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SUSCEPTIBILITY

Antifungal Susceptibility of Emerging Dimorphic Pathogens in the Family *Ajellomycetaceae*

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ABSTRACT The *in vitro* susceptibilities of 24 molecularly identified dimorphic fungi belonging to the genera *Adiaspiromyces*, *Blastomyces*, and *Emergomyces* within the family *Ajellomycetaceae* were tested against 8 standard antifungal agents using CLSI document M38-A2. Amphotericin B and posaconazole had the lowest geometric mean MICs (<0.05 μg/ml) followed by itraconazole (<0.07 μg/ml), voriconazole (<0.15 μg/ml), and isavuconazole (<0.42 μg/ml) while fluconazole was not active. Micafungin demonstrated good *in vitro* antifungal activity against *Emergomyces* (geometric mean minimum effective concentration [GM MEC] 0.1 μg/ml) and *Blastomyces* (GM MEC <0.017 μg/ml).

KEYWORDS dimorphic fungi, *Adiaspiromyces*, *Blastomyces*, *Emergomyces*, antifungal susceptibility testing, CLSI

Fungi in the family *Ajellomycetaceae* are well known systemic dimorphic pathogens. Recent phylogenetic studies resulted in the establishment of two new genera (*Adiaspiromyces* and *Emergomyces*) in addition to the well-known genera *Blastomyces*, *Histoplasma*, and *Paracoccidioides* (1–3). The genus *Adiaspiromyces* now accommodates the former *Emmonsia crescens* and the environmental species *Adiaspiromyces terricola* (2). The genus *Emergomyces* contains *Emergomyces pasteurianus* as a type species (formerly *Emmonsia pasteuriana*) and four newly introduced species, *Emergomyces africanus* (1), *Emergomyces orientalis* (4), *Emergomyces canadensis*, and *Emergomyces europaeus* (2). *Blastomyces* was reorganized to include three new species, *B. silverae*, *B. helicus*, *and B. percursus* along with *Blastomyces parvus* (formerly *Emmonsia parva*), *B. gilchristii*, and the well-known human pathogen *B. dermatitidis* (1, 2).

These genera and their species differ in their pathogenic forms (adiaspores, small yeasts and large yeasts), host range, and clinical manifestation. *Adiaspiromyces crescens* is primarily a causative agent of pulmonary infections in terrestrial mammals (5) but has been reported occasionally as a causative agent of pulmonary infections in humans (6). The pathogenic form is called adiaspore, a large spore with dimensions ranging from 20 to 140 μm (7). Since human infections are rare, there are only few reports on antifungal susceptibility testing (5) or treatment (6).

In contrast, *Emergomyces* species are human pathogens and not yet isolated from the environment or from animals. Almost all infected human hosts were HIV infected. *Emergomyces* species differ from the other species within the same family by producing budding yeasts (less than 5 μm) *in vivo* rather than adiaspores and often disseminate with secondary cutaneous manifestation, referred to as emergomycosis (8). Two species

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have been tested for antifungal susceptibility: *Emergomyces africanus*, the causative agent of an outbreak in South Africa (8, 9), and *Emergomyces pasteurianus* (10). *Emergomyces* infections have been treated mostly with amphotericin B, itraconazole, or fluconazole (4, 11; S. Sanche, A. Wong, L. Sigler, S. Angel, and S. Peterson, presented at Focus on Fungal Infections, Miami, FL, 2005, abstract 87) but no clinical trial has been conducted to date.

The genus *Blastomyces* is more versatile than *Adiaspiromyces* and *Emergomyces*. *Blastomyces dermatitidis*, *B. gilchristii*, and *B. percursus* are human pathogens with broad-based large yeasts (more than 5 μm) as the pathogenic form (2). *Blastomyces dermatitidis* and *B. gilchristii* cause blastomycosis mostly in North America (12), with few cases reported from the Asia-Pacific region (13, 14). There are several antifungal susceptibility studies (15–17) and treatment suggestions (18, 19). *Blastomyces percursus* was recently described as a human pathogen in both immunocompetent and immunocompromised patients (20). The other two species, *B. parvus* (*Emmonsia parva*) and the newly described *B. silverae* cause infections in small animals but rarely in humans (2). No *in vitro* susceptibility data for these two species have been published.

