Submission to Journal of Clinical Microbiology

In vitro **antifungal susceptibility of the yeast- and mould-phases of the dimorphic fungal pathogen, *Emergomyces africanus* (formerly *Emmonsia* species), from HIV-infected South African patients**

Tsidiso G. MAPHANGA[1,2], Erika BRITZ[1], Thokozile G. ZULU[1], Ruth S. MPEMBE[1], Serisha D. NAICKER[1,3], Ilan S. SCHWARTZ[4] and Nelesh P. GOVENDER[1,3,5]

1. National Institute for Communicable Diseases (Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses), a Division of the National Health Laboratory Service, Johannesburg, South Africa
2. Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa
3. Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
4. Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium and Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
5. Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

[#]Corresponding author: Tsidiso G. Maphanga, National Institute for Communicable Diseases – Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, Private Bag X4, Sandringham, 2132, South Africa.

Phone: +27 11 555 0323. Fax: +27 11 555 0435. Email: tsidisom@nicd.ac.za

Running Title: *Emergomyces africanus in vitro* antifungal susceptibility

Keywords: disseminated emmonsiosis; emergomycosis; novel; opportunistic infection; dimorphic fungus; antifungal susceptibility testing

Word Count (Body):

Conflicts of interests: For unrelated work, Nelesh P. Govender received speaker honoraria/ travel grants from Pfizer, Astellas and MSD (Pty) Ltd, has provided educational materials for TerraNova and has acted as a temporary consultant for Fujifilm Pharmaceuticals. For other authors, none was declared.

Funding: The project was funded by the National Institute for Communicable Diseases.

Abstract

Word count: 250

Introduction: Disseminated emmonsiosis is an important AIDS-related mycosis in South Africa caused by *Emergomyces africanus,* a newly-described and -renamed dimorphic fungal pathogen. *In vitro* antifungal susceptibility data can guide management.

Materials and Methods: Identification of invasive clinical isolates was confirmed phenotypically and by sequencing the internal transcribed spacer region. Yeast and mould-phase MICs for fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, anidulafungin, micafungin and flucytosine were determined using custom-made frozen broth microdilution (BMD) panels, as per Clinical and Laboratory Standards Institute recommendations. MICs for amphotericin B, itraconazole, posaconazole and voriconazole were determined by Etest.

Results: Fifty unique *Emergomyces africanus* isolates were tested. Yeast- and mould-phase geometric mean (GM) BMD and Etest MICs values were 0.01 mg/L for itraconazole. Voriconazole and posaconazole GM BMD MICs were 0.01 mg/L for both phases, while GM Etest MICs were 0.001 mg/L and 0.002 mg/L, respectively. Fluconazole GM BMD MICs were 0.18 mg/L for both phases. For amphotericin B, GM Etest MICs for the yeast- and mould-phases were 0.03 mg/L and 0.01 mg/L. 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 (OR 8.6, 95% CI 1.3 –

Journal of Clinical Microbiology

54.4, p = 0.02) or bone marrow (OR 12.1, 95% CI 1.2 – 120.2, p = 0.03) (vs. skin biopsy) was associated with death.

Conclusions: *In vitro* susceptibility data support management of disseminated emmonsiosis with amphotericin B followed by itraconazole, voriconazole or posaconazole. Fluconazole was a relatively less potent agent.

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

INTRODUCTION

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 address 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 multi-system disease with a high case fatality (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).

