European Journal of Clinical Microbiology & Infectious Diseases (2018) 37:1099–1102
https://doi.org/10.1007/s10096-018-3222-x

ORIGINAL ARTICLE

Susceptibility profile of echinocandins, azoles and amphotericin B against yeast phase of *Talaromyces marneffei* isolated from HIV-infected patients in Guangdong, China

H.-L. Lei[1] · L.-H. Li[2] · W.-S. Chen[3] · W.-N. Song[3] · Y. He[4] · F.-Y. Hu[1] · X.-J. Chen[2] · W.-P. Cai[2] · X.-P. Tang[1]

Received: 12 November 2017 / Accepted: 26 February 2018 / Published online: 13 March 2018

Abstract

Talaromyces marneffei (*T. marneffei*) can cause talaromycosis, a fatal systemic mycosis, in patients with AIDS. With the increasing number of talaromycosis cases in Guangdong, China, we aimed to investigate the susceptibility of 189 *T. marneffei* clinical strains to eight antifungal agents, including three echinocandins (anidulafungin, micafungin, and caspofungin), four azoles (posaconazole, itraconazole, voriconazole, and fluconazole), and amphotericin B, with determining minimal inhibition concentrations (MIC) by Sensititre YeastOne™ YO10 assay in the yeast phase. The MICs of anidulafungin, micafungin, caspofungin, posaconazole, itraconazole, voriconazole, fluconazole, and amphotericin B were 2 to >8 μg/ml, >8 μg/ml, 2 to >8 μg/ml, ≤0.008 to 0.06 μg/ml, ≤0.015 to 0.03 μg/ml, ≤0.008 to 0.06 μg/ml, 1 to 32 μg/ml, and ≤0.12 to 1 μg/ml, respectively. The MICs of all echinocandins were very high, while the MICs of posaconazole, itraconazole, and voriconazole, as well as amphotericin B were comparatively low. Notably, fluconazole was found to have a higher MIC than other azoles, and exhibited particularly weak activity against some isolates with MICs over 8 μg/ml. Our data in vitro support the use of amphotericin B, itraconazole, voriconazole, and posaconazole in management of talaromycosis and suggest potential resistance to fluconazole.

Keywords Antifungal susceptibility · *Talaromyces marneffei* · HIV positive · Echinocandin · Azole · Sensititre YeastOne

Introduction

Talaromyces marneffei (formerly *Penicillium marneffei*) is a life-threatening thermal dimorphic fungus endemic to tropical Southeast Asian regions and is the etiological agent of talaromycosis [1]. While primarily affecting people living with HIV, *T. marneffei* case reports have recently been described in HIV-negative, immunocompromised individuals [2–4]. *T. marneffei* has become one of the three most common opportunistic pathogens in Southeast Asia [5] and is the most frequent blood-borne fungus in individuals diagnosed with AIDS living in Southern China [6]. Talaromycosis brings about a substantial public health threat in endemic areas and is now beginning to affect other regions [7].

Talaromycosis is recommended to be treated with amphotericin B for 2 weeks, followed by itraconazole for 10 weeks and then as continuous secondary prophylaxis until $CD4^+$ counts remain above 100 cells/mm^3 for 6 months [8]. However, therapy with amphotericin B and itraconazole can be limited due to drug toxicity and cost. In

H.-L. Lei and L.-H. Li contributed equally to this work.

✉ X.-P. Tang
tangxiaopinggz@163.com

[1] Research Institute of Infectious Diseases, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong Province 510060, People's Republic of China

[2] Department of Infectious Diseases, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong Province 510060, People's Republic of China

[3] Department of Clinical Laboratory, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong Province 510060, People's Republic of China

[4] Department of Medical Ultrasonics, Guangzhou First People's Hospital, Guangzhou, Guangdong Province 510060, People's Republic of China

these cases voriconazole may be a suitable alternative [9]. In addition, fluconazole is an alternative treatment agent in many economically developing countries, including China [10]. Recently, echinocandins have been introduced as first-line drugs to treat invasive candidiasis [11], but whether this class of antifungal agents possesses activity against *T. marneffei* has not been fully investigated. Posaconazole, a newly approved triazole antifungal drug by FDA for treating invasive infections with *Candida*, *Mucor*, and *Aspergillus* in severely immunocompromised patients [12], may also be of significance for possessing activity against *T. marneffei*. Despite there are some other influence factors, early initiation of therapy and selection of empiric antifungal agents according to local susceptibility are greatly important for improving clinical outcomes in systemic fungal infections. In order to investigate the susceptibility profile of *T. marneffei* strains against eight agents, the Sensititre YeastOne™ method, a ready-to-use commercial kit, is adopted and 189 *T. marneffei* clinical strains isolated from HIV-infected patients from January 2013 to December 2016 in Guangdong is determined through their MICs.

