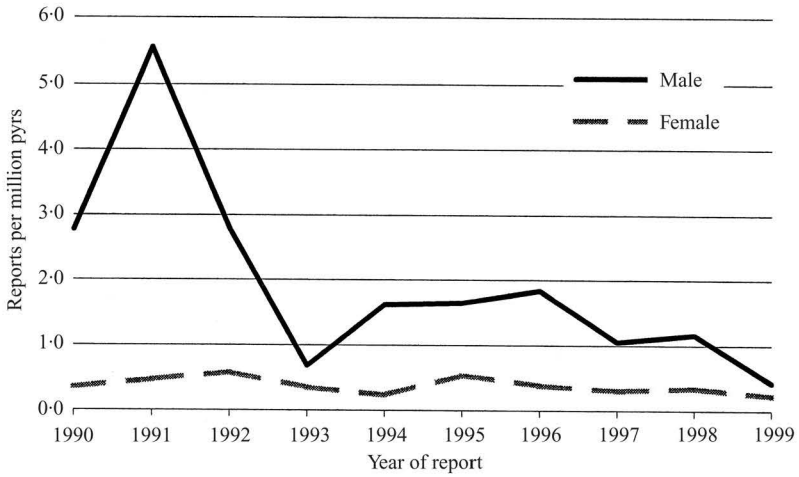

Fig. 9. Annual rates of *Pneumocystis carinii* laboratory reports, by sex (England and Wales: 1990–9).

The age distribution of *P. carinii* reported infections in men and women is shown in Figure 8. Significantly higher rates of *P. carinii* were reported for men than women overall (rate ratio = 4·36, 95% CI 3·47–5·53). Rates of paediatric *P. carinii* were similar in males and females, with the majority of reports being in infants less than 1 year old (2·06 and 2·48 per million population per year, respectively). Reporting rates remained below 0·30 per million per year up to age 14. Adult male and female cases differed markedly, with significantly higher rates in men than women observed in all adult age groups. In 15–44 year olds, rates of 2·39 in men and 0·42 in women per million per year were reported (rate ratio = 5·75, 95% CI 4·18–8·07). The gender discrepancy in *P. carinii* reports was also seen in older age groups, rates in men were 5 times higher than in women in the 45–64 year age range (rate ratio = 5·13, 95% CI 2·98–9·40), and 2 times higher in those aged 65 plus (rate ratio = 2·19, 95% CI 1·12–4·42).

Regional reporting rates of *P. carinii* are shown in Table 6. Annual rates of *P. carinii* showed considerable fluctuation between 1990 and 1999, although reports generally declined in most regions. Cumulative reports received during this period were highest in London (2·86 per million population), with Trent and the South West reporting the highest numbers of infections outside London (2·34 and 1·89 per million population, respectively).

Yearly reporting rates for *P. carinii* for males and females are given in Figure 9. Reporting rates were considerably higher for men than women throughout the period, although reports in males decreased substantially between 1991 and 1993, from 5·56 to 0·67 per million population per year. Reports of *P. carinii* infection in females remained fairly constant over this period.

Other invasive mycoses

A further 121 reports of deep-seated fungal infections, involving 24 different species, were received between 1990 and 1999. The majority of these were fungaemias (77%; 93/121). Other sites from which fungi were isolated were sputum (15), organs, tissue/fluids (5), pulmonary (3), CNS (3) and upper respiratory tract (2). Underlying conditions included leukaemia (10 cases), premature birth (5), HIV infection (5), neutropenia (5), pancytopenia (1), post-surgery/tissue transplant (3) and neoplasms (2). Seven reports of invasive mycoses were in patients receiving total parenteral nutrition.

DISCUSSION

This analysis of laboratory reports of fungal infections has illustrated some important trends in England and Wales, especially with regard to candidosis.

The laboratory reports analysed in this paper are restricted to actual isolations of fungal organisms, which will considerably underestimate the true numbers of invasive mycoses, given the difficulty in isolating the causative organism in many cases of invasive disease. Variations in completeness of reporting between regions will further underestimate the true numbers of isolations made. These limitations primarily affect our ability to estimate the true burden of invasive mycoses but do not entirely prevent any meaningful interpretation of either trends over time or between different subgroups within the population.

Candida species were responsible for the majority of invasive mycoses reported between 1990 and 1999. Rates of candidosis for both males and females were highest in infants. Lack of clinical information accompanying reports prevents full ascertainment of underlying vulnerability in these infants. However, of the candidosis reports with clinical information available, the majority noted use of central venous catheters and prematurity *was* reported in over half of those under 1 year old. Other studies have also documented these risk factors for neonatal candidaemia, along with use of antibiotics, steroids and total parenteral nutrition [19–22]. Central venous catheters have similarly been found to be the source of over 85% of paediatric hospital-acquired bacteraemia [23]. Development of normal flora can be disrupted in neonates admitted to intensive care units, through exposure to different (hospital) flora, the use of antibiotics and methods of feeding, regardless of underlying disease [24]. Colonization by *Candida* species has been shown to be common in low birth weight infants, especially in those delivered vaginally [24], leaving preterm infants susceptible to development of systemic fungal infection [4, 20]. Several outbreaks of invasive *Candida* infection have been reported in neonatal intensive care units, with evidence of transmission facilitated through the hands of health care workers [22, 25].

Markedly higher rates of candidosis were reported in males than females in every year and across most age groups, from 40% higher in infants to 200% higher in those aged 65 plus. Similar male biases in candidosis have been reported in other countries [26]. The bulk of the published literature on the epidemiology of candidosis fails to present sex-specific rates, making our finding difficult to interpret. As the cases presented in this paper were observed through population-based laboratory surveillance, resident population estimates were used as denominators. The majority of these cases are likely to have been acquired in hospital, and as such the higher incidence may reflect either a male bias in the general or a speciality-specific inpatient hospital population at particular risk of opportunistic fungal infection. Hospital Episode Statistics for 1998/99 showed a male bias for many of the common major operations, such as heart bypass (79% male), which could contribute to the higher rates in men in the older age groups [27].

Male sex was associated with a higher likelihood of neonatal candidaemia in a case-control study, matched on birth weight and date of birth, with 80% of cases and 40% of controls being male, although the sample was small and the differences consequently not statistically significant [28]. A small cross-sectional study of candidosis in neonates in intensive care units with normal birth weight (> 2500 g) found 15 of 17 cases to be male, although baseline gender characteristics of neonates in the unit were not given [29]. In this study, nearly all had congenital abnormalities. A further cross-sectional study in India reported 71% of neonatal candidosis cases being male [30], while a cohort study carried out in Finland reported a 30% higher male incidence of paediatric cases of bacteraemia [31]. A cohort study based in neonatal intensive care units failed to find a gender-associated increased risk of candidosis [20], suggesting that the higher rates in male infants observed in this paper could be explained by a higher proportion of male births requiring intensive care. Infant mortality is known to be higher in male than female infants, 6352 deaths per million population for males under 1 compared to 5016 for females in 1998 [32]. Although rare, a number of genetic immunodeficiency disorders affecting cell-mediated immunity are sex linked to the Y chromosome, as shown by mortality rates in infants. Mortality rates due to underlying 'endocrine, nutritional and metabolic diseases and immunity disorders' in infants under one year in England and Wales are 71 *vs.* 48 per million population in males and females respectively [32]. Diabetes, a recognized risk factor for candidosis [6], is also more common in males than females in the United Kingdom [27]. Hyperglycaemia has also been found to be a risk factor for fungal dermatitis in low-birth weight neonates [33]. Investigations using animal models suggest a possible protective effect of oestrogen on fungaemia caused by the dimorphic fungus, *Paracoccidioides brasiliensis* species [34], but there is no evidence that such an explanation could account for the gender bias observed for *Candida* species.

Rates of candidosis reporting varied greatly between regions, being lowest in the Eastern region and highest in the North West. Although this could reflect a genuine difference in burden of disease, it is equally likely to reflect different levels of reporting by different laboratories.

C. albicans was the most common cause of candidosis in all age-groups, although the distribution of non-*albicans* species did vary according to age-group, with *C. parapsilosis* predominating in paediatric cases and *C. glabrata* in adult cases. Similar species-age patterns have been reported in other

countries [7], with some studies reporting a secular shift from *C. albicans* to *C. parapsilosis* as the predominant cause of neonatal candida infection [35, 36].

Marked increases in candidosis reports were seen in the last 10 years, a trend found in other countries in Europe and outside [12, 35]. Rates of candidosis in England and Wales doubled between 1990 and 1999, from 6·76 to 13·70 per million population per year. Rates increased in both males and females, across most age groups and in all regions. The uniformity of the rise would suggest a genuine increase in disease, rather than a reporting artefact. This probably reflects an increase in the pool of susceptible individuals, following the secular trends in more widespread use of treatments inducing immunosuppression, invasive devices facilitating infection and increased survival of vulnerable individuals.

Interpreting laboratory reports of *Aspergillus* species isolated over the past decade presents many problems. Unlike candidosis, aspergillosis is often difficult to diagnose from culture alone, requiring histological examination for proof of infection, which is often not carried out until post-mortem if at all [37]. Laboratory specimens are also particularly prone to contamination with airborne *Aspergillus* conidia. The low sensitivity and specificity of culture for diagnosis of infection by *Aspergillus* species is also likely to result in increasing use of antigen testing as a diagnostic tool rather than attempts at isolating the causative organism [38]. These issues mean that differences in numbers of reports between laboratories or over time are as likely to represent changes in diagnostic techniques utilized and fluctuations in the levels of airborne contaminants as true differences in incidence.

Ascertaining the clinical significance of reports of the laboratory isolation of *Aspergillus* species made over the last decade has also been problematic. Although laboratories were asked to report deep-seated fungal infections only [18], it was clear that superficial infections were reported in great numbers. An attempt was made to differentiate between likely cases of invasive aspergillosis and superficial infections or culture contaminants by applying criteria to *Aspergillus* reports based on specimen site. Isolations from blood culture alone were rejected on the basis that *Aspergillus* fungaemia is rare [10, 39–41], whilst *Aspergillus* species are frequent blood culture contaminants [41, 42]. Insufficient clinical information was provided in most cases to differentiate genuine aspergillus fungaemia from contaminations [42]. Isolations made from sputum specimens were analysed, although not all of these were likely to be indicative of pulmonary aspergillosis [10].

Laboratory reports of aspergillosis observed between 1990 and 1999 rose substantially after 1996 in both men and women, from less than 10 reports per annum to around 50 each for men and women. The rise was restricted to one region, the North West, with over half originating from one laboratory. Further investigations revealed a local change in reporting practice following the introduction of a region-wide automated reporting system around this time.

In both men and women, aspergillosis rates were highest in infants and adults aged 45 plus. A general gender bias in aspergillosis cases was evident, with rates in males outnumbering those in females for most age groups. Due to the small number of cases, rates were only significantly higher in those aged 65 plus. This could be due to a larger male than female population of hospitalised patients vulnerable to opportunistic pathogens, as described for candidosis and also including HIV positive patients, although HIV is very uncommon in those over 65 [43].

P. carinii infections were the second most common invasive mycosis reported by laboratories in England and Wales between 1990 and 1999. Although rates of *P. carinii* infections were relatively high in infants (2 and 2·5 per million per year for males and females respectively), actual numbers of cases were very few (15 altogether). Reported cases of *P. carinii* infection in females were uncommon, totalling 98 throughout the period. Reports of *P. carinii* were four times as common in men than women, probably largely a reflection of pneumocystis pneumonia in HIV-associated immunodeficiency. As for laboratory reports of cryptococcosis, aspergillosis and candidosis, reports of *P. carinii* were more common in men than women in older age groups, five times higher in 45–64 year olds and twice as high in those 65 plus. Other studies of pneumocystis pneumonia in patients with a variety of predisposing factors unrelated to HIV infection, have shown a moderate to large male bias in cases [44–46]. This could indicate that the gender difference seen in our laboratory reports is not entirely due to underlying HIV infection, but could relate to other predisposing conditions in which cellular immunity is impaired [47]. Diagnostic statistics from 1998/9 suggest that more men than women undergo renal, heart, lung and liver transplantation in England [27], operations which necessitate the use of immunosuppressive therapy to prevent organ rejection. Diagnoses of lymphoma, leukaemia

and combined immune deficiency were also more common in men than women, although this was not the case for other known predisposing diseases such as cystic fibrosis and systemic lupus erythematosus. Interestingly, a published case series of pneumocystis pneumonia in patients diagnosed with Wegener's granulomatosis found 9 of 11 cases to be male, despite the similar incidence of Wegener's granulomatosis in males and females (10 of the cases were known to be HIV-negative, 1 remained untested) [48].

Despite the considerable number of *P. carinii* pneumonia cases seen since the advent of the HIV epidemic, there remains a considerable lack of understanding of its natural history. Although pneumocystis pneumonia was long considered to result from reactivation of latent infection in vulnerable individuals, this has been challenged by documented clusters and possible outbreaks of disease [49]. As well as suggesting that recent infections of *P. carinii* can result in pneumocystis pneumonia, this further suggests a common source of infection or person-to-person transmission, something which has never been demonstrated. Given the availability of effective prophylactic treatment against pneumocystis pneumonia, understanding the risk factors for this opportunistic infection will help effective targeting of those at risk.

There is evidence of substantial under-reporting of *P. carinii* by laboratories over the last decade. Data are available on pneumocystis pneumonia from a separate HIV/AIDS surveillance scheme, which records opportunistic infections present at the time of AIDS diagnosis [50]. By June 2000, 3031 diagnoses had been reported to CDSC, for the period 1990–9, in which a definitive diagnosis of *P. carinii* pneumonia had been made for the initial AIDS defining event. Laboratories reported only a fifth of these clinical diagnoses (595) to CDSC. As an unknown number of AIDS patients will have developed pneumocystis pneumonia further into disease progression (i.e. after the initial AIDS defining event), additional clinical diagnoses are likely to have been made in AIDS patients. Although this appears to indicate substantial under-reporting by laboratories, two things should be borne in mind. The definitive pneumocystis pneumonia diagnoses made in these AIDS patients are done through microscopy (histology or cytology) using staining techniques, and not isolations from culture, and as such it would be unclear under current reporting guidelines as to whether they should be included. This is further compounded by *P. carinii* having only been definitively identified as a fungus in recent years and as such it would have been unclear as to which reporting criteria would apply to this organism.

Between 1990 and 1999, 279 laboratory reports of cryptococcosis were made to CDSC. In both males and females, reporting rates were highest in young adults (15–44 years). Rates were significantly higher for males than females across all adult age groups (few paediatric cases were reported), from 4 times higher in 15–44 year-olds rising to 6 times higher in those over 64. The male bias in young adult cryptococcosis cases is likely to reflect the epidemiology of HIV infection in the United Kingdom [43]. Although AIDS cases in the United Kingdom have been concentrated in adults less than 45 [43], sufficient cases are reported in older age groups to explain the male excess of cryptococcosis reports in those aged 45 plus, especially given the small numbers of cryptococcal reports in these age groups.

Other predisposing factors could also contribute to the male excess in older age groups, a case series of cryptococcal disease reported prior to the AIDS epidemic typically described a 2–3 fold male excess of cases [51], as have subsequent studies in HIV-negative individuals [52]. The higher incidence of cryptococcal disease in men than women could relate to higher levels of exposure to environmental reservoirs of cryptococcal yeasts or basidiospores [53], a history of outdoor occupations such as landscaping and building having been associated with increased risk of cryptococcosis [54]. Given that these occupations have traditionally been more common in men than women, reactivation of latent occupationally acquired infection [53] could go some way to explaining the increased incidence in men. Smoking has also been found to increase the risk of cryptococcal infection, independently of occupational risk or male gender [54], a behaviour which has historically been more common in men than women and could exacerbate any existing differences [55]. Another relevant factor could be underlying diabetes mellitus, more common in men than women in the United Kingdom [27], a condition thought to predispose individuals to cryptococcal disease [51], and mycoses in general [6], possibly through associated impairment of cell-mediated immune function and/or the glucose-rich environment. Another possible reason for the higher male incidence of cryptococcal disease is the reported hormonal influence on pathogenesis [52].

An indication of the magnitude of laboratory under-reporting of cryptococcosis comes from the Mycology Reference Laboratories in Leeds and

Bristol on specimens sent for serological detection and cultures sent for identification and/or susceptibility testing. Between 1997 and 1999, cultures and specimens from 135 patients with cryptococcosis were referred to the reference laboratories, only half (70) of these were reported by the source laboratories to CDSC.

There are severe limitations to using this laboratory-based system for surveillance of deep-seated mycoses, arising from sporadic or consistent under-reporting from some laboratories, which hamper any estimates of the burden of infection. For conditions such as aspergillosis, laboratory reports of *Aspergillus* isolation will always greatly underestimate the burden of disease given the difficulty in culturing this species and the reliance on clinical presentation (or post-mortem detection) for diagnosis. This is also possibly the case for *P. carinii* pneumonia, as illustrated by the substantial discrepancy in numbers of clinical diagnoses and laboratory reports received. In the absence of any other national population-based surveillance schemes for invasive mycoses, it is difficult to judge the level of under-reporting or under-estimation from the reporting of infection on CDSC's LabBase.

Although detailed clinical information was often absent from laboratory reports, by analysis of the site from which fungal isolations were made, in conjunction with the known pathogenicity of the particular species, it seems that as many as a third of all reports received between 1990 and 1999 were indicative of superficial or subcutaneous infection. This further illustrates the problems with laboratory reporting of mycoses, given that only deep-seated mycoses should have been reported to CDSC. Coupled with the general under-reporting, it seems unlikely that it is solely resource or motivational constraints which are responsible for the mis/under-reporting, but that there is a lack of clarity as to which fungal infections laboratories should be reporting [56].

Although problems of under-reporting hinder our interpretation of laboratory surveillance data, they do not entirely prevent a cautious analysis of these data. Laboratory reporting of mycoses could provide a reasonably robust means of surveillance for some mycoses in England and Wales, especially for candidosis and cryptococcosis. The value of any laboratory-based surveillance for monitoring *P. carinii* pneumonia and aspergillosis, whose causative organism cannot be readily cultured and are occasionally diagnosed on clinical presentation, or in the case of pneumocystis pneumonia, microscopy only, is likely to remain limited. Clearly hospital acquired infection surveillance schemes will have an important role in monitoring invasive mycoses, but the use of a population-based system could play a useful complementary role in monitoring mycoses occurring in both outpatient and inpatient populations. Laboratory reports to CDSC are generally made shortly after isolation of the organism, making the system particularly valuable in detection of infectious disease outbreaks, as has been the case for mycoses in the United Kingdom [5]. However, without detailed clinical information it will continue to be difficult to interpret the clinical significance of reports to enable colonisation or contamination to be distinguished from infection and disease.

Improvements need to be made to obtain more complete reports from laboratories, and to clarify reporting guidelines such that only invasive mycoses are reported to CDSC. Access to information on underlying disease or immunosuppressive treatments is essential to the interpretation of trends in laboratory reports as it will allow risk groups to be identified. Better completion and expansion of the standardised descriptions available to reporting laboratories to include terms such preterm birth, underlying disease, use of immunosuppressive treatment and whether the report is from an inpatient would greatly help our understanding of laboratory mycoses reports and the epidemiology of mycoses in England and Wales.

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Health hazards posed by feral pigeons

D. Haag-Wackernagel[a,*], H. Moch[b]

[a]*Institute of Anatomy, University of Basel, Basel, Switzerland*
[b]*Institute of Pathology, University of Basel, Basel, Switzerland*

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Summary A comprehensive literature search of epidemiological studies and reports of transmissions of disease from feral pigeons to humans was performed. There were 176 documented transmissions of illness from feral pigeons to humans reported between 1941 and 2003. Feral pigeons harbored 60 different human pathogenic organisms, but only seven were transmitted to humans. Aerosol transmission accounted for 99.4% of incidents. There was a single report of transmission of *Salmonella enterica* serotype Kiambu to humans from feral pigeons, and no reports of transmission of *Campylobacter* spp. The most commonly transmitted pathogens continue to be *Chlamydophila psittaci* and *Cryptococcus neoformans*. Although feral pigeons pose sporadic health risks to humans, the risk is very low, even for humans involved in occupations that bring them into close contact with nesting sites. In sharp contrast, the immunocompromised patient may have a nearly 1000-fold greater risk of acquiring mycotic disease from feral pigeons and their excreta than does the general population.

Introduction

High numbers of feral pigeons live in almost every large city in the world and they are frequently encountered by humans.[1] However, the health hazard that they pose has not been adequately assessed. Clinicians are confronted by the need to educate people about the risk of contracting disease most often when they are clearing feral pigeon excrements or culling them from buildings. However, many people have casual interactions with pigeons that range from feeding them in public parks to handling tamed birds that nest on windowsills. In addition, immunocompromised patients need advice about limiting exposure to potential vectors of zoonotic diseases, even when it concerns seemingly innocuous traditions such as feeding pigeons in the park. Extermination that is intended to greatly reduce pigeons around human populations does not appear to work as well as restricting feeding,[1] are protested by those sensitive to the plight of animals, and most importantly, may pose increased hazards to human health from disturbance of the nesting environment and handling of carcasses. On the other hand, animal protection activists sometimes deny that pigeons pose any health hazard.[2] In spite of the large distribution of feral pigeons and their successful acclimation to humans, there are no reviews of the scientific literature on this topic. This paper aims to give clinicians information that will help them assess the risk to human health from feral pigeons.

*Corresponding author. Address: Pestalozzistrasse 20, CH-4056 Basel, Switzerland. Tel.: +41-61-267-3946; fax: +41-61-267-2791.
E-mail address: daniel.haag@unibas.ch

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Table 1 Pathogenic organisms identified in feral pigeon populations

Viruses
- Western equine encephalomyelitis (WEE)[23]
- Rubella[24,25]
- St Louis Encephalitis[23,26]
- West Nile virus (WNV)[21,22,24]
- Influenza[24]

Bacteria
- *Clostridium perfringens*[12]
- *Listeria monocytogenes*[27]
- *Salmonella enterica*
  - serovar Anatum[28]
  - serovar Anatum var. 15[15]
  - serovar Derby[28]
  - serovar Arizonae[29]
  - serovar 1,4,12:27: g,[m],t:e,n,x[29]
  - serovar Java[30]
  - serogroup E[31]
  - serovar Enteritidis[30]
  - serovar Kiambu[11]
  - serovar Typhimurium[12,16,29–33]
  - serovar Typhimurium Typ 690[25]
  - serovar var. Copenhagen[25,28]
- *Yersinia* spp.[25]
- *Campylobacter jejuni*[13,14,16,17,31,34]
- *Campylobacter coli*[13,17]
- *Escherichia coli* (STEC, VTEC)[35–37]
- *Coxiella burnetti*[24,25,38,39]
- *Chlamydophila psittaci*[8,24,25,30,32–34,38–55]

Fungi
- *Allescheria boydii*[56]
- *Aspergillus* spp.[56,57]
- *Candida albicans*[56,58–63]
- *Candida glabrata*[59,60,63]
- *Torulopsis (Candida) glabrata*[29]
- *Candida guillermondii*[59,60,62,63]
- *Candida humicola*[63]
- *Candida intermedia*[62]
- *Candida krusei*[56,59,60,62,63]
- *Candida lambica*[63]
- *Candida lipolytica*[63]
- *Candida lusitaniae*[60,62]
- *Candida parapsilosis*[62,63]
- *Candida pseudotropicalis*[60,62]
- *Candida rugosa*[60,62,63]
- *Candida tropicalis*[59,60,62,63]
- *Candida zeylanoides*[63]
- *Chrysosporium* spp.[56]
- *Cryptococcus albidus*[62–64]
- *Cryptococcus laurentii*[62,63,65]
- *Cryptococcus neoformans*[29,58,62,63,66–76]
- *Cryptococcus terreus*[62]
- *Cryptococcus uniguttulatus*[65]
- *Debaromyces hansenii*[65]
- *Geotrichum* spp.[59,63]
- *Geotrichum candidum*[56]
- *Histoplasma capsulatum*[77]
- *Hansenula anomala*[63]
- *Kloeckera apiculata*[63]
- *Paeciliomyces* spp.[56]
- *Pichia membranaefaciens*[62]
- *Rhizopus* spp.[56]
- *Rhodotorula* spp.[56]
- *Rhodotorula glutinis*[63]
- *Rhodotorula rubra*[29,62,63]
- *Saccharomyces cerevisiae*[62,63]
- *Saccharomyces oleaginosus*[62]
- *Saccharomyces telluris*[59,60,62]
- *Scopulariopsis* spp.[56]
- *Streptomyces* spp.[56]
- *Torulopsis candida*[62,63]
- *Trichosporon beigelii*[63]
- *Trichosporon capitatum*[63]
- *Trichosporon cutaneum*[56,62,63]
- *Trichosporon pullulans*[63]

Protozoas
- *Toxoplasma gondii*[30,78,79]

Total 60 pathogens as designated by genus and species.

Methods

The review of older literature, which comprises sources not readily accessible to clinicians by standard scientific publication search engines, was accomplished by perusal of bibliographic references in Italian, French, German, Dutch and English publications (Tables 1 and 2). More recently cited sources were collected by searches with key words like 'pigeon', 'dove' and '*Columba livia*' in the medical database PubMed. Only those papers that showed confirmed, or in the case of one large outbreak presumptive diagnosis of disease in humans,[3] are included in this review (Tables 1 and 2). All published reports of transmissions of pathogenic organisms from feral pigeons to humans were included for analysis and tabulation. For the review of food-related infections, the commodity term was used in conjunction with the terms 'food and outbreak and human' (Table 3). Commodities chosen for comparison ranged from extremely common sources, such as milk to infrequently eaten specialty foods such as snake. For this evaluation, each publication was not perused individually for applicability to food related illness in humans, and thus, it only indicates relative frequency of reports of outbreaks for each commodity.

Results

Epidemiological investigations

Seventy-seven epidemiological studies of feral pigeon populations identified human pathogenic organisms coming from 60 cities and regions. Analysis revealed that feral pigeons harbored a

Table 2 Association between human practice and illness contracted from feral pigeons

Pathogen	Number of illnesses[a]	Description of contact
Salmonella enterica	1	Environmental exposure[11]
Chlamydophila psittaci	29[b]	Environmental exposure[41,42,44,45,80–82]
	10	Handling a sick or dead pigeon[40,47,82–84]
	8	Pigeon feeding[42,82,83,85,86]
Histoplasma capsulatum	23	Environmental exposure[3]
	68	Exposure in a hospital setting[77]
Aspergillus spp.	13 (13)	Exposure in a hospital setting[87–90]
Candida parapsilosis	12 (12)	Exposure in a hospital setting[91]
Cryptococcus neoformans	5 (2)	Environmental exposure[67,92–95]
	5 (4)	Exposure in a hospital setting[75,96]
	1 (1)	Wound inflicted by a pigeon[97]
Toxoplasma	1	Environmental exposure[78]
Total cases	176	

[a] Number of patients who were immunoincompetent.
[b] Additional cases are reported, but not enumerated[39,98] (Haag-Wackernagel, unpublished).

total of 60 different human pathogenic organisms (Table 1). Five pathogens were viruses, nine were bacteria, 45 were fungi, and one was a protozoan (Table 1). However, only five pathogens were routinely transmitted to humans (Table 2). There were single case incidences for transmission of *Salmonella enterica* and *Toxoplasma* (Table 2). In general, there was a notable lack of transmission of pathogenic viruses and bacteria to people from feral pigeons.

Confirmation of the link between pigeons and fungal disease

There is a long-recognized link between pigeons and fungal diseases in humans, and, indeed, almost all (17 of 18) investigations of feral pigeon populations were positive for *Cryptococcus* spp. Review of the literature indicated that *Cryptococcus neoformans* and *Chlamydophila psittaci* were the most widespread zoonotic pathogens in feral pigeon populations (Table 2). *C. neoformans* caused diseases in immunocompromised as well as in immunocompetent patients,[4] but primarily in immunocompromised patients (Table 2). *C. neoformans* was acquired by 11 patients of whom seven were immunocompromised (Table 2). The Centers for Disease Control (CDC) reports that in the United States *Cryptococcus* spp. result in 0.2–0.9 cases per 100,000 in the general population per year.[5] However, the CDC also reports that AIDS patients have a 1000-fold higher risk, with an annual reported incidence of 2–4 cases per 1000 persons.[5] The pigeon is not an amplifying host of *Cryptococcus*, but its excrements offer an ideal environment for its growth.[6] Therefore, it appears that the risk of illness associated with pigeons from *Cryptococcus* is now largely a function of the immune status of the human population rather than from people just having contact with birds. This is not a new concept,[7] but the emergence of AIDS over the past 30 years and the associated increase in mycotic disease reiterates that a change in the human population rather than carriage rates by feral pigeons has occurred overtime. Similar to the situation with *Cryptococcus, Aspergillus* and *Candida* infections also appear to implicate a decline in human immune function as the primary risk factor for contraction of illness from feral pigeons (Table 2).

Chlamydia and *Histoplasma* infections in humans did not necessarily show an association with immunocompromise of the host (Table 2), although it cannot be ruled out without full knowledge of each individual case. An outbreak of *Chlamydophila psittaci* infection involved at least 35 persons and it was due to human contact with feral pigeons that

Table 3 PubMed references returned for outbreaks per food commodity

Milk	168	Goat	18
Egg	112	Juice	18
Fish	96	Hamburger	16
Vegetable	96	Horse	14
Shellfish	92	Rabbit	11
Fruit	79	Wild boar	10
Chicken	79	Deer	6
Cheese	73	Duck	5
Beef	66	Pigeon	0
Dairy	64	Alligator	0
Pork	48	Snake	0
Turkey	37	Ostrich	0

Searched on October 22, 2003 at www.ncbi.nlm.nih.gov/entrez/query.fcgi. Search terms were 'commodity and food and outbreak and human'.

varied in intensity (Tables 1 and 2). According to the literature, *C. psittaci* has a worldwide average seroprevalence of 45.8% as determined by review of 33 epidemiological investigations. An earlier 1965 review suggested a seroprevalence of 28.7% in feral pigeon populations in different cities and regions.[8] In preparation for this review, sampling of feral pigeons in Lucerne revealed a mean prevalence of 56% seropositive feral pigeons (H. Wackernagel, unpublished results). All investigations of feral pigeon populations have been seropositive for *C. psittaci* (Table 1), which suggests that this organism is commensally associated with the pigeon host. However, there was no evidence that a high prevalence rate in pigeons is necessarily associated with a high probability of infections in humans that encounter pigeons. Two reports describe infections in humans with *Histoplasma capsulatum* (Table 1), which was the pathogen associated with the highest number of cases in a single outbreak. Because all of the fungal pathogens, including *Cryptococcus*, *Chlamydophila*, and *Histoplasma*, are known to be transmitted by inhalation of aerosolized organisms, the data suggests that 175 of 176 (99.4%) of described transmissions resulted from airborne excreta, which includes dried feces, ocular discharges, and crop milk.[9] However, other methods of transmission are possible if close contact occurs, as evidenced by one report of primary cutaneous cryptococcosis of a HIV-positive drug abuser following injury inflicted by a feral pigeon (Table 1). Contact is sometimes brief, and the patient does not always recall any encounter with birds.[9]

Pigeons are not reported to be a common source of food-borne illness

Only two reports describe transmission of *Salmonella enterica* (*S. enterica*) to humans from either domesticated or feral pigeons, one of which occurred outside the general time frame of 60 years for this review. Illness in 20 soldiers in the Dutch army occurred in 1933 that clearly implicated *S. enterica* serovar Typhimurium variant Copenhagen as the causative agent that contaminated lemon pudding made with eggs from domestic pigeons.[10] It appears to be the only case of food-borne transmission from pigeons to humans in the literature. Feral pigeons resulted in a case of salmonellosis from *S. enterica* serovar Kiambu[11] (Table 2). Given that *S. enterica* serovar Typhimurium variant Copenhagen and *Campylobacter*, which are common food-borne pathogens, are frequently recovered from feral pigeons as well as from domesticated pigeons used for food,[12–17] it is surprising that there are no other reports of food-borne transmission to humans from pigeons in the literature.

To pursue the idea further that there is a low risk of transmission of common food-borne bacteria between pigeons and humans, an independent risk assessment was performed. A feral pigeon population of around 5000–8000 birds that was sampled in Basel, Switzerland in preparation for this review harbored *C. psittaci*, *Campylobacter* spp., *S. enterica* serovar Typhimurium variant Copenhagen, and *Aspergillus fumigatus* (Haag-Wackernagel, unpublished data). However, records from the Department of Internal Medicine of the University Hospital in Basel showed that there were no direct transmissions of disease from feral pigeons to humans between 1997 and 2003 (Flueckiger and Haag-Wackernagel, unpublished data). In addition, a search of the literature revealed that, other than the 1933 outbreak, there were no reports of food-borne illness from either domesticated or feral pigeons since 1933 (Table 3). This finding is unexpected given that contact between pigeon and human is a frequent occurrence as compared to what probably happens with wild boar, snake, ostrich and alligator (Table 3). Illness was noted to occur in the literature base for rabbit, which is another feral animal that is sometimes domesticated and bred for food and kept as a pet. Therefore, it appears that the risk of transmission of food-borne disease from pigeon to human is comparatively low, in spite of pigeons being frequent carriers of common food-borne pathogens.

Other suggested associations between pathogens and feral pigeons

One case of *Toxoplama gondii* is reported (Table 1), which occurred in an elderly woman. Therefore, all immunocompromised patients, including pregnant women who are at higher risk for contracting toxoplasmosis, should be advised to avoid feral pigeon populations and their nesting sites. Currently, there is no experimental evidence that pigeons are an important vector for the recently emerged viral disease of human pathogenic avian influenza[18] and the epidemiological association between feral pigeons and humans in regards to this disease is only speculative.[19] Although it appears that feral pigeons are seropositive for West Nile virus, there is no epidemiological evidence that they are an amplifying vector as are crows.[20–22]

Discussion

Epidemiological screening programs of animal reservoirs often identify pathogens that have never been, or are seldom, transmitted to humans. In regards to zoonotic disease transmitted from the feral pigeon to humans, all viruses, *Yersinia*, *Salmonella*, *Campylobacter* and *Toxoplasma* are in this category. Therefore, the results of many of the epidemiological investigation herein reviewed were only relevant for assessing the health of the feral pigeon populations rather than for assessing their hazard to human health. Thus, the mere isolation of a pathogen from pigeons does not justify their extermination, although people should be educated about the risk factors for acquiring mycotic disease. The low number of 176 reported transmissions in the literature that spans approximately 60 years underestimates risk, because many cases are not published or the sources of the infections are not recognized. However, in spite of the worldwide distribution of feral pigeons, the close and frequent contact they have with humans, their use as food, and the high prevalence of carriage of human pathogens, zoonotic disease caused by feral pigeons is infrequent. Healthy people who are exposed to feral pigeons and their nesting sites, or who have increased exposure from hobbies or occupations involving feral and domesticated pigeons, should be informed of these risks and advised of appropriate ways to protect themselves. These methods include wearing coveralls, face masks, other respiratory protection, washing hands and by disinfection against excreta dust with antiseptics. If possible, sick pigeons should be avoided, or at the very least, handled with great care to prevent exposure to excreta. The literature reiterates that the immunocompromised patient is at considerably higher risk for contracting opportunistic disease from feral pigeon populations. Since opportunistic pathogens have a multitude of vectors besides feral pigeons, disease prevention is probably best attained by educating the immunocompromised patient to limit contact with all avian sources and to follow guidelines for maintaining hygiene under any circumstance where close contact with pathogenic organisms might occur.

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Evolution of Cryptococcal Antigen Testing: What is new?

Elizabeth Nalintya, MBChB[1], **Reuben Kiggundu, MBChB**[1], and **David Meya, MBChB, MMed**[1,2,3]

[1] Infectious Diseases Institute, Mulago Hill Road, Makerere University, Uganda. [2]Department of Medicine, Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, USA. [3] School of Medicine, College of Health Sciences, Makerere University

Abstract

Over the last decade, an upsurge in both the frequency and severity of fungal infections due to the HIV/AIDS epidemic and the use of immunosuppressive therapy has occurred. Even diagnostic methods like culture and microscopy, which have low sensitivity and longer turn-around-times are not widely available, leading to delays in timely antifungal therapy and detrimental patient outcomes. The evolution of cryptococcal antigen (CrAg) testing to develop inexpensive and more sensitive methods to detect cryptococcal antigen is significant. These newer tests employ immunoassays as part of point-of-care platforms, which do not require complex laboratory infrastructure and they have the potential to detect early disease and reduce time to diagnosis of cryptococcal infection. Advocacy for widely available and efficacious life-saving antifungal treatment should be the only remaining challenge.

Keywords

Cryptococcus; antigen; testing; lateral flow; assay; HIV; diagnosis; thermal contrast; Fungus; cryptococcal disease; cryptococcal meningitis

Introduction

Infection with the human immunodeficiency virus (HIV) and increased use of immunosuppressive therapy have led to an increase in both the frequency and severity of fungal infections [1]•. *Cryptococcus neoformans* and *Cryptococcus gatti* are responsible for

Corresponding author Tel: +256 312 307 224 Fax: +256 312 414 289 david.meya@gmail.com.
Elizabeth Nalintya, Infectious Diseases Institute, College of Health Sciences Makerere University, Mulago Hill Road, # 22418,Kampala, Uganda. Tel: +256 312 307 224 / Fax: +256 312 414 289 enalintya@idi.co.ug
Reuben Kiggundu, Infectious Diseases Institute, College of Health Sciences Makerere University, Mulago Hill Road, # 22418, Tel: +256 312 307 224 Fax: +256 312 414 289 reubenkaaja@yahoo.com
David Meya, Infectious Diseases Institute, College of Health Sciences, Makerere University, Mulago Hill Road, # 22418, Kampala, Uganda.

Compliance with Ethics Guidelines

Conflict of Interest

Elizabeth Nalintya, Reuben Kiggundu, and David Meya declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

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an estimated 700,000 cases of cryptococcal meningitis in sub Saharan Africa [2] and 7,800 cases of cryptococcal meningitis in North America in 2006 respectively [3]. Mortality from cryptococcal disease remains high even in the era of ART. Early diagnosis of cryptococcal infection is critical to improving clinical outcomes.

A notable increase in the prevalence of cryptococcal meningitis in the last decade has not been matched by improved diagnostics in resource limited settings where most of the infections occur. The low sensitivity of older test methods and delay in obtaining results has driven research for cheaper and more widely available sensitive diagnostics.

Traditional approaches to diagnosis include direct microscopic examination of clinical samples, histopathology, culture, and serology [1]•. However, new innovative technologies that use molecular and immunoassay point-of-care platforms have the potential to meet the needs of both resource-rich and resource-limited clinical environments [4]•. In this review, we describe the evolution of diagnostic techniques for cryptococcal infection focusing on CrAg testing and outline the current need and gaps in the area of cryptococcal infection testing.

Epidemiology of Cryptococcal meningitis

Cryptococcal meningitis (CM) is a severe life threatening illness caused by cryptococcus species, which mainly occurs in immune compromised individuals and rarely in immunocompetent persons and rarely in immunocompetent persons [5-7].

Cryptococcus is free living encapsulated saprophytic yeast. Human infections are caused by *Cryptococcus neoformans* and *cryptococcus gattii*. The epidemiology, clinical and molecular characteristics of these two species vary. *C. neoformans* is classified into *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D). Also identified are serotypes B and AD.[1]• *Cryptococcus neoformans* var. *grubii* causes most infections among HIV-infected persons [8, 9]. Ecologically, *C. neoformans* is found in soil contaminated with bird droppings, heart wood and homes of HIV-infected persons [10, 11]. *C. gattii* is classified into serotypes B and C. Previously known to be found predominantly in tropical and subtropical regions, outbreaks in Vancouver island, Canada and US Pacific North West have complicated our understanding of its ecological niche [12-14]. *C. gatti* infection predominantly occurs in immunocompetent patients [15].

The global burden of cryptococcal meningitis remains high despite advances in diagnosis and treatment. It is an AIDS defining illness, mainly occurring in patients with CD4 < 100 cells/μL [16, 17]. It is estimated that the mortality occasioned by CM among HIV infected patients is 50-70% [3]. The disease burden of CM parallels the HIV epidemic, with highest incidence and mortality in sub-Saharan Africa, South and Southeast Asia regions with high HIV prevalence and low access to ART [7, 18]. Annually, sub-Saharan Africa, and South and Southeast Asia account for 720,000 and 120,000 cases of CM respectively [3]. In addition to being the leading cause of meningitis in the sub-Saharan Africa region [7, 19-21], it accounts for 13-44% of all AIDS related deaths [22, 23], with mortality rates as high as 50-70% [3, 7, 19]. *C. neoformans* occurred in 5-15% of AIDS patients during the

peak of the HIV epidemic in Europe, United States and Australia, but the incidence of CM in these regions has declined partly due to access to HAART and antifungal use [15, 24-27].

Although HIV is the major risk factor for cryptococcal meningitis, immunosuppressive therapy, sarcoidosis and lymphoproliferative disorders are also associated with increased risk of developing cryptococcal meningitis [28].

Evolution of Cryptococcal Testing

The diagnosis of fungal infections in the past has relied primarily on techniques based on visualisation of the fungus, for example by direct microscopic examination of clinical samples, histopathology, and culture [4]•. These approaches require personnel with relatively high levels of specific mycology training [4]• and hence have a limitation for widespread use in resource limited settings. The increase in pathogenic fungi in the past decade has forced investigators to develop and apply new methods of fungal identification that go beyond classical phenotypic methods [4]•. As a consequence, there is increased emphasis on the use of molecular methods and antigen detection as surrogates for culture for the diagnosis of cryptococcal meningitis. Further still, old testing techniques lacked sensitivity and specificity and take too long to be clinically useful [1]•. We briefly describe the evolution of cryptococcal diagnostic techniques over time.

Direct microscopic examination of Clinical samples, Histopathology, and Culture

Culture has been the gold standard for diagnosis of cryptococcal disease and has characteristic advantages such as growing the specific organism and allowing for sensitivity testing in order to identify the most suitable therapy, however, the yield for most specimens is low and will usually be positive when the fungal burden is high. The turn-around-time using conventional culture media (Sabouraud Dextrose Agar and Mycosel agar; BD Diagnostic Systems) is usually more than 7 days, but could be positive in a few days among patients with high fungal burden and requires laboratory personnel with the requisite expertise [4]•. Fungal culture has evolved from conventional growth media to the use of birdseed (*Guizotia abyssinica*) agar for detection and rapid identification of *C. neoformans* [29]. This media has decreased the time to detection of most strains of *C. neoformans* from about several days to 72 hours - the time it takes for phenoloxidase activity to produce dark brown colored colonies. In a study comparing conventional media and birdseed agar by culture of 35 clinical samples from AIDS patients, the results showed 100% sensitivity and specificity with plates incubated at 30°C [4, 29]•. TOC (tween 80-oxgall-caffeic acid) agar has been used for identification of *C. neoformans* within 24 hours from previously isolated colonies [30]. However, it requires extended incubation of 3–5 days if used as the primary isolation medium. Detection of urease production for rapid recognition of *C. neoformans* [31] has also been attempted, but this method lacks specificity and needs to be followed by a more reliable method.

Microscopy is another fundamental technique whose sensitivity is dependent on the quality of the specimen and the experience of the laboratory personnel. Stains like India ink are used to stain specimens to ease visualisation. India ink staining, however, has limitations as described in a study comparing diagnostic techniques in Uganda. Sensitivity of India ink

microscopy was the lowest (86%) of any test and was highly dependent on fungal burden in CSF[4]. Sensitivity decreased to 42% (19/45) among persons with cerebrospinal fluid (CSF) cultures <1,000 colony forming units (CFU)/mL. Overall, 1 of 7.2 cryptococcal diagnoses was missed by India ink microscopy (negative predictive value of 80%; (95% CI 76%–84%). If India ink microscopy had been the only diagnostic test used, 8.8% of meningitis cases in Uganda would have been misdiagnosed. Among persons in Uganda who had India ink microscopy–negative results, *Cryptococcus* spp. remained the most common pathogen (20%). [32]••. India ink staining is also not suitable for diagnosis of invasive Cryptococcal disease, as this would require deep tissue biopsies.

Histopathology requires several stains enabling a more obvious appearance of *C. neoformans*. Classical stains used in histopathology include Gomori methenamine silver, periodic acid-Schiff, Gridley fungus, and hematoxylin and eosin stains [33]. Alternatively, Calcofluor white (CW) can be used with a fluorescent microscope to observe fungal elements in clinical samples. CW binds b -glycosidic linkages of polysaccharides in the fungal cell wall but also binds non-specifically to keratin and human connective tissue elements [34].

Antigen detection tests

These are tests that detect fungal antigen (*Cryptococcus neoformans and gatti*).

Latex agglutination—Diagnosis of CM was the first application of antigen detection for diagnosis of fungal infection that received widespread clinical use [35]. Antibodies were raised in rabbits against whole cryptococcal cells and passively coated onto latex beads. Termed latex agglutination, the assay detected glucuronoxylomannan (GXM), the major capsular polysaccharide of *C. neoformans*. GXM is shed in large amounts into blood and CSF during the course of cryptococcal meningitis. GXM occurs in four major serotypes: A, B, C, and D and a hybrid serotype AD [1]•. Studies done in 2012 to validate this test against newer test are summarised in Table 1 [32]••.

Most manufacturers (e.g., IMMY, Meridian Biosciences Inc., and Bio-Rad) propose and recommend use of pronase to reduce false-positive results caused by the presence of rheumatoid factors in the specimen especially in serum [4]•.

Lateral flow assay

A ground breaking landmark for cryptococcal antigen testing was the development of a lateral flow immunoassay (dipstick) (CrAg LFA). It was developed using a cocktail of monoclonal antibodies that were formulated to be reactive with all GXM serotypes [1, 37]•. This dipstick test uses gold-conjugated, monoclonal antibodies impregnated onto an immunochromatographic test strip to detect cryptococcal capsular polysaccharide glucuronoxylomannan antigen for all 4 *C. neoformans* serotypes (A–D)[37]. If cryptococcal antigen is present in a specimen, suspended, gold-conjugated antibodies bind to the antigen. The gold-antibody- CrAg complex migrates by capillary action up the test strip, interacts with immobilized monoclonal antibodies against the antigen and forms a band. The LFA kit contains immunochromatographic test strips, positive controls, and assay diluent that can be

stored at room temperature for ≤2 years. To perform the LFA, 1 drop of diluent (≈40 μL) is added to a container with 40 μL of patient specimen. The dipstick is inserted into the container and incubated at room temperature for 10 min [32]••.

In a review article evaluating the LFA, seven conference abstracts and two full-length published articles through August 2012 were reviewed. Six abstracts and the two full-length articles reported data on serum specimens and five abstracts included data on CSF specimens. The median sensitivity using serum was 100% (95.6%, 100%) and the median specificity was 99.5% (95.7%, 100%). Using CSF specimens, the median sensitivity was 100% (96.2%, 100%) and the median specificity was 97.7% (70.4%, 100%) [4]•. In another large scale evaluation of the lateral flow assay method, 1,000 specimens (589 serum and 411 CSF specimens) were tested in parallel at the ARUP laboratories a national reference laboratory under the pathology department of university of Utah. Comparison of Meridian EIA vs IMMY LFA showed 97.8% agreement (positive agreement 71.8, negative agreement 97.7%), kappa 0.82(0.75-0.9). In conclusion, the IMMY assays showed excellent overall concordance with the Meridian EIA. Assay performance differences appear to be related to issues of analytic sensitivity and serotype bias [38]••. Serotype sensitivity of the LFA has previously been assessed and the CrAg LFA found to have high sensitivity for GXM of all four serotypes, with A = B > C > D. The observed sensitivity of the CrAg LFA was greater than was previously reported for currently available CrAg immunoassays in latex agglutination or enzyme immunoassay formats [37].

Another study comparing four assays assessed detection of cryptococcal antigen in serum (n=634) and CSF (n=51). When compared to latex agglutination, the sensitivity and specificity of the Premier EIA, Alpha CrAg EIA and CrAg LFA were 55.6/100, 100/99.7 and 100/99.8%, respectively, from serum samples. There was 100% agreement among the four tests for CSF, with 18 samples testing positive by each of the assays [39]•.

The LFA is a semi quantitative test that can be used to measure disease burden by determining the CrAg titers for positive results. These have been found to be informative for patients with asymptomatic antigenemia and for patients with high titers the risk of cryptococcal meningitis and death is higher than those with lower titers as observed in a recently concluded CrAg screening study in Uganda. Contrary to our earlier understanding regarding the lack of a role for CrAg titers in treatment and risk stratification for symptomatic CM, among asymptomatic CrAg positive patients titers might have a role in improving patient clinical management and hence patient outcome. Further assessment of this can be done with thermal contrast measurement as described below.

Thermal Contrast Measurement of CrAg Titer

This laser thermal contrast method was suggested in 2012 and in a study done in Uganda it was used to provide quantification of the LFA in comparison with semi quantitative CrAg LFA titers by using the heat signature of laser-irradiated gold used in the LFA. To detect gold nanoparticles conjugated to monoclonal antibodies on the LFA line, the line was irradiated with a 0.01 W laser (532 nm, diode pumped; Millenia, Santa Clara, CA, USA) for 30 seconds, and temperature change (thermal contrast) was recorded with an infrared camera (A20; FLIR ThermoVision, Portland, OR, USA), as described [40]•. Three spots on each

horizontal LFA line were irradiated and the average maximum temperature change was calculated. An antigen titer was calculated from the thermal contrast by using a calibration curve established by 2-fold serial dilutions of 3 specimens in triplicate with known CrAg LFA titers ($R^2 = 0.97$). This study demonstrated that a novel technique, laser thermal contrast, had 92% accuracy in quantifying CrAg titers from 1 LFA strip to within <1.5 dilutions of the actual CrAg titer by serial dilutions ($R = 0.91$, $p<0.001$). LFA performance was more sensitive than that of any other diagnostic test. Conversely, the worst performing test was India ink microscopy, which is the most common cryptococcal diagnostic test in Africa, despite missing 1 in 7 cryptococcal diagnoses and having only an 80% negative predictive value in our cohorts [32]••.

Implications for the Future

Testing for cryptococcal disease has evolved over the last few years with particular improvements in CrAg testing. The development of the lateral flow assay could revolutionize diagnosis and management of cryptococcal disease. The test offers the ability to perform cryptococcal antigen testing at point-of-care, without the additional requirement of complex laboratory infrastructure especially in sub-Saharan Africa, where cryptococcal disease is prevalent.

The persistence of cryptococcal antigen remains an issue especially among patients who present with recurrence of symptoms and signs of meningitis (having had a prior episode of cryptococcal meningitis) and are antiretroviral therapy-experienced. In these cases, the clinician should be guided by use of CSF culture to differentiate between relapse (or new infection) and paradoxical cryptococcal immune reconstitution inflammatory syndrome (IRIS), with sterile cultures during IRIS, despite a positive CrAg test.

The experimental methods for cryptococcal antigen testing including thermal contrast could be developed further into cheaper bedside tests that could increase the repertoire of cryptococcal diagnostics in the future.

Finally, the diagnosis of early disease has become an important aspect of cryptococcal antigen testing with high sensitivity of the lateral flow assay providing the capability to detect lower quantities of antigen. An ongoing study on cryptococcal antigen testing among asymptomatic patients with CD4 counts <100 cells/μL in Uganda suggest that patients with higher CrAg titers >1:160 have a higher risk of death [41]. This is important as it presents an opportunity to study tailored therapy if one can determine the antigen titer at the time of CrAg testing. Studies conducted in South Africa have shown that CrAg screening of individuals initiating ART and preemptive fluconazole treatment of CrAg-positive patients resulted in markedly fewer cases of CM compared with historic unscreened cohorts. It has also been found to be a cost effective intervention and several modifications of dose and duration of the recommended Fluconazole therapy has led to improved survival [42-47].

For industry, the future should focus on developing a modified lateral flow assay that can further provide a semi quantitative CrAg titer which could be a separate band, for example >1: 160 that could be read off the test strip at the same time the LFA is being read for qualitative positive results. This would enable clinicians to study different treatment

regimens especially for asymptomatic patients who could benefit from pre-emptive antifungal therapy.

Conclusion

In keeping with new developments in the diagnosis of infectious diseases, the development of the lateral flow assay as a point-of-care test for detecting cryptococcal antigen is a huge leap forward and introduces a new paradigm in the management of cryptococcal disease. The ability to screen for cryptococcal antigen, especially prior to initiating antiretroviral therapy among HIV-infected patients and pre-emptively treating those with ‘early’ cryptococcal disease using antifungal therapy to prevent overt and symptomatic cryptococcal disease will save health care costs especially for resource poor countries by eliminating the need for complex laboratory infrastructure. The focus should now be on making this test more widely available, implementing national CrAg screening programs and advocating for more efficacious antifungal treatment regimens that would minimize mortality and morbidity occasioned by HIV infection and cryptococcal co-infections.

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•• Of major importance

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Table 1

Performance characteristics of cryptococcal diagnostic assays in persons with suspected meningitis, Uganda and South Africa*

		Number positive/Number tested (%)			
Diagnostic test	**N**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
CSF culture	806	459/510 (90.0)	296/296 (100.0)	459/459 (100.0)	296/347 (85.3)
100-µL volume	524	309/328 (94.2)	196/196 (100.0)	309/309 (100.0)	196/215 (91.2)
10-µL volume	282	150/182 (82.4)	100/100 (100.0)	150/150 (100.0)	100/132 (75.8)
India ink microscopy	805	438/509 (86.1)	288/296 (97.3)	438/446 (98.2)	288/359 (80.2)
CrAg LFA	666	435/438 (99.3)	226/228 (99.1)	435/437 (99.5)	226/229 (98.7)
CrAg latex (Meridian)	279	176/180 (97.8)	85/99 (85.9)	176/190 (92.6)	85/89 (95.5)
CrAg latex (Immy)	749	452/466 (97.0)	283/283 (100.0)	452/452 (100.0)	283/297 (95.3)

PPV- Positive predictive value; NPV- Negative predictive value; CrAg- Cryptococcal antigen; LFA- lateral flow assay; CSF- cerebrospinal fluid

Global guideline for the diagnosis and management of cryptococcosis: an initiative of the ECMM and ISHAM in cooperation with the ASM

Christina C Chang, Thomas S Harrison, Tihana A Bicanic, Methee Chayakulkeeree, Tania C Sorrell, Adilia Warris, Ferry Hagen, Andrej Spec, Rita Oladele, Nelesh P Govender, Sharon C Chen, Christopher H Mody, Andreas H Groll, Yee-Chun Chen, Michail S Lionakis, Alexandre Alanio, Elizabeth Castañeda, Jairo Lizarazo, José E Vidal, Takahiro Takazono, Martin Hoenigl, Jan-Willem Alffenaar, Jean-Pierre Gangneux, Rajeev Soman, Li-Ping Zhu, Alexandro Bonifaz, Joseph N Jarvis, Jeremy N Day, Nikolai Klimko, Jon Salmanton-García, Grégory Jouvion, David B Meya, David Lawrence, Sebastian Rahn, Felix Bongomin, Brendan J McMullan, Rosanne Sprute, Tinashe K Nyazika, Justin Beardsley, Fabianne Carlesse, Christopher H Heath, Olusola O Ayanlowo, Olga M Mashedi, Flavio Queiroz-Telles Filho, Mina C Hosseinipour, Atul K Patel, Elvis Temfack, Nina Singh, Oliver A Cornely, David R Boulware, Olivier Lortholary, Peter G Pappas, John R Perfect

Cryptococcosis is a major worldwide disseminated invasive fungal infection. Cryptococcosis, particularly in its most lethal manifestation of cryptococcal meningitis, accounts for substantial mortality and morbidity. The breadth of the clinical cryptococcosis syndromes, the different patient types at-risk and affected, and the vastly disparate resource settings where clinicians practice pose a complex array of challenges. Expert contributors from diverse regions of the world have collated data, reviewed the evidence, and provided insightful guideline recommendations for health practitioners across the globe. This guideline offers updated practical guidance and implementable recommendations on the clinical approaches, screening, diagnosis, management, and follow-up care of a patient with cryptococcosis and serves as a comprehensive synthesis of current evidence on cryptococcosis. This Review seeks to facilitate optimal clinical decision making on cryptococcosis and addresses the myriad of clinical complications by incorporating data from historical and contemporary clinical trials. This guideline is grounded on a set of core management principles, while acknowledging the practical challenges of antifungal access and resource limitations faced by many clinicians and patients. More than 70 societies internationally have endorsed the content, structure, evidence, recommendation, and pragmatic wisdom of this global cryptococcosis guideline to inform clinicians about the past, present, and future of care for a patient with cryptococcosis.

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Department of Infectious Diseases, Alfred Hospital, Melbourne, VIC, Australia (C C Chang PhD)**; Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, VIC, Australia** (C C Chang)**; Centre for the AIDS Programme of Research in South Africa, Durban, South Africa**

Introduction

Cryptococcosis accounts for substantial morbidity and mortality globally. In 2022, WHO listed *Cryptococcus neoformans* as a top fungal priority pathogen.[1] Cryptococcosis often involves the CNS or the lungs, but disseminated disease can affect any organ, yet appear localised. Despite the knowledge gained and improvements in clinical outcomes generated by multiple interventional trials[2–7] done primarily in low-income settings with insufficient resources, mortality from cryptococcal meningoencephalitis is high, ranging from 24 to 47% at 10 weeks.[2,4,7,8] The highest burden of disease is in low-income and middle-income countries, especially in sub-Saharan Africa,[9] where HIV and AIDS are the dominant risk factor, although new non-HIV immunocompromised risk groups and putatively immunocompetent individuals are increasingly reported in high-income settings with sufficient resources.