Emergomyces 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

92 African provinces, including KwaZulu-Natal (13-15) (authors' unpublished data). To date,
93 with 86 laboratory-confirmed cases among HIV-infected persons in South Africa, *E.*
94 *africanus* is far more commonly isolated than other well-described endemic pathogens such
95 as *Histoplasma*, *Blastomyces* or *Sporothrix* (authors' unpublished data).
96
97 Patients with disseminated emmonsiosis often present with a syndrome of fever, widespread
98 skin lesions of varying morphologies, pneumonia, anemia, elevated liver enzymes and weight
99 loss (2, 13, 15). Misdiagnosis is common (15). To date, no clinical trial has been conducted
100 to evaluate treatment options for patients with this disease. A retrospective review suggested
101 improved outcomes for patients treated with amphotericin B followed by triazoles compared
102 to those treated with triazoles alone; however, many of the former patients were incidentally
103 prescribed low doses and short courses of fluconazole to treat presumed esophageal
104 candidiasis (15). Nonetheless, authors have recommended treatment with amphotericin B
105 deoxycholate followed by itraconazole for a minimum of 12 months (5, 13-15) pending
106 immune reconstitution, based on Infectious Diseases Society of America (IDSA) guidelines
107 for HIV-associated disseminated histoplasmosis (16, 17).
108
109 We aimed to compare the *in vitro* antifungal susceptibility of the yeast- and mould-phases of
110 *E. africanus* to several antifungal agents using a reference and commercially-available
111 method and to determine if there was an association between MIC and clinical outcome in
112 order to guide clinical management of patients with disseminated emmonsiosis.

MATERIALS AND METHODS

Isolates and case definition: We obtained cultured isolates of *E. africanus* during passive laboratory-based surveillance conducted by NICD 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 co-infections, clinical presentation at time of diagnosis, diagnostic investigations, management and outcome.

Species confirmation: The identity of 50 stored *E. africanus* was initially confirmed by a detailed description of microscopic and macroscopic characteristics. While *E. africanus* has not yet been formally classified as a BSL3 organism, we prepared all slides and cultures in a class II biosafety cabinet with use of personal protective equipment, including N95 masks. We limited our work with the mould-phase as far as possible. Mould-phase isolates were sub-cultured onto Sabouraud agar (Diagnostic Media Products [DMP] - National Health Laboratory Service, Sandringham, South Africa) and incubated at 25ºC and 30ºC for up to four weeks. The typical microscopic morphology of the mould phase was observed using a lactophenol cotton blue (DMP) slide preparation: septate hyphae, slender conidiophores at right angles to hyphae and two to three round conidia borne on each conidiophore. To convert the fungus to the yeast phase, a piece of mould obtained from Sabouraud agar was sub-cultured on brain-heart infusion (BHI) + 5% sheep blood agar slope or a BHI agar plate (DMP) and incubated for one to two weeks at 35ºC. 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 was performed using ITS1

and ITS4 primers after genomic DNA was extracted from yeast-phase isolates using the Zymo ZR fungal/bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA). *Candida albicans* ATCC 90028 was included as a quality control strain during PCR and sequencing of the ITS region. The sequences were determined using capillary electrophoresis on an ABI 3500 genetic analyser (Applied Biosystems, USA). The species-level identity was obtained from NCBI BLAST database based on pairwise sequence alignment (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antifungal susceptibility testing: We performed susceptibility testing for both yeast- and mould-phase isolates using a reference broth microdilution (BMD) method and the commercial Etest method (bioMérieux, Marcy, l'Etoile, France). The broth microdilution method was performed according to Clinical and Laboratory Standards Institute (CLSI) approved standards: M27-A3 (for the yeast phase) and M38-A2 (for the mycelial form) with a modified inoculum size for the latter (18, 19). Briefly, the inoculum was prepared from fresh cultures and the turbidity adjusted using a turbidometer to the equivalent of a 1 McFarland standard to obtain 2.5 x 10^3 to 5 x 10^3 CFU/mL for the yeast phase and a 2 McFarland standard to obtain 2.5 x 10^5 CFU/mL for the mycelial phase. Customised, round-bottomed frozen 96-well microtitre plates, containing two-fold dilution ranges of itraconazole, voriconazole, posaconazole, fluconazole, flucytosine, anidulafungin, caspofungin and micafungin, were immediately inoculated (TREK Diagnostic Systems, Inc., Cleveland, Ohio, USA). Yeast-phase BMD MIC endpoints were read at 50% inhibition for fluconazole, voriconazole, posaconazole, itraconazole, flucytosine, caspofungin, anidulafungin and micafungin. Mould-phase BMD MIC endpoints were read at 50% inhibition for fluconazole and flucytosine and 100% for voriconazole, posaconazole, itraconazole and amphotericin B. For the mould phase, echinocandin MEC (minimum