Materials and methods

T. marneffei strains

Out of the 189 strains, 41, 39, 38, and 71 strains were collected during 2013, 2014, 2015, and 2016, respectively. The collected specimens included were the blood, bone marrow, and bronchoalveolar lavage fluid. All isolates were identified by conventional culture and microscopic characteristics before being stored at − 80 °C. Internal transcribed spacer (ITS) were amplified with PCR and detected with direct DNA sequencing to determine the species [13].

Antifungal susceptibility testing

Sensititre YeastOne ™ YO10 assay (Thermo Scientific, Cleveland, OH, USA) was conducted to test antifungal susceptibility according to manufacturer's instructions. The disposable Sensititre panels, which are 96-well plates incorporated with alamarBlue for colorimetric determination, contain serial twofold dilutions of the following dried antifungal agents: 0.015 to 8 μg/ml of anidulafungin, 0.008 to 8 μg/ml of micafungin and caspofungin, 0.008 to 8 μg/ml of posaconazole and voriconazole, 0.015 to 16 μg/ml of itraconazole, 0.12 to 256 μg/ml of fluconazole, and 0.12 to 8 μg/ml of amphotericin B.

Prior to testing, strains were sub-cultured onto Sabouraud dextrose agar and incubated at 37 °C for 4 days. Then, a 0.5 McFarland yeast suspension of each strain was prepared using the spectrophotometric method, with final concentrations of $1.5–8 \times 10^3$ CFU/ml. One hundred microliter of the yeast suspension was dispensed into each well of the dried panels for rehydration. Finally, the panels were enclosed with adhesive seals and incubated at 35 °C in a non-CO_2 atmosphere. Quality control strains of *Candida parapsilosis* ATCC® 22019 were included throughout the experiments. MIC endpoints were read visually when the color had obviously changed from blue (with growth) to red (without growth) after incubation.

Results

Triazoles were found to have low MICs against the yeast phase of most isolated *T. marneffei* in vitro, most strains (> 88%) for posaconazole, itraconazole, and voriconazole with MICs ≤ 0.015 μg/ml, and 80.4% strains were found to have MICs≤4 μg/ml for fluconazole. Posaconazole MIC values ranged from ≤ 0.008 to 0.06 μg/ml, the lowest MIC (geometric mean value, 0.013 μg/ml) of all tested azoles, followed by voriconazole and itraconazole. Fluconazole had the highest MIC (4.074 μg/ml) of all tested azoles, and most remarkable is that two (1%) strains were found to have MICs of 16 and 32 μg/ml, respectively. Moreover, the results revealed that the antifungal activity of triazoles was superior to echinocandins against the yeast form of *T. marneffei*. Echinocandins had comparatively lower or almost no activity against *T. marneffei* in vitro. The MICs against all strains were > 8 μg/ml for micafungin and ≥ 2 μg/ml for caspofungin and anidulafungin (not shown). Besides, all strains had MICs ≤ 1 μg/ml for amphotericin B. An overview is shown in Table 1.

Discussion

Talaromycosis, a disease with multiple clinical manifestations and high rates of morbidity and mortality, has increasing incidence among HIV-infected patients [14]. However, data describing antifungal susceptibility of *T. marneffei* is limited [15, 16], and no corresponding data has been previously reported from Guangdong Province. In this study, 189 clinical isolations from patients diagnosed with talaromycosis in the referral hospital of Guangdong Province were included.

Sensititre YeastOne TM YO10 assay has been evidenced to be with high agreement in testing *Candida* compared with broth dilution method and Etest [17]. Our protocols of Sensititre method were modified from those standardized by CLSI for the determination of antifungal susceptibilities in *Candida* species [18], which represent the first time this method has been used to evaluate antifungal susceptibilities of *T. marneffei*. Based on the results observed in our study, we

Table 1 In vitro MICs of 189 *T.marneffei* strains to five antifungals as determined by YeastOne method

