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MYCOLOGY

In Vitro Antifungal Susceptibility of Yeast and Mold Phases of Isolates of Dimorphic Fungal Pathogen *Emergomyces africanus* (Formerly *Emmonsia* sp.) from HIV-Infected South African Patients

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ABSTRACT Disseminated emmonsiosis is an important AIDS-related mycosis in South Africa that is caused by *Emergomyces africanus*, a newly described and renamed dimorphic fungal pathogen. *In vitro* antifungal susceptibility data can guide management. Identification of invasive clinical isolates was confirmed phenotypically and by sequencing of the internal transcribed spacer region. Yeast and mold phase MICs of fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, anidulafungin, micafungin, and flucytosine were determined with custom-made frozen broth microdilution (BMD) panels in accordance with Clinical and Laboratory Standards Institute recommendations. MICs of amphotericin B, itraconazole, posaconazole, and voriconazole were determined by Etest. Fifty unique *E. africanus* isolates were tested. The yeast and mold phase geometric mean (GM) BMD and Etest MICs of itraconazole were 0.01 mg/liter. The voriconazole and posaconazole GM BMD MICs were 0.01 mg/liter for both phases, while the GM Etest MICs were 0.001 and 0.002 mg/liter, respectively. The fluconazole GM BMD MICs were 0.18 mg/liter for both phases. The GM Etest MICs of amphotericin B, for the yeast and mold phases were 0.03 and 0.01 mg/liter. The echinocandins and flucytosine had very limited *in vitro* activity. Treatment and outcome data were available for 37 patients; in a multivariable model including MIC data, only isolation from blood (odds ratio [OR], 8.6; 95% confidence interval [CI], 1.3 to 54.4; $P = 0.02$) or bone marrow (OR, 12.1; 95% CI, 1.2 to 120.2; $P = 0.03$) (versus skin biopsy) was associated with death. *In vitro* susceptibility data support the management of disseminated emmonsiosis with amphotericin B, followed by itraconazole, voriconazole, or posaconazole. Fluconazole was a relatively less potent agent.

KEYWORDS disseminated emmonsiosis, emergomycosis, novel, opportunistic infection, dimorphic fungus, antifungal susceptibility testing

The family *Ajellomycetaceae* (within the order *Onygenales*) includes phylogenetically related dimorphic fungal genera such as *Emmonsia*, *Histoplasma*, *Blastomyces*, and *Paracoccidioides* (1). The family was recently reorganized to include a new genus, *Emergomyces*, to accommodate several emerging *Emmonsia*-like fungi causing disseminated disease, mostly among immunocompromised patients worldwide and to ad-

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dress the polyphyletic nature of fungi previously included in the *Emmonsia* genus (2, 3). Unlike *Emmonsia parva* and *Emmonsia crescens*, which cause adiaspiromycosis, fungi within the genus *Emergomyces* cause disseminated emmonsiosis (or emergomycosis), a multisystem disease with a high case fatality rate (4, 5). In addition, *Emergomyces* differs from classic *Emmonsia* species by producing budding yeasts *in vivo* rather than adiaspores (5, 6). Currently, the genus *Emergomyces* includes at least three species: the type species, *Emergomyces pasteurianus*, which appears to have a cosmopolitan distribution; a rarer species, *Emergomyces orientalis*, reported from China; and *Emergomyces africanus*, a species endemic to southern Africa (2, 7). The first case of *E. pasteurianus* was described in an Italian patient with AIDS (8). Thereafter, several reports followed from Spain, China, India, and more recently, a single case from South Africa (2, 9–12). *E. orientalis* has been reported only from a single immunocompetent Chinese patient (7). At least two other unnamed species exist within *Emergomyces*, including a strain isolated from lung tissue of a man with rheumatoid arthritis in Germany and two isolates from immunocompromised patients in Canada (2).

E. africanus was initially described in 2013 as the causative agent of a disseminated mycosis among 13 HIV-infected South African adult patients, most from the Western Cape province (5). Additional cases have since been described in most South African provinces, including KwaZulu-Natal (13–15; our unpublished data). To date, with 86 laboratory-confirmed cases among HIV-infected persons in South Africa, *E. africanus* is far more commonly isolated than other well-described endemic pathogens such as *Histoplasma*, *Blastomyces*, or *Sporothrix* (our unpublished data).