Complementary diagnostic and management guidelines for cryptococcosis exist.[10–21] This comprehensive management guideline serves primarily to facilitate clinical decision making while also providing an overview of the uncertainties in cryptococcosis management. With contributors across the globe, this guideline gives voice to expertise and challenges from diverse settings in a globally relevant Review. General principles and treatment recommendations are provided, and clinicians are urged to use careful clinical judgement when formulating

Key points

- Accurate delineation of the cryptococcosis clinical syndrome is important as it guides antifungal treatment choice and duration; cryptococcosis syndromes are divided into CNS, disseminated disease, isolated pulmonary disease, or direct skin inoculation (figure 1)
- Liposomal amphotericin B 3–4 mg/kg daily and flucytosine 25 mg/kg four times a day is the most optimal induction therapy option for cryptococcal meningitis, disseminated cryptococcosis, and severe isolated pulmonary cryptococcosis in high-income settings
- In low-income settings, patients with HIV-associated cryptococcal meningitis are best treated with liposomal amphotericin B 10 mg/kg as a single-dose, with 14 days of flucytosine 25 mg/kg four times a day and fluconazole 1200 mg daily as induction therapy; this induction therapy has not been trialled in non-HIV-associated cryptococcal meningitis or other non-CNS cryptococcosis syndromes
- Optimise outcomes by providing the most effective antifungal therapy while preventing, monitoring, and managing potential toxicity; do not stop or switch to an inferior regimen too early or unnecessarily
- Expect and monitor for clinical relapse and investigate thoroughly for causality; review adherence to antifungal therapy and consider drug–drug interactions; during treatment follow-up, do not escalate antifungal therapy for persistent blood antigenemia (blood cryptococcal antigen), persistently positive CSF cryptococcal antigen, visible cryptococci in CSF (without culture positivity), or abnormal CSF microscopy or biochemistry, as they are not necessarily indicators of microbiological failure
- Adapt and adopt these ECMM global guidelines to suit local practices, while constantly advocating for better antifungal access, scrutinising new trial data, and reviewing local data to improve patient outcomes

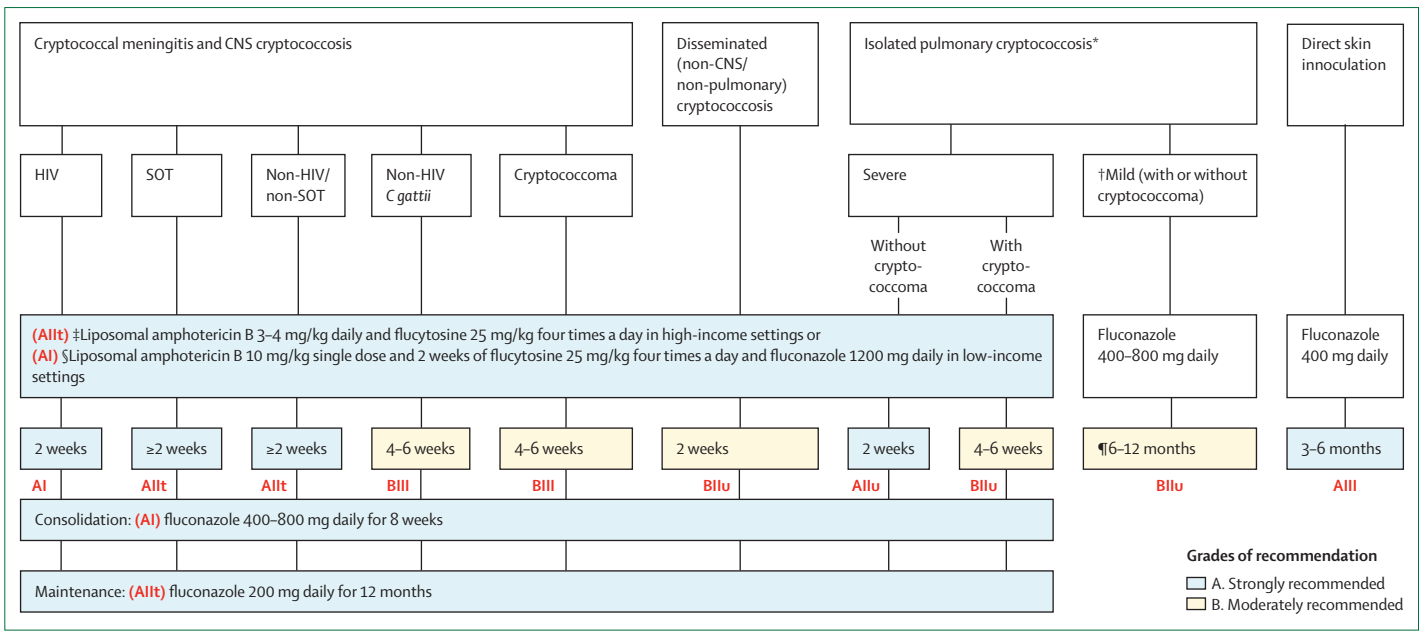

Figure 1: **Recommended first-line antifungal therapy by cryptococcosis syndrome**
Grading of recommendation and level of evidence in bolded red letters. Recommendation grading by shading: blue (strongly recommended; A) and yellow (moderately recommend; B). SOT=solid organ transplantation. *C gattii*=*Cryptococcal gattii*. w=weeks. *Isolated *Cryptococcal neoformans* or *Cryptococcal gattii* pulmonary cryptococcosis, mild is defined as asymptomatic or mildly symptomatic patients or with a solitary small nodule (<2 cm); whereas severe is defined as multiple lesions, large lesions (≥2 cm), lobar consolidation, cavitation, multi-lobar involvement, or hypoxaemic. †If the presence of *Cryptococcus* spp in respiratory specimens is deemed to be airway colonisation after careful evaluation and no treatment is elected, regular follow-up is recommended, especially in the setting of future immunosuppression. ‡Strongly preferred in cryptococcal meningitis and CNS cryptococcosis in SOT and non-HIV non-SOT patient populations, disseminated cryptococcosis, and severe pulmonary cryptococcosis. ‡Has not been directly compared against §. §Has only been trialled in people with cryptococcal meningitis and there are no supporting data of its use in SOT or non-HIV non-SOT patients or in other cryptococcosis syndromes. ¶Can consider a shorter duration (eg, 3 months) in immunocompetent individuals with mild isolated pulmonary cryptococcosis.

(C C Chang); **Institute of Infection and Immunity, St George's University London, London, UK** (Prof T S Harrison MD, Prof T A Bicanic BMBCh, Prof N P Govender MBBCh); **Clinical Academic Group in Infection and Immunity, St George's University Hospitals NHS Foundation Trust, London, UK** (Prof T S Harrison, Prof T A Bicanic); **Medical Research Centre for Medical Mycology, University of Exeter, Exeter, UK** (Prof T S Harrison, Prof T A Bicanic, Prof A Warris MD, Prof N P Govender); **Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand** (Prof M Chayakulkeeree MD); **Sydney Infectious Diseases Institute, University of Sydney, Sydney, NSW, Australia** (Prof T C Sorrell MBBS, Prof S C Chen PhD, Prof J-W Alffenaar PhD, J Beardsley PhD); **Department of Infectious Diseases** (Prof T C Sorrell, Prof S C Chen, J Beardsley),

treatment plans for the individual patient. See the appendix for more detailed text, tables, and panels relevant to each section. A summary of the first-line treatment for the different cryptococcosis syndromes is in figure 1.[3] An explanation of the evidence grading system used for the recommendations throughout is in panel 1.

Populations at high risk, clinical presentations, and outcomes

Primarily acquired via inhalation but occurring mainly upon reactivation after a period of latency, cryptococcosis has protean manifestations, with cryptococcal meningitis being the most common severe presentation. Pulmonary cryptococcosis is underdiagnosed and often subclinical. Disseminated cryptococcosis can involve any organ of the body, thus a thorough clinical assessment is required, even in individuals who appear asymptomatic.[24,25] Although classic patient populations at high risk include people living with HIV and solid organ transplant (SOT) recipients, individuals with other immunosuppressive conditions or receiving immunosuppressant drugs and people putatively immunocompetent are also affected by cryptococcosis (appendix pp 6, 79). Those who survive cryptococcosis report substantial morbidity, ranging from 10–70% depending on the disease syndrome and severity, underlying predisposing conditions of the host, and the health-care system in which the patient is managed[26–29] (panel 2; appendix pp 8, 39).

Yeasts causing cryptococcosis and diagnostic methods

C neoformans species complex is the predominant causative agent of cryptococcosis in people living with HIV, and *Cryptococcus gattii* species complex more commonly causes disease in people who appear immunocompetent. Although both can cause a similarly broad range of cryptococcosis syndromes, *C neoformans* has a predilection for CNS disease and *C gattii* is more often associated with pulmonary disease and large cryptococcomas.[30–32]

Diagnostic methods used to establish the diagnosis, extent, severity, and prognosis of cryptococcosis are constantly evolving (appendix pp 10, 41). Microscopy and culture of cerebrospinal fluid (CSF) pellet after centrifugation and blood culture, accompanied by CSF and blood (ie, serum, plasma, or whole blood) cryptococcal antigen testing (most commonly by lateral flow assay) and radiological studies, are central to the diagnosis of cryptococcosis (panel 3; appendix pp 10, 35, 41).[33,34]

Screening, primary prophylaxis, and pre-emptive therapy

Supportive evidence for cryptococcal screening is limited to people living with HIV and depends on blood cryptococcal antigen by lateral flow assay (panel 4; appendix p 49).

Panel 1: **Grade of recommendation and level of evidence**

This guideline follows the structure and definitions of previous European Confederation of Medical Mycology guidelines on invasive fungal infections,[22,23] which are in accordance with the Grading of Recommendations Assessment, Development and Evaluation and Appraisal of Guidelines for Research & Evaluation systems, as previously described. Strength of recommendation and quality of evidence are provided.

Grade of recommendation

- A: the guideline group strongly supports a recommendation for use
- B: the guideline group moderately supports a recommendation for use
- C: the guideline group marginally supports a recommendation for use
- D: the guideline group supports a recommendation against use

Level of evidence

- I: evidence from at least one well-designed randomised controlled trial (RCT)
- II: evidence from at least one well-designed clinical trial, without randomisation; from cohort or case-controlled analytic studies (preferably from >1 centre); from multiple time series; or from results of uncontrolled experiments
- III: evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

Added index for source of level II evidence

- r: meta-analysis or systematic review of RCT
- t: transferred evidence (ie, results from different patient cohorts or similar immune-status situations)
- h: historical control as control group
- u: uncontrolled trials
- a: for published abstract presented at an international symposium or meeting

Panel 2: **Recommendations for populations at high risk, clinical presentations, and outcomes**

- (AIII) Cryptococcosis should be considered in any patient presenting with compatible symptoms or microbiology, regardless of their immune status.
- (AIII) Among patients without known predisposition to cryptococcosis, exclusion of an underlying immunodeficiency (eg, performing HIV serology and CD4 T-cell count) is recommended

Panel 3: **Recommendations for yeast causing cryptococcosis and diagnostic methods**

(AIIt) All patients with suspected or confirmed cryptococcosis (including cryptococcal antigenemia) require clinical assessment for CNS, pulmonary, and other body site involvement.

Investigations for disseminated disease should include:

- Lumbar puncture with measurement of CSF opening pressure, glucose, protein, cell counts, microscopy, and culture and quantification of CSF cryptococcal antigen
- Quantification of blood cryptococcal antigen and cultures of blood, sputum (or other respiratory specimens), or other affected sites
- Brain imaging (preferably MRI) and chest imaging (preferably CT)

HIV-associated cryptococcal meningitis

Induction therapy

Multiple studies support the successful combination of amphotericin B plus flucytosine as the induction treatment of choice in HIV-associated cryptococcal meningitis. First trialled by van der Horst and colleagues, the addition of flucytosine to amphotericin B showed a trend towards improved CSF sterility at 2 weeks and reduced frequency of relapse.[35] In a subsequent trial, this combination cleared cryptococci (measured as early fungicidal activity [EFA]) more rapidly than either amphotericin B alone or amphotericin B plus fluconazole.[5] Importantly, the combination of amphotericin B 1 mg/kg daily plus flucytosine 25 mg/kg four times a day showed a survival advantage at day 70, compared with amphotericin B alone in the treatment of cryptococcal meningitis.[2] The nephroprotection of liposomal amphotericin B compared with amphotericin B is long recognised and the accessibility of liposomal amphotericin B in high-income settings led to the establishment of liposomal amphotericin B 3–4 mg/kg daily plus flucytosine 25 mg/kg four times a day for 2 weeks as the standard.

In low-income settings, challenges with antifungal access, adverse effects, and difficulty of monitoring and safely managing 2 weeks of amphotericin B induction treatment led to phase 2 studies exploring alternative regimens. Fluconazole monotherapy, even at doses up to 1200 mg daily, was associated with approximately 50% mortality at 10 weeks and up to 75% mortality at 1 year.[36–38] An oral combination of fluconazole 1200 mg daily plus flucytosine 25 mg/kg four times a day was associated with a significant improvement in EFA compared with fluconazole alone.[39] The addition of a short, 5–7 day course of amphotericin B at 1 mg/kg daily to oral fluconazole or combined oral fluconazole and flucytosine showed improved rates of cryptococcal clearance,[40,41] similar to rates observed with 14 days of amphotericin B.

In the phase 3 ACTA trial conducted in centres in Africa the oral combination of fluconazole 1200 mg daily and flucytosine 25 mg/kg four times a day for 2 weeks was compared with 1 week of amphotericin B 1 mg/kg daily and 2 weeks of amphotericin B 1 mg/kg daily as induction therapy, with the amphotericin B groups

and Department of Pharmacy (Prof J-W Alffenaar), **Westmead Hospital, Westmead, NSW, Australia; Department of Infectious Diseases, Great Ormond Street Hospital, London, UK** (Prof A Warris); **Faculty of Science, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, Netherlands** (Prof F Hagen PhD); **Department of Medical Mycology, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands** (Prof F Hagen); **Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, Netherlands** (Prof F Hagen); **Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St Louis, MO, USA** (A Spec PhD); **College of Medicine, University of Lagos, Lagos, Nigeria** (R Oladele PhD); **Department of Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa** (Prof N P Govender); **Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa** (Prof N P Govender); **Centre for Infectious Diseases and Microbiology Laboratory Services, Institute for Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead, NSW, Australia** (Prof S C Chen); **Department of Microbiology, Immunology and Infectious Diseases, Department of Medicine, Snyder Institute for Chronic Diseases, University of Calgary, Calgary, AB, Canada** (Prof C H Mody MD); **Infectious Disease Research Program** (Prof A H Groll), **Center for Bone Marrow Transplantation** (Prof A H Groll), **and Department of Pediatric Hematology/Oncology, University Children's Hospital, Münster, Germany; Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan** (Y-C Chen PhD); **National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes,**

Zhunan, Taiwan (Y-C Chen); **Fungal Pathogenesis Section, Laboratory of Clinical Immunology & Microbiology, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (Prof M S Lionakis MD); **Institut Pasteur, Centre National de Référence Mycoses Invasives et Antifongiques, Groupe de recherche Mycologie Translationnelle, Département de Mycologie, Université Paris Cité, Paris, France** (Prof A Alanio PhD); **Laboratoire de parasitologie-mycologie, AP-HP, Hôpital Saint-Louis, Université Paris Cité, Paris, France** (Prof A Alanio); **Instituto Nacional de Salud, Bogotá, Colombia** (E Castañeda PhD); **Department of Internal Medicine, Hospital Universitario Erasmo Meoz, Faculty of Health, Univesidad de Pamplona, Cúcuta, Colombia** (J Lizarazo MD); **Departmento de Neurologia, Instituto de Infectologia Emílio Ribas, São Paulo, Brazil** (J E Vidal PhD); **Departamento de Moléstias Infecciosas e Parasitárias, Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil** (J E Vidal); **Instituto de Medicina Tropical, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil** (J E Vidal); **Department of Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan** (Prof T Takazono PhD); **Department of Respiratory Medicine, Nagasaki University Hospital, Nagasaki, Japan** (Prof T Takazono); **Division of Infectious Diseases, Translational Medical Mycology Research Unit, European Confederation of Medical Mycology Excellence Center for Medical Mycology, Medical University of Graz, Graz, Austria** (Prof M Hoenigl MD); **BioTechMed, Graz, Austria** (Prof M Hoenigl); **School of Pharmacy, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia** (Prof J-W Alffenaar); **Institute for Health, Environment and Work Research—Irset, Inserm UMR_S 1085, University of Rennes, Rennes, France** (Prof J-P Gangneux PhD); **Laboratory for Parasitology**

***Panel 4*: Recommendations for screening, primary prophylaxis, and pre-emptive therapy**

Adults living with HIV who are antiretroviral therapy (ART)-naive or after a period of ART discontinuation with less than 200 CD4 cells per mm^3 must have:

- (AI) A lateral flow assay of blood cryptococcal antigen for the screening of cryptococcosis and the cryptococcal antigen titre should be measured if positive
- (AIIt) All patients with cryptococcal antigenaemia should be carefully assessed and investigated for cryptococcosis and treated as appropriate
- (AIIu) In people living with HIV who have asymptomatic cryptococcal antigenaemia but without clinical cryptococcosis after thorough investigation (including at least a lumbar puncture), fluconazole 1200 mg daily for 2 weeks (when ART can be initiated), followed by fluconazole 800 mg daily for 8 weeks, and 200 mg daily thereafter for 6 months is recommended (guidance might be updated contingent on results of prospective trials)
- (BI) In clinical settings where cryptococcal antigen lateral flow antigen screening is not available (despite WHO's strong recommendations), universal primary prophylaxis with fluconazole 100 mg daily in people living with HIV in high endemic areas with a CD4 count of less than 200 cells per mm^3 is recommended

In patients without HIV:

- (DIIu) Routine blood cryptococcal antigen screening, primary prophylaxis, and pre-emptive therapy are not recommended

being further randomly assigned to either fluconazole 1200 mg daily or flucytosine 25 mg/kg four times a day.[7] 1 week of amphotericin B 1 mg/kg daily plus flucytosine followed by fluconazole 1200 mg daily in the second week was the best-performing induction group, with a 24% 10-week mortality rate. This regimen was adopted as the preferred 10-week induction regimen by WHO and southern African guidelines until the AMBITION-cm study.[16,18]

In the AMBITION-cm phase 3 study, which had sites across Africa, a single initial 10 mg/kg dose of liposomal amphotericin B with oral fluconazole 1200 mg daily plus flucytosine 25 mg/kg four times a day for 2 weeks was compared with the WHO recommendation of 1 week of amphotericin B 1 mg/kg daily plus flucytosine followed by 1 week of fluconazole 1200 mg daily.[6] This new regimen met non-inferiority criteria (10-week mortality 24·8% *vs* 28·7%) with similar EFAs and was significantly better tolerated. The WHO guidelines now recommend the AMBITION-cm regimen as the preferred antifungal therapy in people living with HIV and cryptococcal meningitis.[10]

The applicability of the ACTA and AMBITION-cm trials to high-income settings and in non-HIV populations is contentious. The standard regimen is liposomal amphotericin B 3–4 mg/kg daily plus flucytosine 25 mg/kg four times a day for 2 weeks, which is different to the comparators used in the trials. Retrospective database reviews in the USA showed low rates of acute inpatient mortality from cryptococcal meningitis (10·5% in HIV-cryptococcal meningitis and 13·3% in non-HIV cryptococcal meningitis) and a remarkably low mortality rate at 1 year of 11·6% in the past two decades.[42,43] The reliance on high-dose fluconazole and flucytosine as the basis of induction therapy in the AMBITION-cm study might not be pragmatic in high-income settings, where more comorbidities occur, potential drug–drug interactions need to be carefully considered, and the risk of hepatotoxicity is less tolerated than in low-income settings. In the USA, only a third of patients completed the 14 days of flucytosine.[44] Although some experts support the inclusion of the AMBITION-cm triple regimen as a primary option in high-income settings, other experts call for further comparative trials in high-income settings to assess the regimen's effect in patients with HIV and patients without HIV (in whom no supporting data exist). Regardless of the induction antifungal regimen used, the complications of cryptococcal meningitis, such as increased intracranial pressure, require intense clinical monitoring, and most patients with cryptococcal meningitis require inpatient care for 1–2 weeks or more.

Mycological success, defined as cryptococcocal culture negativity (also termed CSF sterility) has been associated with improved outcomes and reduced clinical relapse.[45] In people living with HIV and cryptococcal meningitis, CSF sterility before ART commencement has been shown to be associated with reduced occurrence of neurological deterioration, microbiological relapse, and cryptococcosis-associated immune reconstitution inflammatory syndrome (C-IRIS).[45] Some treatment guidelines advocate performing a lumbar puncture after 2 weeks of induction therapy (before changing to consolidation therapy) to assess CSF culture sterility as a marker of successful induction.[11,15,18,20] Other guidelines—particularly those focused on low-income settings—do not.[10,16]

Consolidation and maintenance therapy

There have been no trials of consolidation and maintenance therapy in cryptococcal meningitis within the past two decades. Two early studies established 400 mg daily fluconazole for consolidation therapy.[35,46] With the accumulation of safety data of a 800 mg fluconazole daily dose and evidence of a fluconazole dose-response effect,[36,47] this regimen is the preferred consolidation dose in low-income settings, where suboptimal antifungal regimens are used.[16,18] A gradual rise in median fluconazole minimum inhibitory concentrations (MICs) in cryptococcal isolates collected

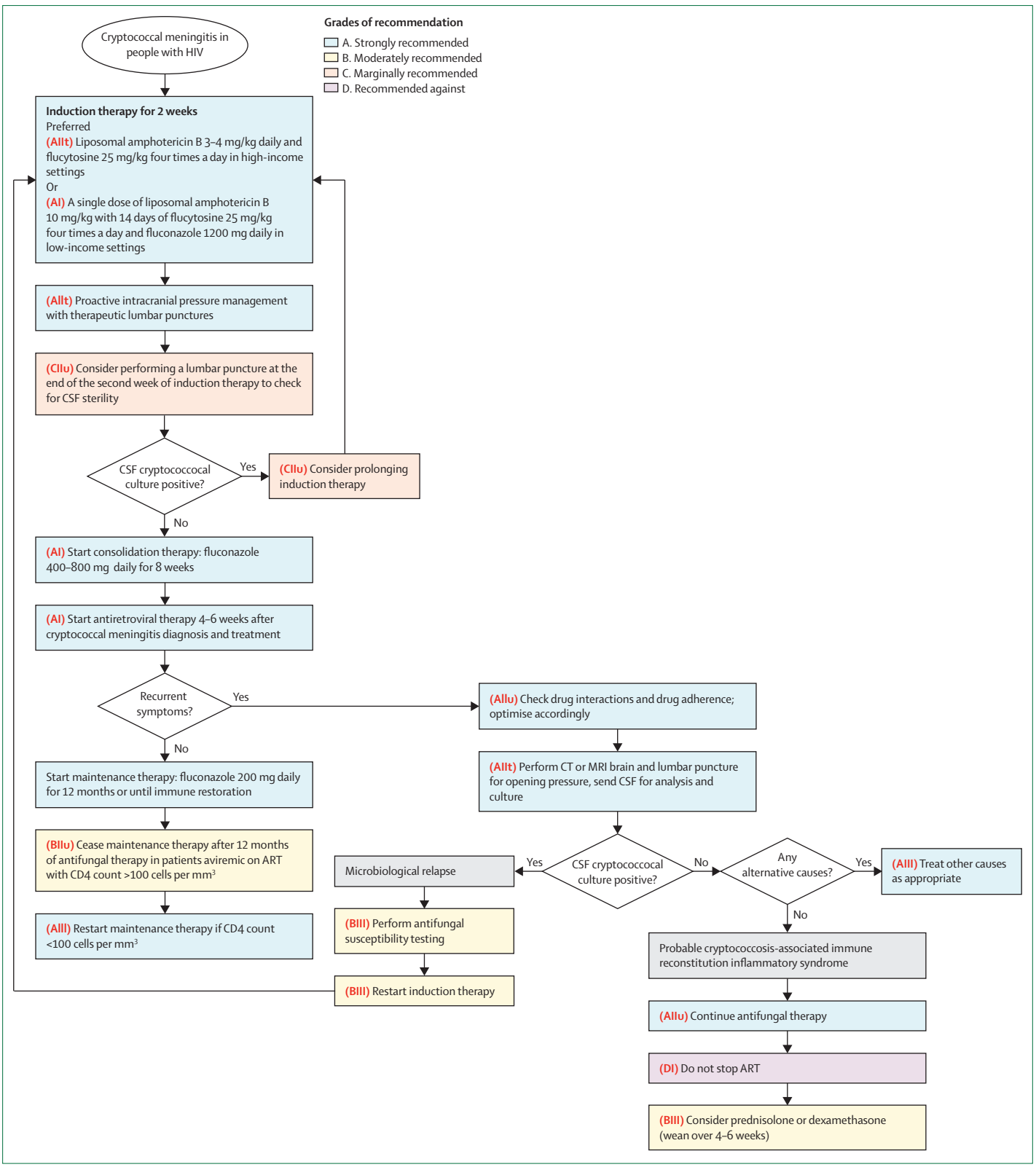

Figure 2: **Management algorithm for cryptococcal meningitis and cryptococcal meningoencephalitis in people with HIV**
ART=antiretroviral therapy. CSF=cerebrospinal fluid.

and Mycology, Centre National de Référence Mycoses Invasives et Antifongiques LA Asp-C, University Hospital of Rennes, Rennes, France (Prof J-P Gangneux); **Jupiter Hospital, Pune, India** (R Soman MD); **Deenanath Mangeshkar Hospital, Pune, India** (R Soman); **Hinduja Hospital, Mumbai, India** (R Soman); **Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, National Medical Center for Infectious Diseases, Huashan Hospital, Fudan University, Shanghai China** (Prof L-P Zhu PhD); **Hospital General de México, Dermatology Service, Mycology section, Universidad Nacional Autónoma de México, Mexico City, Mexico** (Prof A Bonifaz PhD); **Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK** (Prof J N Jarvis PhD, D Lawrence PhD); **Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana** (Prof J N Jarvis, D Lawrence); **Department of Clinical Microbiology and Infection, Royal Devon and Exeter University Hospital NHS Trust, Exeter, UK** (Prof J N Day PhD); **Department of Clinical Mycology, Allergy and Immunology, I Mechnikov North Western State Medical University, Staint Petersburg, Russia** (Prof N Klimko PhD); **Translational Research, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany** (J Salmanton-García PhD, S Rahn PhD, R Sprute MD, Prof O A Cornely MD); **Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf and Excellence Center for Medical Mycology, Department I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany** (J Salmanton-García, S Rahn, R Sprute, Prof Oliver A Cornely); **Partner Site Bonn-Cologne, German Centre for Infection**

Panel 5: Ten principles of cryptococcal meningitis management

The key principles in cryptococcal meningitis management are best read in context (see relevant sections in main text). Although most evidence and recommendations are derived from cryptococcal meningitis in people living with HIV, many of these principles are translatable to non-HIV settings.

1) Selectively screen, risk-stratify, and investigate for cryptococcosis

This principle is specific to people with HIV and cryptococcal meningitis (panel 4).

2) Provide best fungicidal induction regimen possible

- (AIIt) Liposomal amphotericin B 3–4 mg/kg daily plus flucytosine 25 mg/kg four times a day for 2 weeks (preferred in high-income settings and strongly recommended in SOT and non-HIV non-SOT settings); or (AI) a single dose of liposomal amphotericin B 10 mg/kg with 14 days of flucytosine 25 mg/kg four times a day and fluconazole 1200 mg daily (note: only trialled in people living with HIV in low-income settings).
- (CIIu) Consider performing a lumbar puncture at the end of the first or second week of induction therapy to check for CSF sterility before ART commencement.
- (CIIu) Consider prolonging induction therapy if CSF is persistently culture positive at 2 weeks.
- (BIII) In *Cryptococcus gattii* CNS infection occurring in non-HIV patients or CNS cryptococcoma consider extending induction therapy to 4–6 weeks.

3) Monitor for and minimise drug toxic effects

- (AIIu) In-hospital care for the first 1–2 weeks is encouraged to manage the major early complications seen with cryptococcal meningitis management.
- (AIIu) The use of amphotericin B and liposomal amphotericin B should be accompanied by pre-hydration and aggressive potassium and magnesium replacement therapy.
- (AIIu) Frequent (at least every alternate day) complete blood counts, renal function tests, and electrolyte measurements are recommended to assess for therapy-related nephrotoxicity and bone marrow, fluid, and electrolyte changes. Liver function tests at baseline and at least weekly are recommended.
- See appendix pp 16, 30.

4) Manage raised intracranial pressure

- (AIIu) Opening pressure should be measured at every lumbar puncture in patients with cryptococcal meningitis.
- (AIIt) Acute symptomatic elevation of the intracranial pressure (≥20 cm of CSF) should be managed by daily therapeutic lumbar punctures (ie, removal of sufficient CSF, usually around 20–30 mL) to reduce the pressure to 50% of opening pressure or to a normal pressure of ≤20 cm of CSF (documented as a closing pressure).
- (BIIu) Perform a scheduled therapeutic lumbar puncture 48–72 h after initial lumbar puncture or 7 days, regardless of initial opening pressure.
- (AIIt) Persistent raised symptomatic intracranial pressure despite therapeutic lumbar punctures should be managed by surgical decompression via temporary lumbar drainage, shunting, or ventriculostomy, depending on local expertise and resources.

5) Look for an underlying immunosuppressive state

Exploring for an immunosuppressive state—particularly, but not limited to, HIV infection—is important in the management of cryptococcosis.

- (AIII) Among patients without known predisposition to cryptococcosis, exclusion of an underlying immunodeficiency (including performing HIV serology and CD4 T-cell count) is recommended in all patients with cryptococcosis.
- (BIII) Individuals without a known risk factor for disseminated cryptococcosis, particularly those with a history of other atypical fungal, mycobacterial, or bacterial infections, should be considered for evaluation of an undiagnosed immunodeficiency, preferably in consultation with a clinical immunologist (appendix pp 6, 79).

6) Provide and ensure adherence to consolidation and maintenance therapy

- Consolidation (8 weeks): (AI) Fluconazole 400–800 mg daily. 800 mg is preferred in low-income settings.
- Maintenance (12 months or until immune restoration): (AIIt) Fluconazole 200 mg daily.
- (AIIu) Check for drug–drug interactions and adjust the dose as necessary.
- (AIII) Close therapeutic drug monitoring of tacrolimus, cyclosporine, and sirolimus levels and dose reduction of these agents are recommended when azoles are co-administered.[73,74]

7) Optimal commencement of ART in people with HIV

This principle is specific to people with HIV and cryptococcal meningitis.

- (DI) Immediate or very early commencement of ART is not recommended.
- If there is inadequate access to antifungal induction therapy, (AI) delay ART for 4–6 weeks.
- If there is adequate access to antifungal induction therapy, (BIIu) consider further individualisation, taking into consideration resolution of symptoms and signs of cryptococcal meningitis and intracranial pressure (including normalisation of opening pressure), attainment of CSF cryptococcal sterility, successful identification and management of concurrent co-infections and other AIDS-defining illnesses, the patient's readiness for ART, and

(Continues on next page)

(Panel 5 continued from previous page)

local experience of cryptococcal meningitis and cryptococcosis-associated immune reconstitution inflammatory syndrome (C-IRIS) management (usual range is 4–6 weeks).

8) Monitor for clinical relapse and investigate causality

- (AIIt) Investigate thoroughly for causality (ie, CNS and non-CNS and infective and non-infective) in cases of apparent clinical relapse. Investigations should include CT or MRI of the brain, lumbar puncture for opening pressure, and CSF analyses including microscopy and culture.
- (AIIu) Review adherence to antifungal therapy, ART, immunosuppressants, and other medications and consider drug–drug interactions. Perform therapeutic drug monitoring if applicable. Optimise control of underlying diseases.
- (DIIu) The use of follow-up blood or CSF cryptococcal antigen (including monitoring of titres) for clinical decision making is discouraged.
- (DIIu) Do not escalate antifungal therapy for persistent blood antigenemia, persistently positive CSF cryptococcal antigen, visible cryptococci in CSF (without culture positivity), or abnormal CSF microscopy or biochemistry. These are not necessarily indicators of microbiological failure.

9) Evaluate for drug adherence, drug–drug interactions, and drug resistance

This principle is specific to people with culture-positive (microbiological) persistence or relapse.

- (BIII) Antifungal susceptibility testing should be done concurrently on all initial and relapse isolates (if stored and available). An increase in fluconazole minimum inhibitory concentration of >2 dilutions is concerning for the potential development of drug resistance.
- (BIII) Consider recommencing induction therapy with a more optimal regimen that is guided by antifungal susceptibility testing.

10) Carefully exclude alternative diagnoses before attributing clinical relapse to C-IRIS

- (AIIt) For patients with suspected paradoxical C-IRIS, carefully exclude recurrent cryptococcal disease or new infective or non-infective conditions before attributing symptoms and signs to C-IRIS. Perform a brain MRI and lumbar puncture to measure opening pressure and get CSF for microbiological, cellular, and biochemical analyses.
- (AIIu) Treatment of C-IRIS should include therapeutic lumbar puncture and symptomatic therapy, such as analgesia, antiemetics, and antiepileptics if appropriate.
- (AIII) Continue antifungal therapy.
- (BIII) High-dose prednisolone or prednisone (usually 0·5–1·0 mg/kg daily) or dexamethasone (usually 0·2–0·3 mg/kg daily), weaned over 4–6 weeks can be considered in those with persistent symptoms who are unresponsive to therapeutic lumbar punctures. Rarely, a second steroid course with taper is needed.
- (DIII) Do not stop ART.

during initial cryptococcal meningitis presentation have been reported in South Africa and Uganda.[48,49] Although this evidence could lend support for a higher consolidation dose of 800 mg daily of fluconazole in these settings, whether this regimen is required across all patient groups and settings is contentious. Widespread fluconazole use could also perpetuate further rises in MICs.

Maintenance therapy with fluconazole 200 mg daily has been shown to be highly effective at preventing relapse, superior to weekly amphotericin B and itraconazole capsules.[50–52] Rarely, triazoles, such as voriconazole,[53–60] posaconazole,[61–63] or isavuconazole,[64,65] are used as alternatives to fluconazole due to concerns of fluconazole resistance, drug toxicity, or drug–drug interactions. Notably, none of the newer triazoles have been formally trialled in cryptococcosis and none are readily available in low-income settings (appendix p 30).

A low incidence of cryptococcal meningitis relapse is observed after a minimum of 1 year of antifungal therapy in people living with HIV established on ART, who are virologically suppressed or have a CD4 count more than 100 cells/mm³.[66–72]

A management algorithm is described in figure 2 and key principles are discussed in panel 5. Recommendations for cryptococcal meningitis treatment in people living with HIV are based on the availability of antifungal drugs. Preferred and alternative strategies are offered in figure 3 and figure 4A.

Adjunctive therapy

In the past decade, trials of adjunctive treatment in HIV-associated cryptococcal meningitis have all been shown to be ineffective, and in some cases harmful. These include high-dose dexamethasone,[75] sertraline,[76,77] and tamoxifen.[78] The debate regarding adjunctive exogenous interferon(IFN)-γ is unresolved. IFN-γ has been studied in two randomised trials of HIV-associated cryptococcal meningitis, which suggested faster clearance of yeasts in the CSF,[79,80] but further studies are needed. There is no trial evidence supporting its use in non-HIV-associated cryptococcal meningitis (appendix p 65).

Cryptococcal meningitis in SOT recipients

Cryptococcosis is the third most common invasive fungal infection in SOT recipients, with an incidence of 4·5–33·8%[26,28,29] and causing considerable mortality.[12] SOT recipients encompass a third of non-HIV-related cryptococcosis in the USA.[81] The majority of cryptococcosis

Research, Cologne, Germany (J Salmanton-García, S Rahn, R Sprute, Prof Oliver A Cornely); **Histology and Pathology Unit, Ecole nationale vétérinaire d'Alfort, Maisons-Alfort, France** (Prof G Jouvion PhD); **Dynamyc Team, Université Paris Est Créteil and Ecole nationale vétérinaire d'Alfort, Créteil, France** (Prof G Jouvion); **Infectious Diseases Institute, School of Medicine, College of Heath Sciences, Makerere University, Kampala, Uganda** (D B Meya PhD); **Department of Medical Microbiology and Immunology, Faculty of Medicine, Gulu University, Gulu, Uganda** (F Bongomin MMed); **Discipline of Paediatrics, School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW, Australia** (B J McMullan PhD); **Department of Infectious Diseases, Sydney Children's Hospital, Randwick, Sydney, NSW, Australia** (B J McMullan); **Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK** (T K Nyazika); **Pediatric Department, Federal University of São Paulo, São Paulo, Brazil** (Prof F Carlesse PhD); **Oncology Pediatric Institute-IOP-GRAACC, Federal Univeristy of São Paulo, São Paulo, Brazil** (Prof F Carlesse); **Department of Microbiology, Fiona Stanley Hospital Network, PathWest Laboratory Medicine, Perth, WA, Australia** (C H Heath MBBS); **Department of Infectious Diseases, Fiona Stanley Hospital, Perth, WA, Australia** (C H Heath); **UWA Medical School, Internal Medicine, The University of Western Australia, Perth, WA, Australia** (C H Heath); **Dermatology Unit, Department of Medicine, Lagos University Teaching Hospital, University of Lagos, Lagos, Nigeria** (Prof O O Ayanlowo FWACP); **Centre for Respiratory Diseases Research, Kenya Medical Research Institute, Nairobi, Kenya** (O M Mashedi MSc); **Department of Public Health, Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil** (F Queiroz-Telles Filho PhD); **Department of Medicine, Division of Infectious Diseases, University of North Carolina at Chapel Hill School of Medicine,**

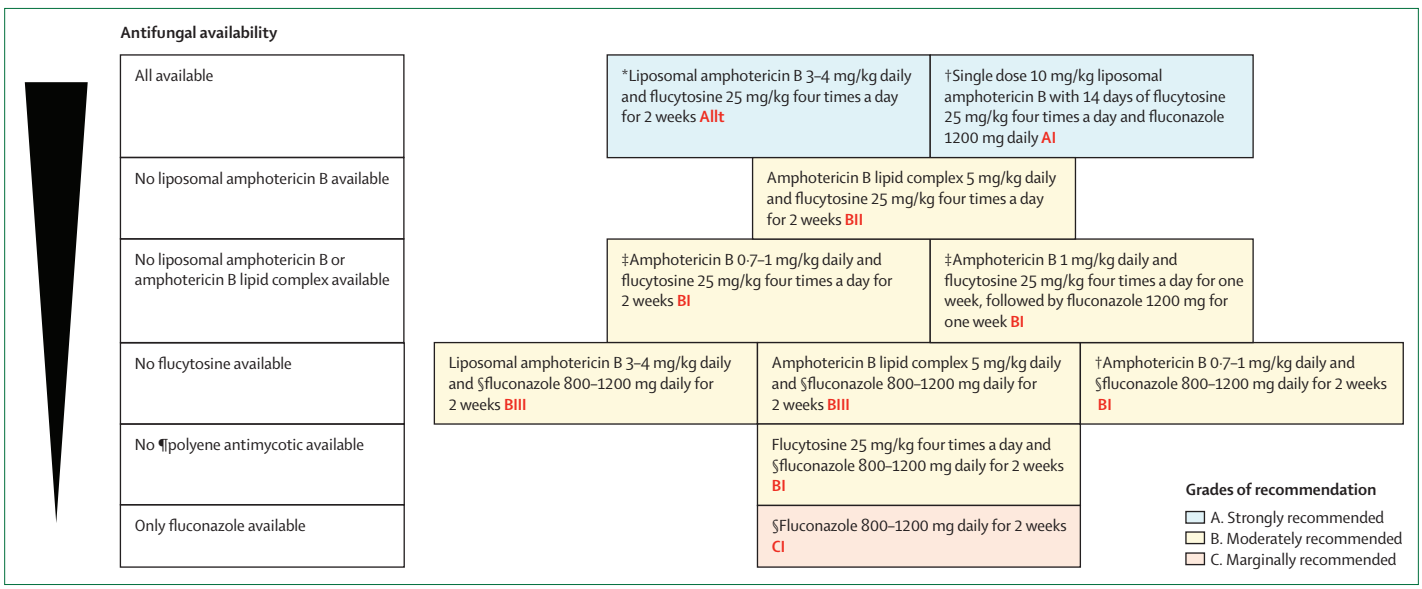

Figure 3: **HIV-cryptococcal meningitis antifungal induction treatment recommendations by antifungal drug availability**
Grading of recommendation and level of evidence in bolded red letters. *Has not been compared with †. †Has only been trialled in HIV-cryptococcal meningitis. ‡Amphotericin B: 1 mg/kg showed earlier fungicidal activity than 0·7 mg/kg, but some institutions use the low dose due to toxicity concerns. §Fluconazole induction doses of up to 1200 mg daily have been trialled but caution is advised; consider drug–drug interaction and liver toxicity. ¶Polyene antimycotic includes amphotericin B formulations such as conventional deoxycholate amphotericin B, liposomal amphotericin B, and amphotericin B lipid complex.

Chapel Hill, NC, USA (Prof M C Hosseinipour MD); **UNC Project Malawi, Lilongwe, Malawi** (Prof M C Hosseinipour); **Department of Infectious Diseases, Sterling Hospitals, Ahmedabad, India** (A K Patel MD); **Africa Centers for Disease Control and Prevention, Addis Ababa, Ethiopia** (E Temfack MD); **Division of Infectious Diseases, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA** (Prof N Singh MD); **Clinical Trials Centre Cologne, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany** (Prof Oliver A Cornely); **Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, MN, USA** (Prof D R Boulware MD); **Université de Paris Cité, APHP, Service des Maladies Infectieuses et Tropicales, Hôpital Necker-Enfants Malades, Centre d'Infectiologie Necker-Pasteur, Institut Imagine, Paris, France** (Prof O Lortholary MD); **Institut Pasteur, CNRS, Unité de Mycologie Moléculaire, Centre National de Référence Mycoses Invasives et Antifongiques, UMR 2000, Paris, France**

in SOT occurs late (ie, months or years after transplantation) and is due to reactivated disease; however, acute donor-derived infections have been described.[14,82,83]

Anti-rejection drugs vary in their degree of immunosuppression and heart and small bowel transplant recipients are at the highest cryptococcal meningitis risk.[84] CNS and pulmonary cryptococcosis dominate but unusual manifestations, including cutaneous disease[85,86] and pericarditis,[87] have been reported. Notably, blood cryptococcal antigen can be negative in SOT recipients with cryptococcosis, particularly those with single pulmonary nodules or in lung transplant recipients.[88]

There are no randomised treatment trials targeted specifically at SOT recipients; hence, recommendations are extrapolated from evidence in people living with HIV. The use of lipid-formulations in SOT recipients with CNS cryptococcosis was independently associated with reduced mortality compared with amphotericin B.[89] The AMBITION-cm regimen has not been studied in non-HIV patients, and the evidence for high dose fluconazole (with the ensuant potential toxicity and drug–drug interactions) in this group is absent. A precipitous reduction in dosing of immunosuppressants, particularly calcineurin inhibitors, can lead to C-IRIS.[90] Figure 4B contains recommendations for treatment in SOT recipients (appendix p 62).

Cryptococcal meningitis in people without HIV or SOT

The group of people without HIV or SOT is heterogeneous, ranging from apparently healthy people to those with haematological malignancies or liver cirrhosis. There is no single therapeutic regimen or duration that meets all patients' needs, but the therapeutic principles mirror cryptococcal meningitis treatment in high-income settings, with liposomal amphotericin B 3–4 mg/kg daily and flucytosine 25 mg/kg four times a day as induction therapy. Induction therapy can be extended in those with persistently positive CSF cultures or persistent symptoms at 2 weeks. In 2022, the combination of liposomal amphotericin B and flucytosine was shown to have a low acute mortality of 6% in a nationwide observational study of non-HIV-associated cryptococcal meningitis in Japan.[91] Figure 4B contains recommendations for treatment in people without HIV or SOT (appendix pp 18, 62).

Pulmonary cryptococcosis

There are no randomised treatment studies in pulmonary cryptococcosis. Case series and clinical knowledge suggest that for patients with cryptococcaemia and evidence of CNS involvement, those with blood cryptococcal antigen titres more than 1:512 by latex agglutination (or ten-fold higher by lateral flow assay),[92] or severe pulmonary disease should be treated as cryptococcal meningitis.[33,35,93,94] Patients with mild isolated pulmonary disease without cryptococcoma have been successfully treated with fluconazole monotherapy of

Figure 4: **Antifungal treatment recommendations for cryptococcal meningitis**
(A) Recommendations for people with HIV. (B) Recommendations for SOT recipients and patients without HIV or SOT. SOT=solid organ transplant. CSF=cerebrospinal fluid. TDM=therapeutic drug monitoring.

A People with HIV

First-line therapies

Induction (2 weeks)
(AIIt) Liposomal amphotericin B 3–4 mg/kg daily plus flucytosine 25 mg/kg four times a day (preferred in high-income settings); or
(AI) Single dose liposomal amphotericin B 10 mg/kg and 14 days of flucytosine 25 mg/kg four times a day and fluconazole 1200 mg daily (recommended in low-income settings)

→

Consolidation (8 weeks)
(AI) Fluconazole 400–800 mg daily (800 mg preferred in low-income settings)

→

Maintenance (12 months or until immune restoration)
(AIIt) Fluconazole 200 mg daily

Alternative therapies

If liposomal amphotericin B is not available:
(BIIt) Amphotericin B lipid complex 5 mg/kg daily plus flucytosine 25 mg/kg four times a day

If liposomal amphotericin B and amphotericin B lipid complex are not available:
(BI) Amphotericin B 0·7–1·0 mg/kg daily plus flucytosine 25 mg/kg four times a day; or
(BI) Amphotericin B 1 mg/kg daily and 5-flucytosine 25 mg/kg four times a day for 1 week, followed by fluconazole 1200 mg daily for 1 week

If flucytosine is not available:
(BIII) Liposomal amphotericin B 3–4 mg/kg daily plus fluconazole 800–1200 mg daily;
(BIII) Amphotericin B lipid complex 5 mg/kg daily plus fluconazole 800–1200 mg daily; or
(BI) Amphotericin B 0·7–1 mg/kg daily plus fluconazole 800–1200 mg daily

If amphotericin B-based therapies are not available:
(BI) Flucytosine 25 mg/kg four times a day and fluconazole 800–1200 mg daily

If only fluconazole is available:
(CI) Fluconazole 800–1200 mg daily

(BIII) Voriconazole 200 mg twice a day (with TDM)

(BIII) Posaconazole 300 mg daily (with TDM)

(BIII) Isavuconazole 200 mg daily

(CIIt) Itraconazole 200 mg twice a day (with TDM)

Comments:
- (AIIu) Opening pressure should be measured at every lumbar puncture in patients with cryptococcal meningitis
- (AIIu) The use of amphotericin B and liposomal amphotericin B should be accompanied by pre-hydration and aggressive potassium and magnesium replacement therapy
- (AIIu) In-hospital care for the first 1–2 weeks is encouraged to manage the early complications of cryptococcal meningitis therapy
- (BIII) Monitoring of flucytosine drug concentration is recommended, where available and if timely; particularly with renal dysfunction
- (AIIu) Check for drug–drug interactions and adjust doses as necessary
- (CIIu) Consider performing a lumbar puncture at the end of the first or second week of induction therapy to check for CSF sterility before antiretroviral therapy (ART) commencement
- (CIIu) Consider prolonging induction therapy if CSF is persistently culture positive at 2 weeks
- (CIIt) Adjunctive recombinant interferon-γ might be considered for persistently positive CSF yeast cultures in people with HIV-associated cryptococcal meningitis who have evidence of poor inflammatory responses or persistently positive cryptococcal CSF culture after prolonged antifungal therapy
- (DI) The routine use of high-dose dexamethasone in cryptococcal meningitis is not recommended
- (CIII) A short course of dexamethasone can be considered for specific indications such as symptomatic space-occupying lesions in the CNS with surrounding oedema or mass effect and cerebral vasculitis
- (BIIu) Cease maintenance therapy after 12 months of antifungal therapy in patients aviraemic on ART with a CD4 count more than 100 cells per mm^3
- (AIII) Restart maintenance therapy if CD4 count drops to less than 100 cells per mm^3

B SOT recipients and people without HIV or SOT

First-line therapies

Induction (minimum 2 weeks)
(AIIt) Liposomal amphotericin B 3–4 mg/kg daily plus flucytosine 25 mg/kg four times a day

→

Consolidation (8 weeks)
(AIIt) Fluconazole 400–800 mg daily

→

Maintenance (12 months)
(AIIt) Fluconazole 200 mg daily

Alternative therapies

If liposomal amphotericin B is not available:
(BIIt) Amphotericin B lipid complex 5 mg/kg daily plus flucytosine 25 mg/kg four times a day

If liposomal amphotericin B and amphotericin B lipid complex are not available:
(BIIt) Amphotericin B 0·7–1·0 mg/kg daily plus flucytosine 25 mg/kg four times a day

If amphotericin B-based therapies are not able to be used:
(CIIt) flucytosine 25 mg/kg four times a day plus fluconazole 800–1200 mg daily

(BIII) Voriconazole 200 mg twice a day (with TDM)

(BIII) Posaconazole 300 mg daily (with TDM)

(BIII) Isavuconazole 200 mg daily

(CIIt) Itraconazole 200 mg twice a day (with TDM)

Comments:
- Recommendations in HIV patient population are also applicable
- (AIII) Induction therapy with liposomal amphotericin B and flucytosine should be considered for any disseminated disease or isolation from a sterile site (even in the absence of CNS manifestations)
- (AIII) Close monitoring of tacrolimus, cyclosporine, and sirolimus concentrations (TDM) and dose reduction of these agents are recommended when azoles are co-administered[73,74]
- (BIII) Immunosuppressant doses need to be carefully adjusted to allow effective killing of yeasts but should be reduced slowly to avoid precipitating cryptococcosis-associated immune reconstitution inflammatory syndrome; consider a sequential or stepwise reduction of immunosuppressants with careful lowering of corticosteroids early and eliminating mycophenolate before considering reduction of the calcineurin inhibitors because of their direct anticryptococcal activity
- (CIII) In a patient treated for cryptococcosis, retransplantation or a new organ transplant can be considered, provided viable yeasts have been cleared from CSF and the patient is asymptomatic after receiving 12 months of anticryptococcal treatment

Grades of recommendation
- A. Strongly recommended
- B. Moderately recommended
- C. Marginally recommended

(Prof O Lortholary); **Mycoses Study Group Central Unit, Division of Infectious Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA** (Prof P G Pappas MD); **Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, NC, USA** (Prof J R Perfect MD); **Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA** (Prof J R Perfect)

Correspondence to: Dr Christina C Chang, Department of Infectious Diseases, Alfred Hospital, Melbourne 3181, VIC, Australia **christina.chang@monash.edu**

or

Prof John R Perfect, Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA **john.perfect@duke.edu**

See Online for appendix

***Panel 6:* Recommendations for pulmonary cryptococcosis**

- Stratify treatment by disease severity and presence of pulmonary cryptococcoma (appendix pp 22, 67, 84)
- Isolated pulmonary cryptococcosis in immunocompetent or immunocompromised host:
  - (AIIu) Severe disease: as for CNS disease
  - (BIIu) Mild disease: fluconazole 400 mg daily for 6–12 months (range guided by symptom resolution)
- Pulmonary cryptococcosis with CNS manifestations or other evidence of dissemination (eg, cryptococcaemia or skin lesions)
  - (AIIt) as for CNS disease
- (AIII) If the presence of *Cryptococcus* spp in respiratory specimen is deemed as airway colonisation after careful evaluation and no treatment is elected, regular follow-up is recommended, especially in the setting of future immunosuppression
- Pulmonary cryptococcoma (see cryptococcoma section)

***Panel 7:* Recommendations for non-pulmonary non-CNS disease**

- (AIIu) The recommendation for cryptococcaemia is to treat the same as for CNS disease
- (AIII) The recommendation for primary cutaneous (skin) cryptococcosis, attributed to direct inoculation without evidence of dissemination, is fluconazole 400 mg daily for 3–6 months or until healed
- (BIIu) For all other non-CNS non-pulmonary disseminated disease treat the same as CNS disease
- (BIIu) Cryptococcal eye disease should be managed in collaboration with an ophthalmologist

400 mg daily.[93,95,96] Some clinicians consider watchful-waiting and elect not to treat asymptomatic immuno-competent people who incidentally culture any *Cryptococcus* spp in their sputum and have no radiological features of pulmonary cryptococcosis, as they consider this presentation to be airway colonisation.[97] Criteria for distinguishing colonisation from infection is uncertain (panel 6; appendix pp 22, 67).

Non-pulmonary non-CNS disease

Cryptococcosis can affect any organ following haematogenous dissemination. Clinical presentation of non-CNS non-pulmonary disease without fungaemia is rare, but possible. The absence of documented fungaemia does not exclude dissemination. Barring direct inoculation into the skin following trauma, extra-pulmonary disease is by definition disseminated disease and generally requires consideration for aggressive induction therapy. There are no clinical treatment trials for non-pulmonary non-CNS cryptococcosis.

Importantly, visual changes noted in cryptococcal meningitis are frequently related to raised intracranial pressure and do not necessarily indicate direct eye involvement. Ocular cryptococcosis can occur[98,99] but is unusual and requires formal ophthalmological documentation and management. Isolated skeletal osteomyelitis is rare and often requires a combined surgical and medical approach.[100–102] Skin lesions might be polymorphic (panel 7; appendix p 67).

Specific management issues

Raised intracranial pressure

Increased intracranial pressure has been associated with a high burden of cryptococci, leading to both acute and chronic symptoms and signs (eg, visual and hearing loss) and decreased short-term survival. Clinical experience has shown that CSF outflow obstruction can be improved by removal of CSF; observational studies suggested that scheduled therapeutic lumbar punctures result in substantial improvement in survival, regardless of opening pressure.[103,104] For prolonged control of acute increased intracranial pressure, use of lumbar drains in cases without hydrocephaly or ventriculostomies in cases with hydrocephaly might be required.[105–107] Medical therapies including acetazolamide, mannitol, and corticosteroids can be detrimental (panel 8; appendix p 79).[108,109]

Timing of ART commencement

The optimal time to commence ART for HIV infection during cryptococcosis is controversial. Four randomised trials[3,110–112] to find out the optimal timing of ART initiation in HIV-cryptococcal meningitis co-infection have been done in low-income settings, using induction regimens that are not currently preferred, including fluconazole (800 mg daily) monotherapy,[110] amphotericin B 0·7 mg/kg daily,[111] and amphotericin B 0·7–1 mg/kg daily and fluconazole 800 mg daily for 2 weeks. These data seem to suggest that initiating ART within 2 weeks of cryptococcal meningitis presentation is too early in the setting of suboptimal antifungal therapy, and that delaying ART initiation for 4–6 weeks reduces the incidence of C-IRIS and death. CSF sterility before ART commencement might be another factor.[45] A retrospective analysis of combined cohorts in high-income settings did not show higher mortality in those receiving early ART in the first two weeks of antifungal therapy compared with those with delayed therapy.[113] Early ART in high-income settings will need careful justification and close monitoring; further randomised studies might be helpful.[114]

There are no studies for timing ART initiation in other forms of cryptococcosis, those with cryptococcal antigenemia, or those recommencing ART after a period of interruption. Early concerns that potent integrase inhibitors pose an increased risk of C-IRIS have been disproven.[115] Whether those presenting with cryptococcal meningitis within 2 weeks of starting ART require withholding of ART is uncertain (panel 9; appendix p 70).[116–118]

Panel 8: **Recommendations for raised intracranial pressure**

- (AIIu) Opening pressure should be measured at every lumbar puncture in patients with cryptococcal meningitis
- (AIII) A brain CT should be done (if CNS imaging not already done) to exclude CNS outflow obstruction
- (AIIt) Acute symptomatic elevation of the intracranial pressure (≥20 cm of CSF) should be managed by daily therapeutic lumbar punctures (ie, removal of sufficient CSF, usually around 20–30 mL), to reduce the pressure to 50% of opening pressure or to a normal pressure of ≤20 cm of CSF (documented as a closing pressure)
- (BIIu) Perform a scheduled therapeutic lumbar puncture 48–72 h after initial lumbar puncture or 7 days, regardless of initial opening pressure
- (AIIt) Persistent raised symptomatic intracranial pressure, despite therapeutic lumbar punctures, should be managed by surgical decompression via temporary lumbar drainage, shunting, or ventriculostomy, depending on local expertise and resources
- (BIII) Consider ventriculoperitoneal (preferential) and lumboperitoneal shunts (alternative) to control both acute and chronic hydrocephalus if temporary measures are not successful. Ideally, insert shunts after institution of effective antifungal therapy

Panel 9: **Recommendations for the timing of ART commencement**

- (DI) Immediate or very early commencement of ART is not recommended.
- (AI) If suboptimal antifungal induction therapy is used, delay ART for 4–6 weeks.
- (BIIu) If optimal antifungal induction therapy was used, consider further individualisation, taking into consideration resolution of symptoms and signs of cryptococcal meningitis, intracranial pressure (including normalisation of opening pressure), attainment of CSF cryptococcal sterility, successful identification, management of concurrent co-infections and other AIDS-defining illnesses, the patient's readiness for ART, and local experience of cryptococcal meningitis and C-IRIS management (usual range is 4–6 weeks).
- (CIIt) If possible, ensure CSF is cryptococcal culture negative before ART commencement.
- (BIII) For people who have had ART who develop cryptococcal meningitis and might need to switch to second-line ART or recommence ART, a delay of 4–6 weeks is recommended.
- (CIII) Pending further studies, consider withholding ART and restarting at 4–6 weeks in those presenting with cryptococcal meningitis within 2 weeks of starting ART.
- (BIII) Patients with isolated pulmonary cryptococcosis or those with asymptomatic cryptococcal antigenemia can commence ART earlier (eg, at 2 weeks).

Panel 10: **Recommendations for antifungal resistance**

For those with fluconazole resistance or emerging fluconazole resistance:

- (BIII) Consider a long (eg, 4 weeks) course of induction treatment with amphotericin B (1 mg/kg daily) or high dose of liposomal amphotericin B (3–6 mg/kg daily) together with flucytosine
- (BIII) Consider amphotericin B 1 mg/kg weekly or liposomal amphotericin B 3–6 mg/kg weekly as consolidation or maintenance therapy. Consider daily voriconazole, posaconazole, isavuconazole, or itraconazole for isolates without evidence of pan-azole resistance, as guided by antifungal susceptibility testing
- (CIII) If amphotericin B or liposomal amphotericin B are not available, adding flucytosine to high-dose fluconazole (1200 mg daily) could be considered

Resistance to antifungals

Developing secondary resistance to flucytosine is common when given as monotherapy, necessitating its use with a partner drug in cryptococcosis. Acquired resistance to polyenes, such as amphotericin B, is rare, but the emergence of fluconazole resistance is concerning.[48,49,119] Fluconazole monotherapy as induction therapy has been associated with secondary resistance.[120–123]

There are no clinical MIC breakpoints for fluconazole against *Cryptococcus* spp and insufficient data to suggest that high MICs imply worse outcomes. Interpretation of epidemiological cutoff values with the Clinical and Laboratory Standards Institute (CLSI) method for fluconazole requires accurate species identification. The epidemiological cutoff values is 8 ug/mL for *C neoformans* VNI, 16 ug/mL for *C gattii* VGI, and 32 ug/mL for *Cryptococcus deuterogattii* VGII.[124] In principle, a higher than two-fold increase in MIC during treatment could suggest development of resistance and the need for closer clinical monitoring. There are no European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cutoff values available for fluconazole (panel 10; appendix p 72).

