





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

Susanna K. P. Lau,<sup>a,b,c,d</sup>  George C. S. Lo,<sup>d</sup> Clare S. K. Lam,<sup>e</sup>  Wang-Ngai Chow,<sup>d</sup> Antonio H. Y. Ngan,<sup>d</sup> Alan K. L. Wu,<sup>f</sup> Dominic N. C. Tsang,<sup>g</sup> Cindy W. S. Tse,<sup>h</sup> Tak-Lun Que,<sup>i</sup> Bone S. F. Tang,<sup>e</sup> Patrick C. Y. Woo<sup>a,b,c,d</sup>

State Key Laboratory of Emerging Infectious Diseases,<sup>a</sup> Research Centre of Infection and Immunology,<sup>b</sup> Carol Yu Centre for Infection,<sup>c</sup> and Department of Microbiology,<sup>d</sup> The University of Hong Kong, Hong Kong, China; Hong Kong Sanatorium Hospital, Hong Kong, China<sup>e</sup>; Department of Pathology, Pamela Youde Nethersole Eastern Hospital, Hong Kong, China<sup>f</sup>; Department of Pathology, Queen Elizabeth Hospital, Hong Kong, China<sup>g</sup>; Department of Pathology, Kwong Wah Hospital, Hong Kong, China<sup>h</sup>; Department of Pathology, Tuen Mun Hospital, Hong Kong, China<sup>i</sup>

**ABSTRACT** We determined the susceptibilities of 57 *Talaromyces marneffe* 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  $\mu\text{g/ml}$  by broth microdilution and  $>32$ ,  $\leq 0.002$  to 0.008,  $\leq 0.002$  to 0.008, and  $\leq 0.002$   $\mu\text{g/ml}$  by Etest, respectively, at yeast phase; MICs at mycelial phase for anidulafungin and posaconazole were 1 to 2 and 0.004 to 0.063  $\mu\text{g/ml}$ , respectively. The results suggest promising activities of posaconazole. Etest can be used for testing of azoles against *T. marneffe*.

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

*Talaromyces marneffe*, previously named *Penicillium marneffe*, 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. marneffe* 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. marneffe* has been reported (12), no systematic evaluation on its activity against *T. marneffe* 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. marneffe* strains (13). Moreover, it remains uncertain if Etest is a reliable alternative to reference broth microdilution methods for *T. marneffe*

Received 9 July 2016 Returned for modification 17 August 2016 Accepted 19 December 2016

Accepted manuscript posted online 28 December 2016

**Citation** Lau SKP, Lo GCS, Lam CSK, Chow W-N, Ngan AHY, Wu AKL, Tsang DNC, Tse CWS, Que T-L, Tang BSF, Woo PCY. 2017. *In vitro* activity of posaconazole against *Talaromyces marneffe* by broth microdilution and Etest methods and comparison to itraconazole, voriconazole, and anidulafungin. Antimicrob Agents Chemother 61:e01480-16. <https://doi.org/10.1128/AAC.01480-16>.

**Copyright** © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Susanna K. P. Lau, [skplau@hku.hk](mailto:skplau@hku.hk), or Patrick C. Y. Woo, [pcywoo@hku.hk](mailto:pcywoo@hku.hk).

S.K.P.L., G.C.S.L., and C.S.K.L. contributed equally to this article.

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<sup>+</sup> 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  $\mu$ l, followed by addition of 100  $\mu$ l of *T. marneffei* suspension ( $1 \times 10^3$  to  $5 \times 10^3$  CFU/ml for yeast and  $0.4 \times 10^4$  to  $5 \times 10^4$  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  $\mu$ g/ml for itraconazole, 0.0005 to 0.5  $\mu$ g/ml for voriconazole, 0.008 to 8  $\mu$ g/ml for anidulafungin, and 0.0002 to 0.25  $\mu$ g/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  $\mu$ g/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  $\mu$ g/ml for itraconazole, voriconazole, and posaconazole, respectively). In contrast, the MICs of anidulafungin against *T. marneffei* yeast phase were much higher (2 to 8  $\mu$ g/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. marneffe* strains as determined by broth microdilution and Etest methods

Antifungal and test method	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>			% MIC agreement <sup>b</sup>
	Range	50%	90%	
<b>Itraconazole</b>				
Broth microdilution (yeast)	0.002–0.004	0.004	0.004	100
Etest (yeast)	$\leq 0.002$ –0.008	0.004	0.006	
<b>Voriconazole</b>				
Broth microdilution (yeast)	0.016–0.063	0.031	0.063	31.58
Etest (yeast)	$\leq 0.002$ –0.008	0.003	0.006	
<b>Posaconazole</b>				
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)	$\leq 0.002$	$\leq 0.002$	$\leq 0.002$	
<b>Anidulafungin</b>				
Broth microdilution (mycelia)	1–2 <sup>c</sup>	1 <sup>c</sup>	2 <sup>c</sup>	
Broth microdilution (yeast)	2–8	4	8	24.56
Etest (yeast)	>32	>32	>32	

<sup>a</sup>50% and 90%, MICs that inhibited 50% and 90% of the strains tested, respectively.

