



In vitro synergy of echinocandins with triazoles against fluconazole-resistant *Candida parapsilosis* complex isolates

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ABSTRACT

Introduction: *Candida parapsilosis* (*C. parapsilosis*) is a common non-*albicans* *Candida* species ranked as the second common cause of bloodstream infections. Azole resistance and elevated echinocandin MICs have been reported for these fungi. This study was conducted to determine the interactions between azoles and echinocandins against *C. parapsilosis* species complex.

Materials and methods: Fifteen fluconazole-resistant clinical isolates of *C. parapsilosis* complex were included: *C. parapsilosis* sensu stricto ($n = 7$), *C. orthopsilosis* ($n = 5$) and *C. metapsilosis* ($n = 3$). The activity of azoles (fluconazole, itraconazole) and echinocandins (anidulafungin, micafungin) alone and in combination was determined using checkerboard broth microdilution. The results were determined based on the fractional inhibitory concentration index (FICI).

Results: *In vitro* combination of fluconazole with anidulafungin was found to be synergistic (FICI 0.07–0.37) and decreased the MIC range from 4–64 $\mu\text{g}/\text{mL}$ to 0.5–16 $\mu\text{g}/\text{mL}$ for fluconazole and from 2–8 $\mu\text{g}/\text{mL}$ to 0.125–1 $\mu\text{g}/\text{mL}$ for anidulafungin. Similarly, interactions of fluconazole with micafungin (FICI 0.25–0.5), itraconazole with anidulafungin (FICI 0.15–0.37) and itraconazole with micafungin (FICI 0.09–0.37) were synergistic.

Conclusion: The combination of fluconazole and itraconazole with either anidulafungin or micafungin demonstrated synergistic interactions against *C. parapsilosis* species complex, especially against isolates with elevated MIC values. However, the use of these combinations in clinical practice and the clinical relevance of *in vitro* combination results remain unclear.

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1. Introduction

In recent decades, a global shift from *Candida albicans* (*C. albicans*) to non-*albicans* *Candida* species has been reported [1]. *Candida parapsilosis* (*C. parapsilosis*) is a non-*albicans* species serving as the second most common cause of bloodstream infections, preceded by

C. albicans [2]. It has also been found to be as common as *C. albicans* among cases of invasive candidiasis in Serbia [3] and as the leading cause of candidaemia before 2010 in Europe [4], Asia [5], South America [6], and after 2010 in Iran [7], Venezuela and Colombia [8]. Using molecular analysis, *C. parapsilosis* was found to be a complex of three cryptic species: *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* [9]. *C. parapsilosis* is able to produce biofilm on catheters and other medical devices, leading to increased resistance to azole antifungal drugs [2,10]. It also tends to persist in the hospital environment and has frequently been isolated from hospital surfaces and the hands of healthcare workers [11,12], which may

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lead to nosocomial transmission of this pathogen. There are reports of *C. parapsilosis* outbreaks in intensive care units, which is indicative of exogenous transmission of this pathogen through direct and indirect contact by healthcare workers' hands [13–15]. Although members of *C. parapsilosis* complex are usually susceptible to azole antifungals [16], resistance has also been reported [17]. In a recent study, 27.6% (55/199) and 4.5% (9/199) of *C. parapsilosis* sensu stricto isolates were resistant and susceptible dose-dependent to fluconazole, respectively [18]. Echinocandins are recommended by the Infectious Diseases Society of America for treatment of candidiasis, while elevated MICs to this class of antifungals have consistently been reported in the *C. parapsilosis* species complex [19,20]. A promising way to deal with drug resistance in fungi is to use combinations of antifungal drugs, particularly those with different mechanisms of action to reduce the toxicity and side effects by shortening the duration of treatment [21]. Combination of echinocandins with azoles could be attractive as they have different targets and mechanisms of action. Therefore, this study investigated the combination of echinocandins with triazoles against fluconazole-resistant *C. parapsilosis* complex clinical isolates.