Cryptococcal persistence, clinical relapse, and culture-positive (microbiological) relapse

Distinguishing clinical relapse from persistent cryptococcal infection is challenging. Clinical relapse can be due to a microbiological relapse, C-IRIS, raised intracranial pressure (whether related to C-IRIS or not), or other infective and non-infective (CNS and non-CNS) causes (figure 2). Cryptococcal antigen persists in the CSF and blood, thus it has little clinical utility in distinguishing clinical responders from non-responders.[125] Most cases of culture-positive (microbiological) relapse occur early and result from inadequate or suboptimal induction therapy or early discontinuation of consolidation or maintenance therapy (figure 2; panel 11; appendix p 74).

Panel 11: **Recommendations for cryptococcal persistence, clinical relapse, and culture-positive (microbiological) relapse**

- (AIIt) Think broadly and investigate thoroughly for causality (CNS or non-CNS and infective or non-infective) in cases of apparent clinical relapse; investigations should include brain CT or MRI, lumbar puncture for opening pressure, and CSF analyses, including microscopy and culture
- (AIIu) Review adherence to antifungal therapy, ART, immunosuppressants, and other medications and consider drug–drug interactions; perform therapeutic drug monitoring if applicable. Optimise control of underlying diseases
- (CIII) Consider escalating antifungal therapy while awaiting CSF results (and de-escalate if culture-negative)
- (DIIu) The use of follow-up blood or CSF cryptococcal antigen (including monitoring of titres) for clinical decision making is discouraged
- (DIIu) Do not escalate antifungal therapy for persistent blood antigenemia, persistently positive CSF cryptococcal antigen, visible cryptococci in CSF (without culture positivity), or abnormal CSF microscopy or biochemistry; these are not necessarily indicators of microbiological failure

For culture-positive (microbiological) persistent or relapsed infection (figure 1):

- (BIII) Antifungal susceptibility testing should be done concurrently on all initial and relapse isolates (if stored and available); an increase in fluconazole MIC of more than two dilutions is considered concerning for the potential development of drug resistance
- (BIII) Consider reinduction with a more optimal regimen (guided by antifungal susceptibility testing)

Panel 12: **Recommendations for C-IRIS**

- (AIIt) For patients with suspected paradoxical C-IRIS, carefully exclude recurrent cryptococcal disease or new infective or non-infective conditions before attributing symptoms and signs to C-IRIS; perform a brain MRI and lumbar puncture to measure opening pressure and get CSF for microbiological and biochemical analyses
- (AIIu) Treatment of C-IRIS should include therapeutic lumbar puncture and symptomatic therapy, such as analgesia, antiemetics, and antiepileptics, if appropriate
- (AIII) Continue antifungal therapy
- (BIII) High-dose prednisolone or prednisone (usually 0·5–1·0 mg/kg daily) or dexamethasone (usually 0·2–0·3 mg/kg daily), weaned over 4–6 weeks, can be considered in those with persistent symptoms who are unresponsive to therapeutic lumbar punctures; rarely a second steroid course with taper is needed
- (DIII) Do not stop ART
- (BIII) Cases of steroid-refractory or recurrent C-IRIS should be discussed with experts in the field
- (BIIu) Steroids could be considered for PIIRS

Panel 13: **Recommendations for *C gatti***

In *C gattii* CNS disease:

- (AIII) Treat the same as *C neoformans* CNS infection
- (BIII) In non-HIV patients, consider extending induction therapy to 4–6 weeks
- (AIII) Early CSF shunting is indicated for obstructive chronic hydrocephalus

Treatment of *C gattii* lung disease is summarised in the appendix (p 17).

C-IRIS

C-IRIS has been described in people with HIV usually between 2 weeks and 3 months after commencement of ART. Patients develop exaggerated symptoms and signs or atypical inflammation, reminiscent of a paradoxical recurrence,[126,127] but C-IRIS can also occur in the setting of immune recovery or withdrawal of immunosuppressants. It has also been observed in seemingly immunocompetent individuals, including in *C gattii* infections, as a post-infectious inflammatory immune response syndrome (PIIRS).[90,128] There is no diagnostic biomarker for C-IRIS. It is diagnosed by diagnosis of exclusion (figure 2).

There have been no therapeutic trials in C-IRIS. Management strategies include therapeutic lumbar puncture and symptomatic therapies. In severe C-IRIS, corticosteroids are commonly used to dampen inflammation, although their efficacy has not been rigorously examined in clinical trials. In steroid-refractory C-IRIS, there are case reports on the use of tumour necrosis factor-α blockers, such as adalimumab[129–132] or thalidomide,[133–135] with mixed success. Corticosteroids can also be beneficial in PIIRS (panel 12; appendix p 76).[136]

C gattii

About 50–70% of *C gattii* infections occur in putatively immunocompetent hosts,[137–139] compared with 2–30% in people with HIV.[140–144] Autoantibodies to granulocyte-macrophage colony-stimulating factor and idiopathic CD4 lymphopenia are reported risk factors.[137,145–147] Notably, not all commercial lateral flow assays are able to detect *C gattii* disease.[148] Antifungal agents used for treatment are the same as for *C neoformans*.[30,32,141] However, 4–6 weeks of induction therapy might be required in some cases of non-HIV-associated meningitis with *C gattii* (panel 13; appendix p 81).[149]

Cryptococcomas

Cryptococcomas occur predominantly in the lungs and brain and are more frequent in *C gattii* infection.[140,150] CNS cryptococcomas can manifest as neurological deficits or raised intracranial pressure,[140] which requires urgent management. Corticosteroids and surgical resection can be of value.[149,151,152] Radiological lesions can persist indefinitely despite clinical and microbiological cure (panel 14; appendix p 84).[32,153] Recommendations for cryptococcomas are in.

Non-*C neoformans* and non-*C gattii* strains of cryptococcus

There are individual case reports and small case series of non-*C neoformans* and non-*C gattii* cryptococcus infections, predominantly in immunosuppressed patients. *Papiliotrema laurentii* (previously *Cryptococcus laurentii*)[154] and *Naganishia albida* (previously *Cryptococcus albidus*)[155] account for about 80% of the invasive infections in this group and usually involve the skin, lungs, bloodstream, or CNS.[156] Colonisation, especially of the skin, respiratory, and

Panel 14: **Recommendations for cryptococcomas**

- (AIII) Perform a biopsy or aspirate to exclude a secondary pathogen or an underlying tumour in non-responding cryptococcomas (particularly in immunosuppressed patients)
- (BIII) Consider surgical resection for accessible brain lesions more than 3 cm, lesions at risk of compressing critical structures, or large lesions not responding to therapy
- (DIII) During follow-up, do not prolong or escalate therapy for persistent radiological findings in the absence of new or worsening symptoms or signs

For CNS cryptococcoma:

- (BIII) Consider prolonging CNS antifungal induction therapy to 4–6 weeks
- (BIII) Consider corticosteroids for large cryptococcomas with surrounding mass effect or if neurological symptoms and cerebral imaging signs worsen despite a good microbiological response

The appendix (pp 22, 84) summarises treatment of lung cryptococcoma.

gastrointestinal tracts must be distinguished from true disease. In some cases, the laboratory might misidentify another yeast as *P laurentii* or *N albida* on the basis of non-definitive commercial identification methods.[157] Elevated MICs against flucytosine, fluconazole, and other azoles for some isolates have been documented but are of uncertain clinical significance (panel 15; appendix p 85).[158,159]

Pregnancy

The majority of cases of cryptococcosis in pregnancy occur in the third trimester or postpartum.[160,161] Maternal mortality from disseminated cryptococcosis is approximately 25%, and less than 50% of women carry their pregnancy to term.[161] Extensive clinical experience suggests that amphotericin B and liposomal amphotericin B are safe during pregnancy (Category B drug), and thus are the cornerstone of treatment.[161,162] Flucytosine is rated by the USA Food and Drug Administration as a Category C drug because of its direct effects on RNA and DNA metabolism. Fluconazole is a Category D drug due to its increased risk of musculoskeletal malformations, tetralogy of Fallot, and spontaneous abortions (panel 16; appendix p 86).[163–167]

Paediatrics

There is a clear need for paediatric-specific studies in cryptococcosis. CNS disease seems to predominate in paediatrics, but non-CNS disease is probably under-reported.[168–174] Clinical efficacy trials and studies to validate diagnostic tests and therapies for cryptococcosis in children are scarce. Recommendations are extrapolated from studies in adult populations. Dosing of antifungal agents needs particular attention for the paediatric patient (panel 17; appendix p 87).

Panel 15: **Recommendations for non-*C neoformans* and non-*C gattii* strains of cryptococcus**

- (AIII) As non-*C neoformans* and non-*C gattii Cryptococcus* spp are rarely pathogenic, careful assessment of the laboratory identification and clinical context is required to ascertain clinical significance
- (CIII) For CNS or disseminated disease, treat the same as *C neoformans* CNS infection

Panel 16: **Recommendations for cryptococcosis in pregnancy**

- (AIII) Use liposomal amphotericin B or amphotericin B in induction, consolidation, and maintenance therapy and for the treatment of isolated cryptococcal antigenemia
- (DII) Avoid the use of flucytosine and fluconazole in pregnancy, particularly in the first trimester; their use in the second and third trimester requires careful individualised risk–benefit assessment
- (BIII) Fluconazole can be used after delivery despite its excretion into breastmilk
- (AIII) Apply clinical judgement when considering initiation of antifungal therapy and duration of therapy, factoring in trimester of pregnancy and severity of illness
- (CIII) For asymptomatic cryptococcal antigen in pregnancy, consider intermittent polyene therapy, especially in the first trimester

Panel 17: **Recommendations for paediatric cryptococcosis**

For the treatment of CNS or disseminated disease:

- (AIIt) Induction: amphotericin B 1 mg/kg daily or liposomal amphotericin B 3–4 mg/kg daily plus flucytosine (100–150 mg/kg daily in 4 divided doses) for 2 weeks
- (AIIt) Consolidation: fluconazole 12 mg/kg (maximum 800 mg) daily for 8 weeks
- Maintenance: fluconazole 6 mg/kg daily (maximum 800 mg) for 6–12 months
  - (AIIt) Should be provided for people who live with HIV and are immunocompromised
  - (BIIt) Can be provided for people who are immunocompetent
- (AIII) For the treatment of severe isolated pulmonary diseases: treat the same as CNS disease
- (AIII) Treatment of mild isolated pulmonary disease: fluconazole 12 mg/kg daily (maximum 800 mg) for 6–12 months
- (AIII) Screening is recommended for children older than 10 years living with HIV in high disease prevalence areas

Conclusions

Cryptococcosis and its management is complex and challenging. Adherence to clinical practice guidelines can improve outcomes.[44,175] Although there has been substantial development of evidence from randomised controlled trials over the past 20 years, there are considerable unmet needs (appendix pp 23, 91). Addressing these challenges is particularly crucial in low-income settings, where the burden of disease is high and access to antifungal therapy is inadequate. Equally, more clinical research needs to be done in high-income settings, where host risk profiles are changing and an increasing array of presentations of cryptococcosis are being recognised, necessitating more nuanced and individualised treatment plans.

Contributors

JRP guided the structure, content, and development of the guideline. OAC contributed to the conceptual planning, management, and supervision of the project. CCC and JRP coordinated the work of the

authors. TAB, CCC, MC, FH, TSH, OL, RO, JRP, TCS, AS, and AW contributed to the coordination of data collection, data visualisation, and participants' contributions and communication and wrote the first manuscript draft. All authors contributed towards the literature review, collection and preparation of data, creation of tabled recommendations, and critical review of the manuscript.

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Cryptococcosis

Eileen K. Maziarz, MD[*] and **John R. Perfect, MD**

Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, DUMC Box 102359, 315 Trent Drive, Durham, NC 27710, USA

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INTRODUCTION

Cryptococcosis is an infectious disease with worldwide distribution and wide array of clinical presentations caused by pathogenic encapsulated yeasts in the genus *Cryptococcus*. Currently, there are 2 species of *Cryptococcus* that commonly cause disease in humans: *Cryptococcus neoformans* and *Cryptococcus gattii*. *C neoformans* was first identified as a human pathogen in the late 19th century, but was not recognized as a common cause of human disease until the late 1970s.[1,2] Over the last several decades, as vulnerable populations have expanded, cryptococcal meningitis became an infection of global importance, with up to 1 million new infections annually and significant attributable morbidity and mortality, especially among patients with human immunodeficiency virus (HIV) infection and AIDS.[3] Although *C neoformans and C gattii* share many features of a highly evolved, environmentally savvy yeast, there are important species- and strain-specific differences with respect to geographic distribution, environmental niches, host predilection, and clinical manifestations that should be emphasized. As molecular techniques of identification have evolved, we have gained further insight into the pathobiology of these encapsulated yeasts, and their capacity to adapt to environmental pressures, exploit new geographic environments, and cause disease in both immunocompromised and apparently immunocompetent hosts.[4] Despite increased availability of and success with antiretroviral therapy (ART), the worldwide burden of and mortality associated cryptococcal disease remains unacceptably high, and novel strategies of screening and preemptive therapy offer great promise at making a sustained and much needed impact on this sugarcoated opportunistic mycosis.

[*]**Corresponding author.** eileen.maziarz@dm.duke.edu.

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THE PATHOGENS: CRYPTOCOCCUS NEOFORMANS AND CRYPTOCOCCUS GATTII

Cryptococcus is a genus of basidiomycetous fungi with more than 30 species ubiquitously distributed in the environment. There are only 2 species commonly known to cause human disease, *C neoformans* and *C gattii*. The epidemiology of *C neoformans* is well-characterized and this organism causes disease in both immunocompromised and apparently immunocompetent hosts. *C gattii*, conversely, has historically been regarded as a pathogen of apparently immunocompetent patients. However, preexisting conditions and immunocompromised states, including subclinical immune defects, are also reported as risk factors for infection with this species.[5–8] These species differences in clinical presentation may be primarily determined by variable host predilections, but may also be better characterized as we further our understanding of molecular subtypes.[9–12]

Historically, the genus was further classified into 3 varieties, 5 serotypes (based on structural differences in the polysaccharide capsule), and 8 molecular subtypes (Table 1). Molecular methods of identification have enhanced our appreciation for the significant genetic diversity among the *C gattii–C neoformans* complex and have called into question the current 2 species classification system. Recent proposed taxonomy changes based on the understanding of molecular studies have divided the pathogenic cryptococcal species from their classic divisions into better-defined molecular and genetic divisions. At present, the following divisions have been proposed: *C neoformans* var. *grubii* (serotype A) with 3 genotypes (VNI, VNII, VNB); *C neoformans* var. *neoformans* (serotype D or VNIV); and 5 other cryptic species, *C gattii*, *C bacillisporus*, *C deuterogattii*, *C tetragattii*, and *C decagattii* (serotypes B/C or VGI-IV).[13] Phylogenetic analyses, combined with recognized heterogeneity with respect to virulence, host preference, and antifungal susceptibility do provide evidence to support further taxonomic classification into a 7-species/4 hybrid species scheme (Table 2). The molecular taxonomy of cryptococcal species is a vibrant area of evolution that has allowed for a greater understanding of specific strain characteristics, including fitness and predilection for certain environmental niches[13]; clinical correlations have yet to match this molecular precision, however, and for this review we will tend to lump the yeasts into their historical species designations, *C neoformans* and *C gattii*.

Approximately 95% of cryptococcal infections are caused by *C neoformans* (serotype A) strains with the remaining 4% to 5% of infections caused by *C neoformans* (serotype D) or *C gattii* (serotypes B/C strains). Whereas *C neoformans* var. *grubii* (serotype A) is found worldwide, *C neoformans* var *neoformans* (serotype D) is primarily observed in European countries and *C gattii* has historically been geographically restricted to tropical and subtropical regions, such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa. More recently, *C gattii* has been identified in temperate climates such as Vancouver Island and the Pacific Northwest region of the United States and parts of Europe, suggesting an ecological shift possibly related to global temperature and moisture changes.[4,10–12] Although *C gattii* causes up to 15% of all cases of cryptococcosis in Australia and New Zealand, *C neoformans* remains the predominant species even in these endemic areas.[14] In certain areas of Africa around Botswana, where *C neoformans and C gattii* live

together in the environment, active sexual recombination has been reported.[15] Although outbreaks of cryptococcosis are ongoing among immunocompromised populations worldwide, to date only *C gattii* strains have been reported to produce a geographically defined outbreak of disease.[4]

C neoformans is found throughout the world in association with excreta from certain birds such as pigeons,[16] environmental scavengers such as ameba and sowbugs,[17,18] and in a variety of tree species in their hollows. *C gattii* is commonly associated with several species of eucalyptus trees in tropical and subtropical climates.[19] However, recently as it has emerged as an important pathogen capable of widespread outbreaks within new geographic niches including British Columbia and the Pacific Northwest United States,[4,10–12] it has been associated with temperate trees, such as firs and oaks.[9,20–22]

The life cycle of *Cryptococcus* involves both asexual and sexual forms.[23] The asexual form is the haploid encapsulated yeast that reproduces by mitosis with narrow-based budding and is found in clinical and environmental specimens. The sexual state is observed at present under certain laboratory conditions, resulting in meiosis between 2 mating types (MATa and MATα) to form clamp connections, basidia and basidiospores. The α mating type strains represent the vast majority of clinical and environmental isolates, probably related to their ability to produce haploid fruiting. Even same sex mating between 2 strains of the same type (MATα–MATα) does occur and is thought to produce the infectious spores that cause human infection.[24,25] This nonclassical mating between 2 α–α strains allows for further genetic diversity and is implicated in the production of hypervirulent, clonal strains responsible for the *C gattii* outbreak on Vancouver Island, suggesting that such mechanisms may confer the yeast the ability to exploit new geographic niches.[26,27] Furthermore, there are locations in Botswana where there are equal proportions of MATα and MATa isolates in both environmental and clinical populations, providing evidence that sexual recombination remains active even with the spread worldwide of relatively clonal strains.[15,28]

EPIDEMIOLOGY AND RISK FACTORS

Cryptococcosis was considered an uncommon infection before the AIDS pandemic; however, it was an awakening mycosis giant in the 1970s because it was associated with malignancy, organ transplantation, and certain immunosuppressive treatments. The incidence of disease increased significantly in the mid 1980s, with HIV/AIDS accounting for more than 80% of cryptococcosis cases worldwide.[29–31] Cryptococcal meningitis preferentially occurs in persons with impaired cell-mediated immunity and is a major AIDS-related opportunistic infection as the $CD4^+$ cell count falls below 100 cells/μL. With widespread implementation of successful antiretroviral therapy (ART), the incidence of HIV-associated cryptococcosis has decreased significantly in most developed nations, although the incidence in other at-risk populations has not changed (Table 3).[32] Furthermore, the prevalence of and morbidity and mortality associated with cryptococcal meningitis remain unacceptably high in settings where access to ART and other necessary health care resources are limited, specifically sub-Saharan Africa and parts of Asia. In fact, mortality peaked at approximately 600,000 deaths per year in the first decade of the 21st century; even today, it is likely that cryptococcal meningitis–related deaths approach several

hundred thousand per year.[3] Although both *C neoformans* and *C gattii* can also cause disease in apparently immunocompetent hosts, the percentage of infections owing to *C gattii* in such patients is significantly higher than for *C neoformans*.

Pathogenesis and Host Immunity

Cryptococcal infection occurs primarily by inhalation of the infectious propagules (either poorly encapsulated yeast cells or basidiospores) from environmental reservoirs with deposition into pulmonary alveoli. Traumatic inoculation into tissues has been described[33] and may occur infrequently. The yeast may potentially enter via the gastrointestinal tract, although this entry is less consistent. Primary pulmonary infection is generally thought to be asymptomatic or minimally symptomatic despite high rates of serologic reactivity in children in certain urban settings.[34] Clearance of the infection by the host may occur. However, in many individuals, after yeasts are deposited in alveoli, they encounter alveolar macrophages, which play a central role in the immune response.[35] Host response to cryptococcal infection primarily involves a helper T cell response with cytokines including tumor necrosis factor (TNF), interferon-γ, and interleukin-2, resulting in granulomatous inflammation.[36] In many circumstances, this yeast will establish a latent infection within phagolysosome, with dormant (yet viable) yeasts within the thoracic lymph nodes or a pulmonary granuloma that can persist in an asymptomatic individual for years. When local immunity is suppressed, the yeast can grow and disseminate outside these pulmonary lymph node complexes similar to the pathophysiology that is observed in cases of reactivation tuberculosis or histoplasmosis.[31,37] In some hosts, *C gattii* disease seems to be more likely than *C neoformans* disease to present as a progressive granulomatous pulmonary infection, but less likely to disseminate to the central nervous system (CNS). This general observation has been made in human outbreaks and characterized in mouse models, but there remains substantial overlap between species.[12,31,38] In a patient with severely compromised cellular immunity, the yeasts reactivate and can proliferate at the site of initial infection and can disseminate within phagocytes or as yeast cells and gain access to other body sites.[39] Both direct invasion of the blood–brain barrier via transcytosis of free yeast forms through a series of mechanisms between yeast and host factors[40] and/or transport via macrophages into the CNS (the “Trojan horse” mechanism) seem to occur.[41–43] Whether certain immune states permit additional body sites of latency (eg, the CNS or prostate) have not yet been elucidated fully.

Advances in the molecular biology of *Cryptococcus* have confirmed multiple yeast virulence factors.[44] The 3 classical and prominent virulence factors of *C neoformans* include capsule formation, melanin pigment production, and thermotolerance.[23,36] The prominent antiphagocytic polysaccharide capsule, which is composed of glucuronoxylomannan, is unique to *Cryptococcus* species and is considered an essential virulence factor that has multiple effects on host immunity and can increase in size with exposure to body tissues and fluids.[45,46] In addition, *C neoformans* possesses an enzyme that catalyzes the conversion of diphenolic compounds to form melanin, which, when expressed, may have a biological role to protect the yeasts from host oxidative stresses and which may partially explain the organism’s neurotropism into sites with high concentrations of the diphenolic catecholamines. Finally, the ability to grow at 37°C is a basic part of the virulence composite

for most pathogenic fungi in humans including *Cryptococcus*, and molecular studies have linked high temperature growth with multiple signaling pathways and enzymes that this yeast has acquired or adapted to over time to retain or enhance its mammalian pathogenicity. Other virulence factors include phospholipase and urease production and multiple enzymes associated with protection against oxidative stresses, conferring survival within the phagolysosome.[44] It is estimated that more than 100 genes are important for optimal fitness of the yeast in mammalian hosts. The yeast has even adapted sophisticated mechanisms to escape the intracellular environment by modifying the permeability of the phagosome membrane and via nonlytic exocytosis (vomocytosis), allowing cell-to-cell or host compartment transfer of yeast ant its virulence factors without damage to the host macrophages.[47,48]

The many factors in the immunologic responses to *Cryptococcus* cannot be covered completely in this review, but several observations can be made. First, exposure is frequent and the healthy immunocompetent individual is generally resistant to cryptococcal disease. In fact, even in this group, some apparently normal hosts with cryptococcosis have been found to possess anti-granulocyte macrophage colony stimulating factor antibodies as a potential immune defect.[7,8] Second, the effective immune response is through a helper T cell–supported reaction and anything that weakens it may let cryptococci survive and thrive. This includes destruction of $CD4^+$ cells by HIV, reduction of TNF activity by anti-TNF inhibitors, or the multifaceted immune suppressant effect of corticosteroids. From activated macrophages and not alternative macrophages to the development of protective antibodies over nonprotective antibodies, immunity changes over the course of cryptococcal infections. In fact, even some of our protective host mechanisms might be used against us as surfactant D may be coopted by *Cryptococcus* to gain entry into the lung.[49] Clearly, cryptococcosis emphasizes the Goldilocks paradigm of immunity. It produces disease when immunity is too little or too much, but when the human host immunity is just right, disease does not appear.

CLINICAL MANIFESTATIONS

C neoformans and *C gattii* have a major predilection for establishing clinical disease in the lungs and CNS. Other less frequent body sites of infection include skin, prostate, eyes, and bone/joints. However, it should be emphasized that this yeast can widely disseminate and infect most organs in severely immunosuppressed patients and thus has the ability to appear at any human body site.

Pulmonary Infection

The respiratory tract serves as the most important portal of entry for *Cryptococcus*. Clinical manifestations of pulmonary cryptococcosis range from asymptomatic colonization of the airways or a simple pulmonary nodule on a chest radiograph to life-threatening pneumonia with the presence of an acute respiratory distress syndrome.[50,51] In a normal host, asymptomatic, isolated pulmonary infection can occur in about one-third of patients and can be identified simply by an abnormal chest radiograph. In fact, the most common radiologic findings of cryptococcosis include well-defined single or multiple noncalcified nodules and pulmonary infiltrates (Fig. 1), although pleural effusions, hilar lymphadenopathy, and lung

cavitation may also be observed. Patients with pulmonary cryptococcosis can present acutely with symptoms of pneumonia.[50] For example, in the recent outbreak of *C gattii* infections in Vancouver Island area, several cases of severe, symptomatic pulmonary cryptococcosis in apparently immunocompetent individuals occurred.[12] In an immunocompromised patient, however, cryptococcal pneumonia is usually symptomatic and in some cases can progress rapidly to acute respiratory distress syndrome, even in the absence of CNS involvement. Pulmonary involvement ranges from 10% to 55% of patients with AIDS-associated cryptococcal meningoencephalitis, although CNS symptoms usually predominate the clinical picture.[51]

Serum cryptococcal polysaccharide antigen testing is usually negative in cases of true isolated pulmonary cryptococcosis, but at times can be positive in the absence of CNS involvement or other apparent sites of infection. In immunocompromised individuals with *Cryptococcus* isolated from the lung or other sterile body site, however, a lumbar puncture to rule out CNS disease should be considered regardless of a patient's symptoms or serum antigen titer results. The only setting wherein a screening lumbar puncture may not necessarily be required is a patient with *Cryptococcus* isolated from the lung in the apparently immunocompetent patient without referable CNS symptoms and disease that clinically seems to be limited to the lungs.

Central Nervous System Infection

Clinical manifestations of CNS cryptococcosis include a myriad of signs and symptoms, such as headache, fever, cranial neuropathies, altered mentation, lethargy, memory loss, and signs of meningeal irritation.[2,30,31] Symptoms usually develop over a period of several weeks. However, on some occasions, patients present more acutely or lack typical features, such as headache. In severely immunocompromised, HIV-infected patients with CNS cryptococcosis, the burden of fungal organisms is usually high and can reach levels of more than 1 million yeasts per milliliter of cerebrospinal fluid (CSF). These patients may consequently have a shorter onset of signs and symptoms, greater CSF polysaccharide antigen titers, and higher intracranial pressures than other more immunocompetent individuals.

Although disease severity is determined primarily by host immune factors, different species and/or strains of *Cryptococcus* may produce unique clinical manifestations, which can have implications for management. For instance, in certain areas of the world, *C gattii* has been observed to cause cerebral cryptococcomas and/or obstructive hydrocephalus with or without large pulmonary mass lesions more frequently than *C neoformans*.[12,52,53] These patients with parenchymal brain involvement may have a high intracranial pressure and present with cranial neuropathies. In such patients, who have been observed to respond poorly to antifungal therapy, early neurosurgical intervention to control pressure or ensure a correct diagnosis and longer antifungal treatment courses may be required for a successful outcome.[9,54]

Skin Infection

Cutaneous infections are the third most common clinical manifestations of cryptococcosis and patients can present with a variety of skin lesions. Lesions are often indistinguishable from those owing to other infections; as such, a skin biopsy with culture and histopathology are absolutely essential for definitive diagnosis. Primary cutaneous cryptococcosis is very rare and is usually associated with skin injury and direct inoculation of the yeasts[33]; thus, the appearance of cutaneous lesions usually heralds the presence of disseminated infection. Solid organ transplant recipients on tacrolimus seem to be more likely to develop skin, soft tissue, and osteoarticular infections owing to *Cryptococcus*.[55] Tacrolimus acts on the temperature signaling molecule calcineurin in *Cryptococcus* and has anticryptococcal activity at high temperatures, but it loses this direct antifungal activity as environmental temperatures decrease; this may in part explain the increased frequency of cutaneous lesions in patients receiving calcineurin inhibitors.[56]

Prostate Infection

The prostate is not a rare site for cryptococcal infection, but prostatic cryptococcosis is usually asymptomatic. For instance, latent *C neoformans* infection has been recognized to disseminate in the bloodstream during urologic surgery on the prostate for other indications.[57] The prostate gland may thus serve as an important reservoir for disease relapse in patients with a high fungal tissue burden.[58] Cultures of urine or seminal fluid may still be positive for *Cryptococcus* after initial antifungal treatment of cryptococcal meningitis in poorly controlled AIDS patients,[59] strongly supporting the need for prolonged antifungal treatment to eradicate infection in sanctuary sites in these severely immunocompromised patients.

Eye Infection

In early reports of cryptococcal meningitis before the AIDS epidemic, ocular signs and symptoms were noted in a substantial proportion of cases,[60] such as ocular palsies and papilledema. Several other ocular manifestations of cryptococcosis have been identified, including extensive retinal disease with or without vitritis, which can lead to irreversible blindness.[61] Visual loss may be owing to optic nerve infiltration by yeasts or vascular compromise from intracranial hypertension. The former process results in rapid visual loss with limited effective treatments, whereas the latter phenomenon results in more gradual visual loss and can be interrupted with aggressive management of increased intracranial pressure.

Infection at Other Body Sites

C neoformans can cause disease in essentially any organ of the human body. In fact, the first identification of this fungus from a clinical specimen was from a patient with tibial osteomyelitis in the 19th century.[1] Bone involvement of cryptococcosis typically presents as circumscribed osteolytic lesions in any bone of the body, but most commonly the vertebrae, and cryptococcal osteomyelitis has been associated with underlying sarcoidosis.[62] Bone marrow infiltration can be observed in severely immunocompromised hosts. Fungal peritonitis[63] and cryptococcuria are also reported in several case series. An appreciation for this yeast's protean clinical manifestations is essential, both at the time of initial diagnosis,

as well as when immune defects are restored during treatment and immune restoration phenomena can present.

Immune Reconstitution Inflammatory Syndrome

Restoration of pathogen-specific immunity can result in a phenomenon known as the immune reconstitution inflammatory syndrome (IRIS), an entity that can occur before ("unmasking IRIS") or during ("paradoxic IRIS") antifungal therapy. Cryptococcal IRIS is best characterized in HIV-infected patients with CNS infection and is associated with significant morbidity and mortality.[64–76] In addition, IRIS is estimated to occur in 5% to 11% of solid organ transplant recipients with cryptococcal infection and is associated with increased risk of allograft failure[77–83] and may also be observed in non-HIV, nontransplant patients.[84] Proposed criteria for IRIS in HIV-associated disease include onset of symptoms within 12 months of ART initiation (with concomitant $CD4^+$ recovery).[85] These criteria are imprecise and do not address all populations at risk (Box 1). As such, it is incumbent upon the treating provider to have a high level of suspicion for this entity, as opposed to alternative diagnoses, which include progressive infection (from inadequate antifungal therapy, direct antifungal drug resistance, or persistent immune deficits), coinfection with other opportunistic infections, malignancy, or drug toxicity.

Cryptococcal IRIS is thought to represent a dysregulated reversal of a Th2 (anti-inflammatory) to a strong helper T cell (pro-inflammatory) immune response in the setting of immune recovery.[86] Multiple factors are thought to be associated with future IRIS episodes, including high yeast burden at baseline, ineffective host immune response to initial infection, and rapid restoration of immunity.[67,73] Host immune responses in various compartments may not be uniform and are likely influenced by baseline parameters at the site.[87] Differences in baseline CSF cytokine and chemokine expression are thought to facilitate the development of cryptococcal IRIS, potentially via myeloid cell trafficking to the CNS and, consequently, production of excessive inflammation.[88,89] In fact, evidence of increased macrophage activation and linked CSF pleocytosis have been observed in patients receiving early ART and may mediate increased mortality, even before recognition of the clinical syndrome of IRIS.[87]

Clinical features of cryptococcal IRIS are similar to active cryptococcal infection itself, most commonly presenting as CNS disease, although lymphadenitis, pneumonitis, multifocal disease, soft tissue involvement, and mediastinitis have all been reported.[85,90] Meningeal disease is the most serious presentation.[85] A hallmark finding is suppurative or necrotic granulomatous inflammation with yeast forms seen on histopathology of infected tissues despite negative cultures.[77,80,90,91] Despite changes in inflammatory markers, there are no reliably specific diagnostic tests for IRIS, and establishing the diagnosis presents a considerable clinical challenge, especially with atypical presentations or manifestations at distant sites.[69,92] CSF opening pressure and white blood cell count[67,68,73] at the time of an IRIS event are significantly higher than baseline values for individual patients, which combined with negative cultures, may help to distinguish IRIS from relapsed infection.[70]

Management of cryptococcal IRIS is largely based on expert opinion.[93] First, ensuring the efficacy of antifungal therapy is essential[94,95]; in the absence of disease relapse or direct

antifungal drug resistance, modification of antimicrobial therapy is generally not indicated.[93] A significant proportion of minor cases simply improve without specific treatment.[65,66,76] Corticosteroids have been shown to decrease the need for hospitalization and improve short-term quality of life and functional status in paradoxic tuberculosis-associated IRIS.[96] Although steroids may be essential in treating a serious life-threatening CNS IRIS episode owing to *Cryptococcus*, they should not be used for prevention of IRIS or to control CNS pressure, and may be harmful in some cases.[97] Immunomodulatory agents including those with anti–TNF-α activity have been used in cases of steroid-refractory IRIS.[65,98–101] Other strategies, including therapeutic lumbar drainage for intracranial hypertension[93,102] and, at times, surgical drainage of suppurative lymph nodes,[86,91] are important adjunctive measures that may be considered in severe disease. Continuation of ART in the setting of IRIS is generally recommended and has been performed safely.[66,71,92,103,104]

LABORATORY DIAGNOSIS

Definitive diagnosis of cryptococcosis is made by isolation of *Cryptococcus* from a clinical specimen or direct detection of the fungus by means of India ink staining of body fluids. There are several other methods used for the diagnosis of cryptococcosis, including histopathology of infected tissues and serologic methods. Molecular methods, although available and extensively used for research purposes, are not used currently in routine clinical practice.

Direct Examination/India Ink

The most rapid method for diagnosis of cryptococcal meningitis is direct microscopic examination for encapsulated yeasts by India ink preparation of CSF. *Cryptococcus* can be visualized as a globular, encapsulated yeast cell with or without budding, ranging in size from 5 to 20 μm in diameter (Fig. 2). The sensitivity of India ink staining of CSF depends on fungal burden and is reported to be 30% to 50% in non–AIDS-related cryptococcal meningitis and up to 80% in AIDS-related disease. False positives can result from intact lymphocytes, other tissue cells and nonviable yeast forms, which further limits the diagnostic utility of direct microscopy of CSF for cryptococcal meningitis.[105]

Culture and Identification

Cryptococcus can be cultured readily from biologic samples such as CSF, sputum, and skin biopsy on routine fungal and bacterial culture media. In adults with HIV-associated cryptococcal meningitis, CSF and blood cultures are positive in up to 90% and 70% of patients, respectively (reviewed in[106]). Colonies are usually observed on solid agar plates after 48 to 72 hours incubation at 30°C to 35°C in aerobic conditions and will appear as opaque, white-to-cream colonies that may turn orange-tan or brown after prolonged incubation. The mucoid appearance of the colony is related to the capsule size around the yeasts. Despite relatively rapid growth for most strains, cultures should be held for up to 4 weeks, particularly for patients receiving antifungal treatment.

Cytology and Histopathology

Cryptococcus can be identified by histologic staining of tissues from the lung, skin, bone marrow, brain, and other organs.[107] Histopathologic staining and cytology of centrifuged CSF sediment and other bodily fluids are more sensitive than the India ink staining method.[108–111] The organism is observed as a yeast that reproduces by narrow-based budding. The yeast is best identified by special stains that label the polysaccharide capsule including mucicarmine, periodic acid-Schiff, and Alcian blue stains.[2] The Fontana–Masson stain identifies melanin in the yeast cell wall. Other fungal stains such as Calcofluor, which binds fungal chitin, or Gomori methenamine silver, which stains the fungal cell wall, are also used to identify the organism from clinical specimens.[2,109]

Serology

The diagnosis of cryptococcosis improved significantly with the development of serologic tests for the cryptococcal polysaccharide capsular antigen (CrAg), which is shed during infection. Latex agglutination and enzyme immunoassay techniques have been available widely (using both serum and CSF), the former of which had been the most commonly used methodology until recently, with overall sensitivities and specificities of 93% to 100% and 93% to 98%, respectively.[112,113] False-positive results of latex agglutination testing usually have initial reciprocal titers of 8 or less,[112] whereas false negatives can be seen owing to a prozone effect in the setting of extremely high antigen titers, which can be overcome with dilution.[114] Low fungal burden, as in chronic low-grade meningitis or in the very early stages of infection, and improper specimen storage can also cause false-negative results in latex agglutination tests.[115] Recently, a lateral flow assay was approved for use in serum and CSF, with sensitivity and specificity of greater than 98% in both specimen types (including whole blood from finger stick samples) and sensitivity of 85% in urine.[116–123] The semiquantitative test offers many advantages over the other serologic methods, including rapid turnaround (approximately 15 minutes), minimal requirements for laboratory infrastructure, stability at room temperature, low cost, and wider capture of *C gattii* polysaccharides.[116] Combined with these advantages, the assay's excellent performance across a broad range of clinical settings, including settings with low burden of HIV infection and high rates of *C gattii* infection,[100–104] make it an attractive option for point-of-care testing in both resource-available and resource-limited settings.[116,117,124]

Baseline cryptococcal polysaccharide antigen titers in serum and CSF correlate with fungal burden and carry prognostic significance in patients with cryptococcal meningitis.[122,125,126] However, there is limited value in serial monitoring of antigen titers acutely in assessing treatment response, because the kinetics of antigen clearance is a slower and less predictable marker of treatment response than quantitative culture.[122,127] Quantitative CSF yeast culture and its serial use for measurement of effective fungicidal activity has become a primary research tool for effectiveness of therapeutic regimens.[128] The quantitative yeast count has been correlated with outcome[129] and effective fungicidal activity has correlated with success of antifungal regimens, including survival.[95,128,130] Despite a decade of use and validation of its effectiveness in clinical studies, the use of quantitative CSF yeast culture for the determination of effective fungicidal activity has not yet become a part of routine clinical practice.

TREATMENT

Basic Principles

Amphotericin B deoxycholate (AmBd) is the cornerstone of treatment for severe cryptococcal infection, including meningoencephalitis. Treatment is summarized in Table 4. A standard induction dose of 0.7 to 1 mg/kg/d is recommended. Liposomal amphotericin B (3–6 mg/kg/d) has become a preferred alternative with similar outcomes and less nephrotoxicity, and is recommended specifically for primary induction in patients at risk for renal dysfunction.[93,131,132] Flucytosine (5-FC) is used in combination therapy with AmBd as first-line therapy in cryptococcal meningitis or severe pulmonary cryptococcosis at a dosage of 100 mg/kg/d in divided doses.[133,134] This combination represents the most potent fungicidal regimen, with faster CSF sterilization and fewer relapses, and is associated with lower attributable mortality.[133–139] Because the interruption of induction therapy is associated with poorer outcome, in resource-available areas the liposomal product has become the preferred polyene. Unfortunately, there are still no comparative studies with 5-FC combined with lipid formulations of amphotericin B as opposed to AmBd. Early mycological failure (defined as persistently positive CSF cultures at day 14) correlates with late treatment failure and poor outcome,[140] and lack of 5-FC is independently associated with both early[141] and late[137] mycological failure. This improved fungicidal activity of combination therapy translates into a direct survival benefit compared with AmBd monotherapy.[135] 5-FC should be dose adjusted for renal dysfunction, with therapeutic drug monitoring to decrease its primary side effect of bone marrow suppression.[142] There are emerging data that lower doses of 5-FC in combination with amphotericin may demonstrate similar fungicidal activity.[138]

Although combination induction therapy remains the recommended first-line therapy for severe cryptococcosis, 5-FC availability is limited in settings where the disease burden and mortality rates are the highest. Alternative combination therapies have been investigated, the most efficacious of which is AmBd plus fluconazole (800 mg/d), which results in improved rates of fungal clearance, neurologic recovery, and survival compared with AmBd alone or in combination with lower doses of fluconazole.[143,144] This combination offers a more feasible and potentially viable option for effective initial therapy in settings where access to 5-FC is limited. Optimizing treatment outcomes without exhausting limited resources is critical in many settings. Standardized fluid and electrolyte supplementation protocols for patients treated with amphotericin B in these resource-limited settings have been associated with improved early survival.[145] Additionally, shorter courses of amphotericin B in combination with other agents may be considered in these settings, although clinical endpoints for such regimens have not been rigorously evaluated.[146,147] An ongoing trial evaluating the combination of intermittent dosing of high-dose of liposomal amphotericin B with high-dose fluconazole in resource-limited settings is underway to address this unanswered question (AmBition-CM, www.controlled-trials.com/ ISRCTN10248064). Additional alternative induction regimens are available in the guidelines but their use is not encouraged based on limited data of the success with these regimens.[148] Fluconazole monotherapy for meningitis is not recommended for induction given its fungistatic nature, poor success, and higher relapse rates as well as increased rates of resistance in relapse.[93,94]

However, in areas without access to AmBd, high doses (≥1200 mg/d) of fluconazole should be commenced.

A 3-stage regimen of induction, consolidation, and maintenance is standard treatment for cryptococcal meningitis in all patients, irrespective of host risk factors.[93,133] In HIV-infected patients, initial induction treatment usually begins with combination therapy as described, followed by consolidation treatment with fluconazole (400–800 mg/d) for 8 weeks in patients who have demonstrated favorable response. Longer courses of both induction (eg, 6 weeks) and consolidation (or "eradication") therapy have been suggested in *C gattii* meningoencephalitis, irrespective of host immune status, owing to the observed severity of neurologic disease in this group of patients,[11,52,53] but this is not certain and in general *C gattii* should be treated similarly to *C neoformans*. After consolidation, long-term suppression is commenced with oral fluconazole (200–400 mg/d). This approach has decreased rates of relapse from approximately 40% to less than 5% in severely immunosuppressed patients.[149] Secondary prophylaxis is discontinued after 1 to 2 years of antifungal therapy in patients who respond to ART with an increase in $CD4^+$ cell counts to greater than 100 cells/μL and a decrease in HIV viral load to undetectable levels for at least 3 months.[93,150,151] The other triazoles (itraconazole, voriconazole, and posaconazole) are active against cryptococcal isolates in vitro and, in combination with AmBd, may have similar fungicidal activity to 5-FC,[144] but owing to differences in bioavailability, CSF penetration, drug interactions, cost, and lack of robust studies in cryptococcosis, these agents are not recommended as first-line agents for consolidation or maintenance therapy. However, they may have a role in refractory cases.[152–155]

Timing of Antiretroviral Therapy

In HIV-associated cryptococcal infection, ART has a major impact on long-term prognosis. However, several studies have suggested an increased risk of IRIS among HIV-infected patients initiated on ART early after the diagnosis of an opportunistic infection.[64,65,156] More contemporary studies have demonstrated conflicting results regarding outcomes of cryptococcal infection based on timing of ART initiation,[103,157,158] and studies in tuberculosis have demonstrated a survival benefit with earlier ART (despite increased rates of IRIS).[159,160] Recently, the Cryptococcal Optimal ART Timing Trial (COAT) provided some definitive guidance to delay initiation of ART in patients with cryptococcal meningitis for a minimum of 4 weeks after starting antifungal therapy. This randomized trial demonstrated improved survival in patients with cryptococcal meningitis in whom ART initiation was deferred for up to 5 weeks after diagnosis as compared with immediate ART (within 1–2 weeks).[161] Although increased rates of IRIS observed with early ART did not attain statistical significance, markers of macrophage activation were increased in this early group, suggesting that subclinical or compartmentalized IRIS may occur and influence mortality.[87,161]

Organ Transplant Recipients

Organ transplant recipients with CNS cryptococcal infection are managed similarly to HIV-infected patients, although lipid formulations of amphotericin B are preferred to limit nephrotoxicity.[93] A longer course of induction therapy is indicated if CSF cultures remain

positive at 2 weeks, because this scenario is associated with an increased 6-month mortality.[162] Relapse rates among organ transplant recipients are lower than in HIV-associated disease, such that a shorter course of maintenance therapy can be pursued following standard consolidation, but generally these patients are treated for 1 year.[93,162] Drug interactions between fluconazole and immunosuppressive agents should be anticipated owing to CYP3A4 inhibition, and a preemptive reduction in calcineurin inhibitors should be considered. Management of immunosuppression in the setting of cryptococcal infection requires recognition of the increased risk of IRIS.[77,80,163] Thus, stepwise reduction in immunosuppression is recommended, although the approach should be individualized for each patient.

Non–HIV-Infected, Nontransplant Patients

Very few prospective data are available on the management of cryptococcal infection in the apparently immunocompetent host lacking classical risk factors for cryptococcosis.[134] This heterogeneous group of patients is diagnosed later, irrespective of disease severity.[32,84] Recommendations for longer induction therapy (≥4 weeks) are based on the recognition of poorer outcomes and higher mortality rates in this group of patients both in early[134,164] as well as contemporary[32] studies. However, in patients with good prognostic factors and excellent antifungal induction response, 2-week induction therapy can be successful. Therapy should be extended further if 5-FC is not included (or there is limited exposure to this drug) in the induction regimen.[93] Recommendations for consolidation and maintenance parallel those for HIV-infected patients and reflect high relapse rates (30%) within the first year before the introduction of consolidation and maintenance antifungal strategies.[93,134] Criteria for stopping treatment in these patients include resolution of symptoms and at least 1 year of suppressive antifungal therapy.

Management of Intracranial Pressure

Along with the optimization of antifungal therapy, management of increased intracranial pressure is critically important in cryptococcal meningoencephalitis. Intracranial hypertension frequently corresponds with CSF fungal burden, potentially mediated by CSF outflow obstruction by clumped yeast forms even during early therapy, and is associated with increased morbidity and mortality.[97,165] Intracranial imaging should be performed before lumbar puncture if impaired mentation or focal neurologic deficits are present. A baseline CSF opening pressure should be obtained in all patients. Aggressive attempts to control increased intracranial pressure should occur when patients are symptomatic, although emerging data suggest there may be benefit to therapeutic lumbar punctures, irrespective of baseline opening pressure in resource-limited settings.[166] Treatment options for managing acutely elevated intracranial pressure include repeated lumbar punctures (daily until pressure and symptoms are stable for >2 days), lumbar drain insertion, ventriculostomy, or ventriculoperitoneal shunt, if obstructive hydrocephalus develops.[97] Consideration of early neurosurgical consultation has been recommended in cases of meningoencephalitis owing to *C gattii* where CNS inflammation is often severe.[52,53] Medical treatments such as corticosteroids (unless IRIS suspected or in cases of severe *C gattii* infection), mannitol, and acetazolamide are generally not recommended.[52,53,129,167] If

shunt placement is necessary, CSF sterilization is not required before insertion, which can be performed once appropriate antifungal therapy has been commenced.[168]

Persistent and Relapsed Infection

Persistent and relapsed infection must be distinguished from IRIS. Persistent disease has been defined as persistently positive CSF cultures after 1 month of antifungal therapy, whereas relapse requires new clinical signs and symptoms and positive cultures after initial improvement and fungal sterilization.[93] Surrogate markers, including biochemical parameters, India ink staining, and cryptococcal antigen titers, are insufficient to define relapse or alter antifungal therapy. General recommendations for management in these persistent or relapsed cases include resumption of induction therapy, often for a longer duration and at increased dosages, if tolerable, and pursuance of comparative antifungal susceptibility testing.[93] Although primary direct antifungal resistance to azoles and polyenes is rare, decreased susceptibility to fluconazole has been observed in some cases of culture-positive relapse.[94] There has not yet been a convincing minimum inhibitory concentration breakpoint for cryptococcal species in antifungal susceptibility testing; thus, the importance of comparative minimum inhibitory concentration testing with the original isolate in cases where resistance is suspected cannot be overemphasized.[169,170]

Nonmeningeal Disease

Although isolation of *Cryptococcus* from respiratory tract specimens can occur in the absence of clinical disease (colonization), it is incumbent upon the treating clinician to assess for subclinical disease or potential for complications when *Cryptococcus* is isolated from any clinical specimen. In the absence of immune compromise, airway colonization carries a low risk for invasive disease and treatment can be deferred; although in most cases, given the safety profile of fluconazole, many clinicians favor treatment in all patients in whom *Cryptococcus* is isolated. In immunosuppressed patients with isolated pulmonary cryptococcosis, however, treatment is recommended to prevent dissemination.[93] This group of patients should be evaluated for systemic disease (including blood and CSF cultures as well as CrAg testing from serum and CSF) to optimize treatment. In any patient in whom cryptococcemia is identified, symptoms are severe, or CSF examination reveals asymptomatic CNS involvement, treatment for cryptococcal meningitis is recommended.[93] The potential for severe pulmonary infection owing to *C gattii* should be appreciated when *Cryptococcus* is isolated from respiratory cultures in settings where this species is endemic[11,12,52,53,171]; however, to date, there are no convincing data that species identification is required to optimally select antifungal therapy, and disease severity remains the critical factor in determining initial treatment. Cerebral cryptococcomas often can be managed with prolonged antifungal therapy without the need for surgical removal unless mass effect or other evidence of obstruction is identified. A longer induction phase with AmBd plus 5-FC, followed by 6 to 18 months of consolidation therapy with fluconazole (400–800 mg/d) is recommended. Localized infection of extrapulmonary nonmeningeal sites can occur occasionally with direct inoculation, but more commonly represents disseminated infection. Suspicion for the latter must be maintained when *Cryptococcus* is identified from a sterile body site, because management strategies differ if disseminated disease is present. Consultation with ophthalmology is indicated in cases of cryptococcal eye disease.[93]

Screening and Prevention

There is no question that early identification of HIV infection and initiation of ART in patients before progression to severe immunodeficiency is the most effective intervention at reducing the global burden of cryptococcosis and other opportunistic infections. However, despite increased access to ART worldwide, late presentations of HIV infection still occur and the burden of severe cryptococcal infection and related mortality remains disproportionately represented in these populations.

Fluconazole prophylaxis has been shown to be effective for preventing cryptococcosis in patients with advanced AIDS in endemic areas[172,173]; however, universal prophylaxis is relatively cost ineffective,[124] has not been shown to offer a survival benefit,[174] and may add to the appearance of azole-resistant strains. As such, this approach is not recommended currently.

Given that mortality from cryptococcal meningitis remains unacceptably high, alternative management strategies have been evaluated and implemented in resource-limited settings, specifically a “screen and treat” approach using serum cryptococcal antigen (CrAg) testing followed by preemptive fluconazole therapy in CrAg-positive patients. CrAg is an early marker of cryptococcal disease, detectable in serum a median of 22 days before the onset of symptoms, and is both highly predictive of incident cryptococcal meningitis and an independent risk factor for death during the first year of ART.[175–177] This approach is associated with a decreased incidence of cryptococcal meningitis and improved survival among patients with advanced HIV disease and has been successfully implemented in several resource-limited settings, with a baseline prevalence of asymptomatic cryptococcal antigenemia of 5% to 13%.[177,178] Moreover, analyses have consistently demonstrated both the cost effectiveness and survival advantage of a “screen and treat” approach, as compared with standard of care or universal fluconazole prophylaxis, at CrAg prevalences as low as 0.6%.[178–180] As access to lateral flow assay testing in these settings is increased, the cost effectiveness is likely to be greater than initially reported. The World Health Organization now recommends implementation of CrAg screening and preemptive fluconazole therapy in ART-naïve adults with a CD4 count of less than 100 cells/mm^3 before initiating ART in endemic settings.[181] Several nations in sub-Saharan Africa have since operationalized programs as a part of the existing HIV infrastructure. Several unanswered questions remain, however, including the feasibility of implementation, the dose and duration of preemptive fluconazole, the criteria for lumbar puncture in asymptomatic patients, and the potential impact on azole resistance. Some data suggest a ‘screen and treat’ would be cost effective, even in resource-rich settings, although this is currently not part of standard practice, despite recent reports of CrAg prevalence of more than 3% in the United States.[176,182] Routine screening for cryptococcal infection and/or prophylaxis are not recommended in solid organ transplant recipients, even when immunosuppression is augmented in patients with previously (appropriately) treated infection.[183]

In the arena of direct immune modulation for cryptococcosis management, aside from the use of ART, progress has been slow. First, although both cryptococcal glucuronoxylomannan–tetanus toxoid conjugate vaccine and specific monoclonal antibodies to cryptococci have been developed, clinical trials have not been initiated to determine their

usefulness in human subjects.[184,185] The use of immune stimulation with recombinant gamma-interferon has both immunologic support and 2 positive clinical trials,[186–189] but has only been used in refractory cases and likely reflects concerns about precisely judging immune stimulation when IRIS can be a deadly problem.

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KEY POINTS

- Cryptococcosis is a major invasive fungal infection that is capable of widespread disease outbreaks in both immunocompromised and apparently immunocompetent hosts.
- Molecular advances continue to enhance our understanding of *Cryptococcus* and provide insight into its evolution into a pathogen of global importance.
- Diagnosis has improved with the introduction of point-of-care diagnostic assays.
- Screening and preemptive antifungal therapy offer great promise in making a significant impact in this highly deadly opportunistic mycosis.

Box 1

Suggested diagnostic criteria for the immune reconstitution inflammatory syndrome

New appearance or worsening of any of the following:

Clinical or radiographic manifestations consistent with an inflammatory process:

> **Central nervous system:** Contrast-enhancing lesions on neuroimaging (computed tomography or MRI); cerebrospinal fluid pleocytosis (ie, >5 white blood cell count per μL); increased intracranial pressure (ie, opening pressure of ≥20 mm H_20), with or without hydrocephalus.
>
> **Pulmonary:** Nodules, cavities, masses or pleural effusions.
>
> **Other:** Lymphadenopathy, skin, soft tissue, osteoarticular lesions.

Histopathology showing granulomatous lesions.

Symptoms occurring during receipt of appropriate antifungal therapy[a] that cannot be explained by a newly acquired infection or another process (neoplasm, etc).

Negative results of cultures, or stable or reduced biomarkers for the initial fungal pathogen during the diagnostic workup for the inflammatory process.

All 3 criteria must be present for a positive diagnosis.

[a] Exclude intrinsic and de novo drug resistance, and suboptimum drug concentrations.

Adapted from Sun H, Alexander B, Huprikar S, et al. Predictors of immune reconstitution syndrome in organ transplant recipients with cryptococcosis: implications for the management of immunosuppression. Clin Infect Dis 2015;60(1):36–44; and Singh N and Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect Dis 2007; 7:398.

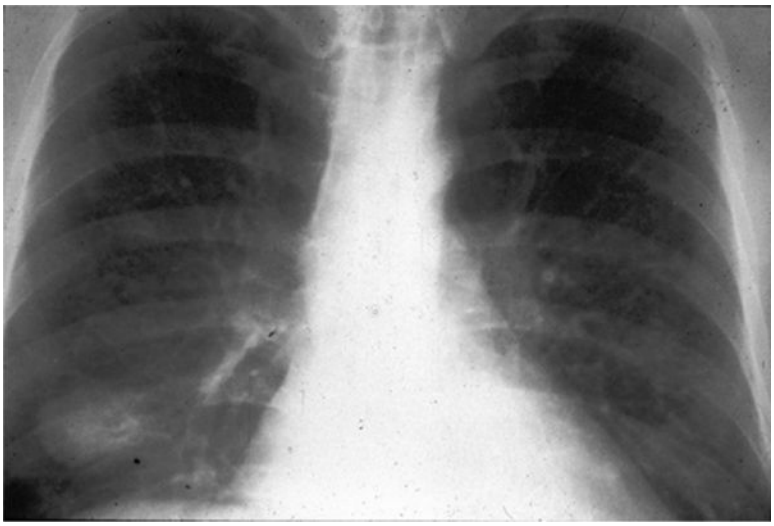

Fig. 1.

Solitary pulmonary nodule. In an asymptomatic patient with isolated pulmonary cryptococcosis. (*Courtesy of* J. R. Perfect, MD, Durham, NC.)

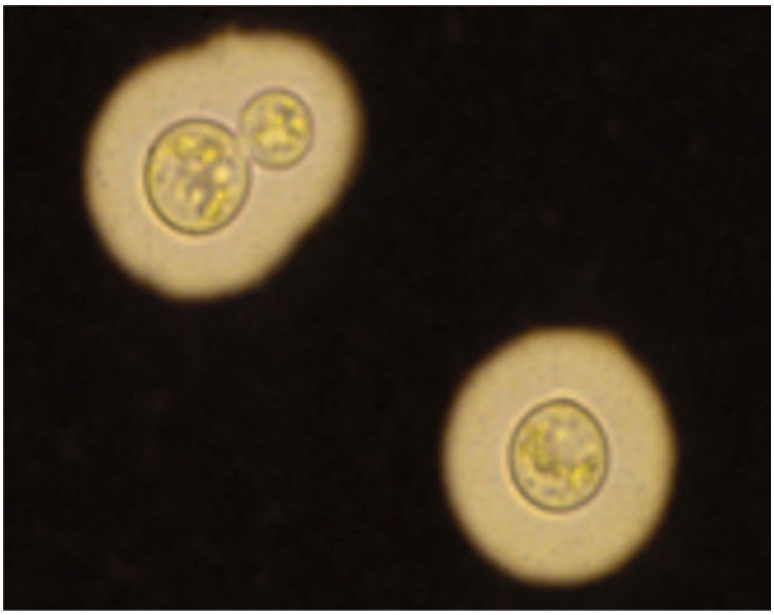

Fig. 2.

India ink staining. Encapsulated yeast seen on India ink preparation of cerebrospinal fluid in a patient with cryptococcal meningitis. (*Courtesy of* J. R. Perfect, MD, Durham, NC.)

Table 1

Current classification of pathogenic *Cryptococcus* species

Serotype	Species and Varieties	Molecular Types
A	*C neoformans* var. *grubii*[a]	VN I, VN II
B	*C gattii*	VG I, VG II, VG III, VG IV
C	*C gattii*	VG I, VG II, VG III, VG IV
D	*C neoformans* var. *neoformans*	VN IV
AD	*C neoformans*	VN III

[a]Responsible for the vast majority of disease owing to C neoformans worldwide.

Adapted from Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gatti/Cryptococcus neoformans* species complex. Fungal Genet Biol 2015;78:17.