163 effective concentration) endpoints were read macroscopically as a lowest concentration that
164 yielded small pellets of granular growth ("microcolonies") compared to the hyphal-type
165 growth seen in the growth control well (20). Etest MICs for amphotericin B, voriconazole,
166 itraconazole and posaconazole were determined using Roswell Park Memorial Institute
167 (RPMI) 1640 plates containing 2% glucose (DMP) according to the manufacturer's
168 recommendations. Based on very high echinocandin MICs observed during preliminary
169 testing, Etest MICs were not determined for these agents. Etest MIC endpoints were read as
170 follows: 80% inhibition for voriconazole, posaconazole, itraconazole (i.e. micro-colonies
171 within the elliptical zone of inhibition were ignored) and 100% for amphotericin B. All
172 plates were incubated at 35ºC and read by three independent observers at seven days.
173
174 Quality control (QC) strains were included in each test run: *Candida parapsilosis* American
175 Type Culture Collection [ATCC] 22019, *Candida krusei* ATCC 6258 and *E. africanus*
176 National Collection of Pathogenic Fungi [NCPF] 4164 for yeast-phase tests and *Aspergillus*
177 *fumigatus* NCPF 7097, *A. fumigatus* NCPF 7100 and *E. africanus* NCPF 4164 for mycelial-
178 phase tests. The MICs for *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258 were within
179 the CLSI-recommended ranges for all runs. Quantitative colony counts were performed to
180 assess the purity and accuracy of the final inoculum. To check the yeast-phase inoculum, we
181 spread 0.02 mL onto a Sabouraud agar plate to determine the number of CFU/mL. Plates
182 were incubated at 35°C and after 7 days, we counted between 50 to 100 colonies. For the
183 mould-phase, we spread 0.002 mL of the inoculum onto the surface of a plate and after
184 incubation, counted up to 500 colonies.
185
186 Statistical methods: We calculated a geometric mean (GM), MIC_{50} and MIC_{90} for each MIC
187 distribution. For each antifungal agent and test method, we used a Wilcoxon ranked sum test

to compare the MICs generated from yeast- and mould-phase tests. We used a multivariable logistic regression model to assess the association of age, sex, province, CD4+ T-lymphocyte (CD4) count, antiretroviral treatment (ART) history, antifungal treatment, specimen type and antifungal MICs for amphotericin B, itraconazole, voriconazole, posaconazole and fluconazole with patient outcome. All analyses were performed using Stata version 14.0 (StataCorp Limited, College Station, Texas, USA). Two-sided p values of <0.05 were considered significant.

Ethics approval

Ethics clearance for the 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).

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 by *E. africanus* were males with a median age of 35 years (interquartile range (IQR), 30-38). Sixty-nine per cent (31/45) of 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 these patients were HIV-infected. Of 44 HIV-infected patients with a recorded CD4 count, the median CD4 count was 12 cells/µl (IQR, 7-27 cells/µl). Of 31 cases with an available ART history, 20 (65%) were ART-experienced and nine (29%) were ART-naïve. Isolates were cultured from skin biopsy (n=23), blood (n=18), bone marrow (n=6), biopsy from an unknown site (n=1) and an unknown specimen (n=1). Demographic and clinical features of *E. africanus* cases with available data are summarized in Table 1.