2. Materials and methods

2.1. Fungal isolates

Fifteen fluconazole-resistant clinical isolates of *C. parapsilosis* complex – including *C. parapsilosis* sensu stricto (n = 7), *C. orthopsilosis* (n = 5) and *C. metapsilosis* (n = 3) – were included in this study. All the isolates were previously identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) and confirmed by molecular methods (i.e. sequencing of internal transcribed spacer ribosomal DNA and D1/D2 regions).

2.2. Antifungal drugs

Stock solutions of antifungal drugs were made by dissolving fluconazole (Pfizer Central Research, Sandwich, UK) in sterile double-distilled water, and itraconazole (Janssen Research Foundation, Beerse, Belgium), anidulafungin (Pfizer Central Research, Sandwich, United Kingdom) and micafungin (Merck Sharp & Dohme, Haarlem, Netherlands) in dimethyl sulfoxide (DMSO). Working

solutions at 4X the final concentrations were prepared in RPMI 1640 with glutamine, but without NaHCO₃ as recommended by the Clinical Laboratory Standards Institute (CLSI) M27-A3 [22]. The final concentrations ranged from 0.125–64 µg/mL for fluconazole, 0.03–16 µg/mL for itraconazole and 0.125–8 µg/mL for echinocandins.

2.3. In vitro combination testing

In vitro interaction of azoles with echinocandins (i.e. fluconazole with anidulafungin, fluconazole with micafungin, itraconazole with anidulafungin, and itraconazole with micafungin) were studied using a microdilution checkerboard technique [23]. For preparation of test microplates, 50 µL of each concentration of azoles (fluconazole or itraconazole) were added to columns 1–10, and then 50 µL of echinocandins (anidulafungin or micafungin) were added to rows A–G. Row H and column 11 contained the azoles and echinocandins alone, respectively. Column 12 was the drug-free well that served as the growth control. Fungal inoculum was prepared following the CLSI M27-A3 [24] and 100 µL of it was dispensed to all the wells of test microplates. After 24 h of incubation at 35 °C, the results were visually read. To assess the interaction outcomes, the fractional inhibitory concentration index (FICI) value was calculated as follows: (MIC of Drug A in combination/MIC of Drug A alone) + (MIC of Drug B in combination/MIC of Drug B alone). The interaction was considered to be a synergistic effect when the FICI was ≤0.5, indifferent at >0.5 to <4.0, and antagonistic at ≥4.

3. Results

The results of *in vitro* combination testing of fluconazole and itraconazole with echinocandins are summarised in Table 1. The MIC ranges of fluconazole when tested alone against *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* isolates were 32–64 µg/mL, 8–16 µg/mL and 4 µg/mL, respectively. The combination of fluconazole with anidulafungin resulted in a synergistic interaction (FICI range 0.07–0.37) and decreased the MIC range of fluconazole to 4–16 µg/mL against *C. parapsilosis* sensu stricto, 0.5–4 µg/mL against *C. orthopsilosis* and 0.5–1 µg/mL against *C. metapsilosis* (Table 1). Similarly, fluconazole combined with micafungin interacted synergistically (FICI range 0.25–0.5) and reduced the MIC range of fluconazole to 2–16 µg/mL against *C. parapsilosis*, 1–4 µg/mL against *C. orthopsilosis* and 0.5–1 µg/mL

Table 1
The MICs of azoles and echinocandins alone and in combination against *Candida parapsilosis* species complex.