Table 2

Proposed taxonomy changes for the *Cryptococcus neoformans*/*C gattii* complex

Current Species Name	Genotype by RFLP	Proposed Species Name
C neoformans var. *grubii*	VNI	*C neoformans*
	VNII	
	VNIII	
C neoformans var. *neoformans*	VNIV	*C deneoformans*
C neoformans intervariety hybrid	VNIII	*C neoformans* × *C deneoformans* hybrid
C gattii	VGI	*C gattii*
	VGIII	*C bacillisporus*
	VGII	*C deuterogattii*
	VGIV	*C tetragattii*
	VGIV/VGIIIc	*C decagattii*
C neoformans var. *neoformans* × *C gattii* AFLP4/VGI hybrid	—	*C deneoformans* × *C gattii* hybrid
C neoformans var. *grubii* × *C gattii* AFLP4/VGI hybrid	—	*C neoformans* × *C gattii* hybrid
C neoformans var. *grubii* × *C gattii* AFLP6/VGII hybrid	—	*C deneoformans* × *C deuterogattii* hybrid

Adapted from Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gatti/Cryptococcus neoformans* species complex. Fungal Genet Biol 2015;78:17.

Table 3

Risk factors for *Cryptococcus* infection

HIV infection	**Rheumatologic diseases[a] Systemic lupus erythematosus Rheumatoid arthritis**
Corticosteroid and/or immunosuppressive therapies	Idiopathic $CD4^+$ lymphopenia
Solid organ transplantation[a]	Chronic liver disease (decompensated)[b]
Malignant and lymphoproliferative disorders[a,b]	Renal failure and/or peritoneal dialysis
Sarcoidosis	Hyper-IgM syndrome or hyper-IgE syndrome
Treatment with monoclonal antibodies (etanercept, infliximab, alemtuzumab)	*Diabetes mellitus*[c]
Anti-GM CSF antibodies	—

Abbreviations: GM CSF, granulocyte macrophage colony stimulating factor; HIV, human immunodeficiency virus; Ig, immunoglobulin.

[a] Immunosuppression for these conditions may influence risk.

[b] Poor prognosis especially among patients with hematologic malignancy.[32]

[c] Historically considered a risk factor but may reflect the frequency of condition rather than specific risk to an individual. Not found to be a risk factor in.[190,191]

Adapted from Casadevall A, Perfect JR. Cryptococcus neoformans. Washington, DC: ASM Press; 1998.

Table 4

Treatment recommendations for HIV-associated cryptococcal meningoencephalitis

	Duration
Induction therapy	
Primary regimen	
AmBd (0.7–1 mg/kg/d) plus flucytosine (5-FC) (100 mg/kg/d)[a]	2 wk
Alternative regimens[b]	
If 5-FC intolerant or unavailable: AmBd (0.7–1 mg/kg/d) or L-AMB[c] (3–4 mg/kg/d) or ABLC (5 mg/kg/d)	4–6 wk
AmBd (0.7–1 mg/kg/d) plus fluconazole (800 mg/d)	2 wk
Fluconazole (≥800 mg/d, preferably 1200 mg/d) plus 5-FC (100 mg/kg/d)	6 wk
Fluconazole (800–2000 mg/d, preferably 1200 mg/d)	10–12 wk
Itraconazole (200 mg BID)	10–12 wk
Consolidation therapy	
Fluconazole (400 mg/d)	8wk[d]
Maintenance or suppressive therapy	
Fluconazole (200 mg/d)	≥1 y[e]
Alternative reqimens[a]	
Itraconazole (200 mg BID)	≥1 y[e]
AmBd (1 mg/kg IV per week)	≥1 y

Abbreviations: 5-FC, flucytosine; ABLC, amphotericin B lipid complex; AmBd, amphotericin B deoxycholate; BID, twice daily; L-AMB, liposomal amphotericin B.

[a] L-AMB, 3–4 mg/kg/d or AmB lipid complex (ABLC; 5 mg/kg/d) for patients predisposed to renal dysfunction.

[b] Can be considered as alternative regimen when primary regimen not available but not encouraged as equivalent substitutes.

[c] L-AMB can be safely administered in doses as high as 6 mg/k/d.

[d] Initiate highly active antiretroviral therapy approximately 4 weeks after beginning antifungal regimen.

[e] After 1 year of therapy, if successful response to antiretroviral drugs (CD4 count ≥ 100 and viral load low or undetectable for >3 months), can consider discontinuation of antifungal therapy. Consider reinstitution if CD4 count is <100.

Adapted from Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guide lines for the management of cryptococcal disease: 2010 update by the Infectious Disease Society of America. Clin Infect Dis 2010;50:291–322.

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The Brief Case: the Cryptic *Cryptococcus*

Anisha Misra,[a] Zachary A. Yetmar,[b] Amber A. Milone,[a] Lydia A. Ruefthaler,[a] Nancy L. Wengenack,[a] Paschalis Vergidis,[b] Elitza S. Theel[a]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[b]Division of Public Health, Infectious Diseases, and Occupational Medicine, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA

KEYWORDS *Cryptococcus*, fungemia, solid-organ transplantation, capsule deficient, antigen, unencapsulated

CASE

A 64-year-old male with a history of nonalcoholic steatohepatitis, alpha-1-antitrypsin deficiency, and an orthotopic liver transplant completed 5 months prior, presented to the hospital with a 1-day history of fever and fatigue. The patient had recently undergone evaluation for rash and pancytopenia and was ultimately diagnosed with graft-versus-host disease (GVHD) affecting his skin, gastrointestinal tract, and bone marrow. High-dose prednisone and twice-weekly etanercept were added to his prior immunosuppressant regimen of tacrolimus and mycophenolate. During a subsequent outpatient evaluation for bone marrow transplantation, he developed fever and fatigue again and was admitted to the hospital for further evaluation.

On presentation the patient was febrile to 38.2°C. His previous GVHD-related rash had resolved, and he denied any other localized symptoms. Initial laboratory studies were significant for a complete blood count showing pancytopenia, with a leukocyte count of 0.1 $\times$ 10^9/L (normal range, 3.4 $\times$ 10^9 to 9.6 $\times$ 10^9/L); electrolytes, renal function, and hepatic function testing were all within normal limits. Serum cytomegalovirus quantitative PCR (Roche Diagnostics, Indianapolis, IN) and *Cryptococcus* antigen (CrAg lateral flow immunoassay [LFA], IMMY Diagnostics, Norton, OK) testing were also performed, and results were negative. Chest X-ray did not show any focal consolidation or other acute findings. Bacterial blood cultures were obtained and grew methicillin-susceptible *Staphylococcus aureus* from the anaerobic bottle after 17 h of incubation. The patient was initially started on vancomycin and cefepime and later transitioned to cefazolin, 2 g every 8 h, once the susceptibility test results were available. However, at 86 h of incubation an aerobic blood culture bottle flagged positive and subculture onto sheep blood agar yielded *Cryptococcus neoformans*, which was identified by the BioFire Blood Culture Identification 2 (BCID2) panel (bioMérieux, Salt Lake City, UT) and confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics) using both the Bruker research-use-only and Mayo Clinic-developed spectrum library databases. Blood cultures obtained on each of the following 2 days also grew *C. neoformans*.

A repeat serum CrAg LFA performed 9 days later was also negative. To rule out postzone effect, the sample was serially diluted to 1:1,280; all dilutions remained negative on all samples tested. The *C. neoformans* isolate was subcultured on Sabouraud's dextrose agar (SDA) at 30°C and 37°C. After 4 days, colonies were examined using India ink staining, which did not demonstrate the presence of a capsule (Fig. 1). Although the absence of capsule production *in vitro* is not definitive evidence of inhibited capsule production *in vivo*, together with the negative serum CrAg result, the findings were highly suggestive of infection with a capsule-deficient *Cryptococcus* isolate.

The patient was initiated on liposomal amphotericin B, 4 mg/kg every 24 h, and flucytosine, 25 mg/kg every 6 h. Due to progressive thrombocytopenia, lumbar puncture

Editor Carey-Ann D. Burnham, Pattern Bioscience

Address correspondence to Elitza S. Theel, theel.elitza@mayo.edu.

The authors declare no conflict of interest.

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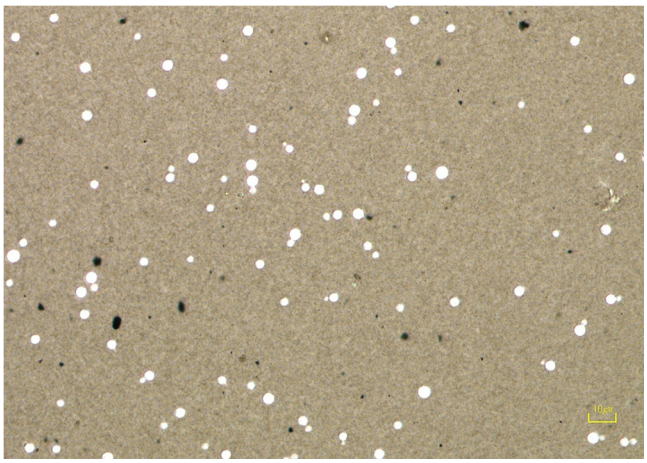

FIG 1 India Ink stain of capsule-deficient *Cryptococcus*. In the presence of a capsule India ink displaces around the capsule, creating a clearing around the yeast cells. Lack of clearing around the cells is indicative of unencapsulated *Cryptococcus*.

was unable to be performed safely to collect cerebrospinal fluid (CSF) for further analysis (e.g., CrAg, fungal culture), although magnetic resonance imaging of the brain was not suggestive of cryptococcosis. The patient received combination antifungal therapy for 14 days, with clinical improvement and blood culture clearance. He was then transitioned to posaconazole, 300 mg daily, for both consolidation therapy and antifungal prophylaxis while neutropenic. Cefazolin was continued for a total of 4 weeks to treat his *S. aureus* bloodstream infection. He underwent matched, unrelated donor stem cell transplantation for GVHD after 3 weeks of antifungal therapy. His posttransplant course was complicated by a vancomycin-resistant *Enterococcus faecium* bloodstream infection and acute invasive pulmonary aspergillosis, although without evidence of relapsed cryptococcosis. As a result of his complicated posttransplant course, the patient passed away 33 days after his allogeneic stem cell transplant.

DISCUSSION

Cryptococcus species are facultative intracellular yeasts. These fungi are frequently encapsulated, and the capsule is primarily composed of the polysaccharides glucuronoxylomannan and glucuronoxylomannogalactan, which are major virulence factors. Historically, *C. neoformans* and *Cryptococcus gattii* represented the predominant pathogens, with multiple serotypes within each species. Recent phylogenetic studies, however, have led to a complete reorganization of the species complex, with *C. neoformans* containing the original serotype A, *C. deneoformans* encompassing serotype D, and at least five different species now recognized within the *C. gattii* complex (*C. gattii*, *C. deuterogattii*, *C. tetragattii*, *C. decagattii*, and *C. bacillisporus*) (1). *C. neoformans* is found worldwide in soil contaminated by bird droppings and decaying organic matter. *C. gattii* was primarily found in the tropical and subtropical areas but is now endemic in British Columbia, the Pacific Northwest, and California and is associated with several different tree species (1, 2).

Cryptococcus species are primarily opportunistic pathogens affecting immunocompromised individuals living with human immunodeficiency virus, cirrhosis, solid-organ transplantation, and hematologic malignancies or stem cell transplants, although infection in immunocompetent persons does occur more commonly with *C. gattii* (1, 3). The patient in this case was severely immunosuppressed due to a liver transplant, which was also complicated by GVHD. This is a rare complication of solid-organ transplantation, which most commonly affects liver or small-bowel transplant recipients and is associated with high mortality rates (4).

The most common clinical presentation for *Cryptococcus* species is pulmonary cryptococcosis, which can range from a solitary pulmonary nodule to severe pneumonitis.

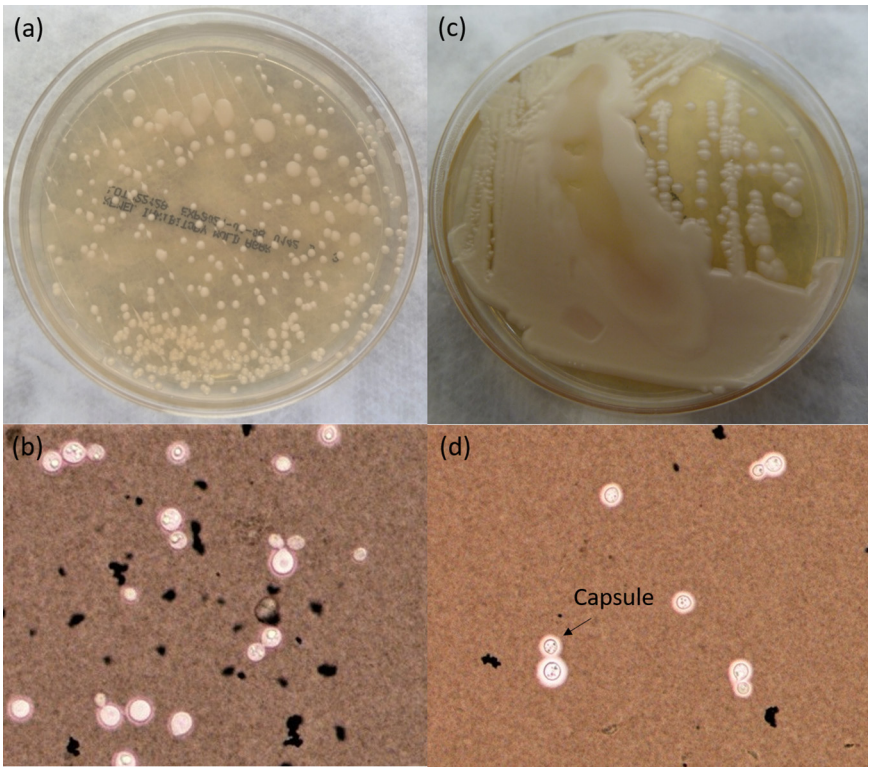

FIG 2 Capsule modulation by *Cryptococcus* over time *in vitro*. (a) Nonmucoid cultures on Sabouraud's dextrose agar (SDA) from original specimen 4 days postculturing; (b) India ink staining of culture indicating no visible capsule production; (c) mucoid *Cryptococcus* colonies following 10 days of subculture on SDA; (d) India ink staining of mucoid colonies, with clearing indicating the presence of a capsule.

Cryptococcus has a predilection for dissemination, which commonly results in meningoencephalitis or fungemia. In immunocompromised patients or those with neurologic symptoms, it is recommended to obtain a lumbar puncture for evaluation, which, if intracranial pressure is elevated, also allows for therapeutic removal of CSF. Unfortunately, thrombocytopenia precluded this assessment in our patient (2).

Cryptococcus species are variably sized, narrow-necked budding yeasts with an average size of 6 μm (range, 2 to 10 μm) (1). Hematoxylin and eosin (H&E) stains do not stain *Cryptococcus* species well, but both the periodic acid-Schiff (PAS) stain and Grocott's methenamine silver (GMS) stain can highlight the organisms in histopathology sections (1, 5). The *Cryptococcus* capsule is best visualized using the mucicarmine stain. The calcofluor white stain uses a fluorochrome that nonspecifically binds to chitin in fungal cell walls and can be used to visualize the organism in direct smears from body fluids and tissues, but it does not aid with visualization of the polysaccharide capsule (5). India ink staining is another rapid and inexpensive, but nonspecific, tool to detect encapsulated yeast cells. However, this stain is no longer routinely used due to its low sensitivity compared to that of CrAg tests (1, 5).

Cryptococcus spp. can be cultured on common mycology media such as Sabouraud's dextrose agar (SDA), inhibitory mold agar (IMA), brain heart infusion (BHI) agar, and bird seed agar (Staib's medium), but they also grow on blood agar plates used in bacteriology laboratories (5). *Cryptococcus* species are, however, sensitive to cycloheximide, and therefore, cycloheximide-containing media should be avoided (1). Colonies on SDA are characteristically white and creamy and typically mucoid due to capsule production. Interestingly, in this case, subculture of the original unencapsulated *Cryptococcus* isolate ultimately led to a mucoid appearance and capsule production after 10 days as demonstrated by positive India ink staining (Fig. 2). This is consistent with other case reports of

unencapsulated *Cryptococcus* species with a negative antigen test (6). Fungal culture for *Cryptococcus* is considered the gold standard diagnostic method, although recovery of the organism from the specimen may take up to a week or longer depending on the initial organism burden. Following growth in culture, methods such as MALDI-TOF MS and Sanger sequencing, using targets such as the D1/D2 region of the large ribosomal subunit gene or the internal transcribed spacer region gene, can be utilized for identification and differentiation between the various *Cryptococcus* species (1, 5).

Detection of CrAg can be achieved via latex agglutination (LA) or the more recently developed lateral flow immunoassay (LFA). Many laboratories have transitioned away from LA assays due to the requirement for reagent refrigeration, longer turnaround time (45 min), and lower sensitivity for non-HIV patients with cryptococcosis and for patients with *C. gattii* infections. In contrast, the CrAg LFA is a rapid ($<$15 min) and reliable method for detecting circulating antigen from either species in serum and CSF (7). The CrAg LFA provides a semiquantitative titer; however, it does not differentiate between *Cryptococcus* species (7). Although the CrAg LFA is highly sensitive and specific (i.e., $>$95%), false-negative results can occur due to high background from hemolyzed blood or postzone effect (7, 8). This immunologic phenomenon indicative of excess of antigen (postzone) in patient samples, as opposed to excess antibody (prozone) levels, ultimately results in the inadequate formation of antibody-antigen complexes and can lead to false-negative results by either agglutination or immunochromatographic methods. In cases in which the CrAg results are discrepant from results of other diagnostic testing, investigations may include performing additional specimen dilutions in an effort to dilute out the target analyte to reach an optimal antigen-antibody proportion, also known as the zone of equivalence (8). In this case, diluting the serum sample still did not result in a positive CrAg test. A false-negative CrAg test may also occur due to extremely low fungal burden or the lack of a capsule, as the CrAg tests detect the capsular glucuronoxylomannan antigen of *Cryptococcus* species.

Cryptococcus can modulate capsule formation based on host response, and thus, an early culture lacking a capsule (e.g., negative by India ink stain) suggests a capsule-deficient *Cryptococcus*, although this is not definitive. Lack of host immune pressures *in vitro*, as well as repeat subculturing, including at different temperatures (i.e., 30°C versus 37°C), can also affect capsule production. Thus, it is notoriously challenging to definitively identify a capsule-deficient *Cryptococcus* infection. False-positive CrAg LFA results are also possible and are reported to occur for patients with *Trichosporon*, *Capnocytophaga*, or *Stomatococcus mucilaginosus* infections (9). Additionally, low positive titers (i.e., $\leq$1:5) should be interpreted with caution for patients at low risk for cryptococcosis (9).

The mainstay antifungal treatment for severe forms of cryptococcosis is combination therapy with amphotericin B and flucytosine (2). In patients with fungemia, meningoencephalitis, or other forms of disseminated infection, induction treatment with combination amphotericin B and flucytosine for at least 2 weeks is recommended. However, a recent clinical trial showed that single high-dose infusion of liposomal amphotericin B followed by fluconazole and flucytosine was noninferior to a longer course of amphotericin B induction (10). In settings where such treatment is unavailable, alternatives include a longer course of amphotericin B monotherapy, high-dose fluconazole with flucytosine, or very-high-dose fluconazole. Induction therapy is followed by consolidation and maintenance therapy with fluconazole. Other triazoles, such as voriconazole and posaconazole, are expected to remain active, although clinical data regarding their efficacy are limited. Posaconazole was used as consolidation therapy in our patient due to concurrent need for antifungal prophylaxis targeting molds such as *Aspergillus* spp. and mucormycoses. Initial monotherapy with fluconazole is typically reserved for patients with mild or asymptomatic localized pulmonary cryptococcal infection.

While it has classically been assumed that capsule-deficient cryptococcal strains are less virulent, it remains unclear how capsule deficiency may affect clinical manifestations and outcomes in cryptococcosis; limited data suggest that these infections are similar to those with normal capsule production (11). Nonetheless, given the reliance on CrAg

testing, the potential diagnostic delay due to capsule-deficient *Cryptococcus* presents a challenge that laboratory personnel and clinicians should be aware of and reinforces the importance of performing fungal culture for patients with suspected cryptococcosis.

SELF-ASSESSMENT QUESTIONS

1. Which stains can best identify capsule production in *Cryptococcus* species?
    a. Hematoxylin and eosin (H&E)
    b. Fontana-Masson stain
    c. Mucicarmine
    d. Calcofluor white stain
2. False-negative CrAg results may occur due to:
    a. Short duration of cryptococcal fungemia
    b. Elevated rheumatoid factor
    c. Immunosuppression
    d. Postzone effect
3. What is the preferred initial treatment for fungemia with *Cryptococcus* species?
    a. Amphotericin B with flucytosine
    b. Itraconazole
    c. Fluconazole
    d. Posaconazole

ANSWERS TO SELF-ASSESSMENT QUESTIONS

1. Which stains can best identify capsule production in *Cryptococcus* species?
    a. Hematoxylin and eosin (H&E)
    b. Fontana-Masson stain
    c. Mucicarmine
    d. Calcofluor white stain

    Answer: c. The aluminum in mucicarmine stains forms a chelating complex with carmine giving it a positive charge which allows it to bind to low density acidic substrates such as the mucin present in the *Cryptococcus* capsule. Hematoxylin and eosin (H&E) does not allow for optimal visualization of *Cryptococcus* yeasts. Periodic acid-Schiff (PAS) and calcofluor white stains can help demonstrate narrow budding yeasts such as *Cryptococcus* but cannot differentiate the presence of a capsule. The Fontana-Masson stain is typically used to detect melanin-producing organisms. Although melanin production is a major virulence factor of neurotropic *Cryptococcus*, melanin is deposited in the cell walls, and therefore, Fontana-Masson does not stain the capsule.

2. False-negative CrAg results may occur due to:
    a. Short duration of cryptococcal fungemia
    b. Elevated rheumatoid factor
    c. Immunosuppression
    d. Postzone effect

    Answer: d. The presence of excessive levels of antigen in the sample (postzone) can lead to inefficient complexing between the CrAg and both the soluble and adhered anti-CrAg antibodies on the lateral flow assay, leading to false-negative results. Other causes of false-negative CrAg results include low fungal organism burden and capsule-deficient *Cryptococcus*.

3. What is the preferred initial treatment for fungemia with *Cryptococcus* species?
    a. Amphotericin B with flucytosine
    b. Itraconazole
    c. Fluconazole
    d. Posaconazole

    Answer: a. The combination amphotericin B and flucytosine is considered first-line therapy for severe cryptococcal infection, fungemia, and cryptococcal meningoencephalitis. High-dose fluconazole is used in later phases of treatment but can be an alternative initial treatment in combination with flucytosine if first-line therapy is unavailable. However, monotherapy with flucytosine should not be administered due to the possibility of rapid development of resistance. Posaconazole and itraconazole have anti-*Cryptococcus* activity but would not be routinely considered for initial treatment of severe or disseminated infection.

TAKE-HOME POINTS

- Causes of false-negative cryptococcal antigen testing include low fungal burden, postzone effect, and the lack of or deficient capsule production. Fungal cultures should always be performed for patients with suspected cryptococcemia.
- *Cryptococcus* species typically cause infection in immunocompromised patients, such as those with HIV and solid-organ transplant and/or stem cell transplant recipients, although infections in immunocompetent persons have been reported.
- *Cryptococcus* is best visualized with periodic acid-Schiff (PAS) stain and Grocott's methenamine silver (GMS) stain, although hematoxylin and eosin (H&E) and calcofluor white staining can be used.
- Cryptococcemia and disseminated cryptococcal infections are typically treated with at least 2 weeks of combination amphotericin B and flucytosine followed by prolonged azole therapy.

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WHO fungal priority pathogens list to guide research, development and public health action

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Abbreviations and acronyms

AG	Advisory Group
AMR	antimicrobial resistance
ANZMIG	Australia & New Zealand Mycoses Interest Group
BWS	best-worst scaling
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus disease
CT	computed tomography
DCE	discrete choice experiment
EAR	emerging antimicrobial resistance
EARS-Net	Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
ECMM	European Confederation of Medical Mycology
ESCMID-EFISG	European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group
EML	WHO Essential Medicines List
GAFFI	Global Action for Fungal Infections
GAP	Global Action Plan (on AMR)
GLASS	WHO Global Antimicrobial Resistance and Use Surveillance System
HSCT	haemopoietic stem cell transplantation
IA	invasive aspergillosis
ICU	intensive care unit
IFD	invasive fungal disease
IPC	infection prevention and control
IRIS	immune reconstitution inflammatory syndrome
ISHAM	International Society for Human & Animal Mycology
LMIC	low- and middle-income country
MALDI-TOF	matrix-assisted laser desorption/ionization time of flight
MCDA	multicriteria decision analysis
MEC	minimum effective concentration
MIC	minimum inhibitory concentration
MRC	Medical Research Council Centre
MSGERC	Mycoses Study Group Education and Research Consortium
PCR	polymerase chain reaction
PJP	*Pneumocystis jirovecii* pneumonia
R&D	research and development
ReLAVRA	The Latin American and Caribbean Network for Antimicrobial Resistance Surveillance (Spanish acronym)
TB	tuberculosis
US CDC	United States Centers for Disease Control and Prevention
WHO	World Health Organization
WHO BPPL	WHO bacterial priority pathogens list
WHO FPPL	WHO fungal priority pathogens list
WHO AG FPPL	WHO Advisory Group on the FPPL

Executive summary

Infectious diseases are among the top causes of mortality and a leading cause of disability worldwide. Drug-resistant bacterial infections are estimated to directly cause 1.27 million deaths and to contribute to approximately 4.95 million deaths every year, with the greatest burden in resource- limited settings.

Against the backdrop of this major global health threat, invasive fungal diseases (IFDs) are rising overall and particularly among immunocompromised populations. The diagnosis and treatment of IFDs are challenged by limited access to quality diagnostics and treatment as well as emergence of antifungal resistance in many settings.

Despite the growing concern, fungal infections receive very little attention and resources, leading to a paucity of quality data on fungal disease distribution and antifungal resistance patterns. Consequently, it is impossible to estimate their exact burden.

In 2017, WHO developed its first bacterial priority pathogens list (WHO BPPL) in the context of increasing antibacterial resistance to help galvanize global action, including the research and development (R&D) of new treatments. Inspired by the BPPL, WHO has now developed the first fungal priority pathogens list (WHO FPPL). The WHO FPPL is the first global effort to systematically prioritize fungal pathogens, considering their unmet R&D needs and perceived public health importance. The WHO FPPL aims to focus and drive further research and policy interventions to strengthen the global response to fungal infections and antifungal resistance.

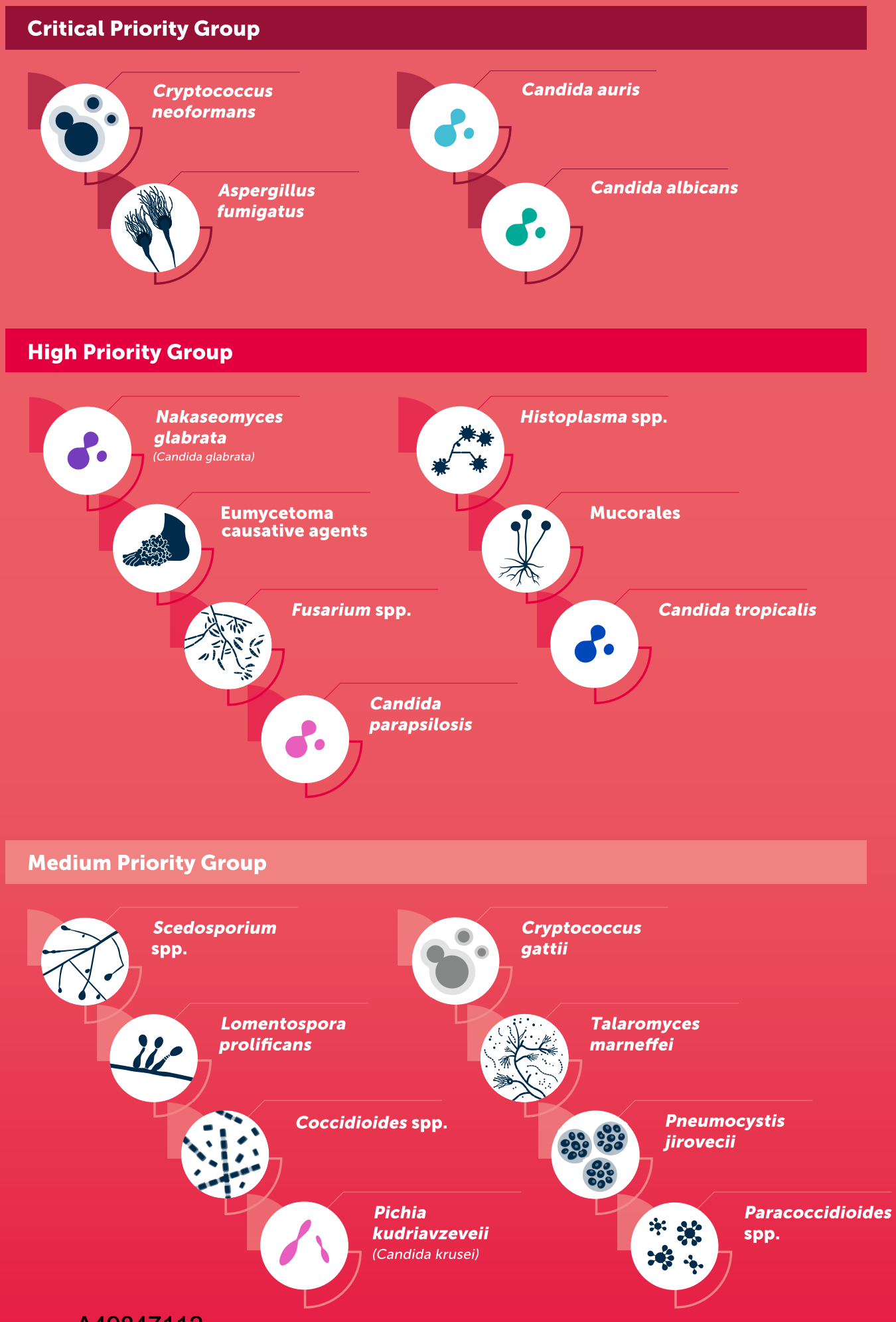
The development of the list followed a multicriteria decision analysis (MCDA) approach. The prioritization process focused on fungal pathogens that can cause invasive acute and subacute systemic fungal infections for which drug resistance or other treatment and management challenges exist. The pathogens included were ranked, then categorized into three priority groups (critical, high, and medium). The critical group includes *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus* and *Candida albicans*. The high group includes *Nakaseomyces glabrata (Candida glabrata)*, *Histoplasma* spp., eumycetoma causative agents, Mucorales, *Fusarium* spp., *Candida tropicalis* and *Candida parapsilosis*. Finally, pathogens in the medium group are *Scedosporium* spp., *Lomentospora prolificans*, *Coccidioides* spp., *Pichia kudriavzeveii (Candida krusei)*, *Cryptococcus gattii*, *Talaromyces marneffei*, *Pneumocystis jirovecii* and *Paracoccidioides* spp.

This document proposes actions and strategies for policymakers, public health professionals and other stakeholders, targeted at improving the overall response to these priority fungal pathogens, including preventing the development of antifungal drug resistance. Three primary areas for action are proposed, focusing on: (1) strengthening laboratory capacity and surveillance; (2) sustainable investments in research, development, and innovation; and (3) public health interventions.

Countries are encouraged to improve their mycology diagnostic capacity to manage fungal infections and to perform surveillance. In most contexts, this might require a stepwise approach. There is a need for sustainable investments in research, development, and innovation. More investments are needed in basic mycology research, R&D of antifungal medicines and diagnostics. Innovative approaches are needed to optimize and standardize the use of current diagnostic modalities globally. In addition, public health interventions are needed to highlight the importance of fungal infections, including through incorporating fungal diseases and priority pathogens in medical (clinical) and public health training programmes and curricula at all levels of training. Similarly, collaboration across sectors is required to address the impact of antifungal use on resistance across the One Health spectrum.

Finally, regional variations and national contexts need to be taken into consideration while implementing the WHO FPPL to inform priority actions.

Fig. 1. WHO fungal priority pathogens list (WHO FPPL)



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1. Background

Fungal pathogens and infections are an increasing global public health concern. People most at risk are those with underlying health problems or a weakened immune system, such as chronic lung disease, prior tuberculosis (TB), HIV, cancer, and diabetes mellitus. Critically ill patients in an intensive care unit (ICU), patients undergoing invasive medical procedures and receiving broad-spectrum antibiotics, and those taking immune-suppressing medicines are also at risk *(1)*.

Cases of invasive fungal disease (IFD) are rising as the at-risk population continues to expand. This is due to many factors, including advancements in modern medicine and accessibility to therapies and interventions that impair the immune system, such as chemotherapy and immunotherapy for cancer, and solid organ transplantation. New groups at risk of IFD are constantly being identified. Examples include patients with chronic obstructive pulmonary disease (COPD), liver or kidney disease, viral respiratory tract infections such as influenza and those with prior non-tuberculous mycobacterial infections. The coronavirus disease (COVID-19) pandemic has been associated with an increase in the incidence of comorbid invasive fungal infections. Three groups of COVID-19 associated fungal infections; aspergillosis; mucormycosis; and candidaemia, were frequently reported, often with devastating consequences *(2)*. Finally, there is evidence to suggest that both the incidence and geographic range of fungal infections are expanding globally due to climate change *(3, 4)*.

The underrecognized and emerging global health threat of invasive fungal diseases is compounded by the rapid emergence of antifungal resistance and, in many settings, limited access to quality diagnostics and treatment *(5, 6)*. Antifungal resistance has major implications for human health. It generally leads to prolonged therapy and hospital stays, and an increased need for expensive and often highly toxic second-line antifungal medicines. These medicines are often unavailable in low- and middle-income countries (LMICs) *(7, 8)*, which can contribute to increased mortality. The challenges posed by the multidrug-resistant pathogen *Candida auris* highlight these issues: not only does *C. auris* cause increased morbidity and mortality for affected individuals but the pathogen is also difficult to eradicate from hospitals, even with intensive infection-prevention strategies *(9, 10, 11, 12)*. Its detection in the hospital environment may result in prolonged ward closures. The emergence of resistance is partly driven by inappropriate antifungal use across the One Health spectrum *(13)*. For example, agricultural use is responsible for rising rates of azole-resistant *Aspergillus fumigatus* infections, with azole-resistance rates of 15–20% reported in parts of Europe and over 80% in environmental samples in Asia *(9, 14, 15, 16)*.

Currently, only four classes of systemic antifungal medicines (azoles, echinocandins, pyrimidines and polyenes) are used in clinical practice, and only a few others are under development *(17, 18, 19, 20)*. Although existing antifungal medicines are effective, they are associated with a plethora of adverse effects. The use of these medicines also requires expertise, and drug–drug interactions are particularly common *(21)*. Such interactions, along with the requirement for lengthy courses of therapy, further impact patient safety and prognosis.

Additionally, affordable access to quality medicines and diagnostic tests is unevenly distributed. This is especially acute in low-resource settings, where the disease burden is highest *(1)*. As a result, many fungal infections go undiagnosed and untreated. Causative pathogens are rarely confirmed microbiologically, and in most settings, surveillance data are of low quality or absent.

Despite posing a growing threat to human health, fungal infections receive very little attention and resources globally *(1)*. This all makes it impossible to estimate the exact burden of fungal infections and consequently difficult to galvanize policy and programmatic action.

In 2017, WHO developed its first bacterial priority pathogens list (WHO BPPL) in the context of increasing antimicrobial resistance (AMR). The aim of the WHO BPPL was to guide private and public investment into the development of new antibiotics by identifying research and development (R&D) priorities *(22)*. Since the WHO BPPL was launched, WHO has regularly used the list to analyse the antibacterial development pipeline *(23, 24)*. These analyses have shown that the WHO BPPL has been instrumental in informing research and investment decisions. Importantly, the list has also emerged as a valuable tool for raising AMR awareness and informing surveillance measures, infection prevention and control (IPC) interventions, and antimicrobial stewardship guidance. Inspired by the WHO BPPL, WHO developed the first fungal priority pathogens list (WHO FPPL).

2. Aims

In response to the rising threat of fungal infections, combined with existing and emerging resistance and treatability issues, WHO developed this first WHO FPPL to:

- Direct and drive research efforts towards the pathogens that pose the greatest public health threat and/or have the greatest gaps in knowledge.
- Facilitate international coordination and inform investment in R&D to discover new and optimize existing therapeutics and diagnostics, and to improve patient outcomes.
- Monitor antifungal development pipeline to track trends and identify gaps.
- Define research and development (R&D) priorities to align investments and funding with identified unmet public health needs.
- Promote knowledge generation to improve global understanding of and the response to fungal infections and antifungal resistance.
- Inform and enable policymakers to design and implement measures to address IFDs and antifungal resistance.

3. Scope

The list is focused on fungal pathogens responsible for acute, subacute systemic fungal infections for which drug resistance or other treatability and management challenges exist. The pathogens included are all associated with serious risk of mortality and/or morbidity. The list is mainly focused on systemic invasive infections. Similar assessments in the future could include other fungi with important economic and health consequences, particularly those causing mucosal, skin and eye infections.

4. Target audience

The target audience for this document includes but is not limited to:

- National and subnational policymakers in the Ministries of Health or equivalent authorities responsible for infectious diseases and AMR monitoring, and developing and implementing infection prevention and control interventions, national actions plan, and public health policies.
- Medical mycologists, public health researchers, general practitioners, and other healthcare providers.
- Healthcare, infectious diseases, medical mycology, and public health professional societies.
- The pharmaceutical and diagnostics industry, academic and public health research institutions.
- Research funders and public-private partnerships which invest in basic research, and the development, and implementation into practice of new antifungal agents and diagnostics.

5. Approach

The heterogeneity of communicable diseases makes it difficult to prioritize pathogens globally *(25)*. Fungal infections, with their complex epidemiology, risk factors, variable global distribution, and disease dynamics, are no exception. In 2020, a scoping literature review conducted by WHO revealed that no global prioritization of fungal infection threats existed. Only one national infectious disease threat priority list that included fungal pathogens was identified, namely the US CDC priority threat list (2019), which highlighted three fungal "groups": *Candida auris*, antifungal-resistant *Candida* and azole-resistant *Aspergillus fumigatus (26)*. In addition, mucormycosis was prioritized by India in 2021 under the notifiable disease category, as a result of the world's largest outbreak thus far, which was associated with the COVID-19 pandemic.

Various approaches can be undertaken to develop priority lists. In 2017 WHO successfully used multicriteria decision analysis (MCDA) to develop the WHO bacterial priority pathogens list *(27)*, and a similar approach has been adopted for the WHO FPPL *(18)*. MCDA makes it possible to combine a diverse range of criteria, qualitative and quantitative evidence, along with the experience and expertise of stakeholders. In addition, MCDA is reproducible, enabling regular reviews of the list to be performed based on new evidence.

The process began by selecting 19 pathogens to prioritize, based on 10 assessment criteria (Tables 1, 2). The list of pathogens and criteria was determined in consultation with the WHO Advisory Group on FPPL (WHO AG FPPL), relevant WHO programmes and regional offices. WHO commissioned 19 systematic reviews of the literature to describe the pathogens with reference to these criteria (Table 2).[1]

The weight of each prioritization criterion was then determined through a discrete choice experiment (DCE) survey, focusing on the perceived R&D need. DCE is a well-established methodology for determining MCDA criteria weights while minimizing bias *(28,29)*. Due to the complexity of the questions, a minimum sample size of 300 clinicians and/or researchers with expertise in medical or public health mycology was required. Participants were recruited by WHO via country and regional offices, medical mycology societies and social media. Ultimately, 376 respondents from across the globe participated.

Next, the perceived public health importance of each pathogen was determined using best-worst scaling (BWS). For this exercise, a minimal sample size of 40 respondents with senior-level expertise and experience in medical mycology and/or public health was required. WHO invited participants based on the advice of the WHO AG FPPL, WHO regional offices, and key contacts in medical mycology societies around the world, with 49 ultimately taking part. In both surveys, efforts were made to ensure gender balance and geographic representativeness in the respondents.

Results from surveys were combined with the systematic reviews to produce a comprehensive ranking to guide R&D and identify strategies to prevent and control the burden of IFD and antifungal resistance (Figs. 1, 2).

[1] Diagnostic and treatment criteria were not included in the systematic reviews but were determined through an alternative approach.

6. Key findings

The MCDA approach used in the prioritization comprised a DCE global survey focused on R&D, and a BWS global surveys on public health importance. The approach considers a diverse range of criteria, qualitative and quantitative evidence, along with the expertise of stakeholders. Thus, the prioritization process revealed important findings that should inform the use of the WHO FPPL.

First, public health importance is a strong determinant of priority. For the overall ranking of pathogens, BWS survey respondents favoured public health importance over unmet R&D need. Furthermore, apart from antifungal resistance, disease-burden-related criteria (mortality, annual incidence, and morbidity) had the highest weights for relative importance in the R&D DCE survey. These features are reflected in the overall ranking, where the 4 'critical threat' pathogens are those ranked highest for perceived public health importance (*Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus* and *Candida albicans*).

Second, antifungal resistance is a top priority. Of all 10 criteria in the DCE on R&D, respondents gave the highest weighting to antifungal resistance. As a result, fungal pathogens that are highly antifungal resistant ranked top in terms of R&D need (e.g. *Lomentospora prolificans*, *Fusarium* spp., Mucorales, and *Scedosporium* spp.).

Third, the systematic reviews revealed major knowledge gaps on the global burden of fungal infections and antifungal resistance. All 19 pathogens included in the prioritization lacked comprehensive data on the burden of disease, especially data relating to morbidity. Although many papers reported susceptibility data from ad hoc laboratory surveillance projects, formal surveillance and data linkage to clinical outcomes were lacking. Furthermore, susceptibility was reported very inconsistently, making comparisons over time or between geographic areas difficult. Susceptibility data were less common from LMICs, likely due to limited access to medical mycology laboratories in resource-limited settings.

Finally, fungal pathogens distribution and epidemiology vary significantly by region. Some pathogens are global, whereas some are endemic to certain areas. The systematic reviews pointed to major variations in the incidence and prevalence of fungal conditions, partly related to underlying disease and local clinical practice. The prevalence of antifungal resistance also varies considerably. Therefore, regions and countries are encouraged to contextualize these findings at the regional, subregional, or country level to inform local priorities in terms of public health importance and potentially R&D.

Table 1. Summary of the prioritization steps (All steps are overlapping and non-sequential)

Step 1	**Selection of stakeholder groups:** these include the WHO AG FPPL, which consists of mycology experts from all WHO regions, and participants in the Global Medical Mycology Expert Respondent Group.
Step 2	**Selection of pathogens to be prioritized:** the fungal pathogens to be prioritized were selected based on consultation and consensus.
Step 3	**Selection of criteria for prioritization:** criteria and levels for profiling fungal pathogens were selected and each criterion was defined, through an iterative process (see Table 2).
Step 4	**Systematic reviews:** 19 systematic reviews were conducted to describe each of the pathogens according to the predefined criteria and levels.
Step 5	**Assignment of levels:** based on the systematic reviews, and expert opinion where needed, levels were assigned to the criteria for each pathogen (see Table 2).
Step 6	**MCDA-DCE R&D survey:** a large DCE-based survey was conducted across six WHO regions to weight each criterion according to perceived R&D priority. The survey was available in three languages (English, French and Spanish).
Step 7	**Best–worst scaling (BWS) survey for public health importance:** a choice-based survey using BWS was conducted to estimate the weight of each pathogen according to perceived public health importance. This survey also included a question to determine the relative weights of unmet R&D vs. perceived public health importance and was used to inform the final overall pathogen ranking.
Step 8	**Final WHO FPPL:** The public health and R&D rankings were combined according to the weight assignment from step 7 to formulate the final FPPL.

WHO AG FPPL: World Health Organization Advisory Group on the Fungal Priority Pathogens List; DCE: discrete choice experiment; MCDA: multicriteria decision analysis; R&D: research and development; WHO: World Health Organization.

Table 2. Prioritization criteria, definitions and levels

Criterion	Definition/description	Level value
 Deaths	Average case fatality rate	**Low:** < 30% **Medium:** 30-70% fatality **High:** > 70% **Unknown:** no reliable data
 Annual incidence	Number of new cases per million population each year	**Low:** < 2/million **Medium:** 2-50/million **High:** > 50/million **Unknown:** no data available
 Current global distribution	Extent of geographic distribution across the globe	**Localized** in ≤ 2 WHO regions **Globally distributed** in ≥ 3 WHO regions **Unknown:** due to inadequate data
 Trends in last 10 years	Evidence of change in incidence/prevalence patterns	**Stable:** no evidence of increasing incidence/prevalence **Increasing:** evidence of increasing incidence/prevalence **Unknown:** due to inadequate data
 Inpatient care	Average length of hospital stay required for treatment following initial diagnosis	**Low:** < 2 days **Medium:** 2 days to 2 weeks **High:** > 2 weeks **Unknown:** no data available
 Complications and sequelae	Proportion of patients suffering long-term complications of disease	**Low:** expected to affect a minority of patients (e.g. < 10%). **Medium:** expected to affect a significant proportion of patients (e.g. 10-50%). **High:** expected to affect the majority of patients (e.g. > 50%).
 Antifungal resistance	Rate (or level) of acquired or intrinsic resistance to antifungal treatment	**Low:** < 10% acquired or intrinsic resistance for all four classes of antifungals. **Medium:** acquired or intrinsic resistance (> 10%) described for agents from one to two classes of antifungals. **High:** acquired or intrinsic resistance (> 10%) described for agents from three to four classes of antifungals. **Unknown:** no reliable data available
 Preventability	Transmission/ acquisition dynamics and availability of evidence-based, effective preventive measures	**Low:** transmission/acquisition dynamics well described, and preventive measures ineffective or of low-quality evidence, and/or not widely available or difficult to implement. **Medium:** transmission/acquisition dynamics are not well described, but preventive measures based on moderate or high-quality evidence are available and effective. **High:** transmission/acquisition dynamics are well described, and preventive measures based on moderate or high-quality evidence are universally available and effective. **Unknown:** transmission/acquisition dynamics not well described. No preventive measures described.
 Access to diagnostic tests	Availability of diagnostics	**Low:** diagnostics are not available in reference laboratories. **Medium:** diagnostics are available in institutional or reference laboratories but not universally available due to cost, distribution or technical issues. **High:** diagnostics are available and have been successfully implemented in institutional diagnostic laboratories, in at least one but not all high-burden/low-resource settings where disease occurs. **Very high:** diagnostics are universally available in institutional diagnostic laboratories where disease occurs.
 Evidence-based treatments	Treatment options are evidence based and accessible	**Very low:** treatment based on expert opinion with limited evidence. **Low:** peer-reviewed, high-quality guidelines available, but first-line treatment options are unaffordable, toxic or unavailable where disease occurs. **Medium:** peer-reviewed, high-quality guidelines with at least one first-line treatment option which is affordable, non-toxic and available where disease occurs. **High:** peer-reviewed, high-quality guidelines with at least one first-line treatment option which is affordable, nontoxic and available where disease occurs, and includes specific recommendations for all main host groups, including paediatrics.

WHO: World Health Organization.

7. Final ranking of pathogens

The 19 fungal pathogens included were ranked and categorized into three priority groups based on their numerical scores, and consensus discussions among the WHO AG FPPL (Table 3).

- **Critical group:** *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus* and *Candida albicans*.
- **High group:** *Nakaseomyces glabrata (Candida glabrata)*, *Histoplasma* spp., eumycetoma causative agents, Mucorales, *Fusarium* spp., *Candida tropicalis* and *Candida parapsilosis*.
- **Medium group:** *Scedosporium* spp., *Lomentospora prolificans*, *Coccidioides* spp., *Pichia kudriavzeveii (Candida krusei)*, *Cryptococcus gattii*, *Talaromyces marneffei*, *Pneumocystis jirovecii* and *Paracoccidioides* spp.

The relative importance weight of each criterion for R&D priorities ranking varied considerably. The most important was antifungal resistance (38.5%), followed by deaths (13.9%), evidence-based treatment (11.9%), access to diagnostics (10.4%), annual incidence (8.5%) and complications and sequelae (8.4%). The remaining criteria had a relative importance of less than 5%.

Annex 1 shows the ranking for each of the 19 pathogens based on the DCE for R&D need, the BWS for perceived public health importance and finally a combined ranking. Respondents in the BWS assigned a relative importance weights of 0.48 for R&D need and 0.52 for public health importance. These weights were used to determine the overall ranking.

There are notable and understandable variations in ranking for some pathogens. For example, *Lomentospora prolificans* was ranked top for R&D need due to lack of effective treatment options but was ranked low for perceived public health importance due to its rarity. Overall, it ranked 13th. In contrast, *Aspergillus fumigatus* and *Candida albicans* were ranked lower for unmet R&D need but ranked highly for their public health burden. Overall, they both ranked in the top four (Annex 1).

Table 3. WHO fungal priority pathogens list

Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzeveii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

8. Important considerations and limitations

- Some pathogens are confined to certain geographical areas and thus are not considered a priority on a global scale (e.g. *Paracoccidioides* spp.). However, in areas where they are endemic, they are associated with a significant burden of disease. As such, these pathogens should be assessed independently and must be considered in the local context. Regions and countries are encouraged to contextualize these findings at the regional, subregional or country level to inform local priorities in terms of public health importance and potentially R&D.
- This is also the case for specific populations. For example, *Pneumocystis jirovecii* is one of the main pathogens causing opportunistic infections in people living with HIV/AIDS, but it ranked low in the global list. For populations at high risk of specific infections (e.g. cancer and immunosuppressed patients, and newborns with HIV infections) both the list, and the interventions outlined in this document must be contextualized and implemented accordingly.
- The MCDA approach made it possible to combine multiple types of criteria (qualitative vs quantitative) and evidence (systematic review vs expert opinion). Future iterations of the list are likely to be informed by stronger, more robust evidence generated in response to the recommendations in this report.
- The predefined criteria and levels were based on the scoping literature reviews and expert opinion from the WHO AG FPPL and were defined before the systematic reviews were conducted. Some criteria levels did not describe any of the pathogens. Although sensitivity analyses showed that these features of the data have minimal impact on the overall ranking, future iterations will build on the evidence collected in this exercise to further optimize criteria and refine levels.
- Combining the rankings of unmet R&D need and perceived public health importance is challenging. Two different surveys were conducted to assess each of them separately. The perceived public health importance on overall ranking was determined from the BWS survey among a smaller group of highly experienced mycologists. Geographic representativeness was observed during recruitment of participants.
- Evidence from the systematic review was minimal for some criteria in the MCDA-DCE. For example, many pathogens lacked data on complications and sequelae of infection and duration of inpatient care. Where data gaps were found, expert consensus was used to profile the pathogens. Therefore, some of the findings may be subject to bias.
- Large multinational prospective cohort studies are needed to fill the gaps on burden of disease criteria. In addition, quality surveillance data on antifungal resistance, and evidence on IPC measures are needed to better inform future iterations of the WHO FPPL.
- The systematic reviews that informed the criteria used in the MCDA-DCE were limited to peer-reviewed publications in English within the last 10 years (2011–2021). It is likely that more data exist, some in other languages, which could have influenced the strength of the evidence. For example, public health reports from individual countries on specific infections were not identified or included unless published in peer-reviewed journals in English. Future iterations should include other data sources (e.g. conference abstracts, grey literature), and non-English publications, especially for endemic mycoses.
- Sensitivity analyses showed that the overall ranking of fungal pathogens was robust and minimally affected by the country of origin of the respondents (LMIC vs. high-income countries) or by changing the levels of the criteria.
- Attempts to ensure representativeness of the surveys were successful. Of note, females made up 45% (n = 181/401), and 55% (n = 27/49) of the respondents to the MCDA-DCE and the BWS surveys, respectively. 42% (168/401) of the respondents worked in LMICs, or primarily in a language other than English.

9. Implementation and use of the WHO FPPL and priority areas for action

The WHO FPPL is the first global effort to systematically prioritize fungal pathogens, considering their unmet R&D needs and perceived public health importance. The WHO FPPL aims to focus and drive further research and policy interventions to strengthen the global response to fungal infections and antifungal resistance. Currently, there is a clear need for an evidence base to inform public health interventions – both in terms of disease impact and improved delivery of care. To address this need, three key broad areas for action were identified: improved surveillance, targeted support for R&D and innovation, and enhanced public health interventions. These inter-linking action areas build on and reinforce each other (Fig. 2).

Fig. 2. Proposed priority areas for action

AMR: antimicrobial resistance; R&D: research and development; WHO FPPL: World Health Organization fungal priority pathogens list.

9.1. Surveillance

Context. Closing the large knowledge gaps identified in burden, both of disease and antifungal resistance, will require coordinated investment in both laboratory-based and clinical surveillance. This must happen at the national, regional, and international levels.

Achieving the goal of improved laboratory surveillance will depend on access to mycology laboratories, which are also essential for optimal patient care and overall patient safety. While many first-line tests (e.g. microscopy and culture) can be readily implemented in standard microbiology laboratories, access to these tests is still limited in many countries around the world. Other tests such as MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) mass spectrometry systems, real-time PCR (polymerase chain reaction), and antifungal therapeutic monitoring are currently too costly and limited mostly to high-income settings. Addressing this limitation requires a stepwise approach, for example as described in a 2015 report by Global Action for Fungal Infections *(30)*.

Large-scale susceptibility data collection with clinical data linkage will facilitate the development of clinical breakpoints for these fungal pathogens, many of which are not currently available. In 2019 the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) initiated a global collaborative effort to compile available data on fungal infections, expanding its original scope from bacterial infections to include fungi. The first pathogen(s) included in the pilot phase are *Candida* spp., focusing on bloodstream infections in hospitalized patients *(31)*.

Improved clinical surveillance will depend on the level of knowledge and education regarding clinical presentation and risk factors for infections cause by these pathogens. Affordable access to diagnostic tools at the point of care is essential for optimal patient care, and for surveillance data generation. Such diagnostic tools include imaging tests (e.g. CT and MRI), advanced sampling (e.g. bronchoscopy, CT guided biopsy) and other technologies for accurate diagnosis.

Finally, diagnostic capacity underpins antibiotic, and antifungal stewardship. Accurate diagnoses promote the rational use of antifungal agents and reduce unnecessary empiric antimicrobial use *(32)*. Access to quality diagnostics for fungal diseases is essential part of the WHO AMR agenda (Box 1).

Box 1: Surveillance actions, interventions and strategies

- **Build mycology diagnostic capacity** to manage fungal infections and to perform **surveillance**, starting at reference microbiology laboratories for **identification and susceptibility testing** of fungi. Such reference laboratories can perform surveillance and provide external quality assessment and training in fungal diagnosis.
- **Integrate fungal diagnostics** that are included on WHO's model list of essential diagnostics into **routine care** or **specialized laboratories** based on local epidemiology, contexts, capacity and needs. Prioritize diagnostic services to serve **populations at greatest risk** of fungal diseases (e.g., cancer, HIV/AIDS, post-TB, COPD, asthma).
- Build capacity in antifungal stewardship to **limit the inappropriate use of antifungals as well as antibiotics**. Develop **standard operating procedures** and algorithms for laboratories to **optimize the diagnosis** of fungal infections, including for pathogens with outbreak potential; build capacity for outbreak detection, reporting and response.
- Encourage the development of **networks at the national and international level** and participate in collaborative global and regional surveillance initiatives (e.g., WHO GLASS-AMR, GLASS-FUNGI, GLASS-EAR, and other regional platforms such as ReLAVRA and EARS-Net). Knowledge transfer through national, regional, and international disease registries, and other global collaborative platforms, **supports understanding of pathophysiology**, especially of rare pathogens, and will **facilitate research** into therapeutics and diagnostics.
- Utilize epidemiological laboratory and clinical **surveillance data** along with other health care data to **quantify** the burden of IFD and **antifungal resistance** to inform public health interventions, and guide IPC measures.
- Follow a stepwise approach in implementing the FPPL beginning with top priority pathogens, starting with **data and evidence generation**, and **tailoring FPPL to regional, national, and local contexts and needs**.

AMR: antimicrobial resistance; COPD: chronic obstructive pulmonary disease; EAR: emerging antimicrobial resistance; EARS-Net: Antimicrobial Resistance Surveillance Network; FPPL: fungal priority pathogens list; GLASS: Global Antimicrobial Resistance and Use Surveillance System; IFD: invasive fungal disease; ReLAVRA: Spanish acronym of The Latin American and Caribbean Network for Antimicrobial Resistance Surveillance; TB: tuberculosis; WHO: World Health Organization.

9.2. R&D and innovation

Context. Currently, fungal infections receive less than 1.5% of all infectious disease research funding. Consequently, the evidence base is weak, and most treatment guidelines are informed by limited evidence and expert opinion. Tackling the problems posed by IFD will require increased research funding, targeted at the key priorities, new antifungal medicines and improved diagnostics.

Pathogens with limited therapeutic options such as *Lomentospora prolificans* or *Fusarium* spp. were clearly prioritized for R&D. Neither currently approved systemic antifungals nor those in the clinical pipeline fully address the problems faced by health care workers, due to treatment inherent limitations, and the rising rates resistance. The massive use of some antifungals (azoles) in agriculture is further compounding the problem. Novel agent classes with different targets and mechanisms of action, and better safety profiles, should be developed and strictly reserved for use in humans. This, and other outcomes, will rely on the support for basic scientific research, including the pathophysiology of fungal infections. Finally, further research and innovation are needed to optimize the way current antifungals are used, including strategies to make therapeutic antifungal monitoring more widely available and optimize combination therapies to prevent further resistance and enhance efficacy.

The availability of accurate diagnostics was considered in the MCDA and impacted the R&D ranking of pathogens. The clinical diagnosis of IFD is often challenging because presentations are nonspecific and many current tests have poor sensitivity or specificity. Even in settings with adequate diagnostic capacity, the turnaround time for confirmatory tests can be days to weeks, hindering the guidance of effective early treatment. Novel antifungals, and accurate rapid diagnostics, are urgently needed. Despite this, the R&D pipeline is hampered by the long development time associated with traditional R&D models (about 10 years), the insufficient return on investment and the scientific challenges of identifying new targets (Box 2).