<sup>b</sup>Percentage of Etest MICs in yeast phase that were within 2 log<sub>2</sub> dilutions of the broth microdilution method MICs.

<sup>c</sup>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  $\mu\text{g/ml}$  and MEC ranges of 1 to 2  $\mu\text{g/ml}$ , respectively.

The Etest method revealed that the MICs of the azoles against *T. marneffe* yeasts were also very low (ranges,  $\leq 0.002$  to 0.008  $\mu\text{g/ml}$  for itraconazole and voriconazole and  $\leq 0.002$   $\mu\text{g/ml}$  for posaconazole). In contrast to the moderate activities of anidulafungin demonstrated by broth microdilution assays, the MICs of anidulafungin by Etest against all *T. marneffe* strains were >32  $\mu\text{g/ml}$ . 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. marneffe*. Moreover, according to the broth microdilution and Etest methods, the MIC<sub>50</sub> of posaconazole against *T. marneffe* yeasts was the lowest among the three tested azoles. While fluconazole is known to have reduced activities against *T. marneffe* yeasts (MIC range, 4 to 8  $\mu\text{g/ml}$ ), itraconazole and voriconazole usually possess good activities, with MICs usually <1  $\mu\text{g/ml}$  (9, 10, 20, 21). However, MICs as high as 50  $\mu\text{g/ml}$  have been reported for itraconazole against yeast forms of *T. marneffe* (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  $\mu\text{g/ml}$ ) against the yeast phase of a *T. marneffe* strain isolated from a Greek HIV-infected patient with travel history to China was shown (12). As for the mycelial phase of *T. marneffe*, 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. marneffe*. However, the performance of Etest for determining the activities of anidulafungin and possibly other echinocandins remains doubtful. Moreover, the apparent resistance of *T. marneffe* 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  $\mu\text{g/ml}$  (13). In another report, micafungin possessed low activities, with MICs ranging from 4 to 16  $\mu\text{g/ml}$  for yeast forms and 0.313 to 2  $\mu\text{g/ml}$  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.

## ACKNOWLEDGMENTS

This work is partly supported by the Research Grants Council Grant, University Grant Council, by the Committee for Research and Conference Grant, Strategic Research Theme Fund, by the University Development Fund, The University of Hong Kong, by the HKSAR Health and Medical Research Fund (commissioned study) (grant HKM-15-M07) of the Food and Health Bureau, Government of the Hong Kong Special Administrative Region, Hong Kong, by Croucher Senior Medical Research Fellowships, by the Shaw Foundation, and by Providence Foundation Limited, in memory of the late Dr. Lui Hac Minh.

We declare no conflicts of interest.

