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In Vitro Activity of Posaconazole against *Talaromyces marneffei* by Broth Microdilution and Etest Methods and Comparison to Itraconazole, Voriconazole, and Anidulafungin

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ABSTRACT We determined the susceptibilities of 57 *Talaromyces marneffei* strains to anidulafungin, itraconazole, voriconazole, and posaconazole with MICs of 2 to 8, 0.002 to 0.004, 0.016 to 0.063, and 0.001 to 0.002 μg/ml by broth microdilution and >32, ≤0.002 to 0.008, ≤0.002 to 0.008, and ≤0.002 μg/ml by Etest, respectively, at yeast phase; MICs at mycelial phase for anidulafungin and posaconazole were 1 to 2 and 0.004 to 0.063 μg/ml, respectively. The results suggest promising activities of posaconazole. Etest can be used for testing of azoles against *T. marneffei*.

KEYWORDS Etest, *Penicillium*, *Talaromyces marneffei*, anidulafungin, itraconazole, posaconazole, susceptibility, voriconazole

Talaromyces marneffei, previously named *Penicillium marneffei*, is the most important thermal dimorphic fungus causing systemic mycosis (penicilliosis) in southeast Asia (1–3). In recent decades, penicilliosis has emerged in HIV-infected and other immunocompromised patients in various countries (3–6). Since the disease carries a high mortality rate and is associated with relapse, finding ways to improve diagnosis and treatment is crucial (7, 8). Moderate or severe penicilliosis is often treated with amphotericin B for 2 weeks, followed by maintenance with prolonged itraconazole to prevent relapse. Primary prophylaxis with itraconazole in susceptible patients from areas in which the disease is endemic is also recommended.

T. marneffei is known to be susceptible *in vitro* to various antifungal agents, including amphotericin B, itraconazole, and terbinafine (9, 10). Besides itraconazole, voriconazole is an effective azole that is well tolerated for the treatment of penicilliosis (9, 11). Posaconazole, a triazole with broad antifungal activities, may offer additional advantages over itraconazole and voriconazole, especially in critically ill patients with organ dysfunction, because no renal or hepatic dosage adjustment is required. Although excellent *in vitro* activity of posaconazole against one strain of *T. marneffei* has been reported (12), no systematic evaluation on its activity against *T. marneffei* has been performed. While echinocandins are another new class of antifungals potentially effective against diverse fungi, only one report has described the potential activity of anidulafungin against three *T. marneffei* strains (13). Moreover, it remains uncertain if Etest is a reliable alternative to reference broth microdilution methods for *T. marneffei*

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susceptibility testing. Therefore, using broth microdilution and Etest methods, we attempted to assess the potential activities of posaconazole against *T. marneffei* compared to those of itraconazole, voriconazole, and anidulafungin.

A total of 57 nonduplicated *T. marneffei* strains, including 56 strains isolated from blood ($n = 48$), lymph node ($n = 1$), bone marrow ($n = 2$), skin biopsy specimen ($n = 1$), sputum ($n = 1$), or pleural ($n = 1$) or bronchoalveolar lavage ($n = 2$) fluid cultures of patients with culture-documented penicilliosis and the type strain ATCC 18224 isolated from a bamboo rat, were included. All *T. marneffei* strains were identified by conventional phenotypic tests and sequencing of three housekeeping genes (mannose phosphate isomerase [MPI], plasma membrane H^+ ATPase [PM-ATPase], and pyruvate kinase [PK]) and by matrix-assisted laser desorption ionization–time of flight mass spectrometry as described previously (7). Yeast and mycelial cultures of *T. marneffei* were grown on Sabouraud dextrose agar (SDA) (Oxoid, Cambridge, United Kingdom) as described previously with modifications (14). A single colony was inoculated into RPMI 1640 medium and incubated at 37°C for yeast cultures or 25°C for mycelial cultures. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as control strains (breakpoints of controls according to CLSI M27-S4 [15]) in each run.