Box 2: R&D and innovation actions, interventions and strategies

- Focus R&D investments on **innovative antifungal agents** (i.e. **no cross-resistance to other antimicrobial classes, new chemical class, new target, and new mode of action-no or minimal drug-drug interaction**) effective against priority pathogens.
- **Improve existing therapies** and generate new knowledge on their optimal use, including pharmacokinetics/pharmacodynamics and therapeutic antifungal monitoring. Optimize combination therapies to **prevent further resistance, enhance efficacy and minimise toxicity**.
- Support research into the development of novel, accurate **rapid diagnostics** for priority pathogens – especially **affordable point-of-care rapid screening tests**, with the potential for widespread roll-out, particularly to **LMICs**.
- Promote research to improve efficacy, efficiency and quality of **fungal identification and susceptibility testing**, including the development of rapid screening tests suitable for LMICs, and to optimize and standardize the use of current diagnostic modalities for comparison locally, regionally and globally.
- Build an evidence base for incorporating **effective clinical care** for fungal disease into **existing health systems**, with the additional aim of informing public health.
- Pursue **public–private partnerships** and **multicountry collaborative research platforms** to support development of new antifungal therapies and diagnostics.

LMICs: low- and middle-income countries; R&D: research and development.

9.3. Public health

Context. IFD and antifungal resistance are important global health issues that impact vulnerable populations globally. Public health interventions must be built on the foundation of surveillance and R&D, with some priorities outlined in this report. A deep, granular understanding of the dynamics of disease burden (incidence, prevalence, mortality and morbidity) and the prevalence of AMR for these priority pathogens will facilitate rational interventions.

The systematic reviews showed that strategies for IPC exist for many of the priority pathogens, albeit with varying levels of evidence and implementation. Concerted efforts must be directed to refining current strategies and implementation plans, and developing new strategies, to contain the expanding burden of disease. Investigations into the role of air quality, the built environment, coinfections and other drivers at the population level should be considered.

With respect to emerging AMR, while the focus of this prioritization was fungal pathogens in human health, environmental contamination with antifungal agents is a problem. Therefore, One Health approaches are required to understand and mitigate these drivers but have thus far been very limited. Interlinked, integrated and innovative multisectoral approaches to surveillance of AMR and antimicrobial use and consumption are needed.

Additionally, in many settings, health care workers are unfamiliar with fungal infections. This results in low clinical suspicion, misdiagnosis, incorrect or delayed treatment and often poor patient outcomes. To address this, fungal infections need to be mainstreamed beyond specialized training programmes as part of early and ongoing medical and public health training. In addition, management of IFD must follow a patient-centred approach that empowers at-risk groups and provides them with the tools they need to look after their own health.

Finally, policy interventions must be implemented to improve access to existing antifungal agents and diagnostics where disease burden is highest (Box 3).

Box 3: Public health actions, interventions and strategies

- Incorporate fungal diseases and FPPL in **medical (clinical) and public health training** programmes and curricula at all medical training levels.
- **Improve global coordination and action** to strengthen and align action on IFD and antifungal resistance **prevention and control.**
- Promote **existing IPC** measures and **develop new** preventive measures at both the health care facility level and in the community.
- **Adopt, adapt and modify** existing and newly developed **health system approaches** to fungal disease care delivery based on fungal diseases epidemiology and local context.
- Promote **rational use** of antifungal agents through antifungal stewardship intervention, promotion existing or development of new evidence-based treatment guidelines and assess impact on outcomes (survival, length of hospital stays, development of resistance, etc.). Ensure availability of quality antifungal drugs as per WHO EML.
- Develop mechanisms and policies to ensure **equitable, affordable access to quality antifungal agents**. Utilize the **WHO EML** and other tools to inform procurement, tailoring to local need and disease epidemiology. Provide affordable access to diagnostics at the point of care for early identification of high risk patients and to improve appropriate and effective treatment.
- Promote **collaboration across sectors** to address the impact of **antifungal use** on **resistance** across the One Health spectrum.

FPPL: fungal priority pathogens list; WHO EML: WHO essential medicines list; IFD: invasive fungal disease; IPC: infection prevention and control; LMIC: low- and middle-income country; WHO: World Health Organization.

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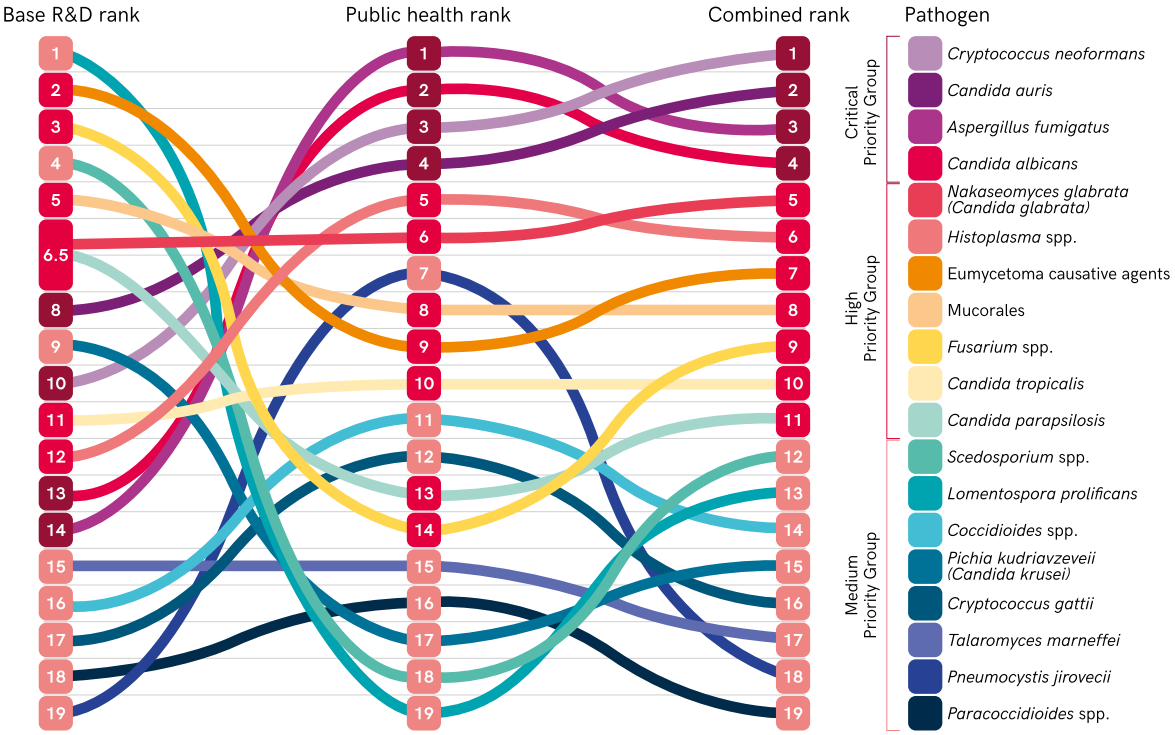
Annex 1. Overall pathogens ranking across the MCDA stages

Overall pathogens ranking across the MCDA stages



Plot showing how pathogens were ranked across three stages of MCDA. From left to right: 1. pathogen ranking based on DCE survey for R&D priorities; 2. pathogen ranking based on BWS scaling survey for public health importance; 3. overall combined ranking. Respondents in the BWS applied the relative importance weights of 0.48 for R&D need and 0.52 for public health importance. These weights were used to determine the overall combined ranking. BWS: best-worst scenario; DCE: discrete choice experiment; MCDA: multicriteria decision analysis; R&D: research and development; spp.: species.

Annex 2. Brief description of each fungal pathogen

(Note: the following description is based on the systematic reviews and evidence extracted to support the prioritization process)

Cryptococcus neoformans

Key facts

- *Cryptococcus neoformans* is an opportunistic fungal pathogen. Cryptococcosis is acquired through the respiratory route when fungi are inhaled from the environment.
- Cerebral cryptococcosis is a life-threatening disease with high mortality despite antifungal therapy.
- Although treatment guidelines are well established for major risk groups (HIV patients), recommended antifungals are unavailable in many countries, and no clear guidelines for non-HIV at risk groups.

Overview

Cryptococcus neoformans is a globally distributed pathogenic yeast which lives in the environment (soil, decaying wood). After inhalation of fungal cells from the environment, *C. neoformans* can infect humans. Cryptococcosis initially affects the lungs but can spread to the central nervous system (cryptococcal meningitis) and blood (cryptococcaemia). Human-to-human transmission does not occur. Most patients are immunocompromised, and the leading risk factor is HIV infection. However, organ transplant patients and others taking medications that weaken the immune system are also at risk, and infection can occur in apparently healthy individuals. Risk factors for invasive cryptococcal disease include HIV infection, iatrogenic immunosuppression, autoimmune disease and decompensated liver cirrhosis.

C. neoformans cryptococcosis is a very serious disease, with mortality ranging from 41% to 61%, especially in patients with HIV infection. Hospital length of stay in patients with *C. neoformans* infection ranged from median of 18 to 39 days, predominantly reported for HIV-positive patients.

Complications due to *C. neoformans* infection and its treatment included acute renal impairment and raised intracranial pressure needing shunts and blindness.

Global annual incidence rates and trends over the last 10 years cannot be assessed due to a lack of studies but are expected to be consistent.

Preventability of invasive cryptococcosis is moderate as there is no vaccine, but prophylactic and pre-emptive therapy in the highest-risk groups significantly reduces the incidence of cryptococcal meningitis.

Access to diagnostics could be rapidly expanded globally, with an effective, fast, cheap and easy-to-perform lateral flow test being available.

Localized cryptococcosis can be treated with fluconazole, while severe and disseminated cases are treated with amphotericin B in combination with flucytosine followed by step-down to fluconazole. Although the treatments are included in the WHO Essential Medicines List (WHO EML), they are still unavailable in many countries. Antifungal resistance is poorly understood and there is only clinical breakpoint for amphotericin B. In addition, reduced susceptibility to fluconazole has been described.

To overcome knowledge gaps, clinical trials aimed at reducing morbidity and mortality are needed. More data on antifungal susceptibility combined with molecular typing of *C. neoformans* would allow better comparison of antifungal resistance rates for different genotypes. In vitro and in vivo synergy tests would allow expansion and optimization of current treatment options for *C. neoformans*. Prospective cohort studies aimed at evaluating long-term complications, risk factors and other clinical outcomes, together with global surveillance data, will better inform the disease burden overall as well as the molecular epidemiology of *C. neoformans* in different patient populations and regions.

Candida auris

Key facts

- *Candida auris* is a yeast that can produce invasive candidiasis. Invasive candidiasis by *C. auris* is a life-threatening disease with high mortality.
- *C. auris* has high outbreak potential and has already produced several hospital outbreaks.
- It is intrinsically resistant to most available antifungal medicines and some strains are pan-resistant.
- Difficult to identify by conventional techniques. Although treatment guidelines are well established, recommended antifungals are unavailable in many countries.
- Preventive measures are not well established. Overall, thermoresistant and partially resistant to commonly use disinfectants.

Overview

Candida auris is a globally distributed pathogenic yeast that can cause invasive candidiasis of the blood (candidaemia), heart, central nervous system, eyes, bones and internal organs. Invasive candidiasis is a serious nosocomial infection that especially affects critically ill and immunocompromised patients, such as cancer or bone marrow and organ transplant patients. Other risk factors include renal impairment, hospital stay longer than 10–15 days, use of mechanical ventilation, central venous catheterization, total parenteral nutrition and sepsis. Previous use of antifungal medicines, especially triazoles, is also associated with increased risk for *C. auris*. *C. auris* has emerged as a cause of hospital outbreaks. This development underscores the importance of adequate infection control preparedness to prevent the spread of *C. auris* within hospitals, although optimal prevention strategies require further study.

The overall mortality of invasive candidiasis with *C. auris* ranged from 29% to 53%. Patients with *C. auris* candidaemia had longer length of stay in hospital or ICU than those with candidaemia caused by other *Candida* spp. The median length of hospital stay was 46–68 days in adult and paediatric *C. auris* candidaemia patients, ranging up to 70–140 days.

Global annual incidence rates cannot be assessed due to the lack of studies. Trends over the last 10 years show an increase in *C. auris* due to outbreaks in many countries. Increase in the numbers of cases during COVID-19 pandemic has been reported by many countries.

Preventability of invasive candidiasis by *C. auris* is moderate. No vaccine is available. Prevention of colonization and surveillance are key in monitoring patients at risk for *Candida* infections.

Access to conventional diagnostics is moderate but overall is difficult to identify by conventional techniques. Availability and affordability of evidence-based treatments is low.

Invasive candidiasis is usually treated with echinocandins, although other antifungals such as azoles might be used following confirmation of in vitro susceptibility. Echinocandins were included in the EML in 2021. Nonetheless, they are still unavailable in many countries. In general, antifungal resistance is moderate. Resistance rates of *C. auris* to fluconazole were as high as 87–100%, while susceptibility to other azoles was variable. *C. auris* isolates showed relatively moderate resistance rates of 8–35% to amphotericin B, and a lower resistance of 0–8% to echinocandins. Unlike other *Candida*, *C. auris* has inherent resistance and in addition pan-resistant isolates have been described.

To overcome the knowledge gap, in vitro and in vivo synergies between antifungal medicines should be evaluated to optimize the current treatment regimens against *C. auris*. Effectiveness and implementation of potential preventative measures need to be explored based on the identified risk factors. Global surveillance studies could better inform the annual incidence rates, distribution and trends in other countries and regions.

Aspergillus fumigatus

Key facts

- *Aspergillus fumigatus* is a ubiquitous environmental mould that can infect humans and cause aspergillosis. It is inhaled from the environment, predominantly causing pulmonary disease, but can disseminate to other sites, such as the brain.
- Aspergillosis is a term used for a wide spectrum of infections that range from allergic reaction, colonization and semi-invasive disease to acute invasive aspergillosis.
- Azole-resistant invasive aspergillosis is a life-threatening disease with very high mortality. Emerging resistance to azoles is concerning.

Overview

Aspergillus fumigatus is a globally distributed ubiquitous environmental mould with pathogenic potential. *A. fumigatus* can produce invasive infections (invasive aspergillosis, IA), mainly in the respiratory system, but can disseminate to other organs, particularly the central nervous system. IA is a serious infection that especially affects the critically ill, those with chronic lung disease and immunocompromised patients, such as those with cancer or transplants. Risk factors for developing IA are well described and include haematological malignancy, chronic lung disease, transplantation (both solid and bone marrow), corticosteroid therapy, neutropenia and chronic liver disease.

Mortality rates in those with azole-resistant *A. fumigatus* infection are high (47–88%) and have been reported to be up to 100% in some studies. Data on overall length of stay in hospital related to IA are limited and range widely (21–532 days); there are no data on the attributable length of stay.

The prevalence of IA is geographically variable, ranging from < 1% to 5–10%; the annual incidence also varies. Azole-resistant *A. fumigatus* infection continues to increase. Trends over the last 10 years could not be established due to a lack of studies.

Preventability is high. No vaccine is available. Antifungal prophylaxis for high-risk groups can prevent IA. Screening for azole resistance is recommended even in azole-naive patients, and especially in high-risk patients such as cancer patients, patients with cystic fibrosis and those in ICU.

Access to conventional diagnostics and availability and affordability of evidence-based treatments are overall low. Azoles are the mainstay of treatment. Other effective agents, such as liposomal amphotericin B, are readily available in high-income countries, but there is limited availability in LMICs.

Antifungal resistance is on the rise. Widespread use of azole fungicides in agriculture to prevent crop losses is contributing to the rising rates of resistant aspergillosis in humans.

To overcome the knowledge gap, cohort studies or sub-analysis evaluating morbidity outcome measures such as length of stay and long-term complications, especially for azole-resistant *A. fumigatus* infections, are needed. In vitro and in vivo synergy studies would also allow optimization of treatment regimens. Global surveillance studies would better inform the distribution of azole resistance, annual incidence rates, and global distribution and trends. Effectiveness and implementation of potential preventative measures need to be explored based on global surveillance studies and other identified risk factors, especially in the setting of newer cancer treatments.

Candida albicans

Key facts

- *Candida albicans* is a fungal pathogen which can be part of the healthy human microbiome but may also cause infections of the mucosae or produce invasive candidiasis.
- Invasive candidiasis is a life-threatening disease with high mortality.
- Treatment is possible and antifungal resistance remains uncommon (low).

Overview

Candida albicans is a globally distributed pathogenic yeast. It is a common member of the human microbiota (mouth, throat, gut, vagina, and skin) and produces no harm in healthy conditions. However, it can multiply in these mucosae or invade other tissues, producing disease. In mucosae, it produces diseases such as oropharyngeal candidiasis, oesophageal candidiasis, vulvovaginal candidiasis and cutaneous candidiasis. More serious, *C. albicans* can produce invasive infections (invasive candidiasis) of the blood (candidaemia), heart, central nervous system, eyes, bones and internal organs with high mortality. Critically ill and immunocompromised patients are especially affected.

Invasive candidiasis has an overall mortality ranging from 20% to 50% despite the availability of active antifungal treatment. Length of stay in hospital is about 2–4 weeks and up to 2 months and is influenced by underlying conditions. It has been suggested that 4% of cases of invasive candidiasis develop secondary growths.

Trends in *C. albicans* over the last 10 years are stable, but in-hospital estimates of incidence and species distribution seem to indicate that infections caused by *C. albicans* are falling relative to other *Candida* species. Recent studies showing higher rates of azole resistance, especially in LMICs, raise concern that resistance is rising.

Preventability of invasive candidiasis by *C. albicans* is low. No vaccine is available. Prevention of colonization and surveillance are key in monitoring patients at risk for *Candida* infections.

Access to diagnostics is high, but availability and affordability of evidence-based treatments is unknown. Some forms of disease are difficult to diagnose, such as abdominal candidiasis whereas blood culture positivity rate is <15%. In these patients, surgical specimen is needed for conventional diagnosis.

Treatment of invasive candidiasis usually includes echinocandins followed by a stepdown to azoles when appropriate. Although echinocandins were included in the EML in 2021, they are still unavailable in many countries. Antifungal resistance is relatively uncommon. Nevertheless, resistance rates especially in non-sterile site isolates seem to be increasing, evidencing the need for more robust and systematic surveillance.

To overcome knowledge gaps, population-based estimates of the incidence of invasive candidiasis in the last 5 years are needed. Stronger data on complications, sequelae and attributable mortality, as well as mitigation strategies should be generated.

Nakaseomyces glabrata (Candida glabrata)

Key facts

- *Nakaseomyces glabrata (Candida glabrata)* is a commensal yeast which can cause invasive candidiasis.
- Mortality from invasive candidiasis due to *N. glabrata* can be as high as 20–50%.
- Preventative measures for invasive disease are not well established. Treatment guidelines are available, although AMR is increasing and poses a challenge.

Overview

Nakaseomyces glabrata (Candida glabrata) is a globally distributed commensal yeast with pathogenic potential. It is a leading cause of candidiasis, usually second only to *C. albicans* in incidence. It can cause invasive candidiasis involving the blood (candidaemia), heart, central nervous system, eyes, bones and/or internal organs. Invasive candidiasis due to *N. glabrata* is a very serious disease, with all-cause mortality at 30 days up to 20–50%. Risk factors for infection include those impacting host immunity.

Little has been reported about complications and sequelae of infection. There are also few data to estimate impact on length of hospital stay, although this is likely similar to other *Candida* species where durations of 2–8 weeks are frequently described. Stay directly attributable to infection is unclear.

Trends over the last 10 years reveal that the prevalence of *N. glabrata* as a proportion of all invasive *Candida* infections is increasing.

Similar to other *Candida* spp., information on the preventability of invasive candidiasis by *N. glabrata* is limited.

Access to conventional diagnostics and availability and affordability of evidence-based treatments are unknown. Invasive candidiasis is usually treated with echinocandins, although other antifungals such as azoles might be used following confirmation of in vitro susceptibility. Echinocandins were included in the EML in 2021 but are still unavailable in many countries.

This species shows high minimum inhibitory concentrations (MICs) to azoles, and in recent years echinocandin resistance seems to have been rising. To overcome the knowledge gap, clinical trials focused on improving outcomes and preventing infection are needed. There is a major lack of data from middle- and especially low-income settings about all aspects of the pathogen. Cohort studies addressing gaps around attributable mortality, complications and sequelae, and length of hospital stay are particularly needed.

Histoplasma spp.

Key facts

- *Histoplasma* spp. are globally distributed pathogens that cause histoplasmosis. Disseminated histoplasmosis particularly affects immunosuppressed patients, but it can also infect healthy individuals.
- *Histoplasma* spp. have the potential to produce outbreaks.
- Disseminated histoplasmosis is a life-threatening disease with mortality ranging from 21% to 53% in HIV patients.
- Treatment is possible, and AMR remains moderate but is rarely measured.

Overview

Histoplasma spp. are globally distributed dimorphic fungi that live as a mould in the environment (soil, and bird and bat droppings) and as a yeast-like form at human body temperature. Histoplasmosis mainly affects the lungs and can expand to the central nervous system, the blood and other parts of the body. It cannot be transmitted between patients (no human-to-human transmission).

Most people that inhale *Histoplasma* spp. do not get ill. Healthy patients usually recover without medication. However, critically ill and immunocompromised patients, such as HIV, cancer and organ transplant patients, may develop severe forms of the disease. A CD4 T-cell count ≤ 50–75 cells/µL is a risk factor for AIDS patients.

Mortality rates in HIV/AIDS patients ranged from 21% to 53%, while lower rates (9–11%) were found in immunosuppressed patients and solid organ transplant recipients. One study reported a mortality rate of 2.7% in children with histoplasmosis. Length of stay in hospital is about 5–7 days in adults and children, with large variability. An average of a month stay was observed in patients with fungal meningitis. The incidence of complications and sequelae is unknown.

Global annual incidence rates cannot be assessed due to a lack of studies. Endemic regions with high incidence in Latin America and Africa have been described, but others reported lower rates. Trends over the last 10 years are stable.

Preventability of invasive histoplasmosis is low. No vaccine is available. Early diagnosis and treatment of HIV would be expected to reduce the burden of disease, although this has not been quantified.

Access to conventional diagnostics is moderate, and availability and affordability of evidence-based treatments is low. Healthy patients usually recover without medication. For severe cases, amphotericin B followed by itraconazole is recommended. Moderate and chronic cases are treated with itraconazole.

Antifungal resistance is moderate. Breakpoints for antifungal resistance are not available. Studies are very limited, but MICs seem to be low for azoles and amphotericin B.

To overcome the knowledge gap, more studies, including in vitro and in vivo synergy tests, are needed to better inform the susceptibility profile of *Histoplasma* spp. in various patient populations and to optimize antifungal regimens. The effectiveness and implementation of potential preventative measures need to be explored based on the identified risk factors. Global surveillance studies could better inform the annual incidence rates, distribution and trends in other countries and regions.

Eumycetoma causative agents

Key facts

- Eumycetoma is a deep tissue infection associated with significant disability. It can be caused by various fungal pathogens, which enter the body through breaks of the skin.
- Global incidence is unknown. Eumycetoma is especially prevalent among the poor in LMICs and appears to have significant geographic variability.
- Behavioural interventions are best described in terms of disease prevention, but their impact has not been comprehensively assessed. Although antifungal treatments are available and resistance is not considered a major issue, amputation of the affected area is frequently needed.

Overview

Eumycetoma is a deep tissue infection caused by fungi found in soil and water. The fungi enter the body through breaks in the skin. Eumycetoma causative agents include *Madurella* spp., *Falciformispora senegalensis*, *Curvularia lunata*, *Scedosporium* spp., *Zopfia rosatii*, *Acremonium* spp. and *Fusarium* spp., although microbiological data are limited. Eumycetoma is a serious infection that especially affects the poor, with many complications and sequelae. Up to 60–80% of mycetoma patients report significant impact on their daily life, and amputation rates as high as 39% have been reported. Risk factors include being a farmer, male and young (11–30 years).

Mortality could not be fully assessed due to the lack of data, but overall is thought to be low. Prevalence varies significantly by geographic location, even within countries. Tropical settings report most cases, but there is limited surveillance data globally, and all estimates are likely a gross under-representation. Trends over the last 10 years suggest no change.

Prevention frequently consists of educational and behavioural hygiene interventions (especially the use of shoes), although data on impact and cost-effectiveness are limited.

Evidence-based treatment guidelines are also limited. There is a serious lack of microbiological data on infecting species and their antifungal susceptibility patterns to inform guidelines. Nevertheless, the evidence that exists suggests considerable heterogeneity in terms of antifungal resistance between species. Treatment is typically with long-term antifungals, and amputation is frequently required for full resolution of infection.

To overcome the knowledge gap, more information on disease burden is necessary, especially in terms of prevalence, morbidity and economic impact. Microbiological data on infecting species and their susceptibility profile are very limited, and more clinical trials to inform treatment and prevention guidelines are needed.

Mucorales

Key facts

- Mucorales is a large group of fungi consisting of different genera. Mucorales are globally distributed and cause a wide spectrum of infection termed mucormycosis.
- Mucorales particularly infect immunocompromised patients but can occur in those with poorly controlled diabetes mellitus and those who have experienced trauma, particularly skin and soft-tissue injuries.
- Invasive mucormycosis is a life-threatening disease with high mortality. Treatments such as surgery and antifungal agents are available.

Overview

Mucorales is a large group (i.e. Order) of globally distributed pathogenic moulds, including *Rhizopus* spp., *Mucor* spp., *Lichthiemia* spp., and others. They can infect the human host after spore inhalation, producing mucormycosis. Therefore, the Mucorales commonly affect the lungs and sinuses, and can spread to the eye, central nervous system and gastrointestinal tract. Fungal invasion can also occur through skin breaks and after burns or other traumatic injuries. Mucorales cannot be transmitted between patients (no human-to-human transmission). Invasive mucormycosis especially affects immunocompromised patients, such as cancer and transplant patients; it has also been well described in those with poorly controlled diabetes mellitus and those who have suffered trauma injuries. Risk factors for mucormycosis include neutropenia and diabetes mellitus. Trauma was also a risk factor for subcutaneous mucormycosis. Invasive mucormycosis is a very serious disease, with mortality ranging from 23% to 80% in adult patients, and up to 72.7% in paediatric patients. Data on length of stay in hospital are limited, but it has been reported to be about 16–17 days. The degree to which the inpatient stay is attributable to mucormycosis has not been determined.

Global annual incidence rates cannot be assessed due to the lack of studies. General population-based incidence rates were poorly described. Trends over the last 10 years show an increase.

Preventability of invasive mucormycosis is challenging. No vaccine is available.

Access to conventional diagnostics and availability and affordability of evidence-based treatments are unknown.

Antifungal resistance is difficult to determine, as clinical breakpoints have not been established. MICs for azoles are generally higher for *Mucor* spp. compared with *Rhizopus* spp. Mucorales are generally susceptible to amphotericin B, although some species/strains can have high MICs. Mucorales are inherently resistant to fluconazole, voriconazole and echinocandins.

To overcome the knowledge gap, development of better diagnostics is needed. Also, more systematic testing (including in vitro and in vivo synergy) of a larger number of isolates per species is needed to establish clinical breakpoints. The susceptibility data need to be correlated with clinical data to establish clinical breakpoints. Preventative strategies, including optimization of antifungal prophylaxis, should be explored in prospective studies, together with evaluation of morbidity outcomes. Global surveillance should generate more consistent measures of incidence rates and prevalence to allow better understanding and comparison of distribution and trends for invasive mucormycosis.

Fusarium spp.

Key facts

- *Fusarium* spp. belong to a large genus of globally distributed filamentous fungi which are found in nature and can infect humans to cause fusariosis.
- Invasive fusariosis is a life-threatening disease, with mortality ranging from 43% to 67%.
- Treatment is difficult due to innate resistance to many of the currently available antifungal agents.

Overview

Fusarium spp. are a group of pathogenic moulds. While globally distributed, they occur mostly in tropical regions. They are saprotrophs, found predominantly in soil, decomposed organic matter and plants. *Fusarium* spp. can cause invasive disease (invasive fusariosis), mainly of the respiratory system and the eyes (keratitis), but can also disseminate to the central nervous system and other organs. They are known to cause fungaemia due to their capacity for adventitious sporulation.

Invasive fusariosis is a serious infection that especially affects immunocompromised patients, such as those with haematological malignancies or post-haemopoietic stem cell transplantation (HSCT). Risk factors for invasive fusariosis include acute myeloid leukaemia, allogeneic HSCT, cytomegalovirus reactivation and presence of skin lesions positive for *Fusarium* spp. at baseline.

Mortality rates (30-day) ranged between 43% and 67% for invasive fusariosis and were especially high for infections involving *F. solani* species complex and *F. proliferatum*. There are no data on length of hospital stay for invasive fusariosis. Endogenous endophthalmitis can complicate invasive fusariosis but is uncommon (< 10%). This can rarely cause visual loss/blindness. Rarely, enucleation is required to prevent blindness.

Global annual incidence rates cannot be assessed due to the lack of studies. Trends over the last 10 years show an increase.

Preventability is low. No vaccine is available. Antifungal prophylaxis has been evaluated in a limited number of studies showing variable results.

Access to diagnostics is moderate, and availability and affordability of evidence-based treatments are low.

Antifungal resistance is high. *Fusarium* spp. seem to be inherently resistant to most antifungal agents, although no clinical breakpoints have been established. Based on MICs, susceptibility to azoles is generally lower than to other antifungal mediciness, such as amphotericin B. *F. solani* showed reduced susceptibility to azoles compared with non-*F. solani* species.

To overcome the knowledge gap, more information on mortality and complications due to invasive fusariosis is needed. Generally low susceptibility was observed for current antifungal medicines. Accordingly, synergy studies and subsequent controlled clinical studies are needed to compare and optimize current drug combinations. Given the limited treatment options, the efficacy of antifungal prophylaxis needs to be established in larger, controlled clinical trials, together with more rigorous risk factor analysis. Surveillance data are needed to understand the global distribution and trends for *Fusarium* spp. infections in various regions other than India and Brazil.

Candida tropicalis

Key facts

- *Candida tropicalis* is a yeast which can be part of the healthy human microbiome but is also capable of causing invasive infections.
- Invasive infection is life-threatening, with mortality ranging from 55% to 60% in adults and 26% to 40% in paediatric patients.
- Specific preventative measures are not well described.

Overview

Candida tropicalis is a globally distributed commensal yeast with pathogenic potential. It is a common member of human and animal microbiota and causes no harm in healthy conditions. However, like other *Candida* species, *C. tropicalis* can produce invasive infections (invasive candidiasis) of the blood (candidaemia), heart, central nervous system, eyes, bones and internal organs. Invasive disease is associated with mortality as high as 55–60% in adults and 26–40% in paediatric patients. Data on complications and sequelae of infection are notably lacking. Risk factors for infection include critical illness and decreased host immunity, and this includes patients in neonatal ICUs.

Invasive candidiasis with *C. tropicalis* has an overall mortality ranging from 55% to 60% in adults and 26% to 40% in paediatric patients. Length of stay in hospital is poorly described, although likely comparable to other *Candida* species.

Global annual incidence rates cannot be assessed due to the lack of studies. Trends over the last 10 years show an increase in *C. tropicalis*.

Access to diagnostics varies, and availability and affordability of evidence-based treatments are still limited globally.

Preventability of invasive candidiasis by *C. tropicalis* is low. No vaccine is available. Infection prevention measures, including care bundles for central venous catheters, likely reduce infection rates, although the impact for this pathogen specifically is not well documented.

Antifungal resistance rates of *C. tropicalis* to azoles, including fluconazole, itraconazole, voriconazole and posaconazole, generally ranged from 0% to 20%, with some studies reporting higher resistance rates of 40–80%. For this reason, invasive disease is usually treated empirically with echinocandins. Echinocandins were included in the EML in 2021 but are still unavailable in many countries.

Overcoming the knowledge gap requires both clinical studies to improve outcomes and cohort studies or sub-analysis to evaluate morbidity outcome measures such as length of stay and long-term complications for invasive *C. tropicalis* infections. More rigorous risk factor analysis from these studies could better inform preventative measures and the need for implementation strategies. Evaluation of potential in vitro and in vivo synergy between antifungal medicines could help optimize the current treatment regimens for *C. tropicalis*. Global surveillance studies could better inform annual incidence rates, distribution and trends in other countries and regions.

Candida parapsilosis

Key facts

- *Candida parapsilosis* is a yeast which can be part of the healthy human microbiome but which also causes invasive infection. Its propensity to form biofilms makes it a particular concern for central venous catheter infections.
- Invasive candidiasis is a life-threatening disease with mortality ranging from 20% to 45%.
- Despite some challenges related to AMR, effective treatments are available. Since infection is equently associated with central venous lines, care bundles to reduce infection are important.

Overview

Candida parapsilosis is a globally distributed commensal yeast with pathogenic potential. It is a normal part of human and animal microbiota and causes no harm in healthy conditions. However, it can produce invasive infection (invasive candidiasis) of the blood (candidaemia), heart, central nervous system, eyes, bones and internal organs, especially in critically ill and immunocompromised patients, such as cancer and bone marrow or organ transplant patients. Concerns have centred around neonatal ICUs.

Invasive disease is associated with mortality ranging from 20% to 45%, despite active antifungal treatment. Data on length of stay in hospital and complications and sequelae of infection are lacking.

Global annual incidence rates cannot be assessed due to the lack of studies. Trends over the last 10 years show an increase in invasive *C. parapsilosis*. In some regions this pathogen is the primary agent for non-*C. albicans* candidaemia.

Preventability of invasive candidiasis by *C. parapsilosis* is low and poorly described. No vaccine is available. Early removal of central lines was shown to reduce incidence of infection. Also, antifungal medication is used in certain patients such as those with cancer or transplants.

Antifungal resistance is moderate. Azole resistance rates in excess of 10% were reported frequently, across multiple regions. Resistance to echinocandins, flucytosine and amphotericin was rare, but overall shows intrinsically higher MICs to echinocandins than other *Candida* species. Studies assessing biofilm mass are concerning for higher rates of resistance to all antifungal agents in biofilm situations (such as central lines, implants and prostheses). Invasive candidiasis is treated empirically with echinocandins, although other antifungals such as azoles might be used once susceptibility has been determined. Echinocandins were included in the EML in 2021 but are still unavailable in many countries.

To overcome the knowledge gap, more data from low-income settings on the incidence, prevalence among candidaemia cases and mortality are needed. Data on complications are very sparse, and it is impossible to assess the impact of this organism on long-term disability. Overall, systematic surveillance is lacking.

Scedosporium spp.

Key facts

- *Scedosporium* spp. are globally distributed fungal pathogens found in nature that can infect humans and produce scedosporiosis.
- Invasive scedosporiosis is a life-threatening disease with mortality rates as high as 42–46%.
- Treatment is threatened by high rates of AMR.

Overview

Scedosporium spp. are globally distributed opportunistic pathogenic moulds. *Scedosporium* spp. can produce invasive infection (invasive scedosporiosis), mainly of the respiratory system, but also blood, central nervous system, other organs, as well as systemic infections, which can be deadly. Risk factors for scedosporiosis include the presence of malignancy, HSCT and severe infection. Mortality rates are as high as 42–46% in adults and children. A more recent study in France reported lower mortality rates (30-day mortality of 9%, 3-month mortality of 19%) in adults and children with invasive scedosporiosis. Patient care length and complications and sequelae are unknown due to a lack of studies. Trends over the last 10 years are stable.

Preventability of invasive scedosporiosis is low. No vaccine is available. Data on preventative measures are lacking.

Access to diagnostics is moderate, and availability and affordability of evidence-based treatments are low. Invasive scedosporiosis is usually treated with voriconazole in combination with other antifungal medicines. In many cases, surgery is needed to remove the infected tissue.

Antifungal resistance is high. There are no pharmacological breakpoints. Reduced susceptibility to amphotericin B, itraconazole, isavuconazole and echinocandins is common. Voriconazole is typically the most active antifungal against these species.

To overcome the knowledge gap, studies reporting on clinical outcomes specific to invasive scedosporiosis are needed in order to better understand mortality rates, hospital length of stay, complications and sequelae. Such studies with larger patient numbers would enable more rigorous risk factor analysis to identify specific preventative strategies. In vitro and in vivo synergy testing would be beneficial to optimize current and emerging treatment options. Surveillance studies at a national or global level are needed to understand the annual incidence and global distribution of *Scedosporium* spp.

Lomentospora prolificans

Key facts

- *Lomentospora prolificans* is a globally distributed pathogen that can cause invasive lomentosporiosis in immunocompromised patients.
- Invasive lomentosporiosis is a life-threatening disease, with mortality ranging from 50% to 71% in adults and 50% in immunocompromised children.
- Treatment is threatened by high AMR rates.

Overview

Lomentospora prolificans is a globally distributed, opportunistic pathogenic mould. It can produce invasive infection (invasive lomentosporiosis) in the respiratory system, blood, central nervous system, other organs, as well as systemic infections, which are usually deadly. Invasive lomentosporiosis is a serious nosocomial infection that especially affects critically ill and immunocompromised patients, particularly those with cancer. The mortality of invasive lomentosporiosis ranges between 50% and 71% in adults and 50% in immunocompromised children. Patient care length and complications and sequelae are unknown due to a lack of studies.

Global annual incidence and trends over the last 10 years cannot be assessed due to the lack of studies.

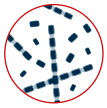
Preventability of invasive lomentosporiosis is unknown. No vaccine is available. In addition, research on the impact of different preventative measures is lacking.

Access to diagnostics is moderate, and availability and affordability of evidence-based treatments are low. Invasive infection is usually treated with voriconazole and terbinafine.

Antifungal resistance is high. Breakpoints for resistance are not defined, but all current licenced antifungals have no in vitro activity against this fungus.

To overcome the knowledge gap, larger studies are needed to better understand the outcomes assessed. Morbidity outcomes such as hospital length of stay and disability need to be defined to understand long-term effects on patients. Current antifungal medicines show low susceptibility rates based on the MIC or MEC (minimum effective concentration), and potential synergy treatment could be explored. New innovative antifungal treatments options are needed. More rigorous risk factor analysis is required to define risk factors and potential preventative strategies. Global surveillance studies could better inform the emergence pattern of the pathogen in a comparable study population.

Coccidioides spp.



Key facts

- *Coccidioides* spp. are some of the most virulent fungal pathogens. Coccidioidomycosis is acquired through the respiratory route when fungi are inhaled from the environment.
- Invasive coccidioidomycosis is a life-threatening disease, especially in vulnerable patients, but it can also infect healthy patients.
- Treatment guidelines are well established but are threatened by high rates of AMR.

Overview

Coccidioides is a genus of pathogenic dimorphic fungi distributed in the Americas, which lives as a mould in the environment (soil, etc.). After inhalation of fungal cells from the environment, *Coccidioides* spp. can infect humans. Coccidiomycosis initially affects the lungs but can expand to the central nervous system, blood, bones and other parts of the body. No human-to-human transmission has been described.

Although it can affect healthy individuals, immunocompromised patients such as cancer and HSCT or organ transplant patients are more affected. Risk factors include people of African descent, including African-Americans, increasing age (over 40–60 years old) and occupation and environmental dust and soil exposure.

Invasive coccidioidomycosis is a very serious disease, with mortality ranging from 2% to 13%. Mortality rates are higher in vulnerable patients. Hospital length of stay in patients with *Coccidioides* spp. infection ranged from a median of 3 to 7 days, with a median of 22.7 days in coccidioidal meningitis.

Global annual incidence rates cannot be assessed due to a lack of studies. Trends over the last 10 years show an increase in *Coccidioides* spp. infections.

Preventability of invasive coccidioidomycosis is low. No vaccine is available. Although many of the risk factors are non-modifiable, increased screening for coccidioidomycosis is suggested in endemic areas, especially in transplant patients, together with optimization of antifungal prophylaxis.

Access to conventional diagnostics is moderate, and availability and affordability of evidence-based treatments in most endemic areas varies.

Primary pulmonary coccidioidomycosis could resolve without antifungal treatment; however, treatment is recommended in risk groups. Disseminated coccidioidomycosis is treated with fluconazole, itraconazole or amphotericin B.

Antifungal resistance is of concern. High MICs for fluconazole and lower MICs for other azoles have been described, but data are still limited. Some studies reported variable MICs for caspofungin, and low MICs for anidulafungin and micafungin. Antifungal susceptibility testing is made even more challenging due to the danger posed by this fungus to laboratory staff.

To overcome the knowledge gap, large cohort studies to allow adequate evaluation of clinical outcomes such as mortality, length of stay and complications are needed, especially in children. Adequately powered prospective studies in larger numbers would allow better evaluation of clinical outcomes. Studies correlating antifungal susceptibility to clinical outcomes, as well as comparative trials for different antifungals, are needed to optimize treatment regimens.

Pichia kudriavzeveii (Candida krusei)

Key facts

- *Pichia kudriavzeveii (Candida krusei)* is a fungal pathogen which can cause infections of the mucosae or produce invasive candidiasis.
- Invasive candidiasis is a life-threatening disease with high mortality.
- Treatment is possible and antifungal resistance is of concern (moderate), as affordable access to effective treatment regimen is still limited.

Overview

Pichia kudriavzeveii (Candida krusei) is a globally distributed opportunistic pathogenic yeast. It is a common member of the human microbiota. However, it can invade mucosae and cause oropharyngeal candidiasis, oesophageal candidiasis, vulvovaginal candidiasis and cutaneous candidiasis. It can also cause invasive candidiasis. Invasive candidiasis is a serious nosocomial infection that especially affects critically ill and immunocompromised patients. The proportion of patients suffering from complications and sequalae is not well known due to a lack of data.

Invasive candidiasis with *P. kudriavzeveii* has an overall mortality ranging from 44% to 67% in adult patients. Limited data are available on length of stay, but it is considered comparable to invasive infections with other *Candida* spp.

The annual incidence is moderate; global annual incidence rates are difficult to assess due to the lack of studies. Trends over the last 10 years are considered to be stable.

Preventability of invasive candidiasis by *P. kudriavzeveii* is low. No vaccine is available. Prevention of colonization and surveillance are key in monitoring patients at risk for *Candida* infections. Reinforcement of hand hygiene on wards could be a general infection control measure to prevent infections, including *P. kudriavzeveii*.

Access to diagnostics is moderate, and availability and affordability of evidence-based treatments are low.

Antifungal resistance is moderate, as *P. kudriavzeveii* is considered intrinsically resistant to fluconazole; but resistance to other azoles and echinocandins is low (0–5%). Invasive candidiasis treatment usually includes echinocandins, although other antifungals, such as azoles, might be used. Echinocandins were included in the EML in 2021 but are still unavailable in many countries.

To overcome the knowledge gap, data on morbidity (hospitalization and disability) and annual incidence are needed. Global surveillance studies and stronger surveillance systems could better inform the distribution pattern of the pathogen in comparable study populations. Specific preventative measures based on risk factors should be explored for their potential benefit and feasibility for implementation.

Cryptococcus gattii

Key facts

- *Cryptococcus gattii* is a globally distributed fungal pathogen, traditionally described more frequently in tropical and subtropical areas, although it can adapt to different temperate settings.
- Invasive disease is life-threatening, and mortality commonly ranges from 10% to 25%. Immunocompromised individuals are at higher risk, but healthy individuals can also be affected.
- Treatment guidelines exist for *C. gattii* infection and resistance remains low, although key medicines are frequently unavailable in LMICs.

Overview

Cryptococcus gattii is a globally distributed pathogenic yeast. It is primarily found in the environment (soil, certain trees, etc.) in tropical and subtropical areas of the world. It can infect the human host after inhalation of spores. Cryptococcosis initially affects the lungs but can spread to the central nervous system (cryptococcal meningitis), the blood (cryptococcaemia) and other parts of the body. There is no human-to-human transmission. Invasive *C. gattii* cryptococcosis is a serious infection, traditionally described as affecting immunocompetent hosts (in contrast to *C. neoformans*). Risk factors include being critically ill, immunocompromised, older age and having pre-existing immunosuppression (e.g. oral corticosteroid use, organ impairment). Neurological sequelae occurred in up to 27% of patients. Immune reconstitution inflammatory syndrome (IRIS) occurred in 9.4% of patients. These complications were reported up to a year after treatment initiation.

Invasive *C. gattii* cryptococcosis is a very serious disease, with mortality reported as 43% for bloodstream infections, though based on limited data. Mortality rates for central nervous system infections and pulmonary infections ranged from 10% to 23% and 15% to 21%, respectively. Length of stay in ICU was about 9 days for adult patients in Australia. Most patients received at least 14 days of therapy requiring hospitalization.

Overall *C. gattii* accounted for 11–33% of cryptococcal infections. Distribution varies by molecular types. Overall annual incidence is low, although some endemic areas (and populations) have higher rates and outbreaks do occur. Trends of *C. gattii* over the last 10 years are unknown due to a lack of surveillance.

Preventability of invasive cryptococcosis by *C. gattii* is low. As with other fungal pathogens, no vaccine is available. Access to diagnostics is very high, with an effective, fast, cheap and easy-to-perform lateral flow test available. Invasive cryptococcosis is usually treated with liposomal amphotericin B in combination with flucytosine (severe lung infection or central nervous system), although fluconazole monotherapy can be used for asymptomatic infection or mild-to-moderate pulmonary infection). Availability and affordability of evidence-based treatments are low. Antifungal resistance is unknown. *C. gattii* isolates showed variable susceptibility data depending on the molecular type, and in general showed higher MICs to fluconazole compared with other azoles, including isavuconazole, itraconazole, posaconazole and voriconazole. MICs for amphotericin B and flucytosine were low.

To overcome the knowledge gap, stronger surveillance systems to understand the global distribution of *C. gattii* and its molecular epidemiology are needed. Such systems may allow more rigorous identification of at-risk populations, dispersion patterns and preventative measures. Better understanding of clinical manifestations and susceptibility profiles for different molecular types is needed and could potentially make it possible to individualize treatment options.

Talaromyces marneffei

Key facts

- *Talaromyces marneffei* is acquired through the respiratory route when spores are inhaled from the environment.
- Invasive talaromycosis is a life-threatening disease, particularly in adults with HIV infection, but it can also infect healthy individuals.
- Treatment guidelines are well established, but recommended antifungals are unavailable in many countries.

Overview

Talaromyces marneffei is a pathogenic dimorphic fungus endemic in South-East Asia and some areas of China. It can be found in the environment (soil, decaying wood, etc.) and may infect the human host after inhalation of spores. Talaromycosis affects the lungs but can expand to the central nervous system, the blood and other parts of the body. There is no human-to-human transmission.

Invasive talaromycosis especially affects critically ill and immunocompromised patients, such as HIV (risk factor being low CD4 count), cancer or organ transplant patients.

Invasive talaromycosis is a serious disease, and mortality ranges between 12%-21% in adults with HIV infection. Hospital length of stay in patients with *T. marneffei* was around 27 days. Complications due to *T. marneffei* infection included respiratory failure, IRIS and wasting syndrome.

Global annual incidence rates cannot be assessed due to a lack of studies. Trends over the last 10 years show an increase.

Preventability of invasive talaromycosis is low. No vaccine is available. Prevention of colonization and surveillance are key in monitoring patients at risk for *T. marneffei* infection.

Access to conventional diagnostics is moderate, and availability and affordability of evidence-based treatments are low. Invasive talaromycosis is usually treated with amphotericin B, itraconazole or voriconazole.

Antifungal resistance is low. There are no established clinical breakpoints for *T. marneffei* isolates to allow studies to define resistance rates to antifungal medicines. Fluconazole showed higher MICs compared with other azoles, such as itraconazole, posaconazole and voriconazole. Anidulafungin and caspofungin showed higher MICs.

To overcome the knowledge gap, prospective studies investigating clinical outcomes such as mortality and morbidity are needed, including paediatric patients. Antifungal susceptibility studies on a larger number of isolates are needed. Such studies should ideally investigate the correlation between in vitro susceptibility and clinical outcome to allow optimization of treatment and establishment of clinical breakpoints. More active surveillance systems are needed to estimate global distribution and trends for talaromycosis, including data on non-HIV/AIDS patients.

Pneumocystis jirovecii

Key facts

- *Pneumocystis jirovecii* is an opportunistic fungal pathogen which is acquired from person to person through the air.
- *P. jirovecii* pneumonia is a life-threatening disease, with substantial but highly variable mortality.
- Treatment is well established, but antifungals are unavailable in many countries.

Overview

Pneumocystis jirovecii is a globally distributed, opportunistic pathogenic fungus. *Pneumocystis jirovecii* pneumonia (PJP) is acquired from person to person through the air. It can be carried by healthy individuals who are asymptomatic.

Although *P. jirovecii* can affect healthy individuals, immunocompromised patients such as those with HIV, organ transplant patients and others taking medications that weaken the immune system are more affected. Risk factors for PJP include AIDS, cancer, iatrogenic immunosuppression with solid organ transplantation (especially renal), autoimmune and inflammatory disease, and nephrotic syndrome.

PJP is a very serious disease, and mortality ranges from 0% to 100% overall (highly variable). Hospital length of stay in patients with *P. jirovecii* infection ranged from 0–123 days (median of 6.6–30 days). Complications include respiratory failure, long-term graft failure and renal failure.

P. jirovecii is globally distributed, but global annual incidence rates are unknown but are expected to be consistent. Trends over the last 10 years indicate stable incidence overall but a decline in some groups, particularly in persons with HIV.

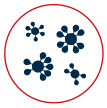
Preventability of PJP is high. No vaccine is available. Preventative measures were widely reported and consisted of drug prophylaxis, which is highly efficacious.

Access to conventional diagnostics is moderate, and availability and affordability of evidence-based treatments are high. PJP is usually treated with cotrimoxazole.

Antifungal resistance is unknown. Phenotypic susceptibility testing is not possible for *P. jirovecii*, breakpoints have not been established, and treatment is with agents different to those used for other fungal infections.

To overcome the knowledge gap, better understanding is needed of factors associated with the risk of PJP acquisition and mortality, especially in non-HIV at risk populations. The significance of molecular mutations remains to be further explored. Also, more and standardized information on annual incidence is needed.

Paracoccidioides spp.



Key facts

- *Paracoccidioides* spp. are fungal pathogens that are acquired through the respiratory route when spores are inhaled from the environment.
- Paracoccidioidomycosis is a life-threatening disease with medium mortality despite antifungal therapy. Immunocompromised individuals are at higher risk, but the pathogen can also infect healthy individuals.
- Treatment is well established, but antifungals are unavailable in many countries.

Overview

Paracoccidioides spp. are pathogenic dimorphic fungi endemic to Central and South America that live in the environment (soil). After inhalation or penetration of the skin by fungal spores from the environment, the pathogen can infect humans. Paracoccidioidomycosis mainly affects the lungs, mucous membranes and skin, and can expand to the lymph nodes and other organs of the reticuloendothelial system. Most people infected with *Paracoccidioides* spp. never develop symptoms. It cannot be transmitted between patients (no human-to-human transmission).

Risk factors include age > 40 years old and male gender.

Paracoccidioidomycosis is a serious disease. Mortality ranges from 3% to 23%, especially in patients with HIV. Hospital length of stay in patients with *Paracoccidioides* spp. infection could not be assessed due to a lack of data. Complications due to *Paracoccidioides* spp. infection and its treatment include low adrenal reserve and lymphedema.

Paracoccidioides spp. are endemic to Central and South America, but global annual incidence rates cannot be assessed due to the lack of studies. Trends over the last 10 years are stable.

Preventability of invasive paracoccidioidomycosis is low. No vaccine is available. Data on prevention strategies are lacking.

Access to conventional diagnostics is moderate, and availability and affordability of evidence-based treatments are very low.

Invasive paracoccidioidomycosis is usually treated with itraconazole, amphotericin B or cotrimoxazole.

Antifungal resistance is unknown.

To overcome the knowledge gap, larger cohort studies evaluating morbidity outcome measures such as length of stay and long-term complications of paracoccidioidomycosis are needed. There have been no antifungal susceptibility data for *Paracoccidioides* spp. in the last 10 years. Susceptibility testing with newer antifungal medicines as well as potential synergy tests should be performed to ensure evidence-based treatment regimens and optimization. Studies predominantly reported paracoccidioidomycosis in Brazil. Global surveillance studies are needed to inform incidence rates, distribution and trends in other countries and regions. These studies could also identify risk factors in different patient populations to enable tailored preventative strategies.

World Health Organization

World Health Organization
Antimicrobial Resistance Division
20 Avenue Appia
1211 Geneva 27
Switzerland
https://www.who.int/antimicrobial-resistance/en/

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Title Page

Estimating the burden of Invasive and Serious Fungal Disease in the United Kingdom

Pegorie M[1], Denning DW[2,3], Welfare W[1,3]

[1]Public Health England North West Health Protection Team (Greater Manchester)

[2] National Aspergillosis Centre, University Hospital of South Manchester and The University of Manchester, Manchester

[3] Manchester Academic Health Sciences Centre, University of Manchester

Correspondence: DW Denning, 2nd Floor Education and Research Centre, Wythenshawe Hospital, Southmoor road, Manchester, M23 9LT, UK. Email: david.denning@manchester.ac.uk

Key words: Candida, Aspergillus, Cryptococcus, mortality, morbidity

ABSTRACT

Background: The burden of fungal disease in the UK is unknown, but was estimated in 2002 by the Health Protection Agency. A few data are systematically collected. We have re-estimated the annual burden of invasive and serious fungal disease.

Methods: We used several estimation approaches including voluntary laboratory reports. We searched and assessed published estimates of incidence, prevalence or burden of specific conditions in various high-risk groups; studies with adequate internal and external validity allowed extrapolation to estimate current UK burden. For conditions without adequate published estimates, we sought expert advice.

Results: The UK population in 2011 was 63,182,000 with 18% aged under 15 and 16% over 65. The following annual burden estimates were calculated: invasive candidiasis (IC) 5,142; *Candida* peritonitis complicating chronic ambulatory peritoneal dialysis 88 and the remainder captured under IC; *Pneumocystis* pneumonia 207 to 587 cases, invasive aspergillosis (IA), excluding critical care patients 2,901 to 2,912, and IA in critical care 387 to 1,345 patients, utilizing different external assumptions, <100 cryptococcal meningitis cases. We estimate 178,000 (50,000–250,000) allergic bronchopulmonary aspergillosis cases in asthma, and 873 adults and 278 children with cystic fibrosis. Chronic pulmonary aspergillosis is estimated to affect 3,600 patients, based on burden estimates post tuberculosis and in sarcoidosis.

Conclusions: Much uncertainty is intrinsic to most burden estimates due to diagnostic limitations, lack of national surveillance systems, few published studies and methodological limitations. The largest uncertainty surrounds IA in critical care patients. Further research is needed to produce a more robust estimate of total burden.

Word count: 250

BACKGROUND

Invasive fungal disease is thought to be increasing in frequency in the United Kingdom (UK) due to a variety of factors including increased survival time from previously lethal illnesses and an increase in prevalence of conditions and treatments leading to immunosuppression. Understanding of the overall burden of invasive fungal disease in the UK is limited as there is no formal systematic or mandatory surveillance programme specific to fungal infections, although active surveillance networks exist for candidaemias (voluntary laboratory reporting[1]) and specifically for candidaemias in neonates (voluntary reporting[2]). In addition, several debilitating chronic and allergic fungal diseases, amenable to antifungal therapy have come to greater prominence. An analysis of laboratory reports of fungal infections was carried out in 2001[3], which highlighted the likelihood of underestimating total burden due to the challenges involved in laboratory diagnosis and the voluntary nature of the laboratory reporting system. In 2008, the UK health Protection Agency issued a report entitled "Fungal Diseases in the UK: The current provision of support for diagnosis and treatment: assessment and proposed network solution"[4]. A rough annual burden estimate of many fungal diseases was made in this report, but not subsequently published. The UK community of experts in this area has been active in developing best practice standards for the UK and beyond for the diagnosis and clinical management of fungal disease[5,6,7,8,9]. A necessary next step for healthcare and research prioritisation is to quantify this burden with improved tools and an expanded range of serious fungal infections.

METHODS

We used the UK Office for National Statistics 2011 Census data[10] to estimate UK population size. We used this as the 2011 census is the most recent census in the UK.

We have estimated the annual incidence of the following invasive fungal infections: cryptococcal disease and meningitis, *Pneumocystis* pneumonia, invasive aspergillosis, candidaemia and *Candida* peritonitis, as well as oesophageal candidiasis. In addition, we have estimated the prevalence of chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS). Information on incidence, prevalence and total burden of these conditions in the UK is limited. Where such information was available for the UK or certain countries within the UK (where UK estimates were not available), we included it in the study. One example is data from the voluntary surveillance of candidaemia in England, Wales and Northern Ireland[1].

Where the information was not available we used a pragmatic approach: for each fungal condition, we considered which populations were most at risk of the condition, sought published estimates for incidence or prevalence measures for the condition in these specific risk populations, and applied these rates to available published estimates of size of these high risk populations in the UK (or certain countries within the UK where UK estimates were not available).

Where multiple estimates of incidence or prevalence were published, we considered both internal and external validity of the studies in deciding on which estimate to use. The methods used for estimating burden of the specific fungal conditions are outlined below.

Selection criteria for published estimates of incidence: for many of the severe fungal infection, there is a paucity of published estimates of incidence, therefore we had to be pragmatic in our approach. Where more than one published estimate was available, we prioritised studies with the best applicability to the UK population (i.e. where UK studies were available we used these, if not we used studies from countries with as comparable a population as possible, where non-UK studies were selected, this is made clear in italics in the fungal infection section of the methods) and those with the largest sample sizes (where multiple studies were considered, this is made clear in the fungal infection section of the methods).

Pneumocystis pneumonia

First method

Prior to March 2013, no published estimates of incidence, prevalence or total burden were available for England except for AIDS patients (PHE HIV in the UK report[16]) therefore our initial approach was pragmatic.

The high risk populations identified and the data source used to estimate their current size included AIDS patients (PHE HIV in the UK report[16]) and patients who had received various transplants (Tx) - Heart Tx, Kidney Tx, Liver Tx and Lung Tx or Heart and Lung Tx patients (Organ donation and Transplantation Activity Report 2013/14[11]).

An estimate of total burden amongst the AIDS patient population for 2011 to 2013 was published in the PHE HIV in the UK report[16], we divided this estimate by three to obtain an average yearly estimate.

The incidence rates specific to transplant high risk populations were found from a variety of studies including: Cardenal et al.[12] for Heart Tx patients; Wang et al.[13] for Kidney Tx, Liver Tx and Lung Tx/ Heart + Lung Tx patients.

Second method

A UK study estimating the incidence of *Pneumocystis* pneumonia over an 11 year period using 4 data sources was published in March 2013[14]. This showed that the incidence had increased significantly over the study period. We aimed to estimate the total burden for the most recent year of the study (2010) based on figures reported in the paper for each of the 4 data sources: Hospital Episode Statistics (HES) data - the paper reported the number of cases in 2010; Routine Laboratory Reporting - the paper reported a range for number of cases in 2008-2010, we used the central point of this range; Death Certificate Data - the paper reported the number of cases in 2010; HIV Surveillance Data - the paper did not report a number or range for total number of cases in the later years of the study, we obtained an estimate by extrapolating from figure 3 of the paper.