Patients with disseminated emmonsiosis often present with a syndrome of fever, widespread skin lesions of various morphologies, pneumonia, anemia, elevated liver enzymes, and weight loss (2, 13, 15). Misdiagnosis is common (15). To date, no clinical trial has been conducted to evaluate treatment options for patients with this disease. A retrospective review suggested better outcomes for patients treated with amphotericin B, followed by triazoles, than for those treated with triazoles alone; however, many of the former patients were incidentally prescribed small doses and short courses of fluconazole to treat presumed esophageal candidiasis (15). Nonetheless, authors have recommended treatment with amphotericin B deoxycholate, followed by itraconazole, for a minimum of 12 months (5, 13–15), pending immune reconstitution on the basis of Infectious Diseases Society of America guidelines for HIV-associated disseminated histoplasmosis (16, 17).

We compared the *in vitro* antifungal susceptibilities of the yeast and mold phases of *E. africanus* to several antifungal agents by reference and commercially available methods and determined if there was an association between MICs and clinical outcomes in order to guide the clinical management of patients with disseminated emmonsiosis.

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RESULTS

Cases and isolates. Fifty-one cases of disseminated emmonsiosis diagnosed from 2008 through to 2016 were included. Fifty-eight per cent (28/48) of the 50 patients infected with *E. africanus* were males with a median age of 35 (interquartile range [IQR], 30 to 38) years. Sixty-nine per cent (31/45) of the cases were diagnosed in the Western Cape province, 22% (10/45) in Gauteng, 7% (3/45) in the Free State, and 2% (1/45) in the Eastern Cape. Of the 49 *E. africanus* cases with clinical data available, HIV status could be surmised for 45; all of these patients were HIV infected. Of 44 HIV-infected patients with a recorded $CD4^+$ T-lymphocyte (CD4 cell) count, the median CD4 cell count was 12 (IQR, 7 to 27) cells/μl. Of 31 cases with an available antiretroviral treatment (ART) history, 20 (65%) were ART experienced and 9 (29%) were ART naive. Isolates were cultured from skin biopsy specimens ($n = 23$), blood samples ($n = 18$), bone marrow samples ($n = 6$), biopsy specimens from an unknown site ($n = 1$), and an

TABLE 1 Demographic and clinical features of 50 patients with emergomycosis (disseminated emmonsiosis)

Demographic or clinical feature (total no. of patients)[a]	Value
Median age, yr (IQR) (45)	35 (30–38)
No. (%) of males (48)	28 (58)
No. (%) in province (45)	
Western Cape	31 (69)
Gauteng	10 (22)
Free State	3 (7)
Eastern Cape	1 (2)
No. (%) HIV infected (45)	45 (100)
Median no. of CD4 cells/μl (IQR) (44)	12 (7–27)
ART history (31)	
No. (%) naive	9 (29)
No. (%) experienced	20 (65)

[a]The number varies owing to missing data.

unknown specimen ($n = 1$). The demographic and clinical features of patients with *E. africanus* cases with available data are summarized in Table 1.

Antifungal susceptibility testing of *Emergomyces* isolates. All isolates grew sufficiently for MIC determination after 7 days of incubation. Tables 2 and 3 summarize the MIC/minimum effective concentration (MEC) distribution, range, GM MIC/MEC, MIC_{50}, and MIC_{90} of nine antifungal agents for the yeast and mold phases of 50 *E. africanus* isolates. The BMD and Etest MICs of itraconazole were higher than those of voriconazole and posaconazole for both the yeast and mold phases (Tables 2 and 3). The yeast and mold phase GM MICs of fluconazole (BMD MICs, 0.19 versus 0.18 mg/liter; $P = 0.06$), amphotericin B (Etest MICs, 0.03 versus 0.01 mg/liter; $P = 0.06$), or any other antifungal agent/method tested (data not shown] were not significantly different. In contrast, the BMD method yielded significantly higher GM MICs than the Etest method for voriconazole (BMD MIC of 0.01 mg/liter versus Etest MIC of 0.001 mg/liter; $P < 0.001$) and posaconazole (BMD MIC of 0.01 mg/liter versus Etest MIC of 0.002 mg/liter; $P < 0.001$) for both the yeast and mold phases. There was no difference between the BMD and Etest GM MICs of itraconazole (data not shown).