Antifungal susceptibility testing for *Emergomyces* isolates

All isolates grew sufficiently for MIC determination after seven days of incubation. Tables 2 and 3 summarise the MIC/ MEC distribution, range, GM MIC/ MEC, MIC_{50} and MIC_{90} for nine antifungal agents and the yeast- and mould-phases of 50 *E. africanus* isolates. The BMD and Etest MICs for itraconazole were relatively higher than those of voriconazole and posaconazole for both the yeast- and mould-phases (Tables 2 and 3). The yeast– and mould-phase GM MICs were not significantly different for fluconazole (BMD MICs: 0.19 mg/L vs. 0.18 mg/L; p=0.06), amphotericin B (Etest MICs: 0.03 mg/L vs. 0.01 mg/L; p=0.06) or any other tested antifungal agent/ method [data not shown]. In contrast, the BMD method yielded

225 significantly higher GM MICs than the Etest method for voriconazole (BMD MIC 0.01 mg/L
226 vs. Etest MIC 0.001 mg/L; $p<0.001$) and posaconazole (BMD MIC 0.01 mg/L vs. Etest MIC
227 0.002 mg/L; $p<0.001$) for both yeast- and mould-phases. There was no difference between
228 BMD and Etest GM MICs for itraconazole [data not shown].
229
230 MIC distribution and patient outcome
231 Of 51 cases of emmonsiosis, the clinical outcome could be ascertained for 37 (73%) and
232 management was known for 34 (67%). The overall case-fatality ratio was 38% (14/37). Both
233 clinical outcome and management could be ascertained for 33 patients (65%). Of the 23
234 patients who survived, management was known for 22: 18 (82%) were treated with
235 amphotericin B, followed by itraconazole (16), fluconazole (1) or a combination thereof (1);
236 three patients were treated with triazole monotherapy (two received itraconazole and one
237 received a low dose of fluconazole for 14 days); and one received fluconazole for >12
238 months (with possibly amphotericin B). In contrast, management details were available for 11
239 of 14 patients who died (79%): three received amphotericin B, two received triazole
240 monotherapy (one each received itraconazole and low-dose fluconazole), and six received no
241 antifungal treatment. In the multivariable model, only isolation from blood (OR 8.6, 95%CI
242 1.3 – 54.4 , $p = 0.02$) or bone marrow (OR 12.1 , 95%CI 1.2 – 120.2, $p = 0.03$) (vs. skin
243 biopsy) was associated with death (Table 4).

244 **DISCUSSION**

245

246 Thermally-dimorphic fungi in the genus *Emergomyces* have emerged as a cause of
247 disseminated, sometimes fatal disease among HIV-infected South Africans with very low
248 CD4+ T-lymphocyte counts. We have reported the antifungal susceptibility profile of a large
249 series of *E. africanus* isolates. Voriconazole, posaconazole, itraconazole and amphotericin B
250 had the most potent *in vitro* activity against both mould- and yeast-phases of *Emergomyces*.
251 While fluconazole is far more easily accessible to clinicians in the South African public
252 health sector, this agent was less potent than other azoles (21). This confirms earlier findings
253 of an antifungal susceptibility report of six *E. africanus* yeast-phase isolates (5).

254

255 There are no well-standardised methods for MIC determination for thermally-dimorphic
256 fungi (22); nevertheless, *in vitro* activities of polyenes (i.e. amphotericin B), azoles and
257 echinocandins have been established for some of these organisms (23). We used CLSI
258 approved standards as a guide to yeast- and mould-phase testing but used a higher inoculum
259 for the mould-phase and a prolonged incubation period to facilitate growth and endpoint
260 determination, in line with previous studies (5, 22, 24-26). Antifungal susceptibility testing
261 for thermally-dimorphic fungi is often limited to the mould phase, results of which may be
262 misleading because the yeast phase is responsible for human disease (23). We tested both
263 phases using reference and commercial methods and found no statistically significant
264 differences. Although conversion of the mould-phase to the yeast-phase increases turn-
265 around time, there are fewer laboratory safety concerns with the yeast phase versus the
266 potentially infectious mould-phase and so we would recommend that this phase be used for
267 susceptibility testing. In contrast, significantly higher BMD MICs for voriconazole and
268 posaconazole (but not itraconazole) were generated for both phases. Since most isolates had