Cryptococcal meningitis:

No published estimates of incidence, prevalence or total burden were found for the UK. We obtained an estimate based on a simple direct question to the largest mycology referral laboratories in the UK (Bristol, Leeds and Manchester) of the frequency of positive cryptococcal antigen test results. One publication was found which reported on trends in incidence and numbers of fungal meningitis[15], but this covered all fungal infections and was not specific to cryptococcal infection.

The high risk populations identified included newly diagnosed HIV infection. We used the PHE HIV in the UK report[16] to estimate the current size of this population. The incident rate for this high risk population was obtained from Patel et al.[17]

Invasive aspergillosis

We took a pragmatic approach to estimating the burden of invasive aspergillosis. The high risk populations identified and the data source used to estimate their current size included: Allogeneic hematopoietic stem cell transplantation (HSCT) and autologous HSCT patients (The British Society of Blood and Marrow Transplantation Registry [18]); Heart Tx, Lung Tx, Liver Tx and Kidney Tx (Organ donation and Transplantation Activity Report 2013/14[11]); AIDS patients (HPA HIV in the UK report[16]); Acute myeloid leukaemia (AML), Acute lymphoblastic leukaemia (ALL), Chronic myeloid leukaemia (CML), Chronic lymphocytic leukaemia (CLL), Non Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and Myeloma patients (UK Cancer Registry [19]); Chronic granulomatous disease (CGD) patients (Jones et al.[20]); Chronic obstructive pulmonary disease (COPD): emergency hospital admissions (Di Santostefano et al.[*21]); Critical Care Patients (Hospital Episode Statistics, Adult Critical Care in England: April 2013 to March 2014[22]); patients with lung cancer (UK Cancer Registry[19])

The incidence rates specific to the above high risk populations were found from a variety of studies: Lortholary et al.[23] (for Allogeneic and Autologous HSCT patients, and for Heart Tx, Lung Tx, Liver Tx, and Kidney Tx patients) –*these estimates were not for the UK population but the French population*; Keshishian C. Health Protection (HPA) Mycology Network - Rapid evaluation of incidence estimates (Unpublished)[24] (for AIDS patients); Pagano et al.[25,†] (For AML, ALL, CML, CLL, NHL, HL and myeloma patients) –*these estimates were not for the UK population but the Italian population*; Beauté et al.[26,‡] (for CGD patients) –*this estimate was not for the UK population but the French population*; Guinea et al.[27] (for COPD: emergency hospital admissions) –*this estimates were not for the UK population but the Spanish population*, another study reporting an incidence estimate was considered (Xu et al[28])

[*] We used the HES-based 4 year study to estimate yearly average number of COPD emergency admissions. We excluded the day cases as these were unlikely to develop invasive aspergillosis. We used the estimated incidence in the last year of the study (2007)

[†] The paper reports total yearly number of cases of invasive mould infections according to malignancy type. It also reports that 90% of mould infections were caused by *Aspergillus* spp. We calculated malignancy-specific incidence rates for invasive aspergillosis by applying the 90% rate to the total number of cases of mould infection per malignancy type and dividing this by the total number of patients with the malignancy.

[‡] The paper reported an overall incidence of invasive fungal disease (IFD) per patient year, and reported that 40% of IFDs were cause by invasive aspergillosis. The overall incidence rate was applied to the estimated population size, and 40% of the resulting estimate of overall IFD burden was used for the burden of invasive aspergillosis.

but the sample size for the study was significantly smaller than that of Guinea et al so we did not include it; A wide range of estimates from different studies[29] for critical care patients, see sensitivity analysis discussion below –*these estimates were not for the UK population but the Belgian and Spanish populations*; Yan X et al.[30] for patients with lung cancer –*this estimate was not for the UK population but the Chinese population*.

Critical care patients: Sensitivity analysis

The largest risk-group population by far for invasive fungal infection in our study was patients in critical care at risk of invasive aspergillosis, regardless of which type of critical care unit is considered. Therefore any variation in incidence rate could lead to a significant change in estimated burden. We carried out a sensitivity analysis to reflect this.

Activity data is available for a broad range of critical care units in England[22]. The most common type of admission to ICU amongst cases of invasive aspergillosis is medical admission, and the most common reasons for admission respiratory and cardiovascular disease[31], therefore we considered two broad groups of critical care units in the sensitivity analysis. The first was medical intensive care units (ICUs) and other ICUs where length of patient stay is likely to be similar to that of medical ICUs[§], the second was all ICUs, excluding spinal units.

There is a wide range of published estimates for incidence of invasive aspergillosis in patients in critical care: from 0.3% to 19%[29]. Key factors include: the type of critical care unit considered, and whether or not studies were autopsy controlled. Further complications include the facts that no non-invasive diagnostic test (for example isolation of *Aspergillus* from respiratory cultures) is sensitive or specific enough to establish definite diagnosis[32], and that it is difficult to distinguish colonisation with *Aspergillus* from infection with *Aspergillus*[32].

We focused on those studies that specifically examined the incidence of invasive aspergillosis in critical care units. Four such studies were found, one had a small sample size (n=24) and did not report an incidence estimate so was not considered here[33]. The other three, from which incidence rates estimates were used, are listed in table 4 with their characteristics and the populations they apply to.

We adjusted estimates of burden to account for double counting of patients already counted in groups were we assumed that the majority of those who developed invasive aspergillosis would require ICU admission (solid organ transplant patients and COPD emergency admissions).

Chronic pulmonary aspergillosis

[§] Critical care episodes were counted from table 14 of the Critical Care report 2013-14[22], critical care unit functions included in this group were: Non-specific general adult critical care, Medical adult patients, Liver patients predominate, Renal patients predominate

Chronic pulmonary aspergillosis complicates a wide spectrum of underlying lung disease of which the commonest conditions are pulmonary tuberculosis (PTB), non-tuberculous mycobacterial lung infection, COPD, sarcoidosis, and allergic aspergillosis complicating asthma[39].

An estimate of the annual number of patients with chronic pulmonary aspergillosis after pulmonary tuberculosis (PTB) has recently been published[34]. For most countries, this was based on a 22% rate of chronic pulmonary aspergillosis after PTB in those with cavities of 2.5cm or greater and 2% in those without a residual cavity, but in the absence of UK data, the assumed rate or residual cavitation after PTB was 12% (range in other countries 21-35%[35,36,37]). To generate a 5 year period prevalence a 15% attrition rate was assumed, accounting for surgical resection and death.

An estimate of the rate of chronic pulmonary aspergillosis complicating sarcoidosis in the UK was also recently published[38]. Numerous other antecedent underlying pulmonary conditions are found in patients with chronic pulmonary aspergillosis[39], and the relative proportions of these were used to estimate the total UK burden.

A separate approach was taken using referrals to the National Aspergillosis Centre from the north west of the UK, based on population and regional variation in directly age-standardised mortality rates (DSR). Just over 100 new patients are referred annually to the National Aspergillosis Centre[40]. It was assumed that referral was near complete in the NW of the UK to the National Aspergillosis Centre because of excellent clinical links and proximity. Using published directly age-standardised respiratory disease mortality rate for under year 75 olds (DSR)[41] and regional populations[42], we derived an annual potential diagnosable burden, based on current respiratory medicine practice, which approximates to an annual incidence (table 3).

Allergic bronchopulmonary aspergillosis (ABPA)

ABPA complicates asthma and cystic fibrosis (CF). The global burden of asthma has been re-estimated recently, a total of 334 million in all ages (4.85% of the global population)[43] and 193 million adults with active asthma[44]. The UK has one of the highest rates of asthma in the world, an estimated 16-18.2% of adults with clinical asthma[45], or nearly 8.2-9 million (age 15 and older) [46]. Other more recent data of asthma prescription data from the UK, put the total rate at ~5.4 million, including children. As the prevalence in children is 88% of the adult rate, we derived an adult number of asthmatics of 4.4 million (our lowest and base case estimate).

There are no population data for ABPA or any surrogate marker such as IgE from the UK. An abstract from one hospital tracking IgE and Aspergillus IgE levels in 330 consecutive referrals to an asthma clinic found a 1.5% rate of probable ABPA with most diagnostic features and 13% with both an elevated total IgE and Aspergillus IgE[47]. A base case estimation of ABPA rates in adults was made, using a median prevalence of 2.5% from referrals to secondary care. This 2.5% rate is derived from rates of 0.78% and 4.1%[44,48] from 6 national studies all done in consecutive referrals over a defined period to a specialist chest physician for problematic asthma. Deterministic sensitivity analyses relating to different asthma populations rates and ABPA rates were also derived.

ABPA is reported in children, but is probably rare[49], and there are no epidemiology studies published to estimate a rate.

We ascertained the number of individuals in the UK over the age of 18 with CF from 2011 annual report. Using the distribution frequency described by Baxter et al[50], we derived the likely numbers of adults with aspergillosis in CF in the UK. ABPA in CF is well recognised in older children and teenagers, and we have used the annual CF report for this purpose[51].

Severe asthma with fungal sensitisation (SAFS)

As SAFS is another distinctive pattern of asthma usually associated with sensitisation to multiple fungi and responsive to antifungal medication[52,53,54,55] we estimated the UK burden of this entity. While recently described in children[56], it is rare, and so not estimated. Severe asthma is defined by a poor level of current clinical control including a risk of frequent severe exacerbations (or death) and/or chronic morbidity. Severe asthma includes untreated severe asthma, difficult-to-treat severe asthma, and treatment-resistant severe asthma. In a multi-country comparison of the role of fungal sensitisation in severe asthma, 21% were defined as severe[57]. In other studies[58] lower frequencies of severity are recorded[59], including a recent estimate of 3.6%, depending on many factors, and we have used an arbitrary figure of 5% as our base case to embrace both severe refractory and compliant difficult to control asthmatics. We have also computed a sensitivity analysis.

Fungal sensitisation becomes more common the worse the asthma, with rates ranging from ~25% of patients referred to a specialist to 75% for those repetitively admitted to hospital. We have used a rate of 60% to be conservative. [60,61,62,63]

Candidaemia

There is a voluntary surveillance system in England that collects laboratory reports of all microorganisms isolated (including fungi) at approximately 400 NHS and other laboratories throughout England, Wales and Northern Ireland. The database which compiles this data is called LabBase2[1]. Surveillance reports are published in PHE weekly Health Protection Reports[64].

Blood culture has a poor sensitivity for detecting Candida species: a 2011 systematic review of the diagnostic accuracy of PCR techniques for invasive candidiasis[65] identified 10 studies reporting the sensitivity of blood cultures. The pooled culture positivity rate in patients with proven or probable invasive candidiasis was 0.38 (95%CI: 0.29 to 0.46)[65]. A more recent US study using PCR and beta 1.3-D-glucan detection derived a similar figure[66]. Therefore we made the assumption that the total number of positive blood culture samples represented 38% cases of proven or probable invasive candidiasis tested by blood culture techniques.

Candida peritonitis

We took a pragmatic approach to estimating the burden of Candida peritonitis.

The two main risk groups for this condition in the UK are: surgical ICU patients and patients on chronic ambulatory peritoneal dialysis (CAPD).

Surgical ICU patients

We assumed that the majority of cases in surgical ICU patients would be counted in the estimate of total number of cases of invasive candidiasis discussed above.

CAPD patients

To estimate the number of patients on CAPD in England every year, we used data from the NICE Clinical Guideline 125: Kidney disease: peritoneal dialysis: Costing report, Implementing NICE guidance[67].

To estimate the incidence of peritoneal candidiasis in patients on CAPD, we used an estimate reported on the Leading International Fungal Education (LIFE) website[68]. This incidence estimate was reported as episode per patient year. In our calculation of attributable burden, we assumed that all CAPD patients in England stay on CAPD for at least a year.

Oesophageal candidiasis

The main risk group for this condition in the UK is probably AIDS patients. Oesophageal candidiasis is an AIDS defining illness. The number of cases reported in the UK between 2011 and 2013 was reported in the PHE HIV in the UK report[16]. We divided this figure by three to obtain a yearly estimate of burden.

Another approach to estimating the burden was also taken using published estimates of yearly incidence amongst HIV patients on anti-retroviral therapy[69] -*this estimate was not for the UK population but the USA population*- and estimates of numbers of HIV patients on anti-retroviral therapy in the UK[16].

Mucormycosis

Occasional cases of mucormycosis occur in the UK, usually highly immunocompromised patients, occasionally in intravenous drug addicts, burn or trauma victims or diabetic patients, and rarely related to hospital transmission (Lancet tongue depressors). Most diagnoses are made histologically or on direct microscopy specimens, culture sensitivity is low. No data are collected systematically.

To estimate the number of mucormycosis cases in the UK, we applied *the French population incidence* found from published studies[70],[71] to the UK population (no UK estimate of incidence available).

Other rarer infections

Other rarer infections are not well tracked in the UK, including imported endemic mycoses (histoplasmosis and coccicioidomycosis for example) and are rare from experience of experts at the national Aspergillus centre. Likewise serious infections related to unusual filamentous fungi such as

Fusarium or Scedosporium do occur, the former in leukaemic patients, the latter in some cystic fibrosis patients and rarely as an invasive pathogen.

Results

RESULTS

The UK population in 2011 was 63,182,000 with 18% aged under 15 and 16% over 6510[10].

Pneumocystis pneumonia:

An average yearly total burden of 157 *Pneumocystis* pneumonia (PCP) diagnoses was found for the AIDS patient population in the UK[16] using our first estimation approach.

The estimates of population size, population-specific incidence rate and yearly burden of disease obtained for patients who had received various transplants in the UK are outlined in table 1

Table 1: Estimates of population size, specific incidence rates and yearly burden of Pneumocystis pneumonia for solid organ transplant populations.

Population	Population size	Incidence rate	Yearly burden of disease
Heart Tx	195	5.5%	11
Kidney Tx	2,244	0.3%	7
Liver Tx	830	1.15%	9
Lung Tx or heart and lung Tx patients	397	5.78%	23
Total			50

The total estimate of burden of PCP for both AIDS patients and solid organ transplant populations in the UK was **207**. This estimate ignores other immunocompromised patients, such as haematological malignancy and severe autoimmune disease.

Second method

Our second estimation approach yielded a total UK burden of **587 cases** of PCP for 2010.

Cryptococcal disease and meningitis:

An estimate of up to **100 cases** per year for the UK was obtained from the reference laboratories

It is unclear whether this is an underestimate or an overestimate as it is estimated that in 2011 there were a total of 51 fungal meningitis cases (all fungi, based on culture). However this 2011 estimate is based on voluntary laboratory reporting and furthermore, there is some evidence that cryptococcal infections are under-reported[15]. Many diagnoses of cryptococcal disease are based on cryptococcal

antigen alone, and while meningitis is the commonest manifestation of disease, other organs are affected. It is likely that the vast majority of these cases were in HIV-infected individuals and in 2013 ~6,000 new HIV infections were diagnosed[16 above].

Invasive aspergillosis

The estimates of population size and (p), population-specific incidence rate (i) and burden of disease (n) obtained for high risk populations in the UK excluding critical care units patients are outlined in table 2

Table 2: Estimates of population size, specific incidence rates and yearly burden of Invasive aspergillosis for well recognised at risk groups

Population	Population size	Incidence rate	Yearly burden of disease
Allogeneic HSCT	1,615	8.1%	131
Autologous HSCT	2,225	0.9%	20
Heart Tx	195	4.8%	9
Lung Tx	397	4.1%	7
Liver Tx	830	0.8%	7
Kidney Tx	2,801	0.3%	8
AIDS patients	320	0.6% to 4%	2 to 13
AML	2,921	7.1%	207
ALL	654	3.8%	25
CML	675	2.3%	15
CLL	3,233	0.5%	16
NHL	12,783	0.8%	103
HL	1,845	0.4%	7
Myeloma	4,792	0.2%	9
CGD	119	$i_{all\ IFD}$= 0.040/patient-years**	$n_{all\ IFD}$= 4.76, n_{CGD}=2
Total			**568 to 579**

Therefore **a total of 568 to 579 patients in well recognised at risk groups**. Some cases in haematological patients will have been prevented with antifungal prophylaxis. Only lung Tx recipients with true IA are included, omitting those with airways infection and colonisation, all of whom are treated.

The estimate for patients with pulmonary disease are outlined in table 3

Table 3: Estimates of population size, specific incidence rates and yearly burden of Invasive aspergillosis for pulmonary disease

Population	Population size	Incidence rate	Yearly burden of disease

** Overall incidence of invasive fungal disease (IFC)

COPD emergency hospital admissions	89,466	1.3%	1,163
Patients with lung cancer	44,488	2.63%,	1,170

Therefore the estimate for the total yearly burden of IA in the UK for the all of the above groups is **2,901 to 2,912**

Sensitivity analysis

Table 4: Sources of estimates of the incidence of invasive aspergillosis in critical care patients

Study	Study characteristics	Population studied
Meersseman et al. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med, 2004[72].	-Sample size: n=127 -Autopsy controlled -Study aim: to determine the incidence of IA in medical ICUs -Retrospective, single centre	Patients in medical critical care units
Garnacho-Montero et al. Isolation of *Aspergillus spp*. from the respiratory tract in critically ill patients: risk factors, clinical presentation outcome. Crit Care 2005[73]	-Sample size: n=1,756 -Not autopsy controlled -Study aim: describing characteristics of patients with positive samples for *Aspergillus* species -prospective, multi-centre (73 mixed ICUs)	Patients in any type of critical care unit
Vandewoude et al. Clinical relevance of *Aspergillus* isolation from respiratory tract samples in critically ill patients. Critical Care 2006[32]	-Sample size: n=172 -Not autopsy controlled -Study aim: describing characteristics of patients with positive samples for *Aspergillus* species -Retrospective, single centre, mixed ICU	Patients in any type of critical care unit

Table 5: Sensitivity analysis for estimation of burden of invasive aspergillosis amongst patients in critical care in the UK

Risk group	Number in Risk Group	Incidence Rate	Number of expected cases
Patients admitted to medical ICUs	166,645	5.8%[72]	9,665
Patients admitted to any ICU (Spinal Units excluded)	248,811	1.1%[73]	2,737
Patients admitted to any ICU (Spinal Units excluded)	248,811	0.33%[32]	821

The results of the sensitivity analysis of IA in critical care are displayed in table 2. The variation between highest and lowest burden estimates for medical type ICUs and all type ICUs (spinal units excluded) was over 10-fold. This highlights the level of uncertainty over this estimate of burden. Our view is that the rate of IA in the UK is probably at the low end of the estimates above, with ~50% of the cases occurring in COPD patients[72], even though IA is the most common missed infectious diagnosis at autopsy[74]. So a total ICU caseload of between 821 and 2,737 is likely, of which 50% is attributable to COPD. Adjusting downwards by 50% for probable double counting of cases of COPD emergency hospital admissions (we assumed most of these would be admitted to ICU), and solid organ transplant recipients (n=24) resulted in adjusted estimates of **387 to 1,345 cases**.

The total estimate of burden of IA amongst the high risk populations is **2,901 to 2,912** (excluding ICU populations) and **3,288 to 4,257** (including ICU populations). This estimate ignores those with solid tumours other than lung tumours, autoimmune disease, liver failure and other conditions treated with corticosteroids.

Chronic pulmonary aspergillosis

Chronic pulmonary aspergillosis complicates a wide spectrum of underlying lung disease of which the commonest conditions are pulmonary tuberculosis (PTB), non-tuberculous mycobacterial lung infection, COPD, sarcoidosis, and allergic aspergillosis complicating asthma[39]. Some estimates of the annual incidence and 5 year period prevalence have been published for pulmonary tuberculosis and pulmonary sarcoidosis complicating an estimated 16,270 cases of pulmonary sarcoidosis in the UK[38]. The anticipated annual incidence of each was 118 and 240 respectively. Together these two conditions account for about 30% of patients with CPA[38] and so an annual diagnosable incidence is around 358 cases for these conditions and a total of **1,193 cases**. We compared this total, with current referral to the National Aspergillosis Centre (Table 3), which is actually 110 per year and should be about **204**, if all cases are diagnosed and referred in the NW of the UK. Either estimate suggests major underdiagnosis.

Computing prevalence and assuming a 15% annual mortality, including 370 cases following PTB[34] and 830 (range 415-1660). Together these 2 conditions account for about 30% of patients with CPA[38], consistent with a total UK burden of CPA of **~3,600 cases**. As many are asymptomatic in the early stages, this number is an over-estimate of those at the more severe end of the spectrum requiring therapy.

Table 6: Estimated maximum annual national referral rates for chronic pulmonary aspergillosis in England (2011).

England Region	**Region population <75s (Mid year estimate 2014, ONS)**	**Age standardised DSR for under 75 mortality respiratory disease as a whole for all persons (per**	**Region DSR as a Proportion of NW DSR**	**Unadjusted Referrals per Year**	**Referrals / Million for NW**	**Estimated Region Referrals per Year Adjusted by DSR**

		100,000)				
North East	2,394,771	41.2	0.954	13		13
North West	6,556,394	43.2	1.000	36	5.491	36
Yorkshire and The Humber	4,924,259	38.6	0.894	28		25
East Midlands	4,254,679	32.1	0.743	23		17
West Midlands	5,242,342	34	0.787	28		22
East	5,489,835	25.7	0.595	30		18
London	8,079,584	31.2	0.722	40		29
South East	8,107,490	27.2	0.630	45		28
South West	4,892,429	26.4	0.611	27		16
TOTAL				**270**		**204**

DSR = directly age-standardised mortality rate

Allergic bronchopulmonary aspergillosis (ABPA)

Using our base case of a rate of 2.5% for ABPA among patients with asthma, **110,667 to 235,070 adults would be expected in the UK.** However, the sensitivity analyses vary by over 10-fold from 34,528 to 385,515 affected patients. The only partial population based studies from southern Ireland and the USA[75,76] suggests rates at the lower estimate of published estimates. Referral and discharge patterns across the UK are not uniform, so ABPA is likely to be diagnosed in some areas more often than others. However ABPA is only one fungal complication of asthma, as discussed below under SAFS.

Table 7: Sensitivity analyses of ABPA prevalence in adults with asthma in the UK

	Asthma in UK adults using different estimates		
	Low	Medium	High
Asthma cases	4,426,699	8,288,978	9,402,809
ABPA prevalence			
0.78%	34,528	64,654	73,342
1.5%	66,400	124,335	141,042
2.5%	110,667	207,224	235,070
3.5%	154,934	290,114	329,098
4.1%	181,495	339,848	385,515
Severe asthma prevalence			
3.6%	95,617	179,042	203,101
5%	132,801	248,669	282,084

10%	265,602	497,339	564,169
Fungal asthma prevalence			
50% overlap	121,734	227,947	258,577
33% overlap	163,124	305,449	346,494
20% overlap	194,775	364,715	413,724

Of the 4933 adults with CF in the UK, we estimate that 873 adults have ABPA (95% CIs 597-1243) and 631 people over 15 years old (12.5% of 5062 patients) were documented, indicative of a diagnostic gap of 242. The annual CF report also described 278 children and adolescents with ABPA (7.4% of 3,732 children). In addition, an estimated 1480 (95% CI 1125-1894) have *Aspergillus* bronchitis. If all patients with ABPA and *Aspergillus* bronchitis benefit from therapy (which needs to be established), this totals 2,353 patients.

Severe asthma with fungal sensitisation (SAFS)

Asthma severity and fungal sensitisation rise in parallel[63]. There are ~65,000 admissions to hospital with asthma annually, ~40,250 in adults[77]. Fungal sensitisation rates are not well studied in the UK, especially as patients may be sensitised to one or more fungi[62]. In a series of 121 patients with severe asthma in the UK, sensitisation rates by either skin prick testing or IgE were *Aspergillus fumigatus* 45%, *Candida albicans* 36%, *Penicillium* spp. 29%, *Cladosporium herbarum* 24%, *Alternaria alternata* 22%, and *Botrytis* spp. 18%; 41 (34%) were not sensitised to any fungus tested[62]. The minimum proportion of poorly controlled asthmatics who would be sensitised to a fungus is about 35%, rising to >75% in the worse patients[60]. Using a uniform estimate of 60% fungal sensitisation of the most severe asthmatics (3.6-10%) between 95,617 and 564,169 UK adults have SAFS or severe asthma with ABPA (Table 4).

There is some duplication between ABPA and SAFS, as sensitisation to *A. fumigatus* is common to both and some ABPA patients have severe asthma. These patients are grouped by some authors as having 'fungal asthma' or 'fungal-associated airways disease'. Part of the definition of severe asthma is continuous use of corticosteroids, which is advocated for ABPA, irrespective of the control of asthma. Therefore the overlap is uncertain, and requires detailed study. However given that 75% of SAFS patients are sensitised to *A. fumigatus* and that only a minority of ABPA patients remain on long term steroids, we show a sensitivity analysis with 20%, 33% and 50% overlap in Table 4, using the mid-point estimates for ABPA (2.5%) and severe asthma (5%).

The overall estimate of adults with 'fungal asthma' varies by 3.4 fold, from **121,734 to 413,724**, primarily dependent on the number of adults with asthma.

Invasive Candidiasis

Candidaemia

A total of 1,700 laboratory reports of candidaemia were reported in 2013. Assuming that these represent 38% cases of proven or probable invasive candidiasis tested by blood culture techniques,

the resulting estimate for the total number of cases in England, Wales and Northern Ireland in 2013 was: **4,473**.

Scotland had a rate of candidaemia of 4.8 cases per 100,000 population per year shortly after the millennium[78], yielding an additional 254 bloodstream and **669** invasive *Candida* cases annually.

The total estimate of invasive candidiasis burden for the UK was therefore: **5,142.**

This estimate of burden of candidaemia is likely to be an underestimate as reporting from laboratories is voluntary, therefore likely to be a degree of under-reporting. Population based estimates have been reported in Northern Ireland and Scotland with rates of 6.1 and 4.8 per 100,000 population[78,79] which if extrapolated to the whole population would suggest 2,995 to 3,806 cases annually, as compared to the 1,700 reported for England and Wales (~90% of the population). Further a six sentinel hospital study in England and Wales found an incidence of 18.7 episodes of candidaemia per 100,000 finished consultant episodes (or 3.0/100 000 bed days) in 1997-1999[80] which translates for 2014-15 for England only to 3,497 as there were 18.7 million Finished Consultant Episodes[81], assuming no substantial change in *Candida* bloodstream rate over time.

Considering that the estimate is likely to be an under-estimate, within the range of UK candidaemia burden estimates between 2,995 and 5, 142, we selected the higher end of the range (5,142) as our estimate.

These data indicate a population rate in the UK of candidaemia and invasive candidiasis of 3.1/100,000 and 10.1/100,000 respectively.

Candida peritonitis

CAPD patients

The estimated number of patients on CAPD in England every year was 1,768 year. The estimated number of episodes per patient year attributable to *Candida* in this patient group was 0.05. The resulting estimate for total yearly burden in England was **88 cases**.

Oesophageal candidiasis

An average yearly total burden of **43 diagnoses** was found as AIDS indicator infections.

Many additional cases occur, and one estimate was 0.5% of those on anti-retroviral treatment annually[69]. If applied to the UK population of 73,300 on anti-retroviral treatment in 2013[16], this would equate to **367 episodes annually**, although these data derive in part from patients without full HIV suppression, so could be an over-estimate. Other patient groups also get oesophageal candidiasis, but modelling is not realistic currently.

Mucormycosis

The UK population in 2011 was 63,182,000 [10], and the estimated population incidence of Mucormycosis in France was 0.09 per 100,000 population per year (averaged over 10 years). This resulted in a UK estimate of **57 cases** per year.

Other rare infections

Based on expert view, there are probably **fewer than 25** such patients annually in the UK.

Totals

Table 5 summarises the estimates for total expected number of cases for each invasive fungal infection and rates per 100,000 population.

Table 8: Total estimates of burden

Invasive Fungal Infection	Risk Group	Number of cases expected	Rates per 100,000 population
Pneumocystis pneumonia	All risk groups	**207 to 587**	**0.33 to 0.93**
Cryptococcal meningitis	Primarily AIDS	**100**	**0.16**
Invasive aspergillosis	All risk groups except Critical Care patients	**2,901 to 2,912**	**4.59 to 4.61**
	Critical Care patients	**387 to 1,345**	**0.61 to 2.13**
Chronic pulmonary aspergillosis - all	All risk groups	**204 to 3,600**	**0.32 to 5.70**
Allergic bronchopulmonary aspergillosis (ABPA)	All risk groups	**110,667 to 235,070**	**175~~.15~~ to 372**
Severe asthma with fungal sensitisation (SAFS)	All risk groups	**121,734 to 413,724**	**192~~.67~~ to 654~~.81~~**
~~**Candidaemia**~~**Invasive candidiasis**	All risk groups	**5,142**	**8.14**
Candida peritonitis	CAPD patients	**88**	**0.14**
Oesophageal candidiasis	AIDS patients	**43 to 367**	**0.07 to 0.58**
Mucormycosis	All risk groups	**57**	**0.09**
Other rare infections	All risk groups	**25**	**0.04**

Total		**241,525 to 662,987**	**382~~.27~~ to 1,049~~.32~~**

The estimated total burden of invasive fungal illness in the UK is between **241,525 to 662,987** cases per year.

DISCUSSION

Estimating the burden of invasive fungal infection accurately is challenging due to the lack of a dedicated mandatory systematic surveillance system, and the wide range of incidence estimates for the largest high-risk populations. This is likely to be compounded by the combination of lack of clinical suspicion and limited sensitivity of traditional diagnostic tests used for invasive fungal illness, making it difficult to obtain laboratory confirmation for a significant number of cases. This issue is exemplified for IA as this was the commonest major error in infection diagnoses missed in critical care patients examined at autopsy[74].

There is a significant level of inaccuracy as our estimation methods have relied on limited published information, and there is a wide range of estimates for some of the published incidence rates. This high level of uncertainty is reflected in the results of our sensitivity analysis for the estimation of the burden of invasive aspergillosis in ICU patients, and in the difference between the estimates of *PCP* burden resulting from the two different calculation methods used.

The estimate of burden for PCP obtained by the first method is likely to be an under-estimate as other high risk populations, notably patients with haematological malignancy and those on high dose corticosteroid regimens were not included as no overall incidence rate of PCP could be found in the literature for this group.

The estimate of burden for PCP obtained by the second method should be considered in the light of methodological limitations outlined in the paper used: laboratories may be under-reporting as samples are not processed for *Pneumocystis* diagnosis unless clinically requested, and cytological techniques can also be used (cases diagnosed in this manner would not be counted in this study) and there is potential for double-counting of cases captured both in the HES data set and the Laboratory reporting data set. In addition many cases are clinically diagnosed and treated, many correctly, without a respiratory sample being obtained to enable laboratory diagnosis.

The estimates of chronic respiratory disorders associated with *Aspergillus* and other airborne moulds is much larger than any prior estimate, even if the more conservative assumptions are made. There is certainly some double counting which we have adjusted for but the population prevalence range of fungal asthma of 121,734 to 413,724 is still substantial. Clearly epidemiological studies done in general practice are required to establish a more precise estimate. Our data excludes children, in whom fungal asthma occasionally occurs[82,56].

The lower end of our estimate of total invasive fungal diseases burden range is likely to be an under-estimate, as some condition-specific estimates are for England only. There was potential for double-

counting of cases, however were this was known to be likely, we attempted to account for it by adjusting total estimates.

Estimates of the burden of serious fungal disease for individual countries have been published for Austria, Belgium, Brazil, Czech Republic, Denmark, Dominican Republic, Germany, Greece, Hungary, Ireland~~, and~~ Israel, Jamaica, Kenya, Mexico, Nepal, Nigeria, Qatar, Russia, Senegal, Sri Lanka, Tanzania, Trinidad and Tobago, Uganda, Ukraine, Vietnam have been published along with estimates of chronic and allergic aspergillosis in India[83]. Burden estimates for many other countries and other prospective epidemiology studies are in press and can be used to compare the relative rates of infections to address strategies for prevention and clinical management.

We have not attempted to estimate mortality related to fungal disease in the UK, although others have done so for other countries. The reasons we have not attempted this is because overall and attributable mortality is not always clearly discernable, the estimates we have provideds have much uncertainty attached to them, and adding mortality in addition is likely to add another layer of uncertainty. However we do know that undiagnosed invasive fungal infections such as PCP and IA are always fatal without specific therapy and Candida bloodstream infections and invasive candidiasis have mortalities in excess of 90%, untreated. With treatment, mortality falls~~,~~ especially with PCP in AIDS (~10% mortality) and ~~3~~50% with IA in non-ICU patients. So an estimate of mortality also requires judgements of specific therapy rates, which is unknown for most of these disorders.

Commented [DD1]: This is where the mortality table goes, if we include it.

Strengths and limitations of the study: We acknowledge that the estimates produced in this paper and the methods reached to achieve them are crude and vulnerable to significant error due to lack of robust surveillance information and paucity of published burden studies in the field. We have however made the best attempt possible by: drawing on surveillance data were available; rigorously identifying the relevant high risk groups, the best available estimates of population size for these, and the best available population-specific incidence rates for these; being explicit about the methods used for each individual estimate; and attempted to account for under and over-estimations well as potential double-counting. We are not aware of any other comprehensive burden study for serious and invasive fungal disease in the UK and therefore would argue that although imperfect, this study is a useful contribution to the limited body of knowledge in this field.

CONCLUSION

There is a high degree of uncertainty around the total estimate of burden due to: diagnostic limitations, the lack of a systematic national surveillance system, the limited number of studies published on the topic and the methodological limitations of calculating the burden.

To our knowledge, this is the first attempt at a comprehensive estimation of burden of invasive fungal infection in the UK. Further studies will likely need to combine methods (pragmatic and surveillance-based), take into account any new published information on specific incidence rates, and consider using alternative data sources such as the Hospital Episodes System (HES). An accurate estimate of total burden will ultimately rely on improved diagnostic testing and laboratory reporting.

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Epidemiological Evidence for Dormant *Cryptococcus neoformans* Infection

DEA GARCIA-HERMOSO, GUILHEM JANBON,* AND FRANÇOISE DROMER

Unité de Mycologie, Institut Pasteur, Paris, France

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To date, the time of acquisition of a *Cryptococcus neoformans* infectious strain has never been studied. We selected a primer, $(GACA)_4$, and a probe, CNRE-1, that by randomly amplified polymorphic DNA (RAPD) analysis and restriction fragment length polymorphism (RFLP), respectively, regrouped strains from control samples of *C. neoformans* var. *grubii* environmental isolates according to their geographical origins. The two typing techniques were then used to analyze 103 isolates from 29 patients diagnosed with cryptococcosis in France. Nine of the 29 patients lived in Africa a median of 110 months prior to moving to France; 17 of the patients originated from Europe. Results showed a statistically significant clustering of isolate subtypes from patients originating from Africa compared to those from Europe. We conclude that the patients had acquired the *C. neoformans* infectious strain long before their clinical diagnoses were made.

Cryptococcus neoformans is a ubiquitous and opportunistic yeast that causes life-threatening meningoencephalitis in 3 to 30% of patients with AIDS (24). This encapsulated basidiomycete exists in three varieties: *C. neoformans* var. *grubii* (serotype A) (14) and *C. neoformans* var. *neoformans* (serotype D), both with worldwide distributions, as well as *C. neoformans* var. *gattii* (serotypes B and C), which is limited to tropical and subtropical regions (21). Cryptococcosis, like many other fungal infections, is thought to begin with inhalation of airborne fungi from an environmental source. Basidiospores, which are smaller, more easily aerosolized, and much more resistant to desiccation than yeast cells, are most likely to be the infectious particles (22, 35). It has been reported that this yeast's most important natural source is weathered pigeon droppings or soil contaminated with avian guano (11, 12). Data reported in the literature indicate that *C. neoformans* can be found as a transient commensal organism on humans or as an incidental colonizer in the respiratory tract or on the skin of healthy subjects or even patients with bronchopulmonary disorders (19, 26).

Several observations converge toward the hypothesis that the infectious particles can be acquired long before the infection develops and is diagnosed. First, a high percentage of healthy subjects have anticryptococcal antibodies, which suggests prior contact with the fungus (7, 18). Second, patients coming from tropical areas can be diagnosed with *C. neoformans* var. *gattii* cryptococcosis long after they have left their countries of origin (8). Finally, unlike French patients, African patients living in France and diagnosed with cryptococcosis are rarely infected with *C. neoformans* var. *neoformans* strains (10). To verify this hypothesis, a well-characterized group of patients and a molecular method able to distinguish between isolates of the same serotype but from different geographical regions should be selected. The molecular typing methods currently available are reported to be unable to regroup *Cryptococcus neoformans* var. *grubii* isolates by their geographical origins (4, 5, 33). On the other hand, Kwon-Chung and Bennett, using standard immunological methods of serotyping, have described a nonrandom distribution of serotypes around the world (21). Although serotyping is not sufficiently discriminative to determine the geographical origin of a cryptococcal isolate, it provides good evidence that a technique capable of clustering strains from the same geographical region might exist.

In this study, we addressed the question of the time of acquisition of the infecting organism, an issue that has never before been raised. Using control samples of environmental isolates and two typing methods capable of clustering strains based on their geographical origins, we were able to demonstrate that patients diagnosed with cryptococcosis in France but born in Africa had acquired their infectious strains a long time ago, prior to emigrating from their countries of origin.

MATERIALS AND METHODS

Patients and strains. Twenty environmental isolates of *C. neoformans* var. *grubii* from different geographical regions were used in this study: Japanese isolates J1, J2, J3, J4, and J5 were kindly provided by S. Kohno (Nagasaki University School of Medicine, Nagasaki, Japan) as M12, SH1311, MT11, SUMO1, and SASO1, respectively (36); African isolates AF1 (Morocco), AF2 (Togo), AF3 (Ivory Coast), AF4 (Burundi), and AF5 (Zimbabwe) were provided by D. Swinne (Institute of Tropical Medicine, Antwerp, Belgium) as RV45718, RV45880, RV46288, RV67312, and RV70273, respectively; American isolates US1, US2, and US3 (Kentucky) as well as US4 (New York) were provided by J. M. Clauson (Western Kentucky University, Bowling Green) and A. Casadevall (Albert Einstein College of Medicine, Bronx, N.Y.) as FE-1, PE-1, SSE-1, and B5 respectively (6); and French isolates F1 through F6 were provided by S. Mathoulin and B. Couprie (Centre Hospitalo-Universitaire, Bordeaux, France) as 115A, 57B, 109B, 13A, 110B, and 122A, respectively (16).

A total of 103 clinical *C. neoformans* var. *grubii* isolates were recovered from 29 patients who had been diagnosed with cryptococcosis in France and whose infections had been reported to the National Reference Center for Mycoses during the first year (1997) of a multicentric clinical study, étude Crypto A/D (Direction Générale de la Santé no. 970089). Detailed information on clinical and epidemiological issues (particularly the patients' trips and stays since childhood) and on all of the isolates recovered at the time of diagnosis and during the course of the infection were collected. Among the 29 patients, 17 had been born in Europe and 9 had been born in Africa (see Table 1). The African patients had been living in France for a median of 110 months before cryptococcosis was diagnosed. The last trip back to Africa had occurred as long as 13 years ago (patient P17).

The identification of all cultured organisms as *C. neoformans* was confirmed by standard biochemical methods. Isolates were identified as *C. neoformans* var. *grubii* by the use of canavanine-glycine-bromothymol medium, D-proline assimilation, and a direct immunofluorescence assay using a monoclonal antibody (9). All strains were stored frozen in 40% glycerol at −80°C and were grown overnight in YPD medium (5 g of yeast extract, 10 g of Bacto Peptone, and 10 g of glucose per liter) at 30°C.

* Corresponding author. Mailing address: Unité de Mycologie, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris Cedex 15, France. Phone: (33) 1 45688356. Fax: (33) 1 45688420. E-mail: janbon@pasteur.fr.

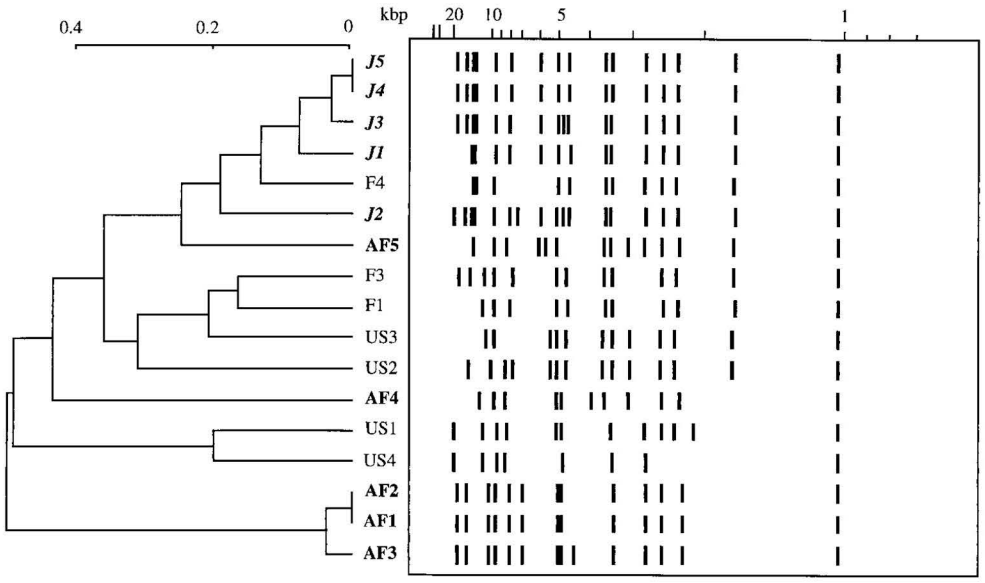

FIG. 1. Schematic representation of and dendogram generated from the Dice coefficient complement computed from the CNRE-1 patterns of environmental isolates from Japan (J), France (F), the United States (US), and Africa (AF).

Randomly amplified polymorphic DNA (RAPD) analysis. *C. neoformans* DNA was extracted as previously described (31). The following primers were chosen from the literature and tested for their discriminatory power on 20 environmental isolates under various annealing temperatures: $(CA)_8$ RY (25), $(GTG)_5$, $(GACA)_4$ (23), the phage M13 core sequence (27), and two enterobacterial repetitive intergenic consensus sequences, ERIC1 and ERIC2 (2). PCR was carried out in a thermal cycler (Omnigene; Hybaid, Teddington, United Kingdom) in 100-μl reaction volumes, each containing 50 ng of genomic DNA, 50 pmol of primer, 200 μM deoxynucleoside triphosphates, and 2 U of recombinant *Taq* polymerase (Pharmacia Biotech, Uppsala, Sweden), with the manufacturer's recommended buffers.

Two primers, ERIC1 and $(GACA)_4$, were then selected and used to study clinical isolates under the following optimized conditions: reactions were cycled 35 times, with a 4-min denaturation at 94°C, 1 min of annealing each at 28 and 48°C, and a 2-min primer extension at 74°C. Amplification products were analyzed by electrophoresis through a 2% agarose gel and visualized under UV light after being stained with ethidium bromide. The reproducibility of this method was confirmed by reanalyzing another DNA preparation from five clinical isolates. In every case, identical profiles were obtained (data not shown).

RFLP analysis. Restriction fragment length polymorphisms (RFLPs) were detected by Southern blot hybridization after restriction enzyme *Sst*I digestion of total-DNA samples. The restriction fragments obtained were then separated by electrophoresis through a 0.8% agarose gel and transferred onto positively charged nylon membranes (Boehringer Mannheim, Mannheim, Germany). The DNA probe (*C. neoformans* repetitive element CNRE-1), generously provided by E. Spitzer and S. Spitzer (28), was labeled with DIG digoxigenin–11-dUTP by using a DIG-High Prime Kit (Boehringer Mannheim). After an overnight hybridization at 68°C and stringent washes, bands were detected and exposed according to the manufacturer's instructions. Reproducibility was confirmed by reanalyzing another DNA preparation from five clinical isolates (data not shown).

Data analysis. DNA fingerprint patterns were analyzed by using the software Taxotron, developed by P. D. Grimont (Institut Pasteur, Paris, France) (17), which automatically identified band positions and compared two profiles by calculating the Dice coefficient complement (number of different bands per total number of fragments in the two profiles). Dendograms were then generated by using the unweighted pair group method of average linkage (17).

Statistical analysis. The distributions of the patients' isolates by subtype according to the European or African origin were analyzed by using Fisher's exact test.

RESULTS

Selection of a typing method for environmental isolates. We tested different typing methods to evaluate their abilities to cluster environmental *C. neoformans* var. *grubii* strains according to their geographical origins. Because RFLP is known to be reproducible and easy to perform, we first tested the ability of CNRE-1 to geographically classify the isolates. Figure 1 shows the Taxotron-derived schematic representation of the RFLP profiles obtained after hybridization of CNRE-1 to *Sst*I-digested genomic DNA. Fifteen different profiles were obtained for the 17 strains tested. Two Japanese (J5 and J4) and two African (AF2 and AF1) isolates yielded identical hybridization profiles with this probe. Partial clustering of the Japanese (four of five) and African (three of five) isolates was obtained, but no specific profile could be associated with a given region. Therefore, we tested another molecular technique to determine it could further differentiate isolates based on their geographical origins.

Previous studies using RAPD techniques demonstrated geographical clustering among isolates of *C. neoformans* var. *gattii* (2, 27). RAPD analysis has been applied in several epidemiological studies of *C. neoformans*; however, some questions have been raised concerning the interpretation of the profiles generated. The advantages RAPD offers over other methods, particularly speed and ease of execution, made it suitable for investigation of the ability to discriminate between *C. neoformans* var. *grubii* isolates according to their geographical origins.

Six different primers previously used in some epidemiological studies of *C. neoformans* were chosen. Using different amplification temperatures, they were tested on the control group of 17 environmental isolates to which 3 more environmental isolates from France were added. Only primers ERIC1 and $(GACA)_4$ revealed roughly the same patterns for strains coming from the same geographical region and different patterns for strains coming from different continents. Representative RAPD profiles obtained with the primer $(GACA)_4$ are shown in Fig. 2. Using this primer, all five Japanese isolates had profile II, five of the six French isolates exhibited profile I, and four of the five African isolates gave profile V or VI. Of the four North American isolates, two exhibited profile I and two showed profile II.

Evaluation of clinical isolates with the selected techniques. One hundred and three *C. neoformans* var. *grubii* clinical isolates were then analyzed with the two primers described above. Four different profiles were obtained with the primer ERIC1.

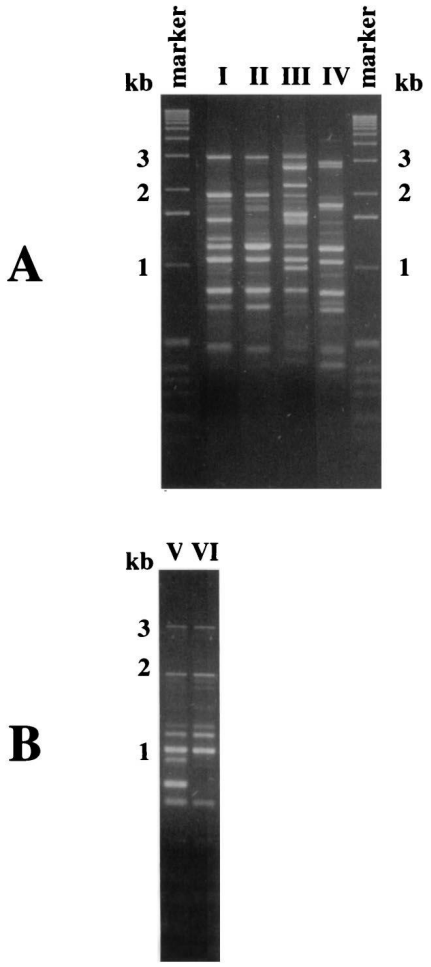

FIG. 2. Representative RAPD profiles of *C. neoformans* isolates generated with the $(GACA)_4$ primer. Profile identifications are indicated as roman numerals (I through VI). Profiles V and VI were obtained only with environmental isolates. Molecular size standards were obtained with the 1-kb ladder (Gibco BRL, Gaithersburg, Md.).

However, no association between a given profile and the corresponding patient's continent of origin could be established (data not shown). Typing of the clinical isolates with primer $(GACA)_4$ revealed four different RAPD profiles, whose distributions are reported in Table 1. All of the strains exhibiting profile III were isolated from European patients. Of the 15 patients with profile I strains, 11 were born in Europe. None of the strains generating profile II or IV were isolated from patients born in Europe. It is important to note that all of the isolates recovered from a particular patient gave the same profile with both primers. Thus, the analysis of clinical isolates showed that the distribution of profiles according to the geographical origin of the patients was not random. This bias of distribution was statistically significant ($P < 0.0005$) when the European and African patients were being compared (Table 2). None of the clinical isolates tested yielded profile V or VI, both of which were characteristic of environmental African isolates. On the other hand, profiles III and IV were specific to clinical isolates in this study. Finally, profile II, which was characteristic of the Japanese environmental isolates, was generated mostly by strains isolated from African patients. These results might be explained by the facts that environmental and clinical African strains did not come from the same country and that no Japanese patients were included in our study. Moreover, the discriminatory power of this RAPD method was low.

TABLE 1. RAPD profiles, generated with primer $(GACA)_4$, among patients of different origins

Patient no.	Sex[a]	HIV status[b]	Country of birth	No. of isolates	$(GACA)_4$ profile	Time since emigration (mo)
P1	M	+	France	8	I	
P2	M	+	France	6	I	
P3	M	+	France	11	I	
P4	M	+	Haiti	4	I	189
P5	F	+	France	1	I	
P6	M	+	France	9	I	
P7	M	+	Algeria	3	I	205
P8	M	+	France	7	I	
P9	M	+	France	14	I	
P10	M	+	Tunisia	2	I	109
P11	M	+	Ivory Coast	2	I	314
P12	M	+	France	1	I	
P13	M	+	Italy	4	I	34
P14	M	+	France	3	I	
P15	M	+	France	1	I	
P16	M	+	Ivory Coast	1	II	0
P17	M	+	DRC[c]	1	II	110
P18	M	+	Colombia	5	II	41
P19	M	+	Gambia	3	II	139
P20	F	+	DRC	2	II	11
P21	M	+	Ivory Coast	1	II	48
P22	M	+	Cambodia	3	II	0
P23	F	+	France	2	III	
P24	M	+	France	4	III	
P25	F	−	France	1	III	
P26	F	−	France	1	III	
P27	M	+	France	1	III	
P28	M	+	France	1	III	
P29	M	−	DRC	1	IV	239

[a] M, male; F, female.
[b] +, HIV positive; −, HIV negative.
[c] DRC, Democratic Republic of Congo.

We then tested the 103 strains with the CNRE-1 probe. As previously described in other reports (29, 32), we found that isolates from the same patient showed identical hybridization patterns, although there were a few examples of microevolution (15, 30). Thus, for the remainder of the analysis, one representative isolate from each patient was chosen, and their hybridization patterns are presented in Fig. 3; 28 different profiles were obtained from 29 isolates studied.

The Taxotron software analysis generated a dendogram in which two boxed clusters, named A and B, can be seen (Fig. 3). Cluster A contained the seven strains with the profile II subtype as determined by the $(GACA)_4$ RAPD technique. Five of these strains were isolated from patients born in Africa, one was from a patient born in Colombia, and one was from a

TABLE 2. Distribution of RAPD profiles obtained with the $(GACA)_4$ primer

Geographical region	Total no. of patients	No. of patients with RAPD profile[a]:			
		I	II	III	IV
Europe	17	11 (65)	0	6 (35)	0
Africa	9	3 (33)	5 (56)	0	1 (11)

[a] The numbers in parentheses are the percentaes of patients whose isolates showed the corresponding profile.

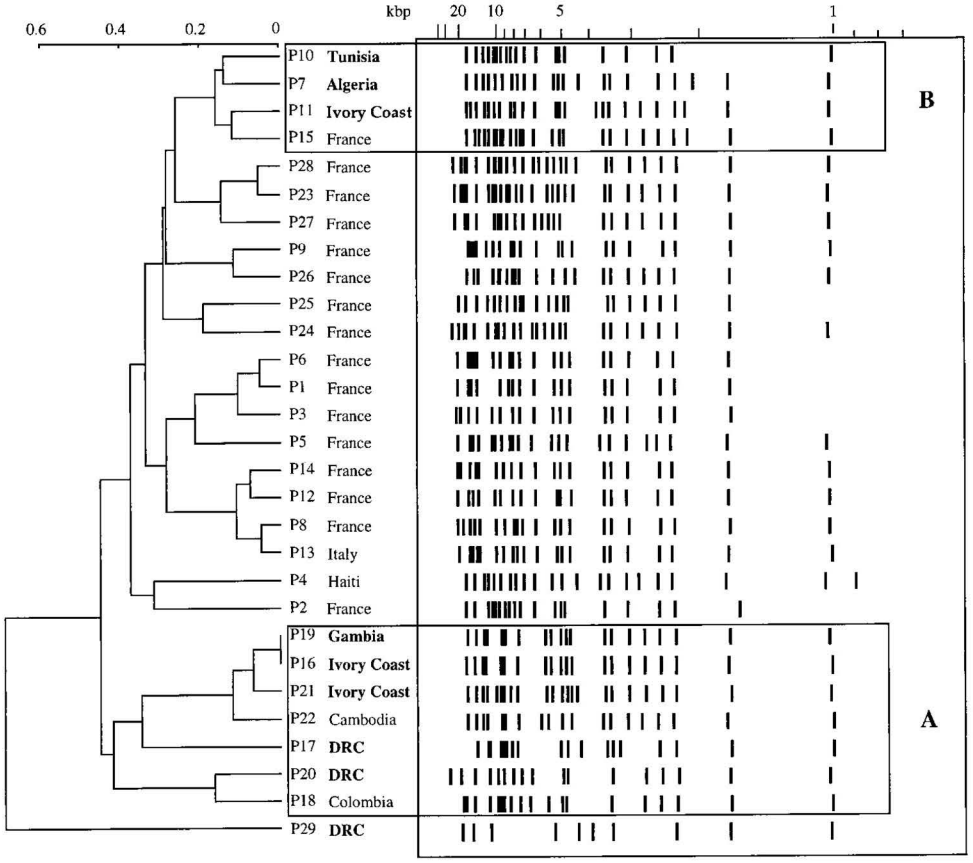

FIG. 3. Schematic representation of and dendogram generated from the Dice coefficient complement computed from the CNRE-1 patterns of 29 clinical isolates. African strains (in boldface) are regrouped in clusters A and B (boxed in this figure). DRC, Democratic Republic of Congo.

patient born in Cambodia. None of the strains from this cluster was isolated from a European patient. Cluster B contained four strains which were all the profile I subtype, as determined by the $(GACA)_4$ RAPD technique. Three of them were isolated from patients born in Africa, and one was from a patient born in Europe. The strain from the African patient P29 seemed to be completely different from the others. It was also the only strain for which the profile IV subtype was generated by the RAPD technique.

DISCUSSION

After analyzing the epidemiology of cryptococcosis in France (10), we were especially interested in learning more about the pathophysiology of this infection. An important issue was the time of acquisition of the infecting isolate compared to the time of diagnosis: were the infectious particles inhaled daily and killed as long as host defense mechanisms were efficient, or, on the contrary, would the immune system normally achieve local control without eradication? Since this latter mechanism has already been evoked or demonstrated for other microorganisms, such as *Leishmania* spp. (1) and *Histoplasma capsulatum* (34), and since several lines of evidence suggest that it could also occur with *C. neoformans*, we looked for a way to verify this hypothesis. To do so required control samples composed of environmental isolates from remote areas (to ascertain their geographical origin), clinical isolates recovered from patients whose travels and clinical histories were known, and the assessment of various typing methods since none has yet been able to correlate geographical origin with a specific pattern.

Indeed, several groups have studied the molecular epidemiology of *C. neoformans* infections and attempted to regroup isolates based on their geographical origins. However, the genetic differentiation generated by CNRE-1 RFLP analysis showed no geographical correlation among strains isolated from Brazil and the United States (13). Using UT-4p, Varma and colleagues reported no obvious clustering of the *C. neoformans* var. *grubii* isolates according to geographical origin (33), although Garcia-Hermoso and coworkers evoked the possibility of geographical clustering (16) when they compared the patterns obtained for French isolates to those observed by Varma et al. (33) and Kohno (20). Finally, using the multilocus enzyme electrophoresis technique, Brandt and colleagues found that some subtypes were more common in some areas of the United States than in others; however, that finding was not confirmed when another typing method (RAPD) was used (3). In light of our results, we think that the lack of clear-cut regional differences in the previously published studies may be due to the population sample from which the clinical isolates were recovered (with its lack of patients coming from remote places and with known travel histories), the lack of environmental isolates from remote areas, and/or the technique selected. Indeed, our data show that depending on the sample chosen (environmental or clinical isolates) and the technique tested (six primers selected for RAPD or CNRE-1 RFLP), we could have concluded that either there was or was not a geographical clustering of isolates and that the isolates tested

exhibited or did not exhibit genetic variability. These discrepancies clearly demonstrate the importance of the sample choice and the technique selected to answer a specific epidemiological question. To address the question of geographical clustering, we needed an epidemiological tool with adequate discriminatory power: not too high (to prevent further strain delineation among isolates from the same geographical origin) and not too low (to enable the differentiation of the isolates based on their geographical origin). The RAPD method using the primer $(GACA)_4$ fulfilled this requirement.

Based on the RAPD profiles obtained, we showed that the distribution of clinical isolates from nine African patients diagnosed with cryptococcosis in France was significantly different from that of clinical isolates recovered from the 17 European patients ($P < 0.0005$). Furthermore, a second, independent typing method (CNRE-1 RFLP) confirmed the results, showing two clusters that contained the isolates from eight of the nine African patients. This finding suggests that the infecting organism can be acquired long before the infection develops, since these patients had been living in France a median of 110 months and had not been in contact with an African environment for as long as 13 years. That African patients were infected with African isolates strongly suggests that these isolates had been sequestered and contained somewhere in the body, most likely the alveolar macrophages. Then, as soon as some kind of immune system defect occurred, which in most of the patients was AIDS, the fungus could multiply, disseminate, and cause infection. The clinical histories of these patients and the demonstration of a geographical clustering of isolates based on the generated profiles are consistent with a dormant phase of *C. neoformans* within all individuals. Why infection would be caused by a dormant strain of *C. neoformans* rather than a newly acquired one, what form the dormant form of *C. neoformans* would assume, and why some isolates would be more virulent than others remain to be determined.

The observation that the infecting organism had been (or at least could have been) acquired long before the infection was diagnosed should be taken into account in the prospective development of prophylactic programs, such as vaccination or antifungal therapy, for populations at particularly high risk of developing cryptococcosis, such as AIDS patients in central or southern Africa, South Africa, or Southeast Asia (24).