Strain No.	MICs alone (µg/mL)				MICs in combination (µg/mL)				FICI/interpretation			
	FLC	ITC	ANF	MCF	FLC/ANF	FLC/MCF	ITC/ANF	ITC/MCF	FLC/ANF	FLC/MCF	ITC/ANF	ITC/MCF
<i>Candida parapsilosis</i> sensu stricto												
TMML 1296	32	4	8	1	4/0.5	8/0.125	0.5/1	0.25/0.125	0.18/SYN	0.37/SYN	0.25/SYN	0.18/SYN
TMML 1297	32	4	8	2	8/0.5	4/0.25	1/1	1/0.25	0.31/SYN	0.25/SYN	0.37/SYN	0.37/SYN
TMML 1298	64	2	2	2	4/0.25	16/0.125	0.5/0.25	0.25/0.25	0.18/SYN	0.31/SYN	0.37/SYN	0.25/SYN
TMML 1299	32	4	4	1	8/0.5	2/0.25	1/0.5	0.5/0.25	0.37/SYN	0.31/SYN	0.37/SYN	0.37/SYN
TMML 1300	64	2	8	2	16/1	16/0.25	0.5/0.5	0.5/0.25	0.37/SYN	0.37/SYN	0.31/SYN	0.37/SYN
TMML 1301	64	4	8	1	4/0.125	8/0.125	0.5/1	1/0.125	0.07/SYN	0.25/SYN	0.25/SYN	0.37/SYN
TMML 1302	32	4	2	2	8/0.25	8/0.25	1/0.25	0.25/0.5	0.37/SYN	0.37/SYN	0.37/SYN	0.31/SYN
<i>Candida orthopsilosis</i>												
TMML 399	8	1	4	4	0.5/0.25	1/0.25	0.06/0.5	0.25/0.25	0.12/SYN	0.18/SYN	0.18/SYN	0.31/SYN
TMML 406	16	1	8	4	4/0.5	4/0.5	0.25/0.25	0.06/0.25	0.31/SYN	0.37/SYN	0.28/SYN	0.12/SYN
TMML 407	16	2	8	2	4/0.25	4/0.25	0.5/1	0.5/0.125	0.28/SYN	0.37/SYN	0.37/SYN	0.31/SYN
TMML 414	8	2	4	4	1/0.25	2/1	0.5/0.125	0.5/0.25	0.18/SYN	0.5/SYN	0.28/SYN	0.31/SYN
TMML 443	16	1	4	4	4/0.5	4/0.125	0.125/0.25	0.03/0.25	0.37/SYN	0.28/SYN	0.18/SYN	0.09/SYN
<i>Candida metapsilosis</i>												
TMML 469	4	1	2	1	1/0.125	1/0.125	0.03/0.25	0.06/0.125	0.31/SYN	0.37/SYN	0.15/SYN	0.18/SYN
TMML 470	4	1	4	1	1/0.5	0.5/0.125	0.06/0.5	0.25/0.125	0.37/SYN	0.25/SYN	0.18/SYN	0.37/SYN
TMML 471	4	1	4	2	0.5/1	1/0.125	0.125/0.25	0.25/0.25	0.37/SYN	0.31/SYN	0.18/SYN	0.37/SYN

Abbreviations: FLC, fluconazole; ITC, itraconazole; ANF, anidulafungin; MCF, micafungin; FICI, fractional inhibitory concentration index; SYN, synergism.

against *C. metapsilosis* (Table 1). The MIC range of itraconazole when tested alone was found to be 2–4 µg/mL against *C. parapsilosis sensu stricto*, 1–2 µg/mL against *C. orthopsilosis* and 1 µg/mL against *C. metapsilosis*. In combination with anidulafungin or micafungin, the MIC range of itraconazole decreased to 0.5–1 µg/mL and 0.25–1 µg/mL against *C. parapsilosis sensu stricto*, 0.06–0.5 µg/mL and 0.03–0.5 µg/mL against *C. orthopsilosis* and 0.03–0.125 µg/mL and 0.06–0.25 µg/mL against *C. metapsilosis*, respectively.

3.1. Discussion

The prophylactic use of azole antifungal drugs, particularly fluconazole, since the late 1990s, significantly reduced the incidence and mortality of systemic *Candida* infections. However, as a consequence, it led to a shift from fluconazole-susceptible to