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SUSCEPTIBILITY

Antifungal Susceptibility Profiles of Olorofim (Formerly F901318) and Currently Available Systemic Antifungals against Mold and Yeast Phases of *Talaromyces marneffei*

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ABSTRACT *In vitro* antifungal susceptibility profiling of 32 clinical and environmental *Talaromyces marneffei* isolates recovered from southern China was performed against olorofim and 7 other systemic antifungals, including amphotericin B, 5-flucytosine, posaconazole, voriconazole, caspofungin, and terbinafine, using CLSI methodology. In comparison, olorofim was the most active antifungal agent against both mold and yeast phases of all tested *Talaromyces marneffei* isolates, exhibiting an MIC range, MIC_{50}, and MIC_{90} of 0.0005 to 0.002 μg/ml, 0.0005 μg/ml, and 0.0005 μg/ml, respectively.

KEYWORDS *Talaromyces marneffei*, olorofim

Talaromyces (formerly *Penicillium*) *marneffei* is the etiological agent of talaromycosis (1), a life-threatening disease that affects immunocompromised hosts, especially those with human immunodeficiency virus (HIV) infection (2). The fungus is a thermal dimorphic microorganism exhibiting a mycelial form at 25°C and a yeast form at 37°C. It may have a natural habitat in soil in areas of southern China (3), and Southeast Asia (including India), where it is endemic (4), and is known to be associated with bamboo rats (5) and dogs (6). Notably, the risk of infection is not restricted to those living in areas where it is endemic. HIV-infected individuals traveling to areas of endemicity have also become infected by *T. marneffei* (7).

Treatment of talaromycosis is typically initiated with amphotericin B, but its use is limited due to toxic side effects and requires a prolonged hospital stay (8). After completing 2 weeks of amphotericin B, patients will be transitioned to consolidation therapy with itraconazole for 10 weeks. For those who cannot take amphotericin B or itraconazole, voriconazole is recommended (8). If untreated or if there is a delay in diagnosis, the mortality rate of *T. marneffei* infections in HIV-infected patients is up to 100% (9). Therefore, the need for new antifungals to treat talaromycosis is urgent.

Several investigational antifungals with novel mechanisms of action that may override both the low susceptibility and adverse side effects are currently under development (10, 11). Among them, ibrexafungerp (12) and fosmagepix (13) demonstrated good *in vitro* activity against the *Scedosporium* species complex and *Lomentospora prolificans*. The new triazole derivative albaconazole (ALBA) (UR-9825) also showed potent activity against these pathogens in both *in vitro* (14) and *in vivo* (15) studies. Olorofim (formerly F901318) is a novel fungicidal drug that selectively inhibits fungal dihydroorotate dehydrogenase (DHODH), a key enzyme in the *de novo* pyrimidine biosynthesis pathway (16). Olorofim has shown potent *in vitro* inhibitory activity against isolates of *Aspergillus* spp., including azole-resistant isolates of *Aspergillus fumigatus* (17) and cryptic aspergilli (18); the *Scedosporium/Pseudallescheria*

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species complex and *Lomentospora* spp. (19, 20); *Madurella mycetomatis* (21); certain species of *Fusarium* and non-*marneffei Talaromyces* spp. (16); as well as the dimorphic human pathogen *Coccidioides* species (22) using both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) methodologies (18). The potent activity of olorofim has also been demonstrated in experimental animal models of disseminated infections caused by *A. fumigatus* (23, 24), *Aspergillus flavus* (25), *Aspergillus nidulans* (23), *Aspergillus tanneri* (23), *Scedosporium apiospermum*, *Pseudallescheria boydii*, *Lomentospora prolificans* (26), and *Coccidioides immitis* (22).

The drug is currently being investigated in phase II clinical studies for the treatment of invasive mold infections (invasive fungal infections [IFIs]) (11). In November 2019, olorofim received breakthrough therapy designation from the U.S. Food and Drug Administration (FDA) for the treatment of IFIs. Currently, a phase IIb clinical trial of oral olorofim is recruiting patients with IFIs and lacking treatment options (ClinicalTrials.gov identifier NCT03583164). The *in vitro* efficacy of olorofim against *T. marneffei* has not been extensively tested yet. We therefore aimed to evaluate the susceptibility of *T. marneffei* to olorofim and other currently available systemic antifungals in its yeast as well as mold phases.