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REVIEW ARTICLE

C. Corey Hardin, M.D., Ph.D., *Editor*

Cryptococcal Disease in Diverse Hosts

David B. Meya, M.B., Ch.B., Ph.D., and Peter R. Williamson, M.D., Ph.D.

THE FUNGUS CRYPTOCOCCUS IS THE MOST COMMON CAUSE OF ADULT meningitis in parts of the world with high rates of human immunodeficiency virus (HIV) infection and persists in many areas where antiretroviral therapy (ART) is widely available.[1,2] The fungus is responsible for up to 180,000 deaths each year worldwide and accounts for up to 68% of HIV-related cases of meningitis.[2] In resource-rich regions, increasing use of immunomodulatory therapy and underlying natural susceptibility have led to a change in epidemiologic factors such that deaths in non–HIV-infected patients account for approximately one third of the deaths related to cryptococcal meningitis or meningoencephalitis.[3] With the advent of vaccines to prevent bacterial meningitides such as those from *Streptococcus pneumoniae* and *Haemophilus influenzae*, cryptococcal meningitis has become one of the most common causes of meningitis in the United States.[4] One study has shown that despite therapy, 10 to 25% of patients in most groups with cryptococcal meningitis die because of delays in diagnosis and treatment challenges.[5] This finding recently led the World Health Organization to assign cryptococcus to the "critical" group of fungal pathogens, with the highest priority for research into better diagnostic approaches and treatment regimens.[6] However, a revolution in new diagnostic, preventive, and therapeutic strategies and the paradigm-shifting recognition of destructive inflammatory syndromes hold promise in efforts to reduce and treat this devastating infection.

From the Infectious Diseases Institute and the Department of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda (D.B.M.); the Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis (D.B.M.); and the Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD (P.R.W.). Dr. Williamson can be contacted at williamsonpr@mail.nih.gov or at the National Institutes of Health, 9000 Rockville Pike, Bldg. 10, Rm. 11C208, Bethesda MD 20892.

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CME

MYCOLOGIC FEATURES

Cryptococcus is a basidiomycetous yeast that is unique among pathogens in humans in that it has an immune-shielding polysaccharide capsule and a cell-wall laccase with broad immunomodulatory properties, which together predispose the organism to neurotropism. The cryptococcus genus is currently undergoing reevaluation but is generally considered to consist of two species complexes, *Cryptococcus neoformans* and *C. gattii*, each of which can be further divided into several molecular genotypes (*C. neoformans*: VNI through VNIV and VNB; and *C. gattii*: VGI through VGVI).[7]

C. neoformans is the predominant cause of infections worldwide both in persons living with acquired immunodeficiency syndrome (AIDS) and in other immunosuppressed populations. The VNI and VNII clades are distributed worldwide,[8] whereas VNB is most often found in sub-Saharan Africa and South America.[9] *C. gattii* has historically been associated with immunocompetent persons, but more recent studies have implicated autoantibodies against granulocyte–macrophage colony-stimulating factor (GM-CSF) in *C. gattii* infections (genotypes VGI, VGII, and VGIII), with an overrepresentation of one clade (VGIV) among persons with AIDS in Africa.[10,11] The two species complexes, *C. neoformans* and *C. gattii*, are currently identifiable by means of matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry in clinical laboratories that have large data sets available for identification.[11] The species distinction is becoming increasingly im-

KEY POINTS

Cryptococcal Disease in Diverse Hosts

- Worldwide, cryptococcal meningitis kills up to 180,000 persons annually and is the most common cause of nonviral meningitis in the United States.
- Besides patients with immunosuppression due to human immunodeficiency virus (HIV) infection, chemotherapy, or immunotherapy, the cryptococcus fungus increasingly causes disease in apparently healthy persons, often without signs such as fevers, which results in diagnostic delays and poor outcomes.
- Despite HIV control in developing countries, expected reductions in the prevalence of cryptococcal disease remain elusive, and therapy is hampered by an inability to secure cost-effective drugs such as flucytosine.
- Prompt diagnosis, fungicidal therapy, and intracerebral pressure control are key for successful treatment of cryptococcal meningitis.
- Inflammatory syndromes such as the immune reconstitution and postinfectious inflammatory response syndromes are major causes of clinical deterioration and may necessitate the use of additional adjunctive therapeutic agents.
- Ending Cryptococcal Meningitis Deaths by 2030 is a strategic framework that must be implemented worldwide in order to reduce deaths from cryptococcal meningitis, with a focus on screening, health care worker education, and shorter, more effective therapies.

portant in clinical practice because of the necessity to rule out autoantibody disease in patients with *C. gattii* infection.

IMMUNE DEFENSE AND HOST–PATHOGEN INTERACTIONS

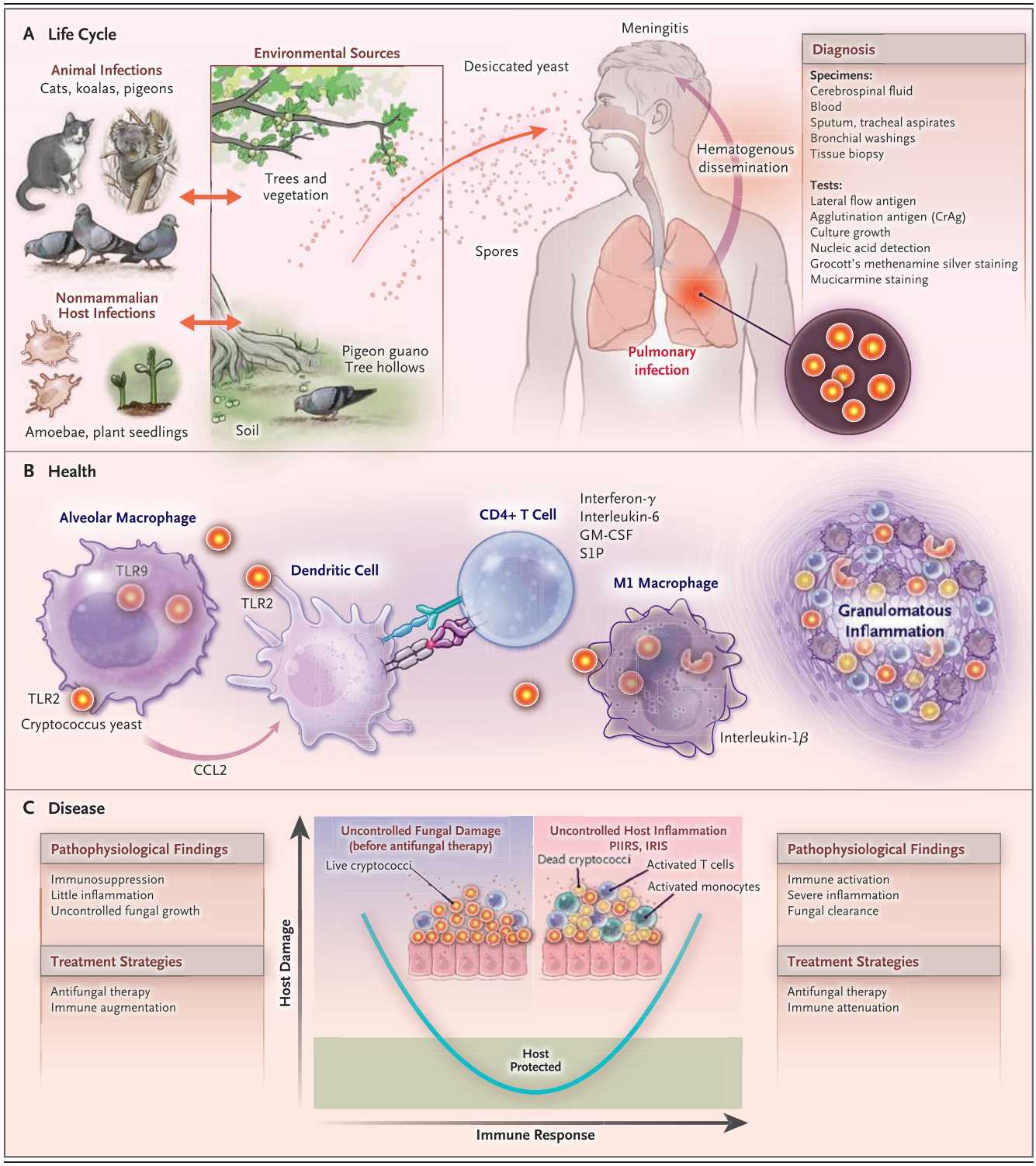
Clinical presentations of cryptococcosis are highly dependent on the nature of the host response. This response has been characterized as a parabola representing the relationship between fungal-mediated damage in persons with high immunosuppression and host-mediated damage in those with more intact immune responses (Fig. 1).[12] A strong adaptive immune response, including CD4+ helper T cells, is required for fungal control because of the unique fungal polysaccharide capsule, which suppresses innate immune recognition of the fungus. This adaptive response is characteristically lacking in patients at risk for cryptococcal infections, such as persons with AIDS and patients undergoing T-cell–depleting chemotherapy (Fig. 1). With an intact adaptive immune response, reduced stimulation of innate immunity is amplified at the dendritic T-cell synapse, which results in differentiation of CD4+ T cells into unique effector subsets with distinctive cytokine profiles, including interferon-γ and GM-CSF from types 1 and 17 helper T (Th1 and Th17) cells. These, in

Figure 1 (facing page). Cryptococcal Life Cycle and Immune Responses.

Cryptococcal yeasts inhabit a number of environmental niches, causing infections in animals and non-mammalian hosts, as well as in humans (Panel A). Infection in humans occurs after the inhalation of desiccated yeast or spores. In healthy persons, responses may be varied, but organisms are recognized by alveolar macrophages by means of pattern-recognition receptors, including toll-like receptors 2 and 9 (TLR2 and TLR9) (Panel B). Activated macrophages then release cytokines such as CCL2 to recruit monocytes and dendritic cells to the lung, which are capable of breaking down fungi and presenting antigen to CD4+ T cells. Activated type 1 helper T cells secrete interferon-γ, interleukin-6, sphingosine-1-phosphate (S1P), and granulocyte–macrophage colony-stimulating factor (GM-CSF), which help to recruit and differentiate classical (M1) macrophages, and these, in turn, facilitate fungal killing and produce cytokines, including interleukin-1β. Secreted cytokines and chemokines activate leukocytes to encapsulate and eliminate cryptococcal organisms within granulomatous lesions. The absence or dysfunction of a healthy immune response may lead to uncontrolled fungal growth, resulting in fungal damage (Panel C, left side of parabola). However, some patients have a skewed hyperimmune response to the pathogen, causing inflammatory damage to the host tissue even after microbiologic control (Panel C, right side of parabola). CrAg denotes cryptococcal antigen, IRIS immune reconstitution inflammatory syndrome, and PIIRS postinfectious inflammatory response syndrome.

turn, activate inflammatory macrophages, resulting in fungal clearance.[13]

The fungus resists macrophage killing by means of capsular shedding, which facilitates survival, replication, and even nonlytic exocytosis to permit brain dissemination.[14] Reconstitution of the adaptive immune system, either by means of tapered T-cell suppressive therapy or successful HIV viral suppression with ART facilitates fungal clearance but can also result in a damaging immune reconstitution inflammatory syndrome (IRIS) (Fig. 1).[15] Clinical deterioration after micro-



biologic control (defined as negative cerebrospinal fluid [CSF] cultures) in previously healthy patients is most likely due to increased exposure to fungal products that are released during fungicidal therapy and is referred to as a postinfectious inflammatory response syndrome (PIIRS).[16] Immunostimulation may improve fungal clearance and reduce host damage in persons with AIDS,[17] whereas immunosuppression with the use of glucocorticoids may benefit patients without HIV infection who have clinical deterioration from host-mediated immune damage.[18]

Since cryptococcus is an opportunistic pathogen that is not transmitted from patient to patient, the predominant evolutionary pressure has optimized various traits necessary for survival in the environment, and these traits have also facilitated its role as a pathogen. For example, the polysaccharide capsule surrounding the cell wall prevents desiccation and killing by free-living amoebas in the environment[19] and is also a key factor that accounts for virulence in infections in humans. Similarly, a cell-wall copper oxidase laccase that facilitates growth and virulence against plants[20] acts as a virulence factor in the host by producing reactive dopamine products and immunomodulatory oxylipids such as prostaglandin E2 (Fig. 1).[13]

EPIDEMIOLOGIC FEATURES AND RISK FACTORS

Serologic studies with the use of cryptococcal antigens or skin tests have shown that cryptococcal exposure varies widely, from approximately 5% among healthy volunteers to double digits among persons at high risk, such as pigeon breeders and a cohort of young children from the Bronx, New York.[21,22] Although some of these exposures are probably eliminated by a healthy immune system, some may result in latent infection. A study focusing on African emigrants to France showed that reactivation of latent disease is a prevalent mode of symptomatic infection in persons with AIDS.[23] Even though these emigrants had not returned to their African home countries for a median of 13 years, the fungal strains causing their infections were similar to those found in Africa rather than those found in France.

The predominant risk factor for cryptococcal disease is HIV infection or AIDS with CD4 counts below 100 cells per cubic millimeter.[24] However, despite reductions in this at-risk population[3] in Botswana, the reduced prevalence of HIV infection was not associated with a substantial reduction in the prevalence of cryptococcal meningitis, possibly because of treatment interruptions in obtaining care or the presence of latent central nervous system (CNS) reservoirs of HIV.[1] HIV-related cryptococcal meningitis is now increasingly diagnosed in persons who have received ART, which may explain the observation that the incidence of cryptococcal meningitis has not decreased substantially in sub-Saharan Africa.[25-27] Furthermore, in 2022, cryptococcosis still accounted for 13 to 24% of all HIV-related deaths, which was not considerably different from the 17% of deaths due to cryptococcosis in a Ugandan cohort of persons with AIDS between 1995 and 1999.[2,28]

Risk factors in persons without HIV infection include glucocorticoid treatment, sarcoidosis, and idiopathic CD4 lymphopenia. Case reports and small case series have implicated immunosuppressive therapy, including anti–tumor necrosis factor α (infliximab), anti–CD52 (alemtuzumab), anti–Bruton's tyrosine kinase inhibitors (ibrutinib), and more recently, agents directed against sphingosine-1-phosphate receptors (fingolimod).[29,30] Typically, infections occur in solid-organ transplant recipients but not in stem-cell recipients, for complex reasons that may include the frequent use of azole prophylaxis in the latter population. Calcineurin-directed therapy (tacrolimus) and therapy directed at the mechanistic target of rapamycin (sirolimus) are also risk factors in both populations.[31]

Approximately 20% of cryptococcal meningitis cases in the United States occur in previously healthy persons without known immune deficits.[3] However, studies have begun to identify immune deficits in such patients. For example, autoantibody disease is relatively common in previously healthy persons in whom cryptococcal disease develops, with approximately half the patients with *C. gattii* infection having antibody to GM-CSF, which is also associated with pulmonary alveolar proteinosis, a severe but treatable pulmonary disease.[10] Indeed, one of the first reported cases of autoantibody-positive cryptococcal meningitis occurred in a patient who had no pulmonary symptoms and had clear computed tomographic (CT) imaging of the chest at the time of fungal

diagnosis but who presented with pulmonary alveolar proteinosis 1 year later, with shortness of breath. The patient became critically ill but had a response to whole-lung lavage and aerosolized GM-CSF therapy.[32] Commercial tests for GM-CSF autoantibodies are increasingly available, and although testing does not appear to influence initial therapy against cryptococcosis, it may be helpful for follow-up, particularly if chronic pulmonary symptoms develop.

In rare cases, cryptococcal disease is due to inborn errors of immunity, including mutations in an autosomal dominant sporadic monocytopenia caused by mutations in the GATA2 zinc finger transcription factor responsible for the monocytopenia and mycobacterial infection syndrome and Emberger's syndrome,[33] the hyper-IgE recurrent infection syndrome,[34] and X-linked hyper-IgM immunodeficiency.[35] In addition, several common polymorphisms involving the FCγ receptor IIB, an FcγR3A allele in White persons with AIDS, a colony-stimulating factor 1 locus polymorphism in African persons with AIDS, and several *PRR* (pseudo-response regulator) genes have been implicated as secondary genetic modifiers of disease susceptibility.[36]

CLINICAL MANIFESTATIONS

Pathogenic cryptococcus species have a strong predilection for the CNS, progressing from asymptomatic cryptococcal antigenemia to meningoencephalitis.[37] A positive cryptococcal antigen test precedes overt symptoms of meningitis by a median of approximately 3 weeks in 90% of persons with AIDS.[28] In Africa, approximately 11% of persons with cryptococcal antigenemia present with cryptococcal meningitis at the time of cryptococcal antigen screening, whereas 8% have progression to cryptococcal meningitis over the next 6 months, despite 10 weeks of fluconazole therapy and ART.[38] Fever is seen in approximately half the patients with HIV-associated disease, but it is less common in persons who were previously healthy, which leads to delays in diagnosis.[39] Visual symptoms can be associated with cranial-nerve involvement (diplopia) or can be related to direct optic-nerve involvement or increased intracranial pressure, which results in reduced perfusion to retinal ganglion axons and subsequent swelling of the axons, with leakage of cellular contents into the extracellular space of the optic disk.[40,41] Hearing impairment is often related to inflammation of cranial nerve VIII within the internal auditory canal.[42]

Isolated lung involvement is more common in transplant recipients and in patients who were previously healthy than in persons with AIDS. Rarely causing colonization, cryptococcosis can be manifested as nodules, hilar lymphadenopathy, or lung cavities that are often misdiagnosed as tumors or as tuberculosis without the characteristic fibrosis and calcifications.[43] Radiographic evidence of lytic lesions is indicative of bone involvement, and skin disease can be manifested as molluscum-like lesions with central necrosis or even chronic ulcers.[29,44] However, concomitant CNS disease is often present, necessitating more intensive therapy. Thus, evidence of local disease from a source outside the lung is suggestive of dissemination and warrants consideration of a lumbar puncture even in the absence of neurologic symptoms.

DIAGNOSIS

Cryptococcal antigens can be identified with high sensitivity in blood and CSF, with sensitivities and specificities exceeding 99%, regardless of HIV infection status.[45,46] Cryptococcal antigen tests can quantify the amount of antigen, although they do not distinguish live organisms from dead ones. Latex agglutination antigen testing of blood and CSF has more recently been supplanted by a lateral flow assay, a dipstick sandwich immunochromatographic assay with a readout similar to that of the common pregnancy test (Table 1). In a large validation study in South Africa and Uganda, the cryptococcal antigen lateral flow assay performed best, with high sensitivity and specificities (>99%), identifying several-culture negative cases.[45] As a point-of-care test that is easy to perform, has a 15-minute turnaround time, and is less expensive than the agglutination test, which requires a 30-minute inoculation, the lateral flow assay is the test of choice worldwide for both screening and diagnosis. The assay can detect both *C. neoformans* and *C. gattii* infections with excellent sensitivity in blood samples obtained from previously healthy patients — a factor that lowers the threshold for testing and thus potentially could reduce diagnostic delays

Table 1. Performance Characteristics of Cryptococcal Diagnostic Assays in Cerebrospinal Fluid (CSF) from Persons with Suspected Meningitis.*

Diagnostic Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
			percent	
Cryptococcal antigen lateral flow immunochromatographic assay	99.3	99.1	99.5	98.7
CSF culture†	90.0	100	100	85.3
100 mm^3	94.2	100	100	91.2
10 mm^3	82.4	100	100	75.8
India ink microscopy	86.1	97.3	98.2	80.2
Cryptococcal antigen latex agglutination assay				
Meridian	97.8	85.9	92.6	95.5
IMMY	97.0	100	100	95.3
Metagenomic next-generation sequencing	93.5	96.0	87.8	98.0
PCR assay‡	82.0	98.0	98.0	79.0

* Data are from Boulware et al.,[45] Dantas et al.,[47] the Clinical and Laboratory Standards Institute,[48] Gan et al.,[49] and Bridge et al.[50] PCR denotes polymerase chain reaction.
† Two quantitative CSF culture procedures were used: one from 2006 to 2009 (input volume, 10 mm^3), and one from 2010 to 2012 (input volume, 100 mm^3).
‡ Sensitivity decreases with a low fungal burden. The PCR assay used was the BioFire FilmArray Meningitis–Encephalitis Panel.

in patients with chronic or progressively worsening symptoms.[46]

Indeed, diagnostic delays result in higher mortality in all patient groups. For example, a study in rural Uganda showed that 70% of patients who died after the development of HIV-associated cryptococcal meningitis had sought care three or more times before the diagnosis was made.[51] In addition, the absence of fevers and obvious immunosuppression in previously healthy patients typically delays consideration of an infectious source, with some of the poorest outcomes of all host groups in more economically developed countries.[5]

Elevated cell counts (with a predominance of lymphocytes) and total protein levels and low glucose levels in CSF are suggestive of cryptococcal meningitis in patients presenting with chronic, progressive neurologic symptoms. CSF fungal cultures are useful not only for establishing the diagnosis but also for differentiating microbiologic failure from inflammatory sequelae during therapy in patients with clinical deterioration. (Microbiologic failure is uncommon with recommended first-line therapies but may be supported by elevated drug minimum inhibitory concentrations.[52]) Imaging with CT or magnetic resonance imaging (MRI), which is more sensitive than CT, is useful during the initial evaluation for identifying structural lesions, including hydrocephalus.[53] In addition, methods such as diffusion-weighted imaging with apparent diffusion coefficient maps identify areas of infarction (and can differentiate lesions from cryptococcomas with serial images), whereas contrast-enhanced fluid-attenuated inversion recovery (FLAIR) imaging provides sensitive detection of inflammatory lesions as well as the extent of inflammation.[54]

ANTIFUNGAL TREATMENT

Asymptomatic disease in persons with AIDS, before the development of overt symptoms of cryptococcal meningitis, may be diagnosed by testing for cryptococcal antigen in blood, which can prompt the initiation of preemptive antifungal therapy.[28] Preemptive fluconazole therapy that

Table 2. Current Approaches to Treatment According to Patient Group and Resource Availability.*

Treatment Phase and Patient Group	Duration
Induction therapy	
In HIV-coinfected patients in resource-rich settings: liposomal amphotericin B, 3–4 mg/kg daily, plus flucytosine, 25 mg/kg 4 times per day	2 wk
In HIV-coinfected patients in resource-limited settings	
Liposomal amphotericin B, 10 mg/kg as a single dose, plus flucytosine, 100 mg/kg/day, and fluconazole, 1200 mg/day	2 wk of flucytosine and fluconazole
Liposomal amphotericin B, 3–6 mg/kg/day, or amphotericin B deoxycholate, 0.7–1.0 mg/kg/day, plus flucytosine, 100 mg/kg/day (for both oral and intravenous formulations)	1 wk
Alternative induction therapy in resource-limited settings	
If flucytosine is not available: amphotericin B deoxycholate, 0.7–1 mg/kg/day given intravenously, plus fluconazole, 800–1200 mg/day	2 wk, although 1 wk of amphotericin B deoxycholate is better than none
If amphotericin B deoxycholate is not available: fluconazole, 1200 mg/day, plus flucytosine, 100 mg/kg/day given orally, if available	2 wk
In organ-transplant recipients: liposomal amphotericin B, 3 mg/kg daily, plus flucytosine, 100 mg/kg daily	2 wk
In previously healthy patients or those who have not received a transplant: liposomal amphotericin B, 3–5 mg/kg daily, or amphotericin B deoxycholate, 0.7–1.0 mg/kg daily, plus flucytosine, 100 mg/kg daily in 4 divided doses	4–6 wk or 2 wk after negative CSF, and flucytosine for first 2 wk
Consolidation therapy	8 wk
Fluconazole, 400–800 mg/day†	
Maintenance therapy	
Fluconazole, 200 mg/day; in HIV-infected patients, start ART at 4–6 wk, and consider discontinuing maintenance therapy after a minimum of 1 yr if CD4+ cell count is >100/mm^3 and HIV viral load is suppressed	12–18 mo

* Data are from Jarvis et al.,[57] Chen et al.,[58] and Perfect et al.[59] ART denotes antiretroviral therapy, and HIV human immunodeficiency virus.
† A dose of 800 mg per day is preferred if second-line induction regimens are used.

is administered over a period of 10 weeks has prevented progression to cryptococcal meningitis in 75% of persons who tested positive for cryptococcal antigen.[38] After the onset of cryptococcal meningitis symptoms, amphotericin B–based regimens form the basis for therapy, with a three-step induction, consolidation, and maintenance approach.[55] For induction therapy, fungicidal amphotericin-based regimens have had good outcomes. Rates of CSF fungal clearance, measured by means of quantitative cultures (early fungicidal activity [EFA]), are associated with mortality and may identify poor antifungal regimens.[56] Although an EFA of at least 0.2 $\log_{10}$ colony-forming units per milliliter of CSF has been suggested as a surrogate measure of antifungal efficacy, further studies are needed to determine the optimal EFA threshold reflecting antifungal efficacy (Table 2).[60]

Amphotericin B deoxycholate is the primary formulation used in resource-limited settings. Although this formulation is strongly associated with renal impairment, renal tubular acidosis, hypokalemia, hypomagnesemia, and anemia,[61] presupplementation with electrolytes and fluids minimizes these adverse effects.[62] However, liposomal amphotericin B is used in more economically developed regions because of its reduced toxicity. The addition of flucytosine to amphotericin B–based regimens results in more rapid fungal clearance and improves survival in resource-limited settings.[63] The Ambition trial successfully

Table 3. Framework for Cryptococcal Inflammatory Syndromes.*

Phase and Immunologic Characteristics	Evidence in Patients
During active cryptococcal infection	
Paucity of appropriate inflammation for cryptococcosis	Decreased TNF-α, GM-CSF, interferon-γ, TNF-α, and interleukin-6 in CSF[17,83]
Compartment-specific cytokine profile†	Elevated plasma interleukin-5 and interleukin-7[84]; elevated CSF interferon-γ, interleukin-4, interleukin-17, CXCL10, CCL3, and CCL2[85]
Before ART	
Inappropriate (Th2 cell) responses resulting in poor antigen clearance before ART in patients with HIV infection	Elevated interleukin-4; higher baseline cryptococcal antigen in patients with and those without HIV infection[86]
Elevated CSF chemokine expression	Elevated CSF CCL2, and CCL3[87]
Humoral system: decreased specific antibody levels	Decreased plasma IgM, Lam-IgG, and GXM-IgM[88]
Increasing proinflammatory signaling from APCs because of persistent antigen burden and failure to clear antigen	Elevated interleukin-6 from macrophages[89] and elevated CRP and interleukin-7 from APCs
Interaction between host genetic factors and pathogen; LTA4H SNPs associated with MCP-1 production and risk of IRIS among persons without HIV infection	LTA4H SNPs increase risk of non-HIV–associated IRIS[86]
IRIS	
Effective response of innate and adaptive immune systems	Elevated Th1 and Th17 cytokines; elevated innate cytokines: interleukin-8 and GM-CSF; with or without CSF pleocytosis; with or without elevated CSF protein; negative CSF culture; elevated interleukin-6+ and TNF-α+ monocytes[90]; in non-HIV–associated IRIS, increased interleukin-1Ra, interleukin-7, CCL2, and TNF-α,[86]
Neuronal-cell activation and damage	Elevated CSF FGF-2[85]
Aberrant innate cell trafficking	Trafficking of proinflammatory monocytes and CD4+ T cells into CSF[91]
PIIRS	
Release of fungal antigens after fungal lysis, aberrant CNS T-cell and monocyte activation and migration, or neuronal-cell damage	Negative CSF fungal cultures; elevated CSF protein and pleocytosis; elevated soluble interleukin-2 receptor; elevated CSF interleukin-6; increased HLA-DR+CD4+ and CD8+ T cells in CSF[92]; and increased NFL1[18]

* Type 1 helper T (Th1) cells that are useful in eliminating cryptococcus include tumor necrosis factor α (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ, and interleukin-6. Type 2 helper T (Th2) cells that may lead to cryptococcal proliferation include interleukin-4, interleukin-5, and interleukin-7. HLA-DR is a marker of cellular activation. The chemokines CCL2, CCL3, and CXCL10 induce the movement of various cell types. APC denotes antigen-presenting cell, CCL chemokine ligand, CRP C-reactive protein, CXCR3 CXC chemokine receptor 3, FGF fibroblast growth factor, interleukin-1Ra interleukin-1 receptor antagonist, IRIS immune reconstitution inflammatory syndrome, LTA4H leukotriene A4 hydrolase, NFL1 neurofilament light chain 1, PIIRS postinfectious inflammatory response syndrome, SNP single-nucleotide polymorphism, and Th17 type 17 helper T cells.

† A compartment-specific cytokine profile occurs in addition to or instead of an uncharacteristically low level of inflammation.

used a single high dose of liposomal amphotericin B (at a dose of 10 mg per kilogram of body weight) plus flucytosine and fluconazole as induction therapy, with continuation of fluconazole alone as consolidation therapy.[57] This regimen was rapidly adopted by the World Health Organization for resource-constrained regions as standard therapy for HIV-related cryptococcal meningitis if liposomal amphotericin B is available.[64] However, more data may be necessary to determine the

equivalence of this regimen to the longer courses (1 to 2 weeks) of liposomal amphotericin B that are traditionally used in more economically developed countries and in HIV-negative persons. The U.S. guidelines are currently undergoing revision, with possible inclusion of this single-dose amphotericin regimen as an option for HIV-associated cryptococcal meningitis.

RAISED INTRACRANIAL PRESSURE AND OTHER COMPLICATIONS

Increased intracranial pressure is an important complication of cryptococcal meningitis, with approximately half of HIV-infected patients having pressures greater than 25 cm of water. High pressures are associated with headache, altered mental status, nausea, cranial-nerve deficits, and cognitive sequelae, with increased short-term mortality.[53] Obstructed CSF reabsorption at superior arachnoid outflow tracks in HIV-coinfected patients preserves ventricular communication, resulting in the relative safety of lumbar puncture–directed drainage in this population, whereas in HIV-negative persons, more frequent choroiditis at the foramen of Monroe, Luschka, or Magendie may result in obstructive hydrocephalus, increasing the risk associated with this procedure when it is performed without brain imaging.[65] Obstructive hydrocephalus can be readily diagnosed on MRI by the presence of increased ventricular size with transependymal flow or sulcal effacement.

Daily therapeutic lumbar punctures reduce intracranial pressures and are associated with reduced mortality.[66,67] Despite an impressive case report describing 76 lumbar punctures in a single survivor of cryptococcal meningitis,[68] increases in the acceptance of lumbar puncture require meaningful patient and community engagement.[69,70] Alternative methods of managing intracranial pressure include lumbar drains and ventriculoperitoneal shunts, with the latter more commonly used in non-HIV–associated cryptococcal meningitis because of persistent obstruction.[71] Besides elevated intracranial pressure, coexisting tuberculosis[72] or cytomegalovirus infection,[73] increased neutrophil counts,[74] a high fungal burden,[75] and hyponatremia[76] are associated with an increased risk of death from cryptococcal meningitis among persons with AIDS.

RELATED INFLAMMATORY SYNDROMES

There has been a growing appreciation of the role of infection-related inflammatory syndromes in diverse infectious diseases.[77-79] Intracranial infections such as cryptococcal meningitis are particularly susceptible to these sequelae because of the subsequent swelling within the restricted confines of the skull. For example, the cryptococcal immune reconstitution inflammatory syndrome (IRIS) in HIV-associated disease was found to have an incidence of 4 to 5% in the Ambition trial. The syndrome occurs 1 to 2 months after cryptococcal meningitis has been diagnosed, usually after the early initiation of ART (<4 weeks after diagnosis).[57,80-82] Risk factors for cryptococcal IRIS include a high initial CSF fungal burden and low initial markers of inflammation, including blood CD4+ counts, CSF cells, and inflammatory markers such as interferon-γ, which are rapidly corrected after ART initiation (Table 3).[93,94]

Related to IRIS are unmasking syndromes, in which previously asymptomatic cryptococcal infection is recognized only with the occurrence of neurologic symptoms after the initiation of ART.[27] A cohort study in Uganda suggested an increased risk of death among patients with unmasking cryptococcal infection in whom ART had been initiated in the preceding 14 days.[27]

Similarly, in non-HIV–related cryptococcal meningitis, reductions in immunosuppression during conditioning for solid-organ transplantation or cancer chemotherapy may be accompanied by IRIS-like reconstitution syndromes and typically respond to similar adjunctive therapies.[95] In the absence of changes in immunosuppression, which accounted for 50% of transplant recipients in one series[96] and accounts for a substantial number of previously healthy persons who have not received immunosuppressive therapy, the release of fungal antigens after fungicidal therapy may precipitate PIIRS, a paradoxical postinfectious inflammatory syndrome.[92] PIIRS is defined by a Montreal Cognitive Assessment (MoCA) score of less than 22 (on a scale of 0 to 30, with lower scores indicating greater impairment) or by the presence of visual or auditory deficits in the context of effective antifungal therapy and microbiologic control, as evidenced by negative CSF fungal cultures.[16] A MoCA score of less than 22 at

diagnosis is also a risk factor for a poor outcome in patients with non–HIV-associated cryptococcal meningitis and is a recommended, convenient prognostic test in this population.[97]

PIIRS shares features with cryptococcal IRIS, including a CNS compartmentalized activation of T-cell inflammatory responses, as shown by increased CSF HLADR+CD4+ T cells and elevated levels of CSF cytokines soluble CD25, interferon-γ, and interleukin-6, but has alternatively activated macrophages.[92] In a small, prospective study, consecutive patients with PIIRS had a response to tapered pulse glucocorticoid therapy. All the patients had prompt improvements in MoCA and Karnofsky scores, as well as in visual and auditory measures, with associated reductions in inflammatory biomarkers, opening pressures, and inflammatory MRI findings and with negative fungal cultures during maintenance fluconazole therapy. These findings offer new promise for the treatment of refractory disease and for patients whose condition deteriorates during therapy.[18]

FUTURE DIRECTIONS AND MAJOR UNSOLVED PROBLEMS

The emergence of new paradigms in cryptococcal disease is likely to lead to new actionable strategies for a disease that still kills a substantial number of patients despite therapy. For HIV-associated disease, screening and preventive strategies at the time of HIV diagnosis, facilitated by more sensitive diagnostic testing, including a semiquantitative cryptococcal antigen test currently in development,[98] offer the promise of early, cost-effective oral preemptive treatment strategies that may prevent symptomatic infections.

Encouraging results from a recent phase 2 trial of an oral nanoparticle cochleate amphotericin B formulation combined with flucytosine, albeit with frequent administration and with gastrointestinal side effects, suggest progress in induction therapeutics.[99] Fosmanogepix, which prevents the biosynthesis of cell-wall mannoproteins by inhibiting the fungal enzyme Gwt1,[100] and a third-generation polyene, SF-001 (Elion Therapeutics), which are under development in preclinical studies, are also promising therapeutic agents.[101] More-abbreviated amphotericin B regimens and increased access to flucytosine in resource-limited settings will also improve outcomes.

An important question regarding all patient groups is why patients die despite microbial success. For non–HIV-associated disease, advances in CSF immunophenotyping have led to the identification of PIIRS, a host-damaging immune syndrome (characterized by the right-hand portion of the microbe–host response parabola shown in Fig. 1). This discovery, in turn, has prompted the development of effective adjuvant therapeutic agents such as glucocorticoids for a disease that has not had reductions in mortality since the 1950s. However, further research is needed to examine more fully the nature and scope of the parabola relationships and inflammation in the full range of host populations in which cryptococcal meningitis develops. Newer adjunctive agents for cryptococcal PIIRS that can antagonize CSF inflammation, such as the interleukin-6R antagonist tocilizumab, which was previously found to be effective in other CNS inflammatory diseases, as well as JAK-STAT (Janus kinase–signal transducer and activator of transcription) inhibitors such as ruxolitinib, are under development.[102] Further development of CSF biomarkers, exemplified by the commercially available CSF interleukin-6 and soluble CD25 assays,[102] and MRI techniques in patients with PIIRS[18] will be critical for monitoring and for the administration and dose levels of immunomodulators so that patients receive effective doses without excessive immunosuppression.

Questions about high intracranial pressure — a strong predictor of a poor outcome — warrant exploration. Is high pressure due to obstructing organisms, inflammation or inhibition of host channels, or something else? In which populations does high intracranial pressure occur and when? Could the use of artificial intelligence help identify additional risk factors that are currently unrecognized?[103]

Much also remains unknown about patients without obvious immunosuppression (who are often labeled as “immunocompetent”) in whom cryptococcal disease develops. To address this knowledge gap, the National Institutes of Health Clinical Center is recruiting previously healthy patients without known immunosuppression in order to identify genetic and immunologic deficiencies and to develop new approaches to understanding and treating infections (ClinicalTrials.gov number, NCT00001352). Genetic deficiencies are increas-

ingly treatable with immunotherapies, and accurate identification of relevant genetic pathways may thus be beneficial.

Vaccine-induced immune responses have been shown in animal models with the use of inactivated cryptococcal mutant strains,[104] as well as glucan particles containing fungal antigens.[105] However, efforts to develop vaccines that prevent cryptococcal disease in humans face challenges in the selection of target populations and facilitation of an immune response in those most at risk. If these challenges can be overcome, the development and commercialization of either recombinant protein vaccines or messenger RNA vaccines could be the next frontier of cryptococcal disease prevention.

SUMMARY

The Ending Cryptococcal Meningitis Deaths by 2030 Strategic Framework recommends priorities to reduce morbidity and mortality from cryptococcal meningitis; implementation should be a global effort.[106] Focusing on preventing or reducing cryptococcal disease in HIV-infected and non–HIV-infected populations requires further investment in the development of adjunctive therapies, new compounds, and a vaccine, as well as in continuing to educate health care providers in order to minimize diagnostic delays.

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Original Article

Cryptococcosis—a systematic review to inform the World Health Organization Fungal Priority Pathogens List

Aiken Dao [1,2,3,†], Hannah Yejin Kim [1,4,5,†], Katherine Garnham [1,6], Sarah Kidd [7], Hatim Sati [8], John Perfect [9], Tania C. Sorrell [1,2,3], Thomas Harrison [10,11], Volker Rickerts [12], Valeria Gigante [8], Ana Alastruey-Izquierdo [13], Jan-Willem Alffenaar [1,3,4], C. Orla Morrissey [14,15], Sharon C-A. Chen [16] and Justin Beardsley [1,2,3]

[1]Sydney Infectious Diseases Institute, The University of Sydney, Sydney, Australia
[2]Westmead Institute for Medical Research, Westmead, Sydney, Australia
[3]Westmead Clinical School, Westmead Hospital, Sydney, Australia
[4]Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia
[5]Department of Pharmacy, Westmead Hospital, Westmead, Australia
[6]Sunshine Coast University Hospital, Birtinya, Qld 4575, Australia
[7]National Mycology Reference Centre, Microbiology and Infectious Diseases, SA Pathology, Adelaide, Australia
[8]AMR Division, World Health Organization, Geneva, Switzerland
[9]Duke University Medical Center, Durham, NC, USA
[10]Institute of Infection and Immunity, St George's University London, London, UK
[11]Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, UK
[12]Robert Koch Institute, Berlin, Germany
[13]Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain
[14]Department of Infectious Diseases, Alfred Health, Melbourne, Australia
[15]Monash University, Department of Infectious Diseases, Melbourne, Victoria, Australia
[16]Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead Hospital, Westmead, Sydney, Australia

To whom correspondence should be addressed. Associate Professor Justin Beardsley MBChB, FRACP, PhD; Sydney Infectious Diseases Institute, The University of Sydney, New South Wales, Australia. Tel: +61 2 9351 2222, E-mail: justin.beardsley@sydney.edu.au
[†]Joint first authors

Abstract

Cryptococcosis causes a high burden of disease worldwide. This systematic review summarizes the literature on *Cryptococcus neoformans* and *C. gattii* infections to inform the World Health Organization's first Fungal Priority Pathogen List. PubMed and Web of Science were used to identify studies reporting on annual incidence, mortality, morbidity, antifungal resistance, preventability, and distribution/emergence in the past 10 years. Mortality rates due to *C. neoformans* were 41%–61%. Complications included acute renal impairment, raised intracranial pressure needing shunts, and blindness. There was moderate evidence of reduced susceptibility (MIC range 16–32 mg/l) of *C. neoformans* to fluconazole, itraconazole, ketoconazole, voriconazole, and amphotericin B. *Cryptococcus gattii* infections comprised 11%–33% of all cases of invasive cryptococcosis globally. The mortality rates were 10%–23% for central nervous system (CNS) and pulmonary infections, and ~43% for bloodstream infections. Complications described included neurological sequelae (17%–27% in *C. gattii* infections) and immune reconstitution inflammatory syndrome. MICs were generally low for amphotericin B (MICs: 0.25–0.5 mg/l), 5-flucytosine (MIC range: 0.5–2 mg/l), itraconazole, posaconazole, and voriconazole (MIC range: 0.06–0.5 mg/l). There is a need for increased surveillance of disease phenotype and outcome, long-term disability, and drug susceptibility to inform robust estimates of disease burden.

Key words: *Cryptococcus neoformans*, *Cryptococcus gattii*, cryptococcosis, cryptococcal meningitis, invasive fungal infection.

Introduction

Invasive fungal infections pose a significant threat to global health. Although their burden is ill-defined, crude estimates suggest they cause over 1.6 million deaths annually.[1] The absence of strong surveillance systems results in clinicians making decisions based on limited information about local epidemiology, antimicrobial resistance, and effective treatment strategies. In response to this growing threat, the World Health Organization (WHO) developed a Fungal Priority Pathogens List (FPPL). This list, published in 2022, was created through a comprehensive international consultation process, using a survey incorporating a discrete choice experiment. The individual fungal pathogens, including *Cryptococcus neoformans* and *C. gattii*, were ranked based on the results of systematic reviews, expert opinion, and data from the discrete choice experiments.

Cryptococcosis is a life-threatening invasive fungal infection, that poses a significant global health challenge. Historically, *Cryptococcus* was described as two species: *C. neoformans* (var. *grubii* and var. *neoformans*) and *C. gattii*. More recently, phylogenetic analyses have distinguished seven clades representing species (VNI-III and VGI-IV), and there are likely

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more, with varying virulence and regional distribution.[2,3] For example, VGI is prevalent in Australia and Asia, VGII is particularly associated with the emergence in North America, VGIII is increasing among immunocompromised individuals in the United States, and VGIV is primarily found in Africa.[3–5] Notably, the terminology of two cryptococcal 'species complexes' remains common in clinical practice as it is the most practicable for management purposes.

Cryptococcosis is best documented in people living with HIV/AIDS. However, it is increasingly recognized in other immunocompromised hosts, and occurs in people with various underlying conditions and even unrecognized risk factors.[6–8] Members of the *C. neoformans* and *C. gattii* species complexes are the predominant causative agents,[9] with species-specific differences in epidemiology: for example, *C. neoformans* species complex has traditionally been observed in HIV/AIDS patients, whilst *C. gattii* species complex infection has a propensity to occur in immunocompetent patients.[10]

Innate and adaptive responses work together to combat *Cryptococcus* spp., with CD4 + T-cells particularly important for an effective adaptive response.[11,12] Symptomatic infection often indicates a compromised immune system, particularly in individuals with reduced CD4 + T-cell counts, such as people living with HIV.[13–15] Latency and dormancy are also important aspects of cryptococcal pathogenesis. The fungus can remain dormant in the host due to both immune pressure and fungal factors,[16–19] and in certain host environments, including granulomas, it can avoid immune detection.[19] Reactivation of dormant cryptococci becomes a concern when the host's immune system becomes compromised, potentially leading to invasive disease.[20] Improving our understanding of these and other factors is crucial for improving diagnostic, therapeutic, and preventive strategies.[21]

Cryptococcus neoformans and *C. gattii* species complexes are acquired via the respiratory tract, where they can cause local infection, although it is their tropism for the central nervous system (CNS) that is associated with the most serious manifestations of infection. Cryptococcal meningitis (CM) remains the most common cause of fungal meningitis worldwide with over 220 000 new cases and 180 000 deaths per annum.[22] Consequently, CM is an infection of global relevance, with most deaths seen in sub-Saharan Africa and in South and Southeast Asia.[23–25]

Treatment options for invasive cryptococcosis are limited, and development of novel anti-cryptococcal agents has been slow in recent decades.[26] Cryptococci are intrinsically resistant to echinocandins.[27] Optimal induction treatment relies on amphotericin B and 5-flucytoscine despite their substantial toxicity and limited access associated with economic and logistical constraints. Prolonged treatment with azoles is required following induction therapy.[28]

In low- and middle-income countries (LMICs) where disease burden is highest, poor access to optimal therapeutics (i.e., 5-flucytosine and amphotericin B lipid formulations) increases the clinical challenges and contributes to the observed persistent poor clinical outcomes of cryptococcosis.[29]

This systematic review evaluates *C. neoformans* and *C. gattii* species complexe infections against a set of criteria, namely: mortality, hospitalization and disability, antifungal drug resistance, preventability, yearly incidence, global distribution, and emergence, based on data published between 2011 and 2021. The purpose is to determine knowledge gaps for both *C. neoformans* and *C. gattii* species complexes in the above areas to highlight research needs and to inform the WHO FPPL.

Materials and methods

Search strategies

We conducted a comprehensive search for studies published in English using the PubMed and Web of Science databases. These databases were chosen due to their extensive coverage of medical and scientific literature. The study was conducted according to PRISMA guidelines.[30] All searches were limited to the last 10 years (from 1st January 2011 to 19th February 2021).

On PubMed, we used medical subject headings (MeSH) and/or keyword terms in the title/abstract for each pathogen and criterion.

For *C. neoformans*, the final search used (*C. neoformans*[Title] OR *C. neoformans*[Title]) combined; for *C. gattii*, the final search used (*C. gattii* [MeSH Terms]) combined, using AND term, with criteria terms including (mortality[MeSH Terms]) OR (morbidity[MeSH Terms]) OR (hospitalization[MeSH Terms]) OR (disability[All Fields]) OR (drug resistance, fungal[MeSH Terms]) OR (prevention and control[MeSH Subheading]) OR (disease transmission, infectious[MeSH Terms]) OR (diagnostic[Title/Abstract]) OR (antifungal agents[MeSH Terms]) OR (epidemiology[MeSH Terms]) OR (surveillance [Title/Abstract]).

On Web of Science, MeSH terms are not available and therefore topic search (TS), title (TI), or abstract (AB) search were used. The final search used [TI=('cryptococcus neoformans/cryptococcus gattii') OR TI=('C. neoformans') OR AB=('cryptococcus gattii')], combined using AND term with criteria terms each as topic search, including (mortality) OR (case fatality) OR (morbidity) OR (hospitalization) OR (disability) OR (drug resistance) OR (prevention and control) OR (disease transmission) OR (diagnostic) OR (antifungal agents) OR (epidemiology) OR (surveillance). Symbol * allows a truncation search for variations of the term (e.g., hospitalization or hospitalization).

Study selection

We imported search results from each database into the online systematic review software, Covidence® (Veritas Health Innovation, Australia), and removed duplicates. The inclusion criteria were retrospective/prospective observational studies, randomized controlled trials, guidelines, epidemiology, surveillance reports, published within the last 10 years (2011–2021), reporting adults and paediatric data, including data on the fungal pathogen, and data on at least one criterion. Exclusion criteria were studies reporting on non-human data (e.g., animals, plants) or non-fungal data (e.g., bacteria), no data on relevant pathogens or criteria, case reports, conferences, abstracts, reviews, papers on drugs without marketing authorization, *in vitro* papers on resistance mechanisms, and papers published in non-English language. Identified articles underwent title and abstract screening based on the inclusion criteria. No reason was provided for exclusion during title and abstract screening. Two independent reviewers (AD and HYK) performed full text screening for the final eligible

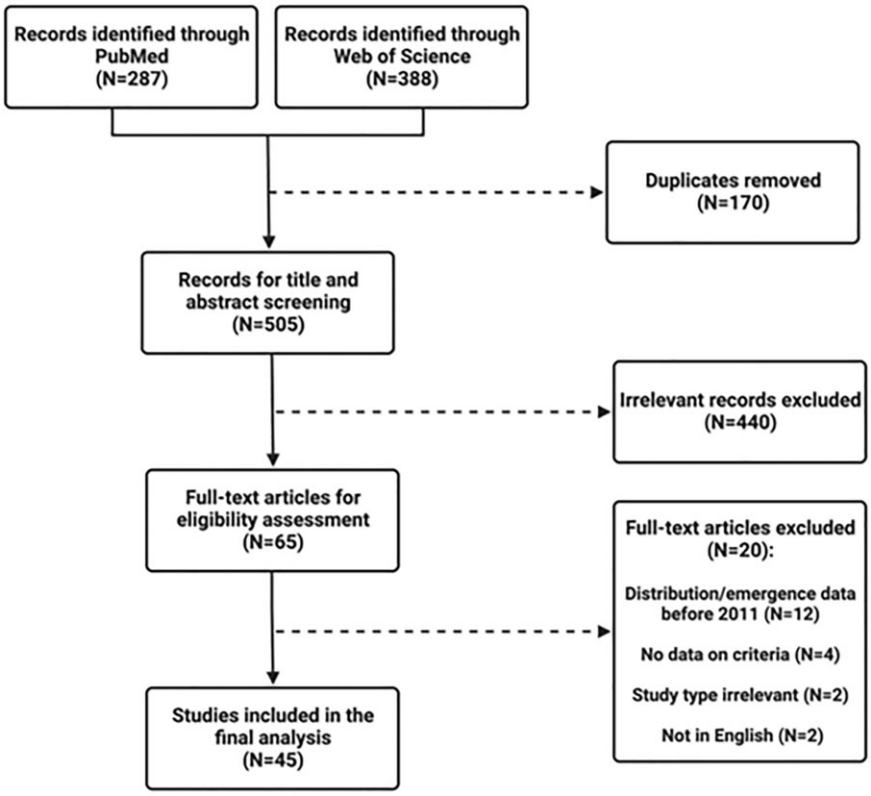

Figure 1. Flow diagram for selection of studies included in the systematic review for *C. neoformans*.

articles on Covidence®. A third reviewer resolved any discrepancies (JWA). Excluded articles were recorded with reasons when excluded during full text screening. If there were any additional articles identified from references of the included articles, these were added. The resulting articles were subject to the final analysis.

Data collection and synthesis

Data from the final included studies were extracted for relevant criteria (AD and HYK). The extracted data were checked by the second reviewer (JWA) (initially 10% check, then expanded to 20% and more if needed, depending on the type of extent of observed errors). The extracted data on the outcome criteria were qualitatively AND/OR quantitatively synthesized, depending on the amount and nature of the data.

Risk of bias assessment

We assessed risk of bias using the risk of bias tool for randomized trials version 2 (ROB 2) tool for randomized controlled trials.[31] The risk of bias in non-randomized studies (RoBANS) tool was used to assess the non-randomized studies.[32] For the overall risk, using ROB 2 tool, the studies were rated 'low', 'high', or 'some' concerns. Using the RoBANS tool, the studies were rated as 'low', 'high', or 'unclear' risk.

For the purposes of this review, we considered each criterion as an outcome of the study and assessed if any bias was expected based on the study design, data collection, and analysis methods for that outcome. Studies that were classified as having an unclear or high overall risk were still eligible for inclusion with cautious interpretation.

Results

Study selection

For *C. neoformans*, PubMed and Web of Science Core Collection databases searched between 1 January 2011 and 19 February 2021 yielded 287 and 388 articles, respectively (Fig. 1). For *C. gattii*, the search yielded 219 and 277 articles, respectively (Fig. 2). A total of 45 (*C. neoformans*) and 14 (*C. gattii*) articles were included in the final analysis.

Risk of bias

For *C. neoformans*, the overall risk of bias for each study is presented in the Table 1A. Of the included studies, 22 studies were classified as low risk of bias in all domains assessed. Twenty-three studies were classified as unclear risk of bias, mostly due to the potential selection biases caused by unclear eligibility criteria or population groups, or unclear confirmation/consideration of confounding variables.

For *C. gattii*, the overall risk of bias for each study is presented in the Table 1B. Of the 14 studies, 5 studies were classified as low risk of bias in all domains assessed. Nine studies were classified as unclear risk of bias, mostly due to the selection biases caused by unclear eligibility criteria or population groups, or unclear confirmation/consideration of confounding variables.

Mortality rates

For *C. neoformans*, 13 studies reported on mortality (Table 2). The mortality rates due to *C. neoformans* were reported to be as high as 41%–61% for patients with HIV in-

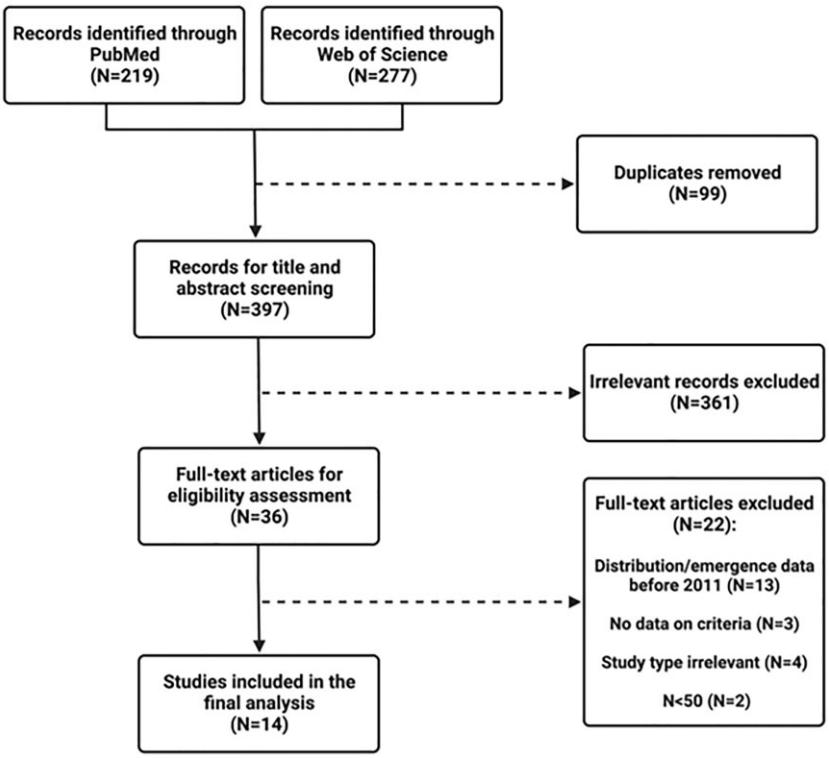

Figure 2. Flow diagram for selection of studies included in the systematic review for *C. gattii*.

fection.[37, 63, 64, 69, 73] Mortality rates specifically reported for HIV-negative patients were lower, ranging from 8% to 20%, but small patient numbers are noted ($N = 12–44$).[63,40,77]

For *C. gattii*, four studies reported on mortality (Table 2). The mortality rate due to *C. gattii*-related bloodstream infection was 43% ($N = 7$) in the study by Smith et al.[85] Other studies reported mortality rates of 10%–23.4% for CNS infections[78, 79, 84, 85] and 14.6%–21% for pulmonary infections, acknowledging the relatively small cohorts.[85,84]

Antifungal susceptibilities

In total, 33 studies reported results of antifungal susceptibility testing on *C. neoformans* isolates (Table 3), and 6 studies for *C. gattii* (Table 4); methodologies included CLSI standard, EUCAST standard, Etest, Vitek 2 YST AST, and Sensititre YeastOne assays. Details of these studies are presented in the appendix (Tables A1 and A2).

Cryptococcus neoformans susceptibility to antifungals

Before 2020, when EUCAST has provided a CBP for amphotericin B only, there were no interpretative clinical breakpoint (CBP) MICs for *C. neoformans*. It is also noteworthy that no causal relationship has been established between MIC and treatment failure.[86] Consequently, interpretive criteria applied to antifungal MIC results for *C. neoformans* in the reviewed publications were highly variable both within and between publications. Examples of interpretive criteria included utilizing *C. albicans* CBPs, or breakpoints suggested with user manuals provided with testing kits, CLSI epidemiologic cutoff values (ECVs), and values selected from previous scientific publications.

Reported susceptibility of *C. neoformans* to fluconazole was variable, with two studies reporting no 'resistance' in their tested isolates[33,42] and some others reporting higher 'resistance' rates of up to 30%.[41,75] Fluconazole MIC_{90} values were variable between studies; however, were as high as 16 to 32 mg/l based on CLSI[33,54, 75, 76] and EUCAST methods for MIC determination.[45] Chen et al. observed significantly increasing numbers of isolates with fluconazole MIC $\geq$ 8 mg/l over the study period 2001–2012 ($P < 0.001$).[41]

Limited numbers of studies reported susceptibility to isavuconazole. Geometric mean MIC values from these studies ranged from 0.011 to 0.065 mg/l[55, 56, 70, 72] and MIC_{90} values ranged from 0.031 to 0.063 mg/l.[56, 70, 72] Reduced susceptibility to itraconazole (0.03–2 mg/l) was uncommon, ranging from 0% to 22%,[42,54] with $\leq$ 1% non-wild type (non-WT) rates.[45,48,51] 'Resistance' rates were lower for ketoconazole (0%–7%)[33,54] and voriconazole (0%).[33,42] For posaconazole and voriconazole, non-WT rates of 1.3%–5.7% were reported.[45,48,51]

For amphotericin B, Andrade-Silva et al. reported a resistance rate of 11% based on 95 isolates from HIV/AIDS patients in Brazil,[33] in contrast to Tewari et al. reporting < 2% resistance rate in their Indian population (80% without HIV infection).[76]

Susceptibility to 5-flucytosine was only reported as non-WT rates of 1%–2%,[45,51] and MIC_{90} values were highly variable between studies but were as high as 8–16 mg/l.[45,70,72,51,38] Selb et al. observed a lower MIC90 of 1 mg/l for serotype A (genotype VNI) compared with MIC90 of 8 mg/l for serotype D (genotype VNIV).[74]

Cryptococcus gattii susceptibility to antifungals

For *C. gattii*, all studies reported MIC values without interpretive CBP MICs.

Studies by Espinel-Ingroff et al. and Lockhart et al. were conducted on large number of isolates (~300) from multiple

Table 1. The risk of bias for each study of *C. neoformans*.

