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In Vitro Activities of Eight Antifungal Drugs against 55 Clinical Isolates of *Fonsecaea* spp.[▽]

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The *in vitro* activities of eight antifungal drugs against clinical isolates of *Fonsecaea pedrosoi* ($n = 21$), *Fonsecaea monophora* ($n = 25$), and *Fonsecaea nubica* ($n = 9$) were tested. The resulting MIC_{90}s for all strains ($n = 55$) were as follows, in increasing order: posaconazole, 0.063 μg/ml; itraconazole, 0.125 μg/ml; isavuconazole, 0.25 μg/ml; voriconazole, 0.5 μg/ml; amphotericin B, 2 μg/ml; caspofungin, 2 μg/ml; anidulafungin, 2 μg/ml; and fluconazole, 32 μg/ml.

Fonsecaea spp., anamorph members of the order *Chaetothyriales* (black yeasts and other melanized fungi), are principal agents of human chromoblastomycosis (16), a chronic cutaneous and subcutaneous infection characterized by slowly expanding skin lesions, a granulomatous immune response, and the presence of meristematic melanized muriform fungal cells in tissue scrapings (4). The last characteristic is a crucial diagnostic indicator that tends to be similar irrespective of the fungal pathogen. Chromoblastomycosis occurs worldwide in tropical and subtropical climates. *Fonsecaea* spp. are recoverable from environmental sources, so the disease is considered to be of traumatic origin (8, 9). The taxonomy of the genus *Fonsecaea* has been reviewed recently (12), and on the basis of sequence data, the following three species are recognized: *Fonsecaea pedrosoi*, *Fonsecaea monophora*, and *Fonsecaea nubica*. These species are morphologically identical, but their clinical spectra differ slightly: *F. pedrosoi* and *F. nubica* appear to be associated strictly with chromoblastomycosis, whereas *F. monophora* has also been isolated from brain abscesses, cervical lymph nodes, and bile (4, 13, 18).

Therapy for chromoblastomycosis is challenging because there is no consensus regarding the treatment of choice. Several treatment options have been applied, but these tend to result in protracted disease, low cure rates, and frequent relapses (5, 9, 10, 16, 18). The therapeutic outcomes are variable and are allegedly dependent on the site of infection, lesion size, the etiological agent, and the patient's health status (4). The specific identification of the causative pathogen is important for epidemiological reasons. The vast majority of cases of chromoblastomycosis in which the pathogen has been identified are caused by *F. pedrosoi*; for example, *F. pedrosoi* was isolated from 94% (66/69 cases) of patients with chromoblastomycosis in Sri Lanka (2) and from 98% (77/78 cases) of patients with culture-positive chromoblastomycosis in Brazil (17).

The present study aimed at determining the *in vitro* susceptibilities of clinical isolates of *Fonsecaea* spp. to seven marketed antifungal drugs and the experimental 1,2,4-triazole antimycotic isavuconazole (11).

Fifty-five *Fonsecaea* strains were obtained from the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) and comprised 21 *F. pedrosoi* strains, 25 *F. monophora* strains, and 9 *F. nubica* strains. Fifty isolates originated from patients with chromoblastomycosis, one isolate was recovered from a patient with a cerebral infection, two isolates were from diseased animals, and two isolates were clinical isolates from unknown sources. Seventeen strains came from southern China, 30 from South and Central America, and 8 from other countries (The Netherlands, Spain, Uruguay, Libya, France, United Kingdom). Strain identities were verified by sequencing the ribosomal internal transcribed spacer (ITS), tubulin (TUB1), and actin (ACT1) regions. *In vitro* susceptibility was determined as described in CLSI document M38-A2 (6). Briefly, the isolates were cultured on potato dextrose agar (35°C) for up to 7 days, and inocula were prepared by gently scraping the surface of the fungal colonies with a sterile cotton swab moistened with sterile physiological saline containing 0.05% Tween 40. Large particles in the cell suspensions were allowed to settle for 3 to 5 min at room temperature, and then the concentration of spores in the supernatant was adjusted spectrophotometrically (530 nm) to a percent transmission in the range 68 to 71, corresponding to 1.5×10^4 to 4×10^4 CFU/ml, as controlled by quantitative colony counts (6). Antifungal drugs were obtained as reagent-grade powders. The final concentrations of amphotericin B (AMB; Bristol-Myers Squibb, Woerden, The Netherlands), itraconazole (ITR; Janssen Research Foundation, Beerse, Belgium), voriconazole

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TABLE 1. Geometric mean MICs, MIC ranges, MIC_{50}s, and MIC_{90}s obtained by susceptibility testing of antimycotic agents against *Fonsecaea* isolates