We report the *in vitro* antifungal susceptibility of eight antifungals of the newly described species *Emergomyces africanus*, *E. orientalis*, *E. canadensis*, *Blastomyces percursus*, *B. helicus*, and *B. silverae*. Although *in vitro* antifungal susceptibility testing of these dimorphic fungi is not standardized, the obtained MIC data might help with treatment decisions for infections caused by them. Of 24 isolates, 14 (58.3%) originated from human patients, 4 (16.6%) from rodents, 2 (8.3%) from soil, and 1 (4.16%) each from a weasel, coyote dung, and a bird's nest; 1 was of unknown origin. It should be noted that these species are still rare, and thus only small numbers of strains per species are available: *Emergomyces pasteurianus* ($n = 3$), *E. africanus* ($n = 5$), *E. orientalis* ($n = 1$), *E. canadensis* ($n = 2$), *Adiaspiromyces crescens* ($n = 4$), *B. gilchristii* ($n = 1$), *B. parvus* ($n = 2$), *B. helicus* ($n = 1$), *B. silverae* ($n = 3$), and *B. percursus* ($n = 1$), with one strain of *Blastomyces dermatitidis* as reference. All strains were initially subcultured on malt extract agar (MEA) (Oxoid, UK) and molecularly identified using multilocus sequencing as previously described (1).

Susceptibility studies were conducted in a class 2 biosafety cabinet in biosafety level 2 (BSL 2) laboratories, except for *B. dermatitidis* and *B. gilchristii*, which were tested using a BSL 3 facility. The isolates were subcultured on potato dextrose agar (PDA) (Oxoid, UK), malt extract agar (MEA) (Oxoid, UK), and Sabouraud's glucose agar (SGA). Sporulation on PDA was most abundant, and up to 10 plates with this medium were used to collect sufficient amounts of conidia. Antifungal susceptibility testing (AFST) was determined by broth microdilution using CLSI document M38-A2 (21). The mold phase on PDA at 24°C was used for inoculum preparation, which was adjusted to an optical density at 530 nm, to present transmission in a range of 76 to 77%, corresponding to 2.5 to 5 $\times 10^5$ CFU per ml and diluted 1:10 in RPMI 1640 medium. Plates were incubated at 24°C for 6 days with the following drug concentration ranges: amphotericin B (Bristol Myers Squib, Woerden, The Netherlands), itraconazole (Janssen Cilag, Beerse, Belgium), voriconazole (Pfizer Central Research, Sandwich, UK), posaconazole (Merck, Sharp & Dome, Haarlem, The Netherlands), and isavuconazole (Basilea Pharmaceutica, Basel), 0.016 to 16 μg/ml; fluconazole (Pfizer), 0.063 to 64 μg/ml; and anidulafungin (Pfizer) and micafungin (Astellas Pharma, Toyama, Japan), 0.008 to 8 μg/ml. The quality of every new batch of MIC plates was tested by using two quality control (QC) strains, *Candida parapsilosis* ATCC 22016 and *Aspergillus flavus* ATCC 204304.

Table 1 summarizes the MICs of 24 isolates from three clinically related genera, *Emergomyces*, *Adiaspiromyces*, and *Blastomyces*. All *Emergomyces* species had MIC values of $\geq$64 μg/ml for fluconazole, except one *Emergomyces canadensis*, which had a MIC of 8 μg/ml. *Adiaspiromyces* species showed high MICs of 16 μg/ml, with one isolate having the highest value of 64 μg/ml. *Blastomyces silverae* showed the highest MIC for fluconazole with $\geq$64 μg/ml, followed by *B. parvus* and *B. helicus* with 32 μg/ml, respectively. *Blastomyces dermatitidis*, *B. gilchristii*, and *B. percursus* had the lowest MICs (2 μg/ml) of all species. For the other azoles, a small degree of variation was found at

TABLE 1 *In vitro* susceptibility of 24 *Emergomyces*, *Adiaspiromyces*, and *Blastomyces* strains