MIC distribution and patient outcome. Of 51 cases of emmonsiosis, the clinical outcome of 37 (73%) could be ascertained and the management of 34 (67%) was known. The overall case fatality ratio was 38% (14/37). Both clinical outcome and management could be ascertained for 33 patients (65%). Of the 23 patients who survived, management was known for 22; 18 (82%) were treated with amphotericin B, followed by itraconazole (16), fluconazole (1), or a combination thereof (1); 3 were treated with triazole monotherapy (2 received itraconazole, and 1 received a small dose of fluconazole for 14 days); and 1 received fluconazole for >12 months (possibly with amphotericin B). In contrast, management details were available for 11 of 14 patients who died (79%); 3 received amphotericin B, 2 received triazole monotherapy (1 each received itraconazole and low-dose fluconazole), and 6 received no antifungal treatment. In the multivariable model, only isolation from blood (odds ratio [OR], 8.6; 95% confidence interval [CI], 1.3 to 54.4; $P = 0.02$) or bone marrow (OR, 12.1; 95% CI, 1.2 to 120.2; $P = 0.03$) (versus a skin biopsy specimen) was associated with death (Table 4).

DISCUSSION

Thermally dimorphic fungi of the genus *Emergomyces* have emerged as a cause of disseminated, sometimes fatal disease among HIV-infected South Africans with very low CD4 cell counts. We have reported the antifungal susceptibility profile of a large series of *E. africanus* isolates. Voriconazole, posaconazole, itraconazole, and amphotericin B had the most potent *in vitro* activity against both mold and yeast phases of *Emergomyces*. While fluconazole is far more easily accessible to clinicians in the South

TABLE 2 Yeast phase MIC distribution of 50 *E. africanus* clinical isolates

Antifungal agent	Test method	No. of isolates with MIC (mg/liter) of:														GM MIC[a]	MIC_{50}[a]	MIC_{90}[a]	MIC range[a]
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64				
Itraconazole	BMD	36	5	4		5										0.01	0.008	0.008	0.008–0.12
Voriconazole	BMD	45	5													0.01	0.008	0.015	0.008–0.015
Posaconazole	BMD	37	6	3	1	2	1									0.01	0.008	0.03	0.008–0.25
Fluconazole	BMD					28	11	10	1							0.18	0.12	0.5	0.12–1
Caspofungin	BMD				1	1	2	2	25	10	7	2				1.18	1	4	0.06–8
Micafungin	BMD					1	4	1	11	15	9	9				1.85	2	8	0.12–8
Anidulafungin	BMD					1	1		20	24	3	1				1.34	2	2	0.12–8
Flucytosine	BMD										1	1	1	1	46	171.74	256	256	4–256
Amphotericin B	Etest	13	1	7	11	7	9	1	1							0.03	0.06	0.25	0.002–1
Itraconazole	Etest	35	5	6	1		3									0.01	0.008	0.03	0.002–0.25
Voriconazole	Etest	49	1													0.001	0.002	0.002	0.002–0.012
Posaconazole	Etest	49	1													0.002	0.002	0.006	0.002–0.012

[a]All MICs are in milligrams per milliliter.

TABLE 3 Mold phase MIC/MEC distribution of 50 *E. africanus* clinical isolates

Antifungal agent	Test method	No. of isolates with MIC/MEC (mg/liter) of:													GM MIC[a]	MIC_{50}/MEC_{50}[a]	MIC_{90}/MEC_{90}[a]	MIC range[a]
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64				
Itraconazole	BMD	35	4	5	2	1	1	2								0.01	0.008	0.06	0.008–0.5
Voriconazole	BMD	37	5	5	3											0.01	0.008	0.03	0.008–0.06
Posaconazole	BMD	32	8	4	4				2							0.01	0.008	0.06	0.008–1
Fluconazole	BMD					34	8	5	1	1		1				0.18	0.12	0.5	0.12–8
Caspofungin	BMD						3		20	23	3	1				1.67	2	2	0.25–8
Micafungin	BMD			1			1		20	11	13	4				1.72	2	4	0.03–8
Anidulafungin	BMD								13	32	5					1.72	2	2	1–4
Flucytosine	BMD														50	208.42	256	256	64–256
Amphotericin B	Etest	24		7	3	6	7	2	1							0.01	0.03	0.25	0.002–1
Itraconazole	Etest	36	1	10	1		1	1								0.01	0.006	0.03	0.002–0.5
Voriconazole	Etest	50														0.001	0.002	0.002	0.002–0.008
Posaconazole	Etest	48	1	1												0.002	0.002	0.004	0.002–0.03

[a]All MICs are in milligrams per milliliter.