Antifungal agent	No. of isolates with MIC (μg/ml)												Geometric mean (μg/ml)
	≤0.015	0.03	0.06	≤0.12	0.25	0.5	1	2	4	8	16	32	
Posaconazole	188		1										0.013
Voriconazole	168	15	6										0.016
Itraconazole	182	7											0.024
fluconazole							5	35	112	33	2	2	4.074
Amphoterocin				5	31	119	34						0.501

can believe this method is suitable to detect the susceptibility of the yeast phase of *T. marneffei*. Past research had shown echinocandins activity against *T. marneffei* differs between the yeast and mycelial phases [16, 19]. Anidulafungin exhibits potential activity (MIC ≤ 2 μg/ml) against the mycelial phase of *T. marneffei* [20], while MICs against the yeast phase of 57 *T. marneffei* strains are over 2 μg/ml [16]. Our findings support previous research demonstrating the limited antifungal activity of anidulafungin against the yeast phase of *T. marneffei*, the parasitic form that can be detected under regular clinical laboratory conditions [21]. Our results also suggest caspofungin and micafungin have similar activity as anidulafungin. *T. marneffei* may well be resistant to the entire echinocandin class while in its yeast phase; however, the relationship between in vitro susceptibility of the yeast phase and clinical outcomes should be further investigated. In contrast to echinocandins, the yeast forms of all *T. marneffei* strains were inhibited by itraconazole and voriconazole. Posaconazole, as another triazole, had been previously used for clinical treatment of other dimorphic fungi infections, including *H. capsulatum* and *Coccidioides* [22, 23]. And it was also observed to have low MICs against the *T. marneffei* strains in our study, which was close to the results released by Lau [16]. Those may collectively indicate posaconazole is a promising agent for treatment of talaromycosis. Furthermore, amphotericin B also possessed good activity against included strains with MICs in the susceptible range. The vast majority of *T. marneffei* strains were sensitive to fluconazole with MICs ≤ 8 μg/ml, but it is noteworthy that four clinical isolates were found to not be within the susceptible MIC range. Additionally, the surprising is the MICs detected by different laboratory are various greatly, we postulate this discrepancy may be due to the absence of standardized protocols for testing.

In brief, our data support the use of amphotericin B, itraconazole, voriconazole, and posaconazole in clinical management of talaromycosis, meanwhile alarm for the potential resistance to fluconazole. However, since clinical breakpoints or epidemiological cutoff values of antifungal agents against *T. marneffei* have not been developed, we only discriminated them as suspected resistant strains according to the reference of *Candida*. Aiming to accurately distinguish resistant and wild-type *T. marneffei* strains, further studies in multiple settings will be required.

Acknowledgements We thank Thomas Fitzpatrick (University of Washington School of Medicine) for the language organization and editing.

Funding The study was supported by the National Natural Science Foundation of China (Grant No. 81301480).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement and informed consent This study was approved by the institutional review board of Guangzhou Eighth People's Hospital, which contained only sub-cultured clinical *T. marneffei* isolates and all patient information were anonymised. Informed consent was not required in this work.

References

1. Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T (2006) Penicillium marneffei infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev 19(1): 95–110. https://doi.org/10.1128/CMR.19.1.95-110.2006
2. Chan JF, Lau SK, Yuen KY, Woo PC (2016) Talaromyces (Penicillium) marneffei infection in non-HIV-infected patients. Emerg Microbes Infect 5:e19. https://doi.org/10.1038/emi.2016.18
3. Peng J, Chen Z, Cai R, Huang X, Lin L, Liang W, Xiong Z, Chen J, Chen H, Yang Y, Liu S, Jiang Q (2017) Recovery from Talaromyces marneffei involving the kidney in a renal transplant recipient: a case report and literature review. Transpl Infect Dis 19(4). https://doi.org/10.1111/tid.12710
4. Luo DQ, Chen MC, Liu JH, Li Z, Li HT (2011) Disseminated Penicillium marneffei infection in an SLE patient: a case report and literature review. Mycopathologia 171(3):191–196. https://doi.org/10.1007/s11046-010-9363-9
5. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, Lam PS, Kozal MJ, Shikuma CM, Day JN, Farrar J (2011) Epidemiology, seasonality, and predictors of outcome of AIDS-associated Penicillium marneffei infection in Ho Chi Minh City, Viet Nam. Clin Infect Dis 52(7):945–952. https://doi.org/10.1093/cid/cir028