No.	Species name	CBS no.	MIC/MEC (μg/ml) for[a]: AMB[b]	FLC	ITC	VOR	POS	ISA	ANID	MICA	Source	Origin
1	*Emergomyces pasteurianus*	101426 (T)	0.031	>64	0.125	0.25	0.125	2	0.5	0.031	Human, HIV, disseminated	Italy
2		140361	0.063	64	0.063	0.25	0.063	0.5	0.5	0.016	Human, HIV, disseminated	S.Africa
3		139522	0.125	>64	0.25	0.25	0.063	1	0.031	0.063	Human, renal transplant, disseminated	China
4	*Emergomyces africanus*	140362	0.25	64	0.125	0.063	<0.016	0.125	1	1	Human, HIV, disseminated	S.Africa
5		136260 (T)	0.031	64	0.063	0.125	0.063	0.5	2	0.031	Human, HIV, disseminated	S.Africa
6		4164	0.063	64	0.063	0.063	0.031	0.125	2	1	Human, HIV, disseminated	S.Africa
7		140363	0.031	64	<0.016	0.063	0.031	0.25	1	0.063	Human, HIV, disseminated	S.Africa
8		139543	0.031	64	0.063	0.25	0.031	2	0.5	0.125	Human, HIV, disseminated	S.Africa
9	*Emergomyces orientalis*	124587	0.063	>64	0.031	0.125	0.031	0.25	1	0.125	Human, diabetes, disseminated	China
10	*Emergomyces canadensis*	139873	0.031	8	0.063	0.125	0.063	0.25	0.5	0.063	Human, transplant, disseminated	Canada
11		139872 (T)	<0.016	>64	0.031	0.125	0.031	0.25	2	0.25	Human, HIV, skin lesion	Canada
12	*Adiaspiromyces crescens*	139864	<0.016	16	0.031	0.063	<0.016	0.125	2	0.125	Rodent	S.Korea
13		191.55	<0.016	16	<0.016	<0.016	<0.016	<0.016	4	0.063	Rodent	Canada
14		177.60 (T)	<0.016	16	0.031	0.063	0.031	0.125	8	8	Rodent	Norway
15		139869	0.125	16	0.031	0.125	0.063	0.25	8	0.125	Environmental	Canada
16	*Blastomyces dermatitidis*	126.33	0.016	2	<0.016	0.063	0.016	0.063	0.031	0.008	No data	no data
17	*Blastomyces gilchristii*	134223	0.031	2	0.031	0.063	0.031	0.125	0.016	0.008	Human	Canada
18	*Blastomyces parvus*	139881 (T)	<0.016	32	0.016	0.125	0.031	0.25	0.5	0.031	Rodent	USA
19		139883	<0.016	32	<0.016	0.063	0.031	0.25	0.25	0.031	Soil	Italy
20	*Blastomyces helicus*	140056 (T)	<0.016	32	<0.016	<0.016	<0.016	<0.016	0.063	0.0031	Human, leukemia	Canada
21	*Blastomyces silverae*	139879 (T)	<0.016	64	0.125	0.125	0.125	0.25	1	0.063	Weasel	USA
22		139885	0.063	64	0.031	0.063	0.063	0.25	1	0.016	Coyote dung	Canada
23		139871	0.063	>64	0.125	0.125	0.063	0.5	0.25	0.031	Bird's nest	Canada
24	*Blastomyces percursus*	139878	<0.016	2	<0.016	0.031	0.031	0.031	0.25	0.031	Human, immunocompetent	Israel

[a]MIC/MEC values determined according to CLSI document M38-A2.
[b]AMB, amphotericin B; FLC, fluconazole; ITC, itraconazole; VOR, voriconazole; POS, posaconazole; ISA, isavuconazole; ANID, anidulafungin; MICA, micafungin.

the generic level. Overall, all MIC values of azole compounds except fluconazole were low. Amphotericin B was the most active antifungal agent and had the lowest MICs against species of *Adiaspiromyces*, *Emergomyces*, and *Blastomyces*. At the genus level, geometric mean (GM) MIC and MIC ranges of amphotericin B were, respectively, 0.027 and <0.016 to 0.125 μg/ml for *Adiaspiromyces*, 0.049 and <0.016 to 0.25 μg/ml for *Emergomyces*, and 0.023 and <0.016 to 0.063 μg/ml for *Blastomyces*. GM MIC values of itraconazole, voriconazole, posaconazole and isavuconazole were comparable to the values of amphotericin B (Table 2).