(This study was partially presented at the 9th Advances against Aspergillosis and Mucormycosis Conference, Lugano, Switzerland, 27 to 29 February 2020 [www.AAAM2020.org] [27], and the 30th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Paris, France, 18 to 21 April 2020 [28]).

A collection of 32 *T. marneffei* strains recovered from southern China was investigated, including 17 isolates of human origin, 11 animal isolates, and 4 environmental strains (Table 1). The 17 clinical strains were isolated from patients who were admitted to Sun Yat-sen Memorial Hospital (3,000 inpatient beds with over 3.02 million outpatient visits per year), Second Affiliated Hospital of Sun Yat-Sen University (SYSU), Guangdong, China, from 1995 to 2014. The 11 animal isolates were obtained from bamboo rats captured in the Jiangxi, Fujian, and Guangdong provinces of China. The 4 environmental strains were isolated from bamboo root and soil in the area where bamboo rats lived. The identity of each strain was confirmed at the species level via PCR amplification and sequence-based analysis of the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) region and β-tubulin gene, as described previously (29).

In vitro antifungal susceptibility testing was performed using CLSI broth microdilution M38-ED3:2017 and M27-ED4:2017 guidelines (33, 34) for mycelial and yeast growth forms, respectively. The mold conidial suspensions were obtained from *T. marneffei* strains cultured on malt extract agar for 7 to 14 days at 25°C. The yeast suspensions were obtained from *T. marneffei* strains cultured on brain heart infusion agar for 4 to 5 days at 37°C. The drugs were provided by F2G, Ltd., Eccles, Manchester, United Kingdom (olorofim), or purchased from Sigma, St. Louis, MO (all other agents). The final concentration ranges of antifungal agents were 0.0313 to 16 μg/ml for amphotericin B, itraconazole, voriconazole, posaconazole, and terbinafine; 0.031 to 32 μg/ml for 5-flucytosine and caspofungin; and 0.00025 to 0.25 μg/ml for olorofim. The MIC was defined as the lowest concentration that completely inhibited growth as assessed by visual inspection in comparison with the control (drug-free well). For caspofungin in mycelial-form cultures of *T. marneffei* only, the MEC (minimum effective concentration) was defined as the lowest concentration in which abnormal, short, and branched hyphal clusters were observed, in contrast to the long, unbranched hyphal elements that were seen in the well.

A. flavus (ATCC 204304) and *A. fumigatus* (ATCC 204305) were used as quality control strains in all experiments.

All experiments on each strain were performed using two independent replicates on different days. The data were analyzed using GraphPad Prism, version 9.0, for Windows (GraphPad Software, San Diego, CA). The MIC/MEC distributions between the isolates originating from different locations were compared using Student's *t* test and the Mann-Whitney-Wilcoxon test; differences were considered statistically significant at a *P* value of $\leq$0.05 (two tailed).