Since no existing guidelines were available for susceptibility testing of *T. marneffei*, broth microdilution assays were performed according to CLSI methods for testing yeasts or filamentous fungi but with a prolonged incubation time of 72 h (yeast cultures) or 96 h (mycelial cultures) (16, 17). Briefly, stock solutions of anidulafungin (Pfizer, NY, USA), itraconazole (Janssen Pharmaceuticals, NJ, USA), voriconazole (Pfizer), and posaconazole (Schering-Plough, NJ, USA) were prepared from standard powder by dissolving them in 100% dimethyl sulfoxide (DMSO) and were serially 2-fold diluted in RPMI 1640 medium (Life Technologies, Carlsbad, CA, USA). Drugs were inoculated onto 96-well microplates, at 100 μl, followed by addition of 100 μl of *T. marneffei* suspension (1×10^3 to 5×10^3 CFU/ml for yeast and 0.4×10^4 to 5×10^4 CFU/ml for conidia) in RPMI 1640 medium, diluted from stock suspensions of 0.5 McFarland standard for yeasts, or 1.0 McFarland standard for conidia. Final drug concentration ranges were 0.001 to 2 μg/ml for itraconazole, 0.0005 to 0.5 μg/ml for voriconazole, 0.008 to 8 μg/ml for anidulafungin, and 0.0002 to 0.25 μg/ml for posaconazole. These figures were mathematically rounded to three or four digits after the decimal point for ease of interpretation. Etest was performed using RPMI 1640 agar with *T. marneffei* yeast suspensions of 0.5 McFarland standard according to manufacturer instructions (18). Etest using mycelial forms was not performed, because growth was too slow to allow testing. The following Etest strips were used: anidulafungin, itraconazole, posaconazole, and voriconazole (concentration ranges were 32 to 0.002 μg/ml for anidulafungin, itraconazole, posaconazole, and voriconazole) (bioMérieux, Marcy-l'Étoile, France). MICs or minimal effective concentrations (MECs) (for anidulafungin against mycelial cultures) were determined according to CLSI guidelines for the microdilution method (16, 17) and manufacturer instructions for the Etest (18). The percent MIC agreement ($\pm$2 2-fold dilutions) between broth microdilution and Etest was calculated as described previously (19). MICs determined by Etest that fell between the 2-fold dilutions of broth microdilution MICs were elevated to the next drug concentration so that they matched the 2-fold dilution scheme.

The MICs as determined by the broth microdilution and Etest methods are summarized in Table 1. All MICs of control strains were within the expected range for each strain. When the broth microdilution method was used, the MICs of the azoles against *T. marneffei* yeast phase were very low (ranges, 0.002 to 0.004, 0.016 to 0.063, and 0.001 to 0.002 μg/ml for itraconazole, voriconazole, and posaconazole, respectively). In contrast, the MICs of anidulafungin against *T. marneffei* yeast phase were much higher (2 to 8 μg/ml). The results suggest that the azoles itraconazole, voriconazole, and posaconazole possessed good activities against the 57 strains of *T. marneffei*, whereas anidulafungin had only moderate activities against *T. marneffei* at yeast phase. While yeast forms represent the infective stage of *T. marneffei in vivo* and are easier and safer to handle in clinical laboratories, we also attempted to assess the potential activities of

TABLE 1 *In vitro* MIC determinations of four antifungals against 57 *T. marneffei* strains as determined by broth microdilution and Etest methods

Antifungal and test method	MIC (μg/ml)[a]			% MIC agreement[b]
	Range	50%	90%	
Itraconazole				
Broth microdilution (yeast)	0.002–0.004	0.004	0.004	100
Etest (yeast)	≤0.002–0.008	0.004	0.006	
Voriconazole				
Broth microdilution (yeast)	0.016–0.063	0.031	0.063	31.58
Etest (yeast)	≤0.002–0.008	0.003	0.006	
Posaconazole				
Broth microdilution (mycelia)	0.004–0.063	0.016	0.031	
Broth microdilution (yeast)	0.001–0.002	0.002	0.002	100
Etest (yeast)	≤0.002	≤0.002	≤0.002	
Anidulafungin				
Broth microdilution (mycelia)	1–2[c]	1[c]	2[c]	
Broth microdilution (yeast)	2–8	4	8	24.56
Etest (yeast)	>32	>32	>32	