BMD MICs for these agents at or below the lower limit of testing range, we speculate that this difference may have merely been an artefact caused by testing different ranges of antifungal concentrations with the two methods. Therefore, we would 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} values for 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 was high in our series, and consistent with previous reports (5, 15). Only culture of the fungus from blood or bone marrow (vs. skin biopsy) was significantly associated with death on multivariable analysis. There are two possible explanations for this finding. Firstly, we speculate that a positive blood or bone marrow culture is a proxy for a higher *in vivo* fungal burden. Secondly, 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 outcome 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 disease caused by other dimorphic fungal infections (17, 27, 28), 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 owing to the fact that this agent is cheaper and included on hospital-level essential medicines lists (versus posaconazole, voriconazole or

294 itraconazole which are far more expensive and require a formal application for procurement
295 by the treating physician). Moreover, itraconazole is sometimes avoided because of
296 interactions with rifampin among patients with co-morbid tuberculosis, and the unavailability
297 of therapeutic drug monitoring in South Africa. Based on the limited *in vitro* susceptibility
298 data presented here, we believe that itraconazole, voriconazole or posaconazole may be
299 superior to fluconazole for the oral step-down phase following amphotericin B therapy for
300 disseminated emmonsiosis.
301
302 This study had some limitations. Clinical data could not be obtained for some patients. We
303 could not exclude the possibility that prescription of antifungals by outside clinicians would
304 evade our data capture and could influence our findings of clinical effect of antifungals. A
305 higher-than-recommended inoculum was used for MIC determination to allow us to read
306 endpoints by 7 days. Despite this, we found very low MICs for most antifungal agents. There
307 are no currently published interpretative clinical breakpoints for any dimorphic fungus,
308 including *Emergomyces*. Nevertheless, given the paucity of published clinical experience
309 with these newly-recognized pathogens, knowledge of *in vitro* MIC data should inform
310 management decisions of clinicians caring for patients with emmonsiosis.
311
312 In conclusion, *in vitro* susceptibility data support management of disseminated emmonsiosis
313 with amphotericin B followed by itraconazole, voriconazole or posaconazole. Fluconazole
314 was a relatively a less potent agent. When indicated for epidemiological purposes in a
315 reference laboratory, we recommend that the yeast phase and either the commercial Etest or a
316 reference BMD method be used to generate MICs for *E. africanus*.

317 **ACKNOWLEDGEMENTS**

318 This work was presented, in part, at the EMBO AIDS related-mycoses meeting, 13-15 July

319 2016, Cape Town, South Africa.

320

321 **AUTHOR CONTRIBUTIONS**

322 Data collection: TGM, ISS, NPG

323 Antifungal susceptibility testing: TGM, TGZ, RSM

324 Data analysis and manuscript writing: TGM, EB, NPG

325 Critical review of manuscript: TGM, ISS, EB, SDN, NPG

326 **REFERENCES**

327 1. **Schwartz IS, Kenyon C, Thompson III GR.** 2016. Endemic Mycoses: What's New
328 About Old Diseases? Curr Clin Micro Rpt **3:**71–80.

329 2. **Dukik K, Muñoz JF, Jiang Y, Feng P, Sigler L, Stielow JB, Freeke J, Jamalian**
330 **A, van den Ende GB, McEwen JG, Clay OK, Schwartz IS, Govender NP,**
331 **Maphanga TG, Cuomo AC, Moreno L, Kenyon C, Borman AM, de Hoog S.**
332 2017. Novel taxa of thermally dimorphic systemic pathogens in the *Ajellomycetaceae*
333 (Onygenales). Mycoses **00:**1-14. doi:10.1111/myc.12601.