TABLE 1 *Talaromyces marneffei* strains tested in this study

Origin (no. of isolates) and strain no.	GenBank accession no.	Origin of isolation[a]	Source of isolation	Geographical origin of isolate	Yr of isolation
Human (17)					
SUMS0047	AB353906	AIDS patient	Skin lesion	Guangdong	1995
SUMS0174	AB353915	AIDS patient	Skin lesion	Guangdong	2002
SUMS0217	JX036541	AIDS patient	Stool	Guangdong	2004
SUMS0304	KR902349	SLE patient	Bone marrow	Guangdong	2007
SUMS0326	MN700104	AIDS patient	Skin lesion	Guangdong	2007
SUMS0486	JQ585633	MM patient	Skin lesion	Guangdong	2010
SUMS0565	MN700095	DM patient	Skin lesion	Guangdong	2011
SUMS0573	MN700102	TB patient	Sputum	Guangdong	2011
SUMS0579	MN700101	SLE patient	Skin lesion	Guangdong	2011
SUMS0590	MN700100	COPD patient	Sputum	Guangdong	2011
SUMS0598	MN700096	AIDS patient	Blood	Guangdong	2011
SUMS0687	MN700099	ALL patient	Blood	Guangdong	2012
SUMS0688	MN700097	SLE patient	Blood	Guangdong	2012
SUMS0743	MN700103	AIDS patient	Blood	Guangdong	2013
SUMS0751	KT121405	AIDS patient	Blood	Guangdong	2013
SUMS0765	MN700105	AIDS patient	Blood	Guangdong	2014
SUMS0766	MN700106	AIDS patient	Blood	Guangdong	2014
Animal (11)					
SUMS0265	MN700098	Bamboo rat	Liver	Jiangxi	2006
SUMS0272	FJ009555	Bamboo rat	Lung	Jiangxi	2006
SUMS0347	FJ009564	Bamboo rat	Liver	Fujian	2007
SUMS0349	FJ009552	Bamboo rat	Liver	Guangdong	2007
SUMS0547	JN679219	Bamboo rat	Liver	Guangdong	2011
SUMS0556	JN679223	Bamboo rat	Lung	Guangdong	2011
SUMS0603	JQ910936	Bamboo rat	Liver	Guangdong	2011
SUMS0608	JQ910941	Bamboo rat	Liver	Guangdong	2011
SUMS0612	JQ910945	Bamboo rat	Liver	Guangdong	2011
SUMS0623	JQ912271	Bamboo rat	Liver	Guangdong	2011
SUMS0629	JQ912277	Bamboo rat	Spleen	Guangdong	2011
Environmental (4)					
SUMS0602	JQ910935	Near the bamboo rat hole	Bamboo root	Guangdong	2011
SUMS0615	JQ910948	Far from the bamboo rat hole	Soil	Guangdong	2011
SUMS0624	JQ912272	Near the bamboo rat hole	Bamboo root	Guangdong	2011
SUMS0630	JQ912278	Bamboo rat hole	Soil	Guangdong	2011

[a]MM, multiple myeloma; DM, dermatomycosis; TB, tuberculosis; SLE, systemic lupus erythematosus; COPD, chronic obstructive pulmonary disease; ALL, acute lymphoblastic leukemia.

The geometric mean (GM) MICs/MECs, the MIC/MEC ranges, and the MIC_{50}/MEC_{50} and MIC_{90}/MEC_{90} distributions of the eight antifungals against 32 *T. marneffei* strains are listed in Table 2. The MIC/MEC distributions of all tested antifungals are presented in Fig. 1. In summary, the GM MICs/MECs of the antifungals against the mold growth form of all *T. marneffei* strains were as follows (in increasing order): 0.0005 μg/ml for olorofim, 0.016 μg/ml for itraconazole and posaconazole, 0.05 μg/ml for voriconazole, 0.08 μg/ml for 5-flucytosine, 0.1 μg/ml for terbinafine, 0.4 μg/ml for caspofungin, and 2 μg/ml for amphotericin B. The GM MICs/MECs against the yeast phase were as follows: 0.0007 μg/ml for olorofim, 0.016 μg/ml for posaconazole, 0.016 μg/ml for itraconazole, 0.017 μg/ml for voriconazole, 0.12 μg/ml for terbinafine, 0.13 μg/ml for amphotericin B, 0.25 μg/ml for 5-flucytosine, and 4.5 μg/ml for caspofungin.

Overall, olorofim showed the lowest MIC values among antifungals tested in both mold and yeast phases of all *T. marneffei* strains, independent of the source of isolation. No statistically significant differences in the olorofim susceptibility profiles were detected between the clinical and environmental isolates of *T. marneffei*. In several

TABLE 2 *In vitro* susceptibility results for cultured mycelial and yeast forms of 32 *Talaromyces marneffei* strains against eight antifungal agents