Anidulafungin had a low GM minimum effective concentration (MEC) and a MEC range of 0.68 and 0.031 to 2 μg/ml, respectively, for the genus *Emergomyces*. The GM MEC and MEC ranges were 0.18 and 0.016 to 1 μg/ml, respectively, for *Blastomyces*. Micafungin showed consistent low values with GM MEC and MEC ranges of 0.1 and 0.016 to 1 μg/ml, respectively, for *Emergomyces*. GM MEC and MEC ranges for *Blastomyces* were 0.017 and 0.016 to 0.063 μg/ml, respectively.

For all 11 strains of the newly described genus *Emergomyces*, posaconazole had the lowest GM MIC values followed by amphotericin B, itraconazole, voriconazole, isavuconazole, anidulafungin, and micafungin (Table 2).

We reported the antifungal susceptibility of 24 isolates from three genera within the *Ajellomycetaceae* family, *Adiaspiromyces*, *Blastomyces*, and *Emergomyces*. All three genera analyzed in this study sporulated slowly. This feature was also reported previously for *Paracoccidioides* (22), *Emergomyces africanus* (7), and *Emmonsia pasteuriana* (23). In contrast to *Blastomyces dermatitidis* and *B. gilchristii*, *Emergomyces* spp. do not consti-

TABLE 2 MIC/MEC range, GM MIC/MEC, MIC/MEC_{50}, and MIC/MEC_{90} values for *Adiaspiromyces*, *Emergomyces*, and *Blastomyces* to eight antifungal drugs

Genus (no. of strains)	Susceptibility parameter[b]	Susceptibility values for[a]:							
		AMB[c]	FLC	ITC	VOR	POS	ISA	ANID	MICA
Adiaspiromyces (4)	MIC/MEC range	<0.016–0.125	16–64	<0.016–0.031	<0.016–0.125	0.016–0.063	<0.016–0.125	2- 8	0.125–8
	GM MIC/MEC	0.027	22.6	0.026	0.05	0.026	0.088	4.75	0.29
Emergomyces (11)	MIC/MEC range	<0.016–0.25	8–>64	<0.016–0.25	0.063–0.25	<0.016–0.125	0.125–2	0.031–2	0.016–1
	GM MIC/MEC	0.049	46.7	0.063	0.133	0.043	0.414	0.68	0.1
	MIC/MEC_{50}	0.031	64	0.063	0.125	0.031	0.25	1	0.063
	MIC/MEC_{90}	0.125	64	0.125	0.25	0.063	2	2	1
Blastomyces (9)	MIC/MEC range	<0.016–0.063	2– >64	<0.016–0.125	<0.016–0.25	<0.016–0.063	0.125–0.5	0.016–1	0.016–0.063
	GM MIC/MEC	0.023	16	0.029	0.062	0.036	0.125	0.18	0.017

[a]MIC/MEC values determined according to CLSI document M38-A2.
[b]MIC values were determined for amphotericin B and azole drugs; MEC values were determined for anidulafungin and micafungin.
[c]Abbreviations are the same as in Table 1.

tute biosafety level 3 (BSL3) organisms, and therefore work was done in a class 2 biosafety cabinet. *Blastomyces* was handled, according to existing recommendations, in a BSL3 facility. Antifungal susceptibility testing of thermally dimorphic fungi is often limited to the mold phase, in which results may be misleading because the yeast phase is responsible for human disease. We limited antifungal susceptibility testing to the mold phase for the following reasons: (i) the pathogenic forms of these fungi differ greatly in the size and (ii) conversion time for some was very long and prone to contamination, with culturing for enough material taking longer than 1 month. Although it is a limitation that only the mold phase was tested, to our assurance recent studies of different endemic fungi using the mold and yeast phase for susceptibility testing found no differences in MICs between the two phases (8, 24).