334 3. **Schwartz IS, Kenyon C, Feng P, Govender NP, Dukik K, Sigler L, Jiang Y,**
335 **Stielow JB, Muñoz JF, Cuomo CA, Botha A, Stchigel AM, de Hoog SG.** 2015. 50
336 Years of *Emmonsia* Disease in Humans: The Dramatic Emergence of a Cluster of
337 Novel Fungal Pathogens. PLoS Pathog **11:**e1005198.

338 4. **Peterson SW, Sigler SL.** 1998. Molecular Genetic Variation in *Emmonsia crescens*
339 and *Emmonsia parva*, Etiologic Agents of Adiaspiromycosis, and Their Phylogenetic
340 Relationship to *Blastomyces dermatitidis* (*Ajellomyces dermatitidis*) and Other
341 Systemic Fungal Pathogens. J Clin Microbiol **36:**2918–2925.

342 5. **Kenyon C, Bonorchis K, Corcoran C, Meintjes G, Locketz M, Lehloenya R,**
343 **Vismer HF, Naicker P, Prozesky H, van Wyk M, Bamford C, du Plooy M, Imrie**
344 **G, Dlamini S, Borman AM, Colebunders R, Yansouni CP, Mendelson M,**
345 **Govender NP.** 2013. A Dimorphic Fungus Causing Disseminated Infection in South
346 Africa. N Engl J Med **369:**1416-1424.

347 6. **Drouhet E, Gueho E, Gori S, Huerre M, Provost F, Borgers M, Dupont B.** 1998.
348 Mycological, ultrastructural and experimental aspects of a new dimorphic fungus
349 *Emmonsia pasteuriana* sp. nov. isolated from a cutaneous disseminated mycosis in
350 AIDS. J Mycol Med **8:**64-77.

7. **Wang O, Kenyon C, de Hoog GS.** 2017. A novel dimorphic pathogen, *Emergomyces orientalis* (Onygenales), agent of disseminated infection. Mycoses **00:**1-10 doi: 10.1111/myc.12583.

8. **Gori S, Drouhut E, Gueho E, Huerre M, Lofaro A, Parenti M, Dupont B.** 1998. Cutaneous disseminated mycosis in a patient with AIDS due to a new dimorphic fungus. J Mycol Med **8:**57-63.

9. **Pelegrin I, Alastruey Izquierdo A, Ayats J, Cuenca-Estrella M, Cabellos C.** 2014. A second look at *Emmonsia* infection can make the difference. Transpl Infect Dis **16:**519-520.

10. **Feng P, Yin S, Zhu G, Li M, Wu B, Xie Y, Ma H, Zhang J, Cheng C, Sijbrand G, de Hoog GS, Lu C, Lai W**. 2015. Disseminated infection caused by *Emmonsia pasteuriana* in a renal transplant recipient. J Dermatol **42:**1179-1182.

11. **Tang XH, Zhou H, Zhang XQ, Han JD, Gao Q.** 2015. Cutaneous Disseminated Emmonsiosis Due to *Emmonsia pasteuriana* in a Patient With Cytomegalovirus Enteritis. JAMA Dermatol **151:**1263-1264.

12. **Malik R, Capoor MR, Vanidassane I, Gogna A, Singh A, Sen B, Rudramurthy SM, Honnavar P, Gupta S, Chakrabarti A.** 2016. Disseminated *Emmonsia pasteuriana* infection in India: a case report and a review. Mycoses **59:**127-132.

13. **van Hougenhouck-Tulleken WG, Papavarnavas NS, Nel JS, Blackburn LY, Govender NP, Spencer DC, Lippincott CK.** 2014. HIV-associated disseminated Emmonsiosis, Johannesburg, South Africa. Emerg Infect Diseases **20:**2164-2165.