Strain type (no. of isolates) and drug	MIC/MEC (μg/ml)[a] in mycelial form				MIC (μg/ml)[a] in yeast form			
	Range	MIC_{50}/MEC_{50}	MIC_{90}/MEC_{90}	Geometric mean	Range	MIC_{50}/MEC_{50}	MIC_{90}/ME_{90}	Geometric mean
All strains (32)								
Olorofim	0.0005–0.001	0.0005	0.0005	0.0005	0.00025–0.002	0.0005	0.002	0.0007
Amphotericin B	0.5–4	2	4	1.9152	0.031–1	0.125	0.475	0.1331
Itraconazole	≤0.016	0.016	0.016	0.0160	≤0.016–0.031	0.016	0.016	0.0163
Voriconazole	≤0.016–0.063	0.063	0.063	0.0453	≤0.016–0.031	0.016	0.0295	0.0174
Posaconazole	≤0.016	0.016	0.016	0.0160	≤0.016	0.016	0.016	0.0160
Caspofungin	0.5–4	1	4	1.3543	0.25–32	8	16	4.5552
5-Flucytosine	0.031–1	0.062	0.125	0.0755	0.031–2	0.25	0.95	0.2443
Terbinafine	0.125–0.25	0.125	0.25	0.1393	0.031–0.5	0.125	0.25	0.1168
Clinical (17)								
Olorofim	0.0005–0.001	0.0005	0.0005	0.0005	0.00025–0.002	0.0005	0.02	0.0007
Amphotericin B	0.5–4	2	4	2	0.031–1	0.125	0.5	0.1252
Itraconazole	≤0.016	0.016	0.016	0.016	≤0.016	0.016	0.016	0.016
Voriconazole	≤0.016–0.063	0.063	0.063	0.0419	≤0.016–0.031	0.016	0.031	0.0173
Posaconazole	≤0.016	0.016	0.016	0.016	≤0.016	0.016	0.016	0.016
Caspofungin	0.5–4	2	4	1.8434	0.25–32	2	16	2.6606
5-Flucytosine	0.031–1	0.063	0.125	0.0834	0.031–2	0.25	1	0.2825
Terbinafine	0.125–0.25	0.125	0.25	0.1471	0.031–0.5	0.125	0.25	0.1252
Animal (11)								
Olorofim	0.0005–0.0005	0.0005	0.0005	0.0005	0.00025–0.002	0.0005	0.002	0.0006
Amphotericin B	1–4	2	2	1.7631	0.063–0.5	0.125	0.25	0.1512
Itraconazole	≤0.016	0.016	0.016	0.016	≤0.016–0.031	0.016	0.016	0.017
Voriconazole	0.031–0.063	0.063	0.063	0.0519	≤0.016–0.031	0.016	0.031	0.018
Posaconazole	≤0.016	0.016	0.016	0.016	≤0.016	0.016	0.016	0.016
Caspofungin	0.5–4	1	2	1.065	1–16	16	16	8.5203
5-Flucytosine	0.063–0.125	0.063	0.125	0.0714	0.031–0.5	0.125	0.5	0.1714
Terbinafine	0.125–0.25	0.125	0.125	0.1331	0.031–0.25	0.063	0.25	0.0971
Environmental (4)								
Olorofim	0.0005–0.0005			0.0005	0.0005–0.002			0.0008
Amphotericin B	2–2			2	0.063–0.25			0.1252
Itraconazole	≤0.016			0.016	≤0.016			0.016
Voriconazole	0.031–0.063			0.0442	≤0.016			0.016
Posaconazole	≤0.016			0.016	≤0.016			0.016
Caspofungin	0.5–1			0.7071	2–32			8
5-Flucytosine	0.031–0.125			0.0626	0.125–1			0.3536
Terbinafine	0.125–0.125			0.125	0.063–0.25			0.1489

[a]MEC, minimal effective concentration; MIC_{50}/MEC_{50}, minimal concentration that inhibits 50% of isolates; MIC_{90}/MEC_{90}, minimal concentration that inhibits 90% of isolates. MECs were used for caspofungin.

recent studies, a similar *in vitro* potency of olorofim was observed for several other molds (16, 17, 19–22), including non-*marneffei Talaromyces* species and multiazole-resistant *Penicillium* spp. (30). Consistent with previous reports, our study also showed that itraconazole, posaconazole, and voriconazole were potent against all *T. marneffei* isolates, with higher MICs of fluconazole than other azoles (31). Caspofungin showed relatively high MICs (MIC ranges, 0.5 to 4 μg/ml and 0.25 to 32 μg/ml against mold and yeast forms, respectively) against all strains tested, which is in agreement with a previous report from China (32). For all tested strains, 5-flucytosine and terbinafine had low MIC values, whereas amphotericin B exhibited higher MIC values against the mycelial phase of all isolates (MIC range, 0.5 to 4 μg/ml). Our results agreed with a previous report (24) that the range of amphotericin B MICs for the mold phase was 0.5 to 4 μg/ml.