Strain (no. of strains) and drug	MIC (μg/ml)			
	Geometric mean	Range	50%	90%
All *Fonsecaea* strains (n = 55)				
Amphotericin B	1.013	0.5–2	1	2
Fluconazole	19.08	8–64	16	32
Itraconazole	0.082	0.031–0.25	0.063	0.125
Voriconazole	0.29	0.125–1	0.25	0.5
Posaconazole	0.041	0.016–0.063	0.031	0.063
Isavuconazole	0.196	0.063–1	0.25	0.25
Caspofungin	2.15	1–4	2	2
Anidulafungin	3.43	1–8	4	2
Fonsecaea pedrosoi (n = 21)				
Amphotericin B	0.967	0.5–2	1	2
Fluconazole	22.25	8–32	32	32
Itraconazole	0.0817	0.031–0.25	0.063	0.125
Voriconazole	0.336	0.125–0.5	0.5	0.5
Posaconazole	0.0497	0.031–0.063	0.063	0.063
Isavuconazole	0.226	0.063–0.25	0.25	0.25
Caspofungin	2.43	2–4	4	4
Anidulafungin	3.5	2–8	8	8
Fonsecaea monophora (n = 25)				
Amphotericin B	1.11	0.5–2	1	2
Fluconazole	19.91	8–64	16	32
Itraconazole	0.0783	0.031–0.25	0.063	0.125
Voriconazole	0.257	0.125–1	0.063	0.125
Posaconazole	0.0369	0.016–0.063	0.031	0.063
Isavuconazole	0.184	0.063–1	0.125	0.25
Caspofungin	1.94	1–4	2	2
Anidulafungin	3.78	1–8	4	8
Fonsecaea nubica (n = 9)				
Amphotericin B	0.925	0.5–2	1	2
Fluconazole	18.66	16–32	16	32
Itraconazole	0.099	0.031–0.25	0.125	0.25
Voriconazole	0.314	0.25–0.5	0.25	0.5
Posaconazole	0.0362	0.031–0.063	0.031	0.063
Isavuconazole	0.17	0.063–0.5	0.125	0.5
Caspofungin	2.16	2–4	2	4
Anidulafungin	2.51	2–8	2	8

(VOR; Pfizer Central Research, Sandwich, United Kingdom), posaconazole (POS; Schering-Plough, Kenilworth, NJ), and caspofungin (CAS; Merck, Sharp & Dohme, Haarlem, The Netherlands) ranged from 0.016 to 16 μg/ml; the fluconazole (FLU; Pfizer) assay range was 0.063 to 64 μg/ml; and the isavuconazole (ISA; Basilea Pharmaceutica International AG, Basel, Switzerland) and anidulafungin (ANI; Pfizer) assay ranges were 0.008 to 8 μg/ml. After 72 h of incubation at 35°C, MICs and minimum effective concentrations (MECs) were determined visually by comparison of the growth in the wells containing the drug with the drug-free control. The MICs of AMB, ITR, VOR, POS, and ISA were defined as the lowest drug concentration that prevented any discernible growth (100% inhibition), whereas for FLU, the MIC was taken as the lowest concentration supporting ≥50% growth inhibition compared to the growth in the control wells. For CAS and ANI, MECs were determined microscopically as the lowest concentration of drug promoting the growth of small, round, compact hyphae relative to the appearance of the filamentous forms seen in the control wells. Quality control strains *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019), and *Candida krusei* (ATCC 6258) were included in each assay run.

The geometric mean MICs, MIC ranges, MIC_{50}s, and MIC_{90}s for the *Fonsecaea* isolates are presented in Table 1. For each drug-species pair, the MIC_{50} and geometric mean MIC values differed by <1 $\log_2$ dilution step, indicating that in all cases the MIC_{50} obtained by inspection reasonably reflected the central tendency of the antifungal susceptibility of the population. All isolates had low MICs (MIC_{90}s $\leq$ 0.5 μg/ml) for POS, ITR, ISA, and VOR; less active drugs (MIC_{90}s $\geq$ 2 μg/ml) were AMB, CAS, ANI, and FLU. There were no significant differences in the activities of the surveyed drugs against *F. pedrosoi*, *F. monophora*, and *F. nubica*. The MICs obtained in this study were similar to those obtained in other studies of *Fonsecaea* isolates (1, 3, 7, 14–16, 21).

Treatment of chromoblastomycosis is difficult. In cases caused by *Cladophialophora carrionii* and *Phialophora verrucosa*, patients generally respond well to relatively low doses of most antimycotics. The *in vitro* susceptibilities of *C. carrionii*

strains to antifungal drugs (20) were similar to those of the *Fonsecaea* spp. In this study, using unique clinical isolates of *Fonsecaea* from patients with chromoblastomycosis, we demonstrated differences in the activities of the compounds. ITR has frequently been used to treat chromoblastomycosis attributed to *Fonsecaea* spp., although elevated ITR MICs have been encountered in sequential isolates during ITR treatment (1).

POS is a new oral triazole that is used for the treatment of invasive fungal infections (19), including infections caused by the species associated with chromoblastomycosis (14). In the present study, POS had the lowest MICs among all the drugs examined, although the MIC_{90}s for ITR and ISA were only 1 and 2 $\log_2$ dilution steps higher, respectively. The experimental drug ISA possesses potent, broad-spectrum activity against the yeasts and molds implicated in serious mycoses (11). POS, ITR, ISA, and VOR all seem to be potential candidates for use for the treatment of chromoblastomycosis, whereas echinocandins will probably have only a limited role in treatment for this indication due to their relatively high MICs and the lack of oral formulations. However, the *in vitro* results presented here need to be confirmed in studies with the appropriate animal models of chromoblastomycosis.

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