[a]50% and 90%, MICs that inhibited 50% and 90% of the strains tested, respectively.
[b]Percentage of Etest MICs in yeast phase that were within 2 $\log_2$ dilutions of the broth microdilution method MICs.
[c]MEC was used for anidulafungin against mycelial cultures according to CLSI guidelines (M38-A2).

posaconazole and anidulafungin against mycelial forms; we found MIC ranges of 0.004 to 0.063 μg/ml and MEC ranges of 1 to 2 μg/ml, respectively.

The Etest method revealed that the MICs of the azoles against *T. marneffei* yeasts were also very low (ranges, ≤0.002 to 0.008 μg/ml for itraconazole and voriconazole and ≤0.002 μg/ml for posaconazole). In contrast to the moderate activities of anidulafungin demonstrated by broth microdilution assays, the MICs of anidulafungin by Etest against all *T. marneffei* strains were >32 μg/ml. The values for percent agreement between the MICs by broth microdilution and the MICs by Etest against yeast forms are shown in Table 1. For itraconazole and posaconazole, 100% agreement within two 2-fold dilutions was found. However, the percent agreement was lower for voriconazole (31.58%) and anidulafungin (24.56%). Despite the low percent agreement for voriconazole, both methods demonstrated very low MICs, supporting the usefulness of Etest for testing the azoles.

The present results show promising activities of posaconazole against the yeast and mycelial phases of *T. marneffei*. Moreover, according to the broth microdilution and Etest methods, the MIC_{50} of posaconazole against *T. marneffei* yeasts was the lowest among the three tested azoles. While fluconazole is known to have reduced activities against *T. marneffei* yeasts (MIC range, 4 to 8 μg/ml), itraconazole and voriconazole usually possess good activities, with MICs usually <1 μg/ml (9, 10, 20, 21). However, MICs as high as 50 μg/ml have been reported for itraconazole against yeast forms of *T. marneffei* (22). While systematic studies evaluating the activities of posaconazole were lacking, the present results are in line with those described in a previous case report in which the potential activity of posaconazole (MIC, 0.001 μg/ml) against the yeast phase of a *T. marneffei* strain isolated from a Greek HIV-infected patient with travel history to China was shown (12). As for the mycelial phase of *T. marneffei*, posaconazole also exhibited good activities with very low MICs, consistent with previous findings on itraconazole (21).

Etest methods may serve as a more convenient alternative to broth dilution for susceptibility testing of azoles against *T. marneffei*. However, the performance of Etest for determining the activities of anidulafungin and possibly other echinocandins remains doubtful. Moreover, the apparent resistance of *T. marneffei* yeasts to anidulafungin by Etest and the relatively high MICs of anidulafungin by broth microdilution against both yeast and mycelial forms suggest that anidulafungin is likely less active

than the azoles against *T. marneffei*. In one of the few published studies on the activities of echinocandins against *T. marneffei*, the authors claimed promising activities of anidulafungin against three *T. marneffei* strains in mycelial phase, with MICs ranging from 0.5 to 2 μg/ml (13). In another report, micafungin possessed low activities, with MICs ranging from 4 to 16 μg/ml for yeast forms and 0.313 to 2 μg/ml for mycelial forms of *T. marneffei* (21). Although the MICs by broth microdilution against mycelial phase were lower than those against yeast phase in this study, the values were still relatively high compared to those of the azoles. Further studies should be performed to better assess the potential activities of echinocandins for treatment of penicilliosis.

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