14. **Lochan H, Naicker P, Maphanga T, Ryan A, Pillay K, Govender NG, Eley B.** 2015. A case of emmonsiosis in an HIV-infected child. S Afr J HIV Med **16:**1-4.

15. **Schwartz IS, Govender NP, Corcoran C, Dlamini S, Prozesky H, Burton R, Mendelson M, Taljaard J, Lehloenya R, Calligaro G, Colebunders R, Kenyon C.**

Journal of Clinical Microbiology

2015. Clinical Characteristics, Diagnosis, Management, and Outcomes of Disseminated Emmonsiosis: A Retrospective Case Series. Clin Infect Dis **61:** 1004-1012 doi:10.1093/cid/civ439.

16. **Chapman SW, Rogers PD, Rinaldi MG, Sullivan DC.** 1998. Susceptibilities of Clinical and Laboratory Isolates of *Blastomyces dermatititidis* to Ketoconazole, Itraconazole, and Fluconazole. Antimicrob Agents Chemother **42:**978-980.

17. **Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, Kauffman CA.** 2007. Clinical Practice Guidelines for the Management of Patients with Histoplasmosis: 2007 Update by the Infectious Diseases Society of America. Clin Infect Dis **45:**807-825.

18. **Clinical and Laboratory Standard Institute.** 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 2nd Edition. Clinical and Laboratory Standard Institute M38-A2, Wayne, PA, USA

19. **Clinical and Laboratory Standard Institute.** 2012. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th Informational Supplemenet. Clinical and Laboratory Standards Institute M27-A3, Wayne, PA, USA

20. **Espinel-Ingroff, A.** 2003. Evaluation of Broth Microdilution Testing Parameters and Agar Diffusion Etest Procedure for Testing Susceptibilities of *Aspergillus* spp. to Caspofungin Acetate (MK-0991). J Clinl Microbiol **41:**403-409.

21. **Motsoaledi A.** 2014. Republic of South Africa. Essential Drugs Programme. Primary Healthcare Standard Treatment Guidelines and Essential Medicines. Fifth Edition. Health Republic of South Africa: Department of Health

22. **Goughenour KD, Balada-Llasat JM, Rappleye CA.** 2015. Quantitative Microplate-Based Growth Assay for Determination of Antifungal Susceptability of *Histoplasma capsulatum* Yeasts. J Clin Microbiol **53:**3286-3295.

23. **Goughenour KD, Rappleye CA.** 2016. Antifungal therapeutics for dimorphic fungal pathogens. Virulence **0:**1-11.

24. **Espinel-Ingroff A.** 1998. Comparison of *In Vitro* Activities of the New Triazole SCH56592 and the Echinocandins MK-0991 (L-743,872) and LY303366 against Opportunistic Filamentous and Dimorphic Fungi and Yeasts. J Clin Microbiol **36:**2950-2956.

25. **Kathuria S, Singh PK, Meis JF, Chowdhary A.** 2014. *In Vitro* Antifungal Susceptibility Profile and Correlation of Mycelial and Yeast Forms of Molecularly Characterized *Histoplasma capsulatum* Strains from India. Antimicrob Agents Chemother **58:**5613-5616.

26. **Nakai T, Uno J, Ikeda F, Tawara S, Nishimura K, Miyaji M.** 2003. *In Vitro* Antifungal Activity of Micafungin (FK463) against Dimorphic Fungi: Comparison of Yeast-Like and Mycelial Forms. Antimicrob Agents Chemother **47:**1376–1381.

27. **Chapman SW, Dismukes WE, Proia LA, Bradsher RW, Pappas PG, Threlkeld MG, Kauffman CA.** 2008. Clinical Practice Guidelines for the Management of Blastomycosis: 2008 Update by the Infectious Diseases Society of America. Clin Infect Dis **46:**1801–12.