6. Nong S, Liang J (2013) Bone marrow Penicillium marneffei infection in acquired immunodeficiency syndrome patients: report of 35 cases. Trop Biomed 30(1):89–91
7. Cao C, Liang L, Wang W, Luo H, Huang S, Liu D, Xu J, Henk DA, Fisher MC (2011) Common reservoirs for Penicillium marneffei infection in humans and rodents, China. Emerg Infect Dis 17(2): 209–214. https://doi.org/10.3201/eid1702.100718
8. Masur H, Brooks JT, Benson CA, Holmes KK, Pau AK, Kaplan JE, National Institutes of H, Centers for Disease C, Prevention, America HIVMAotIDSo (2014) Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: updated guidelines from the Centers for Disease Control and Prevention, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 58(9):1308–1311. https://doi.org/10.1093/cid/ciu094
9. Ouyang Y, Cai S, Liang H, Cao C (2017) Administration of voriconazole in disseminated Talaromyces (Penicillium) marneffei infection: a retrospective study. Mycopathologia 182(5–6):569–575. https://doi.org/10.1007/s11046-016-0107-3
10. Li HR, Cai SX, Chen YS, Yu ME, Xu NL, Xie BS, Lin M, Hu XL (2016) Comparison of Talaromyces marneffei infection in human immunodeficiency virus-positive and human immunodeficiency virus-negative patients from Fujian, China. Chin Med J 129(9): 1059–1065. https://doi.org/10.4103/0366-6999.180520
11. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD (2016) Clinical practice guideline for the Management of Candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 62(4):e1–50. https://doi.org/10.1093/cid/civ933
12. Clark NM, Grim SA, Lynch JP 3rd (2015) Posaconazole: use in the prophylaxis and treatment of fungal infections. Semin Respir Crit Care Med 36(5):767–785. https://doi.org/10.1055/s-0035-1562902
13. Vanittanakom N, Vanittanakom P, Hay RJ (2002) Rapid identification of Penicillium marneffei by PCR-based detection of specific sequences on the rRNA gene. J Clin Microbiol 40(5):1739–1742. https://doi.org/10.1128/JCM.40.5.1739-1742.2002
14. Chaiwarith R, Supparatpinyo K (2016) Talaromyces (Penicillium) marneffei and HIV.1–9. https://doi.org/10.1007/978-1-4614-9610-6_419-1
15. Liu D, Liang L, Chen J (2013) In vitro antifungal drug susceptibilities of Penicillium marneffei from China. J Infect Chemother 19(4):776–778. https://doi.org/10.1007/s10156-012-0511-7
16. Lau SKP, Lo GCS Lam CS, Chow WN, Ngan AH, Wu AK, Tsang DN, Tse CW, Que TL, Tang BS, Woo PC (2017) In vitro activity of posaconazole against Talaromyces marneffei by broth microdilution and Etest methods and comparison to itraconazole, voriconazole, and anidulafungin. Antimicrob Agents Chemother 61 (3). https://doi.org/10.1128/AAC.01480-16
17. Alexander BD, Byrne TC, Smith KL, Hanson KE, Anstrom KJ, Perfect JR, Reller LB (2007) Comparative evaluation of Etest and sensititre yeastone panels against the clinical and laboratory standards institute M27-A2 reference broth microdilution method for testing Candida susceptibility to seven antifungal agents. J Clin Microbiol 45(3):698–706. https://doi.org/10.1128/JCM.01840-06
18. Clinical and Laboratory Standards Institute (2013) Reference method for broth dilution antifungal susceptibility testing of yeasts. Fourth Informat Suppl 32:1–23
19. 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(4):1376–1381. https://doi.org/10.1128/aac.47.4.1376-1381.2003
20. Zekaver O, Paetznick VL, Rodriguez JR, Enuo C, Ostrosky-Zeichner L (2004) In vitro activity of anidulafungin against selected clinically important mold isolates. Antimicrob Agents Chemother 48(5):1912–1915. https://doi.org/10.1128/AAC.48.5.1912-1915.2003
21. Sapmak A, Kaewmalakul J, Nosanchuk JD, Vanittanakom N, Andrianopoulos A, Pruksaphon K, Youngchim S (2016) Talaromyces marneffei laccase modifies THP-1 macrophage responses. Virulence 7(6):702–717. https://doi.org/10.1080/21505594.2016.1193275
22. Goncalves D, Ferraz C, Vaz L (2013) Posaconazole as rescue therapy in African histoplasmosis. Braz J Infect Dis 17(1):102–105. https://doi.org/10.1016/j.bjid.2012.06.027
23. Schein R, Homans J, Larsen RA, Neely M (2011) Posaconazole for chronic refractory coccidioidal meningitis. Clin Infect Dis 53(12): 1252–1254. https://doi.org/10.1093/cid/cir734