In conclusion, olorofim is an antimycotic that is potent against both growth phases of *T. marneffei in vitro*, and further studies are warranted to evaluate its *in vivo* efficacy.

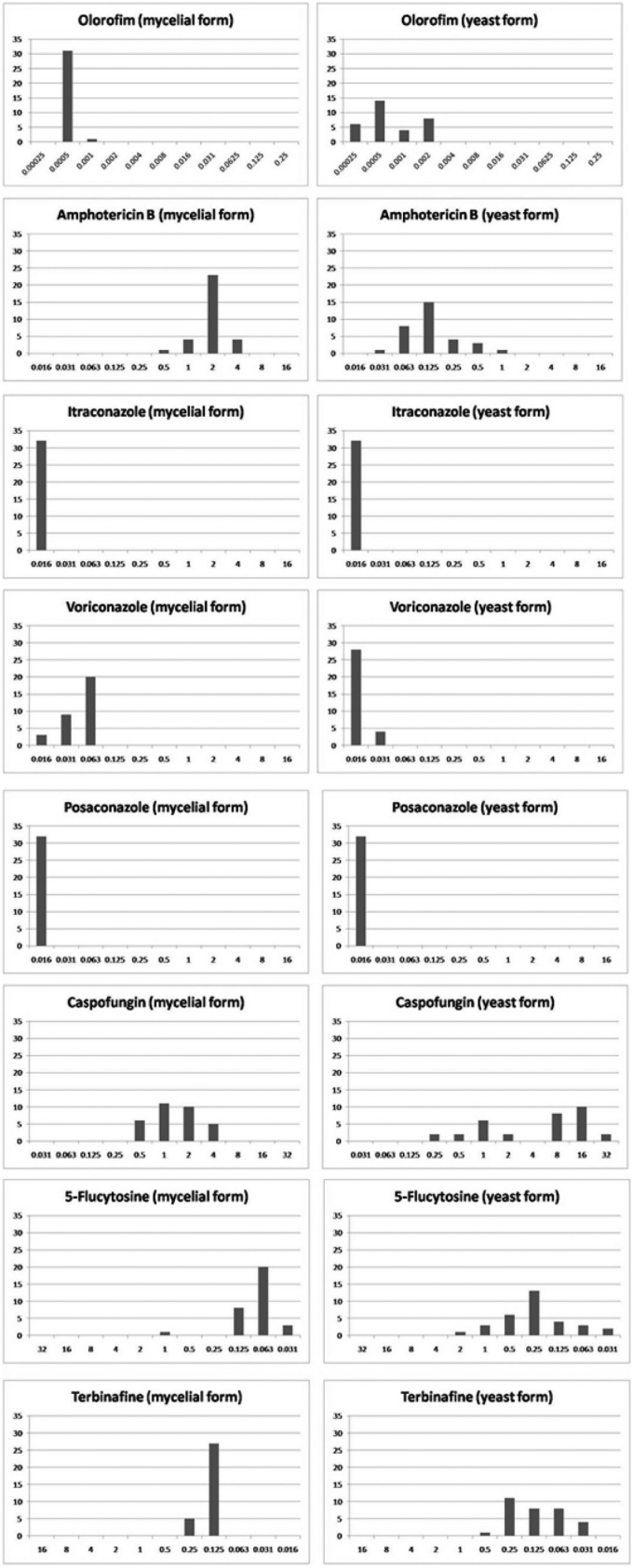

FIG 1 MIC/MEC distributions for 32 *Talaromyces marneffei* strains of clinical and environmental origins. The *x* axis shows the MICs/MECs (in micrograms per milliliter), and the *y* axis shows the number of strains in the set with the given MIC/MEC.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.01 MB.

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We declare no conflicts of interest related to this publication.

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