28. **Kauffman CA, Bustamante B, Chapman SW, Pappas PG.** 2007. Clinical Practice Guidelines for the Management of Sporotrichosis: 2007 Update by the Infectious Diseases Society of America. Clin Infect Dis **45:**1255–65.

421 **Table 1:** Demographic and clinical features of cases of emergomycosis (disseminated
422 emmonsiosis), n=50

Demographic and clinical features	**N***	**n (%)**
Median age (IQR), years	45	35 (30-38)
Male sex	48	28 (58)
Province	45	
Western Cape		31 (69)
Gauteng		10 (22)
Free State		3 (7)
Eastern Cape		1 (2)
HIV-infected	45	45 (100)
Median CD4 count (IQR), cells/ µl	44	12 (7-27)
Antiretroviral treatment (ART)	31	
ART-naïve		9 (29)
ART-experienced		20 (65)

423 *Denominator varies owing to missing data

424 **Table 2:** Yeast-phase MIC distribution of 50 *E. africanus* clinical isolates

		Number of isolates with MIC (mg/L) of:																
Antifungal agent	**Test method**	**≤0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**	**GM MIC[a]**	**MIC_{50} (mg/L)**	**MIC_{90} (mg/L)**	**Range**
Itraconazole	BMD[b]	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

425 [a]GM=Geometric mean; [b]BMD=Broth microdilution

Journal of Clinical Microbiology

426 **Table 3:** Mould-phase MIC/MEC distribution of 50 *E. africanus* clinical isolates

		Number of isolates with MIC/MEC (mg/L) of:																
Antifungal agent	**Test method**	**≤0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**	**GM MIC[a]**	**MIC_{50}/ MEC_{50} (mg/L)**	**MIC_{90}/ MEC_{90} (mg/L)**	**Range**
Itraconazole	BMD[b]	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

427 [a]GM=Geometric mean; [b]BMD=Broth microdilution

428

429 **Table 4:** Patient characteristics associated with outcome, n=37

	Outcome		Univariate analysis		Multivariable analysis	
Variables	**Survived** n/N (%)	**Died** n/N (%)	**OR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Age						
Median, years (IQR)	33 (28-36)	35 (31-38)	1.0 (0.9 – 1.2)	0.59		
Sex						
Male	15/23 (65.2)	5/14 (35.7)	reference		reference	
Female	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)	-	-		
CD4+ T-lymphocyte count						
Median, cells/µl (IQR)	13 (9-32)	14 (6-27)	1.0 (0.98 – 1.04)	0.50		
Antiretroviral treatment (ART)						
ART-naïve	6/21 (28.6)	3/10 (30)	reference			

	Outcome		Univariate analysis		Multivariable analysis	
Variables	**Survived** n/N (%)	**Died** n/N (%)	**OR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**
ART-experienced	15/21 (71.4)	7/10 (70)	0.93 (0.17-4.87)	0.94		
Any antifungal treatment						
No	0/22 (0)	6/11 (54.6)	reference			
Yes	22/22 (100)	5/11 (45.5)	1	-		
Specimen type						
Skin biopsy	15/23 (65.2)	2/14 (14.3)	reference		reference	
Blood culture	6/23 (26.1)	8/14 (57.1)	**10 (1.62 – 61.47)**	**0.01**	**8.57 (1.35 – 54.33)**	**0.02**
Bone marrow aspirate	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 (Etest, yeast phase)	0.06	0.06	0.16 (0.001 – 20.82)	0.47		
Posaconazole (BMD, mould phase)	0.008	0.008	1.91 (0.10-34.82)	0.66		
Itraconazole (BMD, mould phase)	0.008	0.008	0.26 (0.0004-140.69)	0.67		
Voriconazole (BMD, mould phase)	0.008	0.008	72.3 (9.89-5.30)	0.87		
Fluconazole (BMD, mould phase)	0.12	0.20	0.75 (0.29-1.92)	0.54		

430

431