Author	Publication year	Risk of bias (low, high, and unclear)	Reference
A			
Andrade-Silva et al.	2013	Unclear	33
Andrade-Silva et al.	2018	Unclear	34
Ashton et al.	2019	Unclear	35
Bariao et al.	2020	Low	36
Beale et al.	2015	Low	37
Bertout et al.	2012	Unclear	38
Cao et al.	2019	Low	39
Chan et al.	2014	Low	40
Chen et al.	2015	Low	41
Chen et al.	2018	Low	42
Chowdhary et al.	2011	Unclear	43
Cogliati et al.	2018	Unclear	44
Córdoba et al.	2016	Low	45
de Oliveira et al.	2017	Low	46
Desnos-Ollivier et al.	2015	Unclear	47
Espinel-Ingroff et al.	2012	Unclear	48
Espinel-Ingroff et al.	2012	Unclear	49
Espinel-Ingroff et al.	2015	Unclear	50
Fan et al.	2016	Low	51
Gonzalez et al.	2016	Low	52
Govender et al.	2011	Unclear	53
Gutch et al.	2015	Unclear	54
Hagen et al.	2016	Unclear	55
Herkert et al.	2018	Unclear	56
Hurtado et al.	2019	Low	57
Kassi et al.	2016	Low	58
Lahiri et al.	2020	Unclear	59
Lin et al.	2015	Unclear	60
Mahabeer et al.	2014	Low	61
Mahabeer et al.	2014	Low	62
Martins et al.	2011	Low	63
Mdodo et al.	2011	Unclear	64
Miglia et al.	2011	Unclear	65
Naicker et al.	2020	Unclear	66
Nascimento et al.	2017	Low	67
Nishikawa et al.	2019	Low	68
Nyazika et al.	2016	Low	69
Pan et al.	2012	Unclear	70
Pfaller et al.	2011	Unclear	71
Prakash et al.	2020	Low	72
Rakotoarivelo et al.	2020	Unclear	73
Selb et al.	2019	Low	74
Smith et al.	2015	Low	75
Tewari et al.	2012	Unclear	76
Yoon et al.	2020	Low	77
B. The risk of bias for each study of *C. gattii*			
Chen et al.	2012	Low	78
Chen et al.	2013	Low	79
Espinel-Ingroff et al.	2012	Unclear	48
Espinel-Ingroff et al.	2012	Unclear	49
Espinel-Ingroff et al.	2015	Unclear	50
Firacative et al.	2016	Unclear	80
Harris et al.	2011	Low	81
Hurtado et al.	2019	Unclear	57
Kassi et al.	2016	Unclear	58
Lahiri et al.	2020	Unclear	59
Lee et al.	2019	Unclear	82
Lockhart et al.	2012	Unclear	83
Phillips et al.	2015	Low	84
Smith et al.	2014	Low	85

Table 2. The mortality rates due to *C. neoformans* and *C. gattii* infections.

Author	Study period	Pathogen species	Country	Study design	Level of care	Population description	Patients (*N*=)	Mortality type, N/N, %
Desnos-Ollivier et al. [47]	1997 to 2001	*Cryptococcus neoformans*	France	Qualitative data and lab surveillance study (MC)	ND	Patients enrolled during the CryptoA/D study or the nationwide survey on cryptococcosis in France	181	% Patients who died within 90 days after diagnosis/total Serotype A: 21/82 (26%) Serotype D: 7/22 (32%) Serotype AD: 7/25 (28%)
Chan et al. [40]	1999 to 2007	*Cryptococcus neoformans*	Singapore	RCS (SC)	Tertiary	HIV with CD4 counts < 200 cells/mm3	62	HIV+ 15/45 (33%) HIV- 1/12 (8%) OR (95% CI) 5.5 (0.65–46.69) *P*-value = 0.118 HIV+ Deaths at less or equal to 30 days = 9/46 (20%) HIV- Deaths at less or equal to 30 days = 1/12 (8%) OR (95% CI) 2.68 (0.30–127.77) *P*-value = 0.670
Nascimento et al. [67]	2000 to 2011	*Cryptococcus neoformans*	Brazil	LSS (SC)	Tertiary	Patients with CM	61	6/61 (10%) deaths (*C. neoformans* var. *grubii*) in AIDS patients
Govender et al. [53]	2002 to 2008	*Cryptococcus neoformans*	South Africa	PBS (MC)	Tertiary	Patients who had been diagnosed with the first episode of laboratory-confirmed cryptococcosis. Only 1033 out of 8439 met the selection criteria.	1033	Case fatality (non-30-day mortality) 2002–2003: 62/238 (26%) 2007–2008: 84/249 (36%)
Yoon et al. [77]	2005 to 2017	*Cryptococcus neoformans*	United States	RCS (SC)	Tertiary	All patients ≥ 18 years old with the diagnosis of cryptococcosis at Montefiore Medical Centre	126	30-day mortality: HIV+ 4/68 (6%) HIV- 9/44 (20%) 1-year mortality HIV+ 7/55 (13%) HIV- 10/42 (24%) Cause of death due to cryptococcosis HIV + 3/7 (43%) HIV- 6/10 (60%)
Naicker et al. [66]	2007 to 2008 and 2017	*Cryptococcus neoformans*	South Africa	Prospective cohort study (MC)	ND	Patients with the first episode of culture-confirmed cryptococcal disease at 37 South African hospitals	249 and 204	In-hospital deaths 2007–2008: 84/249 (34%) 2017: 62/204 (30%)
Mdodo et al. [64]	2008 to 2009	*Cryptococcus neoformans*	Kenya	LSS (MC)	Tertiary	HIV-positive patients from Kenyatta National Hospital and Mbagathi District Hospital in Nairobi Kenya	67	In-hospital mortality 38/62 (61%)

Table 2. Continued

Author	Study period	Pathogen species	Country	Study design	Level of care	Population description	Patients (*N*=)	Mortality type, N/N, %
Martins et al. [63]	2008 to 2010	*Cryptococcus neoformans*	Brazil	LSS (SC)	Tertiary	Patients diagnosed with mycological CM	63	Deaths occurred in 49% of the cases HIV+ 18/37 (49%) HIV- 13/26 (50%) The number is higher for patients infected by *C. neoformans* VNI genotype. *Cryptococcus neoformans* VNI predominated in HIV + patients
Smith et al. [75]	2010 to 2014	*Cryptococcus neoformans*	Uganda	LSS (MC)	Tertiary	HIV infected and was presenting with his or her first episode of CM.	198	Day 60 deaths: FLU susceptible: 29/58, 50% FLU dose-dependent: 11/27, 41% FLU resistant: 1/5, 20% AMB susceptible 41/89, 46% AMB resistant 0/1
Nyazika et al. [69]	2013 to 2014	*Cryptococcus neoformans*	Zimbabwe	LSS (MC)	ND	HIV-infected adult inpatients from Parirenyatwa Group of Hospitals presenting signs and symptoms of meningitis.	100	Overall mortality rate 56% (30/54) AFLPI/VNI genotype 22/39 (56%) AFLPIA/VNB/VNII genotype 5/8 (63%) AFLPIB/VNII 3/7 genotype (43%)
Hurtado et al. [57]	2013 to 2015	*Cryptococcus neoformans*	Brazil	Autopsy study (MC)	Tertiary	284 deceased patients; Cause of death assigned to a cryptococcal infection	284	Seventeen died from fatal cryptococcal infections. 7/17 patients (41%) died within the first 72 hours of admission.
Rakotoarivelo et al. [73]	2014 to 2016	*Cryptococcus neoformans*	Madagascar	CSS (MC)	Tertiary	Consecutive HIV-infected adults presenting with CD4 cell counts ≤ 200/µl	129	90-day mortality: 30/129 (23%) 90-day mortality with CM: 8/14 (57%) 90-day mortality without CM: 22/115 (19%)
Beale et al. [37]	2005 to 2010	*Cryptococcus neoformans*	South Africa	RCS (MC)	ND	HIV infected individuals prior to the initiation of antifungal treatment	230	The overall mortality of 27% at 10 weeks, 41% at one year
Phillips et al. [84]	1999 to 2007	*Cryptococcus gattii*	British Columbia	RCS (MC)	ND	Patients with *C. gattii* infection, reported to BC Centre for Disease Control	152	Death due to *C. gattii* or where *C. gattii* contributed 11/47 (23.4%) (patients with CNS disease), 13/89 (14.6%) (with lung infection only)

Table 2. Continued

Author	Study period	Pathogen species	Country	Study design	Level of care	Population description	Patients (*N*=)	Mortality type, N/N, %
Chen et al. [79,78]	2000 to 2007	*Cryptococcus gattii*	Australia	RCS (MC)	Tertiary	Adults with *C. gattii* infection	86	11/85 (13%) (10 from *C. gattii*): within 4 months of diagnosis, 10/73 (13.6%) in CNS infection, 11% in CNS + lung infection, 17% in CNS infection only, (7/24) immunocompromised vs. (3/62) healthy hosts, 6/31 (19%) death at 12 months, patients with raised ICP
Smith et al. [85]	2004 to 2011	*Cryptococcus gattii*	United States Pacific Northwest (PNW)	RCS (MC)	ND	Patients with invasive *C. gattii* infection reported to CDC	70	3-month mortality in patients 13/70 (19%) in all patients, 3/7 (43%) in bloodstream infections, 7/33 (21%) pulmonary infections, 3/30 (10%) CNS infections
Harris et al. [81]	2004 to 2011	*Cryptococcus gattii*	United States	RCS (MC)	Tertiary	Patients with *C. gattii* reported to the CDC, US	76	Died of or with infection 19/57 (33%)

CSS = Cross sectional study; LSS = Lab surveillance study; MC = Multi-centre; ND = Not determined; PBS = Population-based surveillance; RSC = Retrospective cohort study; SC = Single centre

Table 3. Antifungal susceptibility of *C. neoformans*.

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Andrade-Silva et al. [33]	CLSI M27-A3	MIC GM 9.7 MIC range 2–32 MIC_{50} 8 MIC_{90} 16 %R 0% 97.9% MIC ≤ ECV 16	NA	MIC GM 0.30 MIC range 0.06–2 MIC_{50} 0.5 MIC_{90} 1 %R 22% (21/95) 100% MIC ≤ ECV 1	MIC GM 0.16 MIC range 0.03–0.5 MIC_{50} 0.12 MIC_{90} 0.5 %R 0% 100% MIC ≤ ECV 0.5	NA	MIC GM 0.11 MIC range 0.06–0.25 MIC_{50} 0.12 MIC_{90} 0.25 %R 0% 97.9% ≤ ECV 1	MIC GM 0.69 MIC range 0.12–4 MIC_{50} 1 MIC_{90} 2 %R 11% (10/95) 97.9% ≤ ECV 2	NA
Bariao et al. [36]	CLSI M27-A3	MIC GM 1.369 MIC range 0.25–16 MIC_{50} 1 MIC_{90} 4	NA	MIC GM 0.092 MIC range 0.031–0.25 MIC_{50} 0.125 MIC_{90} 0.125	NA	NA	MIC GM 0.089 MIC range 0.031–1 MIC_{50} 0.062 MIC_{90} 0.25	MIC GM 0.107 MIC range 0.031–1 MIC_{50} 0.125 MIC_{90} 0.25	MIC GM 1.079 MIC range 0.125–4 MIC_{50} 1 MIC_{90} 2
Bertout et al. [38]	Sensititre YeastOne	MIC GM 3.22 MIC range 0.5–32 MIC_{50} 2 MIC_{90} 16	NA	MIC GM 0.05 MIC range < 0.008–0.12 MIC_{50} 0.015 MIC_{90} 0.12	MIC GM 0.08 MIC range < 0.008–0.25 MIC_{50} 0.015 MIC_{90} 0.12	MIC GM 0.10 MIC range 0.008–0.5 MIC_{50} 0.06 MIC_{90} 0.5	MIC GM 0.06 MIC range < 0.008–0.12 MIC_{50} 0.015 MIC_{90} 0.12	MIC GM 0.31 MIC range 0.06–1 MIC_{50} 0.06 MIC_{90} 0.5	MIC GM 2.76 MIC range 0.5–16 MIC_{50} 2 MIC_{90} 8
Chen et al. [41]	CLSI M27-A3	MIC range 2–64 30/89 (34%) fluconazole MIC ≥ 8	NA	NA	NA	NA	NA	NA	NA
Chen et al. [42]	ATB™ FUNGUS-3 kit	No isolates with MIC ≥ 16 MIC range 1–8	NA	No isolates with MIC ≥ 1 MIC range 0.125–0.5	NA	NA	No isolates with MIC ≥ 1 MIC range 0.06–0.25	No isolates with MIC ≥ 2 MIC range < 0.5–1	No isolates with MIC ≥ 32 MIC range < 4–4
Chowdhary et al. [43]	CLSI M27-A3	MIC GM 2.190 MIC range 0.5–8 MIC_{50} 2 MIC_{90} 4	NA	MIC GM 0.099 MIC range 0.031–0.250 MIC_{50} 0.125 MIC_{90} 0.250	NA	NA	MIC GM 0.053 MIC range 0.015–0.125 MIC_{50} 0.062 MIC_{90} 0.125	MIC GM 0.235 MIC range 0.031–1 MIC_{50} 0.250 MIC_{90} 0.5	MIC GM 1.450 MIC range 0.031–64 MIC_{50} 2 MIC_{90} 4
Cogliati et al. [44]	Yeast nitrogen base (YNB) broth microdilution method	MIC GM 1.962 MIC range 0.12–16 MIC mode 4 98.6% MIC ≤ ECV 8	NA	MIC GM 0.096 MIC range 0.03–1 MIC mode 0.03 or 0.06 99.7% MIC ≤ ECV 0.5	NA	NA	MIC GM 0.049 MIC range 0.03–0.5 MIC mode 0.03 96.9% MIC ≤ ECV 0.12	NA	MIC GM 0.297 MIC range 0.12–64 MIC mode 0.25 MIC mean 0.297 97.8% MIC ≤ ECV 1
Córdoba et al. [45]	EUCAST	MIC range 0.13–128 MIC_{50} 8 MIC_{90} 32 MIC mode 8 ECV_{95} 32 (2.2% non-WT) ECV_{99} 64 (0.7% non-WT)	NA	MIC range 0.015–1 MIC_{50} 0.03 MIC_{90} 0.25 MIC mode 0.015 ECV_{95} 0.5 (0.6% non-WT) ECV_{99} 0.5 (0.6% non-WT)	NA	MIC range 0.015–0.13 MIC_{50} 0.015 MIC_{90} 0.06 MIC mode 0.015 ECV_{95} 0.06 (1.3% non-WT) ECV_{99} 0.13 (0% non-WT)	MIC range 0.015–2 MIC_{50} 0.13 MIC_{90} 0.25 MIC mode 0.13 ECV_{95} 0.5 (1.9% non-WT) ECV_{99} 1 (0.3% non-WT)	MIC range 0.015–1 MIC_{50} 0.25 MIC_{90} 0.5 MIC mode 0.25 ECV_{95} 0.5 (3.8% non-WT) ECV_{99} 1 (0% non-WT)	MIC range 0.13–128 MIC_{50}8 MIC_{90} 16 MIC mode 4 ECV_{95} 32 (2.4% non-WT) ECV_{99} 128 (0% non-WT)

Table 3. Continued

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
de Oliveira et al. [46]	EUCAST	NA	NA	NA	NA	NA	NA	MIC GM 0.4 MIC range 0.12–1 MIC mode 0.5 $MIC_{50/90}$ 0.5	NA
Espinel-Ingroff et al. [48]	CLSI M27-A3	MIC range ≤ 0.12–≥ 64 MIC mode 4–8 98.3% MIC ≤ ECV 16	NA	MIC range ≤ 0.008–≥ 4 MIC mode 0.12 98.9% MIC ≤ ECV 0.5	NA	MIC range ≤ 0.008–≥ 2 MIC mode 0.12 94.3% MIC ≤ ECV 0.25	MIC range ≤ 0.008–≥ 4 MIC mode 0.06 96.5% MIC ≤ ECV 0.25	NA	NA
Espinel-Ingroff et al. [49]	CLSI M27-A3, Etest	NA	NA	NA	NA	NA	NA	MIC range ≤ 0.03–4 MIC mode 0.25 ECV_{95} 1 ECV_{99} 2	MIC range 0.06–to ≥ 64 MIC mode 4 ECV_{95} = 16 ECV_{99} = 32
Espinel-Ingroff et al. [50]	CLSI M27-A3	NA	MIC range 0.008–0.5 MIC mode 0.03 ECV_{95} 0.06–0.12 $ECV_{97.5}$ 0.12	NA	NA	NA	NA	NA	NA
Fan et al. [51]	Sensititre YeastOne	MIC GM 4.28 MIC range 0.5–64 MIC_{50} 4 MIC_{90} 8 WT 92.4% Non-WT 7.6%	NA	MIC GM 0.057 MIC range 0.015–0.5 MIC_{50} 0.06 MIC_{90} 0.12 WT 99% Non-WT 1%	NA	MIC GM 0.084 MIC range 0.008–0.5 MIC_{50} 0.06 MIC_{90} 0.25 WT 97.7% Non-WT 2.3%	MIC GM 0.034 MIC range 0.008–0.5 MIC_{50} 0.03 MIC_{90} 0.12 WT 98.3% Non-WT 1.7%	MIC GM 0.60 MIC range 0.25–1.0 MIC_{50} 0.5 MIC_{90} 1.0 WT 100% Non-WT 0%	MIC GM 3.42 MIC range 0.06–16 MIC_{50} 4 MIC_{90} 8 WT 98.7% Non-WT 1.3%
Gonzalez et al. [52]	CLSI M27-A3	MIC GM 1.335 MIC range 0.5–4 MIC_{50} 2 MIC_{90} 2	NA	NA	NA	NA	MIC GM 0.061 MIC range 0.03–0.125 MIC_{50} 0.06 MIC_{90} 0.125	MIC GM 0.343 MIC range 0.125–1 MIC_{50} 0.25 MIC_{90} 1	NA
Govender et al. [53]	CLSI M27-A3	2002–2003 MIC range 0.5–16 MIC_{50} 1 MIC_{90} 2 2007–2008 MIC range 0.25–8 MIC_{50} 1 MIC_{90} 2	NA	2002–2003 MIC range 0.03–1 MIC_{50} 0.12 MIC_{90} 0.25 2007–2008 MIC range 0.015–0.5 MIC_{50} 0.06 MIC_{90} 0.12	NA	2002–2003 MIC range 0.03–0.5 MIC_{50} 0.12 MIC_{90} 0.25 2007–2008 MIC range 0.03–1 MIC_{50} 0.06 MIC_{90} 0.12	2002–2003 MIC range 0.008–0.25 MIC_{50} 0.015 MIC_{90} 0.06 2007–2008 MIC range 0.008–0.25 MIC_{50} 0.015 MIC_{90} 0.03	2002–2003 MIC range 0.012–0.38 MIC_{50} 0.094 MIC_{90} 0.19 2007–2008 MIC range 0.008–0.94 MIC_{50} 0.094 MIC_{90} 0.19	2002–2003 MIC range 0.25–16 MIC_{50} 1 MIC_{90} 4 2007–2008 MIC range 0.05–8 MIC_{50} 1 MIC_{90} 2

Table 3. Continued

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Tewari et al. [76]	Vitek 2, Etest, CLSI M27-A3	CLSI MIC range 0.25–32 MIC_{90} 16 Etest MIC range 1– >256 MIC_{90} 16 Vitek 2 MIC range < 1–>64 MIC_{90} 8 80%–90% of isolates MIC ≤ 8	NA	NA	NA	NA	NA	CLSI MIC range 0.06–5 MIC_{90} 0.5 Etest MIC range 0.047–0.38 MIC_{90} 0.25 Vitek 2 MIC range < 0.25–2 MIC_{90} 1 98%–100% of isolates MIC < 1	NA
Gutch et al. [54]	CLSI M27-A	MIC range 0.063–64 MIC_{50} 8 MIC_{90} 32 Mean 6.93 MIC ≥ 64, 8.6% MIC 16–32, 31.1% MIC ≤ 8, 60.3%	NA	MIC range 0.03–1 MIC_{50} 0.125 MIC_{90} 0.5 Mean 0.124 MIC ≥ 1, 5.2% MIC 0.25–0.5, 24.1% MIC ≤ 0.125, 70.7%	MIC range 0.03–0.25 MIC_{50}0.064 MIC_{90} 0.064 Mean 0.051 MIC ≥ 0.125, 6.9% MIC 0.0625, 55.2% MIC < 0.0625 37.9%	NA	NA	NA	NA
Hagen et al. [55]	EUCAST	MIC GM 8.96 MIC range 0.5–> 32 MIC_{50} 4	MIC GM 0.065 MIC range < 0.03–0.25 MIC_{50} 0.06	NA	NA	NA	MIC GM 0.104 MIC range < 0.03–0.5 MIC_{50} 0.06	MIC GM 0.180 MIC range < 0.03–1 MIC_{50} 0.125	MIC GM 8.80 MIC range 1–> 32 MIC_{50} 8
Herkert et al. [56]	CLSI M27-A3	MIC GM 0.516 MIC range 0.125–8 MIC_{50} 0.5 MIC_{90} 0.5	MIC GM 0.011 MIC range < 0.016–0.063 MIC_{50} < 0.016 MIC_{90} 0.031	MIC GM 0.027 MIC range < 0.016–0.25 MIC_{50} 0.031 MIC_{90} 0.063	NA	MIC GM 0.027 MIC range < 0.016–0.125 MIC_{50} 0.031 MIC_{90} 0.063	MIC GM 0.021 MIC range < 0.016–0.125 MIC_{50} 0.031 MIC_{90} 0.031	MIC GM 0.098 MIC range < 0.016–0.125 MIC_{50} 0.125 MIC_{90} 0.125	MIC GM 2.42 MIC range 0.25–8 MIC_{50} 2 MIC_{90} 4
Hurtado et al. [57]	Sensititre YeastOne	MIC range 4–16	NA	MIC range 0.03–0.12	NA	MIC range 0.06–0.25	MIC range 0.06–0.25	MIC range 0.5–1	MIC range 1–16
Kassi et al. [58]	CLSI M27-A3	MIC range 0.125–8	NA	NA	NA	NA	NA	MIC range 0.125–1	MIC range 0.5–16
Mahabeer et al. [61]	CLSI M27-A3, Etest, Vitek 2	CLSI MIC range 0.25–4 MIC_{50} 1 MIC_{90} 2 Etest MIC range 0.25–4 MIC_{50} 1 MIC_{90} 2 Vitek 2 MIC range ≤ 1–16 MIC_{50} ≤ 1 MIC_{90} 2	NA	NA	NA	NA	CLSI MIC range ≤ 0.002–0.064 MIC_{50} 0.004 MIC_{90} 0.016 Etest MIC range ≤ 0.002–0.064 MIC_{50}0.008 MIC_{90} 0.016	CLSI MIC range ≤ 0.008–1 MIC_{50} 0.125 MIC_{90} 0.25 Etest MIC range ≤ 0.008–0.25 MIC_{50} 0.06 MIC_{90} 0.125 Vitek 2 MIC range ≤ 0.25–0.5 MIC_{50} ≤ 0.25 MIC_{90} 0.5	CLSI MIC range ≤ 0.125–4 MIC50 1 MIC_{90} 2 Etest MIC range ≤ 1–8 MIC_{50} ≤ 1 MIC_{90} 2

Table 3. Continued

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Mahabeer et al. [62]	CLSI M27-A3, Etest, Vitek-2	CLSI MIC range 0.25–4 MIC_{50} 1 MIC_{90} 2 Etest MIC_{50} 1 MIC_{90} 2 MIC range 0.06–4 Vitek-2 MIC range ≤ 1–16 MIC_{50} ≤ 1 MIC_{90} 2	NA	NA	NA	NA	CLSI MIC range ≤ 0.002–0.064 MIC_{50} 0.004 MIC_{90} 0.016 Etest MIC range ≤ 0.002–0.064 MIC_{50} 0.008 MIC_{90} 0.016	CLSI MIC range ≤ 0.008–1 MIC_{50} 0.125 MIC_{90} 0.25 Etest MIC range ≤ 0.008–0.25 MIC_{50} 0.06 MIC_{90} 0.125 Vitek-2 MIC range ≤ 0.25–0.5 MIC_{50} ≤ 0.25 MIC_{90} 0.5	CLSI MIC range ≤ 0.125–4 MIC_{50} 1 MIC_{90} 2 Vitek-2 MIC range ≤ 1–8 MIC_{50} ≤ 1 MIC_{90} 2
Mdodo et al. [64]	CLSI M27-A3	MIC range 0.25–16 MIC_{50} 4 MIC_{90} 8	NA	NA	NA	NA	MIC range 0.015–0.25 MIC_{50} 0.06 MIC_{90} 0.25	MIC range 0.5–1 MIC_{50} 1 MIC_{90} 1	MIC range 1–16 MIC_{50} 2 MIC_{90} 4
Naicker et al. [66]	CLSI M27-A3	2007–2008 MIC GM: 2.08 MIC range 0.25–8 MIC_{50} 1 MIC_{90} 2 2017 MIC GM: 4.11 MIC range 0.5–64 MIC_{50} 4 MIC_{90} 8	NA	NA	NA	NA	NA	NA	NA
Nascimento et al. [67]	CLSI M27-A2, E–test	CLSI MIC GM 0.30 MIC range 1–16 MIC_{50} 0.25 MIC_{90} 0.5 Etest MIC GM 0.20 MIC range 0.047–0.5 MIC_{50} 0.19 MIC_{90} 0.38	NA	CLSI MIC GM 0.13 MIC range 0.03–1.0 MIC_{50} 0.06 MIC_{90} 0.25 Etest MIC GM 0.44 MIC range 0.016–2.0 MIC_{50} 0.38 MIC_{90} 0.75	NA	NA	CLSI MIC GM 0.27 MIC range 0.03–0.5 MIC_{50} 0.25 Broth MIC_{90} 0.50 Etest MIC GM 0.14 MIC range 0.016–0.75 MIC_{50} 0.094 MIC_{90} 0.25	CLSI MIC GM 0.30 MIC range 0.13–0.5 MIC_{50} 0.25 Broth MIC_{90} 0.50 Etest MIC GM 0.20 MIC range 0.047–0.5 MIC_{50} 0.19 MIC_{90} 0.38	NA

Table 3. Continued

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Nishikawa et al. [68]	CLSI M27-A3, Etest, Vitek 2	CLSI MIC range 2–8 MIC_{50} 4 MIC_{90} 4 MIC mode 4 Etest MIC range 2–32 MIC_{50} 4 MIC_{90} 16 MIC mode 8 Vitek 2 MIC range ≤ 1–2 MIC_{50} 1 MIC_{90} 2 MIC mode 2	NA	CLSI MIC range 0.06–0.5 MIC_{50} 0.125 MIC_{90} 0.125 MIC mode 0.125 Etest MIC range 0.032–1 MIC_{50} 0.125 MIC_{90} 0.75 MIC mode 0.125	NA	NA	CLSI MIC range 0.015–0.25 MIC_{50} 0.06 MIC_{90} 0.06 MIC mode 0.06 Etest MIC range 0.016–0.38 MIC_{50} 0.047 MIC_{90} 0.094 MIC mode 0.06 Vitek 2 MIC range ≤ 0.125 MIC_{50} ≤ 0.125 MIC_{90} ≤ 0.125 MIC mode ≤ 0.125	CLSI MIC range 0.5–2 MIC_{50} 1 MIC_{90} 1 MIC mode 1 Etest MIC range 0.012–0.25 MIC_{50} 0.094 MIC_{90} 0.125 MIC mode 0.094 or 0.125 Vitek 2 MIC range 1–2 MIC_{50} 1 MIC_{90} 1 MIC mode 1	CLSI MIC range 1–8 MIC_{50} 2 MIC_{90} 4 MIC mode 2, 4 Etest MIC range 0.125–>32 MIC_{50} 4 MIC_{90} 8 MIC mode 4 Vitek 2 MIC range ≤ 1–2 MIC_{50} ≤ 1 MIC_{90} 2 MIC mode ≤ 1
Pan et al. [70]	CLSI M27-A3	MIC GM 2.294 MIC range 0.125–32 MIC_{50} 2 MIC_{90} 4	MIC GM 0.027 MIC range < 0.016–0.125 MIC_{50} 0.031 MIC_{90} 0.063	MIC GM 0.063 MIC range < 0.016–0.5 MIC_{50} 0.063 MIC_{90} 0.25	NA	MIC GM 0.061 MIC range < 0.016–0.5 MIC_{50} 0.063 MIC_{90} 0.125	MIC GM 0.049 MIC range < 0.016–0.5 MIC_{50} 0.063 MIC_{90} 0.125	MIC GM 0.251 MIC range 0.063–1 MIC_{50} 0.25 MIC_{90} 0.5	MIC GM 3.483 MIC range < 0.063–>64 MIC_{50} 4 MIC_{90} 8
Pfaller et al. [71]	CLSI M27-A3	MIC range 0.25–32 Mode 4 ECV 8 96.9% MIC ≤ ECV 8	NA	NA	NA	MIC range 0.03–0.5 Mode 0.12 ECV 0.25 96.5% MIC ≤ ECV 0.25	MIC range 0.008–0.5 Mode 0.06 ECV 0.12 95.1% MIC ≤ ECV 0.12	NA	NA
Prakash et al. [72]	CLSI M27-A3	MIC GM 3.575 MIC range 0.06–64 MIC_{50} 4 MIC_{90} 8	MIC GM 0.03136 MIC range 0.016–0.25 MIC_{50} 0.03 MIC_{90} 0.063	MIC GM 0.517 MIC range 0.016–0.5 MIC_{50} 0.06 MIC_{90} 0.125	NA	MIC GM 0.06658 MIC range 0.016–0.5 MIC_{50} 0.06 MIC_{90} 0.125	MIC GM 0.051 MIC range 0.016–1 MIC_{50} 0.06 MIC_{90} 0.125	MIC GM 0.228 MIC range 0.03–4 MIC_{50} 0.25 MIC_{90} 0.5	MIC GM 4.660 MIC range 0.25–64 MIC_{50} 4 MIC_{90} 16
Rakotoarivelo et al. [73]	Etest	MIC range 0.5–>256 MIC mode 12 ECV 32	NA	NA	NA	NA	MIC range 0.004–0.5 MIC mode 0.047 ECV 0.5	MIC range 0.032–0.5 MIC mode 0.250 ECV 1	MIC range 4–>32 MIC mode > 32 ECV 16

Table 3. Continued

Author	MIC methods	Fluconazole	Isavuconazole	Itraconazole	Ketoconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Selb et al.[74]	CLSI M27-A3	Serotype A	NA	NA	NA	Serotype A	Serotype A	Serotype A	Serotype A
		MIC range 0.5–16				MIC range 0.03–0.5	MIC range 0.03–0.125	MIC range 0.125–0.5	MIC range
		MIC_{50} 1				MIC_{50} 0.06	MIC_{50} 0.03	MIC_{50} 0.5	0.25–>64
		MIC_{90} 2				MIC_{90} 0.125	MIC_{90} 0.03	MIC_{90} 0.5	MIC_{50} 1
		MIC mode 1				MIC mode 0.06	MIC mode 0.03	MIC mode 0.5	MIC_{90} 1
		Serotype D				Serotype D	Serotype D	Serotype D	MIC mode 1
		MIC range 0.125–0.5				MIC range	MIC range 0.03	MIC range 0.25–0.5	Serotype D
		MIC_{50} 0.5				0.03–0.125 MIC_{50}	MIC_{50} 0.03	MIC_{50} 0.5	MIC range 1–>64
		MIC_{90} 2				0.03	MIC_{90} 0.03	MIC_{90} 0.5	MIC_{50} 4
		MIC mode 0.5				MIC_{90} 0.06	MIC mode 0.03	MIC mode 0.5	MIC_{90} 8
						MIC mode 0.03			MIC mode 2
Smith et al.[75]	CLSI	MIC range 0.125–64	NA	NA	NA	NA	NA	MIC range 0.125–2	NA
		MIC mode 8						MIC mode 0.5	
		MIC_{50} 8						MIC_{50} 0.5	
		MIC_{90} 32						MIC_{90} 1	
		69% isolates MIC < 16							

Data are reported as they appear in source documents. Susceptibility is expressed as mg/l unless indicated otherwise. ECV = epidemiological cutoff value, GM = geometric mean, MIC = minimum inhibitory concentration, NA = not available, MIC_{50} = MIC required to inhibit the growth of 50% of isolates, MIC_{90} = MIC required to inhibit the growth of 90% of isolates.

countries.[48,49,50,83] Reported MICs for fluconazole were generally high (range: 0.5–32 mg/l), although variable, with isolates of molecular type VGII showing the highest modal or geometric mean MIC of > 8 mg/l compared with other molecular types (1.7–4.0 mg/l for VGI and VGIII).[48,83] Modal MICs of itraconazole, posaconazole, and voriconazole for *C. gattii* ranged from 0.06 to 0.5 mg/l for both molecular-typed and non-typed isolates.[48]

For amphotericin B, modal or geometric mean MICs ranged from 0.25 to 0.5 mg/l for both typed and non-typed isolates.[49,80,82] Susceptibility results for flucytosine were variable with modal or geometric mean MICs of 0.5–2 mg/l, and with higher values reported (> 64 mg/l) for molecular types VGI and VGII.[49,80,82] No susceptibility data were available for echinocandins, but *Cryptococcus* species, like all basidiomycetes are intrinsically resistant to this class.

Annual incidence and global distribution

Annual global incidence rates for *C. neoformans* and *C. gattii* could not be assessed due to lack of denominator from all included studies. However, at a population level, there were estimated 220 000 cases of CM globally in 2014 (about 3 in 100 000 population).[22] Chen et al. reported the annual incidence of *C. gattii* infections was 6 in 100 000 between 2000 and 2007 in Australia,[78] but higher (nearly 10-fold) annual incidence rate was reported in Aboriginal Australians.[78]

Although its proportional contribution to total cases of cryptococcal disease varies by geographic region, it was evident that *C. neoformans* was globally distributed.[87] The prevalence of *C. neoformans* among isolates causing CM was reported in three multi-centre studies from African countries[73,58,65] and one single-centre study from India (Table 5).[59] In Madagascar during 2014–2016, the proportion of cryptococcal infection caused by *C. neoformans* var. *grubii* (serotype A) in HIV-infected patients was 13.2%.[73] A multi-centre lab surveillance study conducted in South Africa during 2005–2006 reported a high prevalence (82%) of *C. neoformans* serotype A (VNI) and a lower prevalence (0%–10%) of serotype A (VNB, VNII), serotype AD (VNIII), and serotype D (VNIV) among paediatric patients with cryptococcosis.[65] Similarly, in Ivory Coast during 2012–2014, a study showed 86% of HIV-associated CM was caused by *C. neoformans* VNI genotype.[58] In India, the majority of the CNS cryptococcosis patients were from Bangalore Urban, Karnataka, which is in the southern part of India; 80% of the clinical strains were *C. neoformans* VNI and 8.75% were *C. neoformans* VNII.[59]

There was limited data available to assess the global distribution of *C. gattii,* four studies informed prevalence of *C. gattii* in patients with cryptococcal infections in different study locations, including Australia, India, Brazil, and Africa (Table 5). Overall, *C. gattii* accounted for 11%–33% of cryptococcal infections.[82,59,57] In contrast, the earlier study conducted in Ivory Coast reported only one case of *C. gattii* infection in 61 HIV-positive patients with cryptococcal infections.[58] Like *C. neoformans*, the distribution of *C. gattii* molecular types seems to vary across regions, although it was difficult to assess as few regions were represented. In Australia, genotype VGI caused the majority of the *C. gattii* cases,[82] whereas in India, VGIV was the most commonly observed genotype.[59]

Table 4. Antifungal susceptibility of *C. gattii*.

Author	Year	MIC method	Fluconazole	Isavuconazole	Itraconazole	Posaconazole	Voriconazole	Amphotericin B	Flucytosine
Espinel-Ingroff et al. [48]	2012	CLSI M27A-3	MIC mode Non-typed 4 VGI 4 VGII 8 VGIIa 4 VGIII 4 VGIV 8	NA	MIC mode Non-typed 0.12 VGI 0.25 VGII 0.12 VGIII 0.25 VGIV 0.5	MIC mode Non-typed 0.12 VGI 0.12	MIC mode Non-typed 0.06 VGI 0.12 VGII 0.12 VGIIa 0.12	NA	NA
Espinel-Ingroff et al. [49]	2012	CLSI M27A-3	NA	NA	NA	NA	NA	MIC mode (range) Non-typed 0.5 (0.06–1) VGI 0.25 (0.03–1) VGII 0.5 (0.125–2) VGIIa 0.25 (0.06–1)	MIC mode Non-typed 1 (0.25–8) VGI 2 (0.125–>64) VGII 2 (0.25–≥64)
Espinel-Ingroff et al. [50]	2015	CLSI	NA	MIC mode (range) 0.03 (0.008–0.5)	NA	NA	NA	NA	NA
Firacative et al. [80]	2016	Sensititre YeastOne	Clinical isolates GM MIC 5.384	NA	Clinical isolates GM MIC 0.0453	Clinical isolates GM MIC 0.06 987	Clinical isolates GM MIC 0.04421	Clinical isolates GM MIC 0.2726	Clinical isolates GM MIC 1.927
Lee et al. [82]	2019	Sensititre YeastOne	VGI GM MIC 1.46 MIC range 0.25–2	NA	VGI GM MIC 0.02 MIC range 0.015–0.06	VGI GM MIC 0.04 MIC range 0.008–0.12	VGI GM MIC 0.02 MIC range 0.008–0.06	VGI GM MIC 0.39 MIC range 0.12–1	VGI GM MIC 0.47 MIC range 0.25–2
Lockhart et al. [83]	2012	CLSI	All isolates GM MIC 5.51 MIC range 0.5–32 VGI GM MIC 1.69 MIC range 0.5–8 VGII GM MIC 8.60 MIC range 1–32 VGIII GM MIC 3.48 MIC range 1–16 VGIV GM MIC 4.79 MIC range 0.5–32	NA	All isolates GM MIC 0.30 MIC range 0.03–2 VGI GM MIC 0.19 MIC range 0.03–1 VGII GM MIC 0.36 MIC range 0.06–2 VGIII GM MIC 0.28 MIC range 0.06–0.5 VGIV GM MIC 0.30 MIC range 0.06–2	All isolates GM MIC 0.31 MIC range 0.008–1 VGI GM MIC 0.20 MIC range 0.03–1 VGII GM MIC 0.33 MIC range 0.008–1 VGIII GM MIC 0.34 MIC range 0.12–1 VGIV GM MIC 0.34 MIC range 0.12–1	All isolates GM MIC 0.10 MIC range 0.008–1 VGI GM MIC 0.03 MIC range 0.008–0.25 VGII GM MIC 0.13 MIC range 0.015–1 VGIII GM MIC 0.07 MIC range 0.03–0.25 VGIV GM MIC 0.10 MIC range 0.03–0.5	NA	NA

Data are reported as they appear in source documents. Susceptibility is expressed as mg/l unless indicated otherwise. ECV = epidemiological cutoff value, GM = geometric mean, MIC = minimum inhibitory concentration, NA = not available, MIC_{50} = MIC required to inhibit the growth of 50% of isolates, MIC_{90} = MIC required to inhibit the growth of 90% of isolates.

Table 5. The incidence and global distribution of *C. neoformans* and *C. gattii*.

Study period	Pathogen	Country	Study design	Level of care	Population description	Patients (*N*=)	Incidence	References
2005 to 2006	*Cryptococcus neoformans*	South Africa	LSS (MC)	ND	Paediatric and adult patients with cryptococcosis during a 2-year period in South Africa	199	Paediatric cases Serotype A, VNI: 67/82 (82%) Serotype A, VNB: 8/82 (10%) Serotype A, VNII: 6/82 (7%) Serotype AD, VNIII: 1/82 (1%) Serotype D, VNIV: 0/82	Miglia et al. [65]
2008 to 2017	*Cryptococcus gattii*	Australia	RCS (MC)	ND	Patients with cryptococcal infections	ND	13/55 (24%) *C. gattii* complex (majority VGI (11), VGII (2) uncommon)	Lee et al. [82]
2012 to 2014	*Cryptococcus neoformans and C. gatii*	Ivory Coast	LSS (MC)	Tertiary	Patients with HIV positive, and none of them received a systemic antifungal treatment	61	High prevalence of CM (86%) due to *C. neoformans* VNI among HIV-infected patients. The results show the prevalence (95%) of serotype A in Ivory Coast. 1/61 (*C. gattii* (VGII))	Kassi et al. [58]
2013 to 2015	*Cryptococcus gattii*	Mozambique and Brazil	Autopsy study (MC)	Tertiary	Deceased patients (for diagnostic autopsies)	223 (Mozambique) and 61 (Brazil)	5/15 (33%) *C. gattii* (VGI and VGIV molecular types) out of fatal cryptococcal infections	Hurtado et al. [57]
2014 to 2016	*Cryptococcus neoformans*	Madagascar	CSS (MC)	Tertiary	Consecutive HIV-infected adults presenting with CD4 cell counts ≤ 200/μl	129	The overall prevalence of cryptococcal infection was 13.2% (17/129, 95% CI7.9-20.3), and that of CM was 10.9% (14/129, 95% CI 6.1–17.5).	Rakotoarivelo et al. [73]
ND	*Cryptococcus neoformans and C. gatii*	India	Epidemiology study (SC)	Tertiary	CNS cryptococcosis patients attending the neurological and neurosurgical services of National Institute of Mental Health and Neurosciences.	160	146/160 (91%) from Karnataka 14/160 (9%) from Tamil Nadu, Andhra Pradesh, West Bengal, Orissa, Bihar, and Pondicherry. 80% *C. neoformans* VNI, 8.75% VNII and 22.5% *C . gattii* (VGI), 8.75% *C. gattii* (VGIV).	Lahiri et al. [59]
ND	*Cryptococcus gattii*	India	PCS (SC)	Tertiary	Patients with CNS cryptococcosis	160	18/160 (11.25%) *C. gattii* (Of these, 14 (8.75%) were *C. gattii* genotype AFLP7/VGIV (serotype C), and 4 (2.5%) *C. gattii* AFLP4/VGI (serotype B).	Lahiri et al. [59]

CSS = Cross sectional study; LSS = Lab surveillance study; MC = Multi-centre; ND = Not determined; PCS = Prospective cohort study; RSC = Retrospective cohort study; SC = Single centre

Inpatient care and the length of stay in hospital

The median hospital length of stay in patients with *C. neoformans* infection ranged from 18 to 39 days,[40,69,73,39] with only Cao et al. 2019 reporting on HIV-negative patients (Table 6). Although Chan et al. reported a greater length of stay for HIV-negative patients with cryptococcosis (predominantly involving *C. neoformans* var. *grubii* VNI) compared with HIV positive cryptococcosis patients (31 days vs. 18.5 days), this difference was based on only 12 HIV-negative patients and was not statistically significant.[40]

Only one study reported on the hospital length of stay in patients with *C. gattii* infection (Table 6). This nationwide retrospective study conducted in Australian hospitals described average intensive care unit (ICU) stay related to *C. gattii* infection in 18 adult patients as 9.1 days with a wide range of 1–29 days.[78] It did not report overall hospital length of stay. Notably, 90% of patients in this study received amphotericin B for the first 14 days, which typically requires inpatient therapy.

Complications, sequelae, and disabilities

Both *C. neoformans* and *C. gattii* infections can lead to severe complications, sequelae, and disabilities (Table 7).

A 2017 review highlighted that neurosensorial impairment and disability are common sequelae 6 months to 1 year after diagnosis in *C. neoformans* infections. Symptoms mainly include residual headache, motor deficit, and vertigo.[88] Other common complications may include anaemia, hypokalaemia, elevated aminotransferase levels, neutropenia, hypercreatinemia, and opportunistic infections.[89]

A study ($n = 50$) described complications from *C. neoformans* infection and treatment in HIV-positive individuals (mostly infected with *C. neoformans* var. *grubii* VNI genotype), including acute renal impairment, likely associated with antifungal therapies (28% of patients), raised intracranial pressure (ICP) needing shunts (18%), and blindness (12%).[40] Cao et al. reported a higher rate of unfavourable clinical outcome (defined as death, vegetative status, or severe to moderate disability) in CM patients with pulmonary nodules compared with those without the pulmonary nodule involvement (72.5% vs. 48%, $P = 0.019$).[39]

Day et al. (2013) found that baseline fungal count and Glasgow Coma Scale (GCS) were independent predictors of 6-month survival for CM. Furthermore, the choice of therapy regimen affects the survival rate and complications. For instance, it was found that neutropenia was more frequent among patients receiving amphotericin B with fluconazole or flucytosine than patients receiving amphotericin B monotherapy. Also, fewer patients had severe anaemia and visual deficit when combined therapy of amphotericin B with fluconazole/flucytosine than amphotericin B therapy alone.[89]

Neurological sequelae at 12 months of treatment were reported in 17%–27% of patients with *C. gattii* infections, and included signs and symptoms of visual impairment, hearing loss, limb weakness or balance disturbance, and cognitive impairment.[78,84]

Immune reconstitution inflammatory syndrome (IRIS) was observed in 9.4% of patients with *C. gattii* infections from 6 weeks to as long as 12 months after the initiation of azole eradication therapy, and these patients presented with new or enlarging brain lesions.[78]

Preventability

Risk factors for *C. neoformans* infection were documented in two studies. HIV/AIDS, cell-mediated immunity-suppressive regimens without calcineurin inhibitors, and decompensated liver cirrhosis were risk factors for CM (adjusted OR of 181.4, 15.9, and 8.5, respectively) and cryptococcemia (adjusted OR of 216.3, 7.3, and 23.8, respectively).[60] Autoimmune diseases (adjusted OR = 9.3) were an additional risk factor for cryptococcemia.[60]

HIV-infected patients and immunocompromised individuals are particularly vulnerable to cryptococcal infections and CM. Although not specific to *C. neoformans*, a retrospective review of routine cerebrospinal fluid laboratory records ($N = 4702$) between 2000 and 2014 in Botswana, South Africa, determined that antiretroviral therapy access alone did not lead to a significant decrease in the incident rate of HIV-associated CM.[90] Furthermore, several systematic reviews have quantified the preventative effect of pre-emptive therapy on CM: Relative risk of 0.19 ($P < 0.0001$)[91]; incidence reduced from 21% to 5% in patients with CD4 < 100, relative risk 0.23[92]; and incidence reduced from 5% to 3% in patients with CD4 < 200, relative risk 0.6.[93]

A study by Harris et al. observed that patients with *C. gattii* outbreak strain infections had more pre-existing conditions compared with patients with non-outbreak strain infections (86% vs. 31%; $P < 0.0001$).[81] The pre-existing conditions mainly involved immunosuppression or previous use of oral corticosteroids (during the year before infection) in 50% of patients and existing lung, renal, heart disease, or diabetes in 20%–30% of patients. It was also observed that patients with outbreak strain infections were older [median (range) of 56 (2–95) vs. 45 (18–56) years, $P = 0.007$].

Discussion

Cryptococcosis is particularly common in HIV/AIDS patients. However, antiretroviral therapy (ART) access alone has not always decreased the incidence of HIV-associated CM significantly.[90] This observation may be associated with late presentation and cumulative default from care by HIV/AIDS patients, suggesting that integrated interventions beyond simply providing ART are required to prevent cryptococcosis and CM.

Cryptococcosis can lead to prolonged hospitalization. The long length of stay in hospital may be partially attributed to treatment recommendations involving 14 days induction therapy with amphotericin B for most of the study period (although current WHO treatment recommendations for HIV-associated CM now favour shorter courses of amphotericin). Amphotericin B must be administered intravenously and, in most settings, is delivered as in-patient therapy. Although CM clearly causes significant morbidity and has a long-term impact on patients, the effect is poorly quantified, and future CM studies should continue to expand the evidence on short- and longer-term disability and quality of life.

There is clear evidence that cryptococcosis is associated with high mortality. Baddley et al. stated that the all-cause mortality rates were 18.8% at 3 months and 25.5% at 12 months.[94] The rates described in this review are higher than those observed in clinical trials. For example, some studies have reported mortality rates for CM of around 20%.[95–99] In trials, patients with significant co-morbidities or very

Table 6. The hospital length of stay due to *C. neoformans* and *C. gattii* infections.

Study period	Pathogens	Study design	Country	Level of care	Population description	Patients (*N*=)	Length of stay	References
1999 to 2007	*Cryptococcus neoformans*	RCS (SC)	Singapore	Tertiary	HIV with CD4 counts < 200 cells/mm^3	62	HIV+ 18.5 days (13–33) (median IQR) HIV- 31 days (17.5–44.5) OR (95% CI) 0.99 days (0.97–1.01), *P*-value 0.192	Chan et al. [40]
2000 to 2007	*Cryptococcus gattii*	RCS (MC)	Australia	Tertiary	Adults with *C. gattii* infection	86	mean ICU stay: 9.1 days (range 1–29) (n = 18)	Chen et al. [79,78]
2010 to 2016	*Cryptococcus neoformans*	RCS (SC)	China	Tertiary	CM patients	90	Pulmonary nodule (PN) positive: 39 days (2–180) PN negative: 37 days (5–210) *P*-value 0.768	Cao et al. [39]
2013 to 2014	*Cryptococcus neoformans*	LSS (MC)	Zimbabwe	ND	HIV-infected adult inpatients from Parirenyatwa Group of Hospitals with signs and symptoms of meningitis.	100	17.5 days of hospital stay IQR (10–22 days)	Nyazika et al. [69]
2014 to 2016	*Cryptococcus neoformans*	CSS (MC)	Madagascar	Tertiary	Consecutive HIV-infected adults presenting with CD4cell counts ≤ 200/μl	129	Hospital stay, days, median, (IQR): 22 (11.0–35.0)	Rakotoarivelo et al. [73]

CSS = Cross sectional study; LSS = Lab surveillance study; MC = Multi-centre; ND = Not determined; RSC = Retrospective cohort study; SC = Single centre

Table 7. The complications, sequelae and disabilities caused by *C. neoformans* and *C. gattii*.

Study period	Pathogens	Study design	Country	Level of care	Population description	Patients (*N*=)	Complications, sequelae, and disabilities	References
1999 to 2007	*Cryptococcus neoformans*	RCS (SC)	Singapore	Tertiary	HIV with CD4 counts < 200 cells/mm^3	62	Complications in 50 HIV+ patients: Raised ICP needing shunts (18%) of patients, blindness (12%), acute renal impairment (28%)	Chan et al. [40]
1999 to 2007	*Cryptococcus gattii*	RCS (MC)	British Columbia	ND	Patients with *C. gattii* infection, reported to BC Centre for Disease Control	152	Persistent neurological symptoms at the end of 12-month follow-up in 8/47 (17%) of CNS patients: including gait or balance disturbance ($n = 3$), partial hearing loss ($n = 2$), cognitive impairment ($n = 2$), blindness ($n = 1$), and seizure disorder ($n = 1$)	Phillips et al. [84]
2000 to 2007	*Cryptococcus gattii*	RCS (MC)	Australia	Tertiary	Adults with *C. gattii* infection	86	20/73 (27%) with neurological sequelae at 12 months, including: visual impairment ($n = 8$), deafness ($n = 3$), limb weakness ($n = 2$), dysphasia ($n = 2$), IRIS ($n = 8$) after 6 weeks to 12 months	Chen et al. [79,78]
2010 to 2016	*Cryptococcus neoformans*	RCS (SC)	China	Tertiary	CM patients	90	Unfavourable clinical outcome in pulmonary nodule (PN)-positive patients vs. PN-negative patients (72.5% vs. 48%, $P = 0.019$): Glasgow Outcome Scale score (on discharge) of 1 to 4, which indicates death, vegetative status, severe and moderate disability, was considered unfavourable clinical outcomes. [40/90 (44%) patients was PN-positive and 50/90 (56%) was PN-negative]	Cao et al. [39]

MC = Multi-centre; ND = Not determined; RSC = Retrospective cohort study; SC = Single centre

advanced disease may be excluded, and interventions and investigations follow a strict protocol. These factors may contribute to the lower mortality.[26,100] Furthermore, diagnoses such as toxoplasmosis, *Pneumocystis jirovecii* pneumonia, or other opportunistic infections may be more thoroughly screened for and managed in trial settings. This hypothesis is supported by Tenforde et al. (2020), who found that in sub-Saharan Africa, short-term mortality rate was 44% in observational studies and only 21% in randomized control trials.[101] Regardless, the mortality rate is unacceptably high, and global research to improve outcomes is needed.

A detailed summary of antifungal susceptibility data is presented in this review. We observed rising MICs to azoles (e.g., itraconazole, ketoconazole, and voriconazole), including *in vitro* 'resistance' to fluconazole in up to 30%,[41] with an increasing number of isolates with MIC ≥8 μg/ml between 2001 and 2012. However, the data are limited, and there is yet no clear association between MIC and clinical outcomes. Nonetheless, this observation calls for ongoing surveillance globally and investigation into the cause. Since *Cryptococcus* spp. are not transmitted from human to human, an environmental selection pressure for azole resistance could hypothetically be at play, as described for other fungal pathogens such as *Aspergillus*.[102] Two studies reported that in patients with HIV/AIDS, 11% of the *Cryptococcus* strains showed non-WT MICs to amphotericin B. A much lower percentage (< 2%) of the *Cryptococcus* strains showed non-WT MICs to amphotericin B in HIV-negative patients.

Cryptococcus gattii susceptibility data varied with molecular type and, in general, showed higher MICs to fluconazole compared with other azoles, including isavuconazole, itraconazole, posaconazole, and voriconazole. MICs for amphotericin B (0.25–0.5 mg/l) and 5-flucytosine (0.5–2 mg/l) were low. Therefore, future studies should continue tracking antifungal susceptibility and resistance for *C. gattii*, and their correlation with clinical outcomes.

There have been significant developments in prevention of CM over the past decade. Strong evidence has emerged for the cost-effectiveness of screening for *C. neoformans* cryptococcal antigenaemia with point-of-care antigen tests and treating positive cases, especially in low-resource settings or high-prevalence areas with high number of HIV cases.[103,104] However, there are no data on high-income countries, for *C. gattii*, or for patient groups outside of HIV/AIDS.

The systematic reviews of *C. neoformans* and *C. gattii* infections were characterized by sparse, frequently inconsistent data. For instance, there were few studies determining the incidence of infections in specific countries. However, it is known that *C. neoformans* is globally distributed, with some geographic variation between members of the species complex as the causative agent. For example, in Madagascar, 13.2% of HIV-infected patients had cryptococcal infection due to *C. neoformans* var. *grubii* (serotype A). Studies in South Africa, Ivory Coast, and India reported high prevalence of *C. neoformans* serotype A (VNI) (80%–86%) in adult and paediatric patients with cryptococcosis. *C. gattii* accounted for 11%–33% of cryptococcal infections overall in countries such as Australia, India, Brazil, and Africa.

Trends over the last 10 years for *C. neoformans* were difficult to assess due to incomplete data. However, the prevalence of *C. neoformans* serotype A VNI reported in two African countries and India was comparable and was consistently high (80%–86%) over the period of 2011–2020.[58,59,65] Apart from that, there was also a lack of country-level or global surveillance studies reporting the emergence of *C. gattii* infections in the last 10 years. The studies reporting the prevalence of *C. gattii* did not provide adequate data to assess global trends. Although studies conducted in African countries (Ivory Coast and Mozambique, respectively) showed a greater prevalence of 33% in 2019 compared with 1.6% in 2016,[58,57] these data are confounded by environmental and study population-related variables. Thus, it is not possible to make a conclusive statement about the trend in this region.

Our review has several limitations. In particular, we were unable to include non-English-language studies. We only included data from peer-reviewed and indexed publications and may therefore have missed valuable data.

Future perspectives

Future research on *C. neoformans* and *C. gattii* should focus on several key areas: (1) obtaining more robust clinical and microbiological data to support diagnosis and treatment; (2) developing new diagnostic tools and treatments; (3) understanding the genetic and molecular mechanisms of these pathogens; (4) understanding host-pathogen interactions and host's immunological response to the infection; (5) understanding the epidemiology of these pathogens in different regions and populations to identify high-risk groups and develop targeted prevention and control strategies.

Stronger surveillance systems and epidemiology studies would better inform the disease burden and the global distribution of *C. neoformans* and *C. gattii*. These may allow more rigorous identification of at-risk populations, dispersion patterns, and preventative measures. Better understanding of clinical manifestations and susceptibility profiles for different molecular types is needed and could potentially inform individualized treatment options. Conducting trials in cryptococcosis is complex because disease is rare, and it is difficult to recruit sufficient patients into clinical trials to detect impacts on clinical outcome, especially in non-HIV populations. Several groups have investigated surrogate markers of treatment effect (such as early fungicidal activity)[105,106] to allow smaller trials. Additional work in this area is needed.

Conclusion

Cryptococcus neoformans and *C. gattii* are important fungal pathogens. Both are globally distributed with significant incidence and mortality rates. Although rising MICs to antifungals have been reported, these are yet to show a clear impact on clinical outcomes. Careful ongoing systematic observations are warranted alongside detailed work to better define burden of infection in terms of both death and disability.

The knowledge gaps identified through this systematic review open avenues for future research studies to elucidate the genetic and molecular mechanisms underlying *C. neoformans* and *C. gattii* infections. Understanding host-pathogen interactions, the role of host immune responses, and the impact of specific molecular characteristics on disease outcomes can guide the development of targeted therapies and interventions. Furthermore, the observed disparities in global distribution and prevalence among different regions and populations emphasize the importance of region-specific surveillance and

tailored public health strategies. By addressing these research gaps, the disease burden of cryptococcosis can be reduced, and the health outcomes of affected individuals across the globe can be improved.

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Author contributions

Aiken Dao (Data curation, Investigation, Project administration, Writing – original draft), Hannah Yejin Kim (Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing), Katherine Garnham (Data curation, Formal analysis, Writing – original draft, Writing – review & editing), Sarah Kidd (Data curation, Validation, Writing – review & editing), Hatim Sati (Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing), John Perfect (Conceptualization, Formal analysis, Methodology, Writing – review & editing), Tania C. Sorrell (Conceptualization, Methodology, Writing – review & editing), Thomas Harrison (Conceptualization, Methodology, Writing – review & editing), Volker Rickerts (Conceptualization, Methodology, Writing – review & editing), Valeria Gigante (Data curation, Project administration, Writing – review & editing), Ana Alastruey-Izquierdo (Conceptualization, Formal analysis, Methodology, Project administration, Writing – review & editing), Jan-Willem Alffenaar (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing), C. Orla Morrissey (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing), Sharon C-A. Chen (Data curation, Formal analysis, Writing – review & editing), and Justin Beardsley (Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing)

Supplementary material

Supplementary material is available at *Medical Mycology* online.

Conflict of interest

AA-I has received personal fees for educational talks on behalf of Gilead and Pfizer.

None.

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SCOTTISH HOSPITALS INQUIRY

Bundle of documents for Oral hearings commencing from 19 August 2024 in relation to the Queen Elizabeth University Hospital and the Royal Hospital for Children, Glasgow

Bundle 24 – Documents referred to in the Expert Report By Allan Bennett regarding Cryptococcus, and Supporting Documentation – Volume 4

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