This is the first comparative *in vitro* susceptibility study of five newly described taxa of dimorphic pathogens, *Emergomyces orientalis*, *Emergomyces canadensis*, *B. percursus*, *B. helicus*, and *B. silverae*, and of *B. parvus* and *B. gilchristii*. *In vitro* susceptibility studies of *Adiaspiromyces crescens*, *Emergomyces africanus*, *Emergomyces pasteurianus*, and *Blastomyces dermatitidis* have been performed previously (5, 8–10). For *Adiaspiromyces crescens*, our results confirm the previous findings of low MICs when the mold phase was tested, with MIC values of 0.06, 0.25, 0.06, and 64 μg/ml for amphotericin B, itraconazole, voriconazole, and fluconazole, respectively (5). Furthermore, the low MIC ranges of amphotericin B and azoles and high MIC range of fluconazole for *Emergomyces pasteurianus* and *Emergomyces africanus* were in agreement with previous findings (9, 10). In addition, the GM MIC, MIC_{50}, and MIC_{90} of *Emergomyces africanus* are in agreement with results described for 50 *E. africanus* strains, except for fluconazole and with slightly higher values for echinocandins (8).

Treatment of patients infected by members of the genus *Emergomyces* (formerly *Emmonsia*) has been done with amphotericin B deoxycholate (25), liposomal amphotericin B (26), combinations of amphotericin B and itraconazole (9, 10) or caspofungin (27), and voriconazole (28). In one case report, a patient infected with *Emergomyces canadensis* strain CBS 139873 (UAMH 10370) was treated with liposomal amphotericin B (S. Sanche et al., presented at Focus on Fungal Infections, Miami, FL, 2005, abstract 87). A patient suffering from rheumatoid arthritis who was infected with *Emergomyces europaeus* (*Emmonsia* sp.) was treated with oral itraconazole (11). A case of *Emergomyces orientalis* was treated with amphotericin B and itraconazole after shifting due to clinical failure with fluconazole (4). These successful results were in agreement with our *in vitro* findings for this genus.

Members of the genus *Blastomyces* had consistently low MIC/MEC values for amphotericin B, itraconazole, voriconazole, posaconazole, isavuconazole, and micafungin. Our results matched previously published data with low MIC ranges for itraconazole and high ranges for fluconazole (15). In a comparative study of 304 strains belonging

to *Blastomyces*, *Histoplasma*, and *Coccidioides*, amphotericin B, itraconazole, and voriconazole were effective *in vitro*, with the MIC_{90} being the lowest for itraconazole, followed by voriconazole and amphotericin B (16). We also report low anidulafungin GM MEC and MEC ranges for *Emergomyces* and *Blastomyces.* Micafungin exhibited potent activity against the mold phases of *Emergomyces* and *Blastomyces* but is weakly active against the yeast forms of *Histoplasma capsulatum*, *B. dermatitidis*, and *Coccidioides immitis* (17). In contrast there was no difference between mold and yeast phase testing of *Histoplasma capsulatum* for caspofungin (24). At present, based on *in vitro* susceptibility testing, it cannot be judged whether echinocandins (anidulafungin and micafungin) have clinical usefulness for dimorphic fungal infections, since for most fungi it remains uncertain which growth form correlates better with therapeutic outcome.

Our results with *Blastomyces* testing agree with recommended treatment of blastomycosis (18). Currently, there are no published treatment guidelines for patients with *Adiaspiromyces* and *Emergomyces*. Conidia of *Adiaspiromyces* do not multiply in host tissues, and it is generally a self-limiting disease. In few immunocompromised patients, antifungal treatment has been attempted apparently with successful outcome (29). According to published cases and international guidelines for the management of patients with dimorphic fungal infections (30, 31), the recommended treatment is amphotericin B, followed by an azole drug (either itraconazole or fluconazole). Our *in vitro* data are in agreement with these guidelines for amphotericin B and itraconazole but not for fluconazole when testing the mold phase. In conclusion, amphotericin B appears highly active against the mold phases of all tested strains within *Ajellomycetaceae* followed by posaconazole, itraconazole, voriconazole, and isavuconazole. The results of this study warrant further investigations of micafungin as a therapeutic agent for infections caused by dimorphic fungi.

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