## REFERENCES

- Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. 1994. Disseminated *Penicillium marneffei* infection in southeast Asia. *Lancet* 344:110–113. [https://doi.org/10.1016/S0140-6736\(94\)91287-4](https://doi.org/10.1016/S0140-6736(94)91287-4).
- Yuen KY, Wong SS, Tsang DN, Chau PY. 1994. Serodiagnosis of *Penicillium marneffei* infection. *Lancet* 344:444–445. [https://doi.org/10.1016/S0140-6736\(94\)91771-X](https://doi.org/10.1016/S0140-6736(94)91771-X).
- Vanittanakom N, Jr, Cooper CR, Fisher MC, Sirisanthana T. 2006. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev* 19:95–110. <https://doi.org/10.1128/CMR.19.1.95-110.2006>.
- Sekhon AS, Stein L, Garg AK, Black WA, Glezos JD, Wong C. 1994. Pulmonary penicilliosis marneffei: report of the first imported case in Canada. *Mycopathologia* 128:3–7. <https://doi.org/10.1007/BF01104271>.
- Woo PC, Lau SK, Lau CC, Chong KT, Hui WT, Wong SS, Yuen KY. 2005. *Penicillium marneffei* fungaemia in an allogeneic bone marrow transplant recipient. *Bone Marrow Transplant* 35:831–833. <https://doi.org/10.1038/sj.bmt.1704895>.
- Chan JFW, Lau SKP, Yuen KY, Woo PCY. 2016. *Talormyces (Penicillium) marneffei* infection in non-HIV-infected patients. *Emerg Microbes Infect* 5:e19. <https://doi.org/10.1038/emi.2016.18>.
- Lau SKP, Lam CSK, Ngan AHY, Chow WN, Wu AKL, Tsang DNC, Tse CWS, Que TL, Tang BSF, Woo PCY. 2016. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid identification of mold and yeast cultures of *Penicillium marneffei*. *BMC Microbiol* 16:36. <https://doi.org/10.1186/s12866-016-0656-0>.
- Chen YS, Liu YH, Teng SH, Liao CH, Hung CC, Sheng WH, Teng LJ, Hsueh PR. 2015. Evaluation of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry Bruker biotyper for identification of *Penicillium marneffei*, *Paecilomyces* species, *Fusarium solani*, *Rhizopus* species, and *Pseudallescheria boydii*. *Front Microbiol* 6:679. <https://doi.org/10.3389/fmicb.2015.00679>.
- Liu D, Liang L, Chen J. 2013. In vitro antifungal drug susceptibilities of *Penicillium marneffei* from China. *J Infect Chemother* 19:776–778. <https://doi.org/10.1007/s10156-012-0511-7>.
- McGinnis MR, Nordoff NG, Ryder NS, Nunn GB. 2000. In vitro comparison of terbinafine and itraconazole against *Penicillium marneffei*. *Antimicrob Agents Chemother* 44:1407–1408. <https://doi.org/10.1128/AAC.44.5.1407-1408.2000>.
- Supparatpinyo K, Schlämm HT. 2007. Voriconazole as therapy for systemic *Penicillium marneffei* infections in AIDS patients. *Am J Trop Med Hyg* 77:350–353.
- Filiotou A, Velegriaki A, Giannaris M, Pirounaki M, Mitroussia A, Kaloterakis A, Archimandritis A. 2006. First case of *Penicillium marneffei* fungemia in Greece and strain susceptibility to five licensed systemic antifungal agents and posaconazole. *Am J Med Sci* 332:43–45. <https://doi.org/10.1097/0000441-200607000-00009>.
- Odabasi Z, Paetznick VL, Rodriguez JR, Chen E, Ostrosky-Zeichner L. 2004. In vitro activity of anidulafungin against selected clinically important mold isolates. *Antimicrob Agents Chemother* 48:1912–1915. <https://doi.org/10.1128/AAC.48.5.1912-1915.2004>.
- Woo PC, Lam CW, Tam EW, Leung CK, Wong SS, Lau SK, Yuen KY. 2012. First discovery of two polyketide synthase genes for mitorubrinic Acid and mitorubrinol yellow pigment biosynthesis and implications in virulence of *Penicillium marneffei*. *PLoS Negl Trop Dis* 6:e1871. <https://doi.org/10.1371/journal.pntd.0001871>.
- Clinical and Laboratory Standards Institute. 2012. Reference method for broth dilution antifungal susceptibility testing of yeasts; fourth informational supplement. M27–S4. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. 3rd ed. M27–A3. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. 2nd ed. M38–A2. Clinical and Laboratory Standards Institute, Wayne, PA.
- AB BIODISK. 2000. Etest for antifungal susceptibility testing package insert edition 1.
- Lau SKP, Wong GKM, Poon RWS, Lee LC, Leung KW, Que TL, Woo PCY, Yuen KY. 2009. Susceptibility patterns of clinical and fish isolates of *Laribacter hongkongensis*: comparison of the E-test, disc diffusion and broth microdilution methods. *J Antimicrob Chemother* 63:704–708. <https://doi.org/10.1093/jac/dkp010>.
- Imwidthaya P, Thipsuvan K, Chaiprasert A, Danchaiwajitra S, Sutthent R, Jearanaisilavong J. 2001. *Penicillium marneffei*: types and drug susceptibility. *Mycopathologia* 149:109–115. <https://doi.org/10.1023/A:1007245226495>.
- 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. <https://doi.org/10.1128/AAC.47.4.1376-1381.2003>.
- Sekhon AS, Garg AK, Padhye AA, Hamir Z. 1993. In vitro susceptibility of mycelial and yeast forms of *Penicillium marneffei* to amphotericin B, fluconazole, 5-fluorocytosine and itraconazole. *Eur J Epidemiol* 9:553–558.