Antifungal susceptibility profiles of Olorofim (formerly F901318), and currently available systemic antifungals against mold and yeast phases of *Talaromyces marneffei*

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Introduction

Talaromyces marneffei is a thermal dimorphic fungus and is the etiologic agent of talaromycosis, a life-threatening disease which affects immunocompromised host especially those with HIV infection. The fungus is endemic in Southeast Asia and is known to be associated with bamboo rats. Talaromycosis is initially treated with amphotericin B but its use is limited due to toxic side effects. Therefore, the need for new antifungals to treat talaromycosis is urgent. Olorofim is a novel fungicidal drug which targets dihydroorotate dehydrogenase in the de novo pyrimidine biosynthesis pathway. It is highly active against *Aspergillus* and other filamentous *Ascomycetes*. However, the *in vitro* efficacy of olorofim against *T. marneffei* has yet to be reported.

We therefore aimed to evaluate the susceptibility of *T. marneffei* to olorofim and other currently available systemic antifungals in its yeast as well as in mold phases.

Methods

We tested 32 clinical and environmental *T. marneffei* strains recovered from southern China against 8 different antifungals according to the Clinical and Laboratory Standards Institute M38-A2 and M27-A3 guidelines. The final concentration ranges of antifungal agents were 0.0313-16 μg/mL for amphotericin B, itraconazole, voriconazole, posaconazole, and terbinafine; 0.031-32 μg/mL for 5-flucytosine and caspofungin; and 0.00025-0.25 μg/mL for olorofim.

The mold conidial suspensions were obtained from *T. marneffei* strains which have been cultured on malt extract agar for 7-14 days at 25 ℃. The yeast suspensions were obtained from *T. marneffei* strains which have been cultured on brain heart infusion agar for 4-5 days at 37 ℃.

Results

The geometric means of the minimum inhibitory concentrations/ minimum effective concentrations (MICs/MECs) of the antifungals against mold phase of all *T. marneffei* strains were (in increasing order): olorofim (0.0005 μg/mL), itraconazole and posaconazole (0.016 μg/mL), voriconazole (0.05 μg/mL); 5-flucytosine (0.08 μg/mL), terbinafine (0.14 μg/mL), caspofungin (1.35 μg/mL) and amphotericin B (1.92 μg/mL).

The geometric means MICs/MECs against the yeast phase were, as follows: olorofim (0.0007 μg/mL), posaconazole and itraconazole (0.016 ug/mL); voriconazole (0.017 μg/mL), terbinafine (0.12 μg/mL), amphotericin B (0.13 ug/mL), 5-flucytosine (0.25 μg/mL), and caspofungin (4.5 μg/mL).

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 0.00025-0.002 μg/mL, 0.0005 μg/mL, and 0.0005 μg/mL, respectively.

Table 1. *In vitro* susceptibility results of mycelial form cultures of 32 *T. marneffei* strains

	MIC/MEC (μg/mL)			
	Range	MIC_{50}/MEC_{50}	MIC_{90}/MEC_{90}	Geometric mean
Amphotericin B	0.5-4	2	4	1.92
5-flucytosine	0.031-1	0.062	0.125	0.08
Itraconazole	0.016	0.016	0.016	0.016
Voriconazole	0.016-0.063	0.063	0.063	0.05
Posaconazole	0.016	0.016	0.016	0.016
Caspofungin	0.5-4	1	4	1.35
Terbinafine	0.125-0.25	0.125	0.25	0.14
Olorofim	0.0005-0.001	0.0005	0.0005	0.0005

MIC: minimum inhibitory concentration; MEC: the minimal effective concentration for caspofungin; MIC_{50}/MEC_{50}: minimal concentration that inhibits 50 % of isolates; MIC_{90}/MEC_{90}: minimal concentration that inhibits 90 % of isolates.

Table 2. *In vitro* susceptibility results of yeast form cultures of 32 *T. marneffei* strains

	MIC/MEC (μg/mL)			
	Range	MIC_{50}/MEC_{50}	MIC_{90}/MEC_{90}	Geometric mean
Amphotericin B	0.031-1	0.125	0.5	0.13
5-flucytosine	0.031-2	0.25	1	0.25
Itraconazole	0.016-0.031	0.016	0.016	0.016
Voriconazole	0.016-0.031	0.016	0.03	0.017
Posaconazole	0.016	0.016	0.016	0.016
Caspofungin	0.25-32	8	16	4.5
Terbinafine	0.031-0.5	0.125	0.25	0.117
Olorofim	0.00025-0.002	0.0005	0.002	0.0007

Conclusion

- **Overall, olorofim demonstrated potent and consistent activity against all *T. marneffei* strains *in vitro,* and its activity was maintained in two different growth phases.**
- **Further studies are warranted to evaluate the *in vivo* efficacy of olorofim against this fungus.**

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