TABLE 4 Outcome-associated characteristics of 37 patients

Variable	Outcome		Univariate analysis		Multivariable analysis	
	Survival	Death	OR (95% CI)	*P* value	aOR[a] (95% CI)	*P* value
Median age (yr)	33 (28–36)	35 (31–38)	1.0 (0.9–1.2)	0.59		
Sex						
No. (%) of males	15/23 (65.2)	5/14 (35.7)	Reference		Reference	
No. (%) of females	8/23 (34.8)	9/14 (64.3)	3.4 (0.8–13.6)	0.08	2.14 (0.44–10.36)	0.34
Province						
Gauteng	1/22 (4.6)	4/12 (33.3)	Reference			
Western Cape	19/22 (86.4)	8/12 (66.7)	0.11 (0.01–1.1)	0.06		
Eastern Cape*						
Free State	2/22 (9.1)	0/12 (0)				
Median no. of CD4 cells/μl (IQR)	13 (9–32)	14 (6–27)	1.0 (0.98–1.04)	0.50		
ART history						
No. naive/total (%)	6/21 (28.6)	3/10 (30)	Reference			
No. experienced/total (%)	15/21 (71.4)	7/10 (70)	0.93 (0.17–4.87)	0.94		
Any antifungal treatment						
No. without/total (%)	0/22 (0)	6/11 (54.6)	Reference			
No. with/total (%)	22/22 (100)	5/11 (45.5)	1			
Specimen type						
No. of skin biopsy samples/total (%)	15/23 (65.2)	2/14 (14.3)	Reference		Reference	
No. of blood cultures/total (%)	6/23 (26.1)	8/14 (57.1)	10 (1.62–61.47)	0.01	8.57 (1.35–54.33)	0.02
No. of bone marrow aspirates/total (%)	2/23 (8.7)	4/14 (28.6)	15 (1.58–142.18)	0.02	12.1 (1.21–120.13)	0.03
Antifungal MIC_{50}						
Amphotericin B[b]	0.06	0.06	0.16 (0.001–20.82)	0.47		
Posaconazole[c]	0.008	0.008	1.91 (0.10–34.82)	0.66		
Itraconazole[c]	0.008	0.008	0.26 (0.0004–140.69)	0.67		
Voriconazole[c]	0.008	0.008	72.3 (9.89–5.30)	0.87		
Fluconazole[c]	0.12	0.20	0.75 (0.29–1.92)	0.54		

[a]aOR, adjusted OR.
[b]Determined by Etest, yeast phase.
[c]Determined by BMD, mold phase.

African public health sector, this agent was less potent than other azoles (18). This confirms the findings of an earlier antifungal susceptibility study of six *E. africanus* yeast phase isolates (5).

There are no well-standardized methods for MIC determination for thermally dimorphic fungi (19); nevertheless, *in vitro* activities of polyenes (i.e., amphotericin B), azoles, and echinocandins have been established for some of these organisms (20). We used Clinical and Laboratory Standards Institute (CLSI)-approved standards as a guide to yeast and mold phase testing but used a larger inoculum for the mold phase and a prolonged incubation period to facilitate growth and endpoint determinations, in line with previous studies (5, 19, 21–23). Antifungal susceptibility testing for thermally dimorphic fungi is often limited to the mold phase, results of which may be misleading because the yeast phase is responsible for human disease (20). We tested both phases by reference and commercial methods and found no statistically significant differences. Although conversion of the mold phase to the yeast phase increases the turnaround time, there are fewer laboratory safety concerns with the yeast phase versus the potentially infectious mold phase and so we recommend that this phase be used for susceptibility testing. In contrast, significantly higher BMD MICs of voriconazole and posaconazole (but not itraconazole) were generated for both phases. Since, for most of these isolates, the BMD MICs of these agents were at or below the lower limit of the testing range, we speculate that this difference may have merely been an artifact caused by testing different ranges of antifungal concentrations with the two methods. Therefore, we recommend that either the Etest or BMD method be used for susceptibility testing. In this study, we also report relatively high MIC_{50} and MIC_{90} of the various

echinocandins and flucytosine. These agents are likely of no value in the management of patients with dimorphic fungal infections and need not be included in a susceptibility testing panel.

The case fatality rate was high in our series and consistent with previous reports (5, 15). Only culture of the fungus from blood or bone marrow (versus a skin biopsy specimen) was significantly associated with death on multivariable analysis. There are two possible explanations for this finding. First, we speculate that a positive blood or bone marrow culture is a proxy for a greater *in vivo* fungal burden. Second, isolation from blood or bone marrow alone (and not skin tissue) implies that a skin biopsy may not have been performed and the diagnosis of a deep fungal infection may not have been considered early enough. Antifungal MICs were not associated with outcomes in our current series, although a larger study may be needed to detect such an association. Currently, there are no published treatment guidelines for patients with disseminated emmonsiosis (14). On the basis of retrospective data and international guidelines for the management of immunocompromised hosts with disseminated diseases caused by other dimorphic fungal infections (17, 24, 25), some authors have recommended that patients with suspected disseminated emmonsiosis be treated with amphotericin B, followed by an azole (either itraconazole or fluconazole), after reporting good clinical outcomes among patients treated with these agents (15). Among the triazoles, fluconazole is much more accessible in South Africa because it is cheaper and included in hospital level essential medicine lists (versus posaconazole, voriconazole, and itraconazole, which are far more expensive and require a formal application for procurement by the treating physician). Moreover, itraconazole is sometimes avoided because of interactions with rifampin among patients with comorbid tuberculosis and the unavailability of therapeutic drug monitoring in South Africa. On the basis of the limited *in vitro* susceptibility data presented here, we believe that itraconazole, voriconazole, or posaconazole may be superior to fluconazole for the oral step-down phase following amphotericin B therapy for disseminated emmonsiosis.

This study had some limitations. Clinical data could not be obtained for some patients. We could not exclude the possibility that prescription of antifungals by outside clinicians would evade our data capture and could influence our findings on the clinical effects of antifungals. A larger-than-recommended inoculum was used for MIC determination to allow us to read endpoints by 7 days. Despite this, we found very low MICs of most antifungal agents. There are no currently published interpretative clinical breakpoints for any dimorphic fungus, including *Emergomyces*. Nevertheless, given the paucity of published clinical experience with these newly recognized pathogens, knowledge of *in vitro* MIC data should inform the management decisions of clinicians caring for patients with emmonsiosis.

In conclusion, *in vitro* susceptibility data support the management of disseminated emmonsiosis with amphotericin B, followed by itraconazole, voriconazole, or posaconazole. Fluconazole was a less potent agent. When indicated for epidemiological purposes in a reference laboratory, we recommend that the yeast phase and either the commercial Etest or a reference BMD method be used to generate MICs for *E. africanus*.

MATERIALS AND METHODS

Isolates and case definition. We obtained cultured isolates of *E. africanus* during passive laboratory-based surveillance conducted by the National Institute for Communicable Diseases from 2008 through to 2016 at nine diagnostic medical public- and private-sector laboratories in South Africa. We defined a case of disseminated emmonsiosis as a patient of any age with an isolate cultured from any normally sterile site and confirmed as *E. africanus* by phenotypic and molecular methods. We abstracted patient charts to obtain clinical details, which included demographics, history of medical conditions including HIV and coinfections, clinical presentation at the time of diagnosis, diagnostic investigations, management, and outcome.

Identification to the species level. The identities of 50 stored *E. africanus* isolates were initially confirmed by a detailed description of microscopic and macroscopic characteristics. While *E. africanus* has not yet been formally classified as a biosafety level 3 organism, we prepared all slides and cultures in a class II biosafety cabinet with the use of personal protective equipment, including N95 masks. We limited our work with the mold phase as far as possible. Mold phase isolates were subcultured on

Sabouraud agar (Diagnostic Media Products [DMP], National Health Laboratory Service, Sandringham, South Africa) and incubated at 25°C and 30°C for up to 4 weeks. The typical microscopic morphology of the mold phase was observed with a lactophenol cotton blue (DMP) slide preparation: septate hyphae, slender conidiophores at right angles to hyphae, and two or three round conidia borne on each conidiophore. To convert the fungus to the yeast phase, a piece of mold obtained from Sabouraud agar was subcultured on a brain heart infusion (BHI)–5% sheep blood agar slope or a BHI agar plate (DMP) and incubated for 1 to 2 weeks at 35°C. A Gram stain was prepared to observe the typical morphology of small, oval, budding yeast cells. PCR and sequencing of the internal transcribed spacer (ITS) regions of the ribosomal gene were performed with ITS1 and ITS4 primers after genomic DNA was extracted from yeast phase isolates with the Zymo ZR fungal/bacterial DNA MiniPrep kit (Zymo Research, Irvine, CA). *Candida albicans* ATCC 90028 was included as a quality control (QC) strain during PCR and sequencing of the ITS region. Sequences were determined by capillary electrophoresis on an ABI 3500 genetic analyzer (Applied Biosystems, USA). Identification to the species level was done with the NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) on the basis of pairwise sequence alignment.

Antifungal susceptibility testing. We performed susceptibility testing of both yeast and mold phase isolates by a reference broth microdilution (BMD) method and the commercial Etest method (bioMérieux, Marcy, l'Etoile, France). The BMD method was performed in accordance with CLSI-approved standards M27-A3 (for the yeast phase) and M38-A2 (for the mycelial form) by using a modified inoculum size for the latter (26, 27). Briefly, the inoculum was prepared from fresh cultures and the turbidity was adjusted with a turbidimeter to the equivalent of a 1 McFarland standard to obtain 2.5×10^3 to 5×10^3 CFU/ml for the yeast phase and a 2 McFarland standard to obtain 2.5×10^5 CFU/ml for the mycelial phase. Customized, round-bottom, frozen, 96-well microtiter plates containing 2-fold dilution ranges of itraconazole, voriconazole, posaconazole, fluconazole, flucytosine, anidulafungin, caspofungin, and micafungin were immediately inoculated (TREK Diagnostic Systems, Inc., Cleveland, OH). Yeast phase BMD MIC endpoints were read at 50% inhibition for fluconazole, voriconazole, posaconazole, itraconazole, flucytosine, caspofungin, anidulafungin, and micafungin. Mold phase BMD MIC endpoints were read at 50% inhibition for fluconazole and flucytosine and 100% for voriconazole, posaconazole, itraconazole, and amphotericin B. For the mold phase, echinocandin MEC endpoints were read macroscopically as the lowest concentration that yielded small pellets of granular growth (microcolonies) compared to the hyphal-type growth seen in the growth control well (28). Etest MICs of amphotericin B, voriconazole, itraconazole, and posaconazole were determined with Roswell Park Memorial Institute (RPMI) 1640 medium plates containing 2% glucose (DMP) in accordance with the manufacturer's recommendations. On the basis of the very high echinocandin MICs observed during preliminary testing, the MICs of these agents were not determined by Etest. Etest MIC endpoints were read as follows: 80% inhibition for voriconazole, posaconazole, and itraconazole (i.e., microcolonies within the elliptical zone of inhibition were ignored) and 100% for amphotericin B. All plates were incubated at 35°C and read by three independent observers at 7 days.

The QC strains included in each test run were *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, and *E. africanus* NCPF 4164 for yeast phase tests and *Aspergillus fumigatus* NCPF 7097, *A. fumigatus* NCPF 7100, and *E. africanus* NCPF 4164 for mycelial phase tests. The MICs for *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were within the CLSI-recommended ranges for all runs. Quantitative colony counts were performed to assess the purity and accuracy of the final inoculum. To check the yeast phase inoculum, we spread 0.02 ml onto a Sabouraud agar plate to determine the number of CFU per milliliter. Plates were incubated at 35°C, and after 7 days, we counted 50 to 100 colonies. For the mold phase, we spread 0.002 ml of the inoculum onto the surface of a plate, and after incubation, we counted up to 500 colonies.

Statistical methods. We calculated a geometric mean (GM) MIC, MIC_{50}, and MIC_{90} for each MIC distribution. For each antifungal agent and test method, we used a Wilcoxon ranked-sum test to compare the MICs generated from yeast and mold phase tests. We used a multivariable logistic regression model to assess the association of age; sex; province; CD4 cell count; ART history; antifungal treatment; specimen type; and MICs of amphotericin B, itraconazole, voriconazole, posaconazole, and fluconazole with patient outcome. All analyses were performed with Stata version 14.0 (StataCorp Limited, College Station, TX). Two-sided *P* values of <0.05 were considered significant.

Ethics approval. Ethics clearance for this study was obtained from the Health Sciences Research Ethics Committee, University of the Free State, Bloemfontein (13/2016), and the Human Research Ethics Committee of the University of Cape Town (704/2013 and 138/2014).

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T.G.M., I.S.S., and N.P.G. collected the data. T.G.M., T.G.Z, and R.S.M. performed antifungal susceptibility testing. T.G.M., E.B., and N.P.G. analyzed the data and wrote the manuscript. T.G.M., I.S.S., E.B., S.D.N., and N.P.G. critically reviewed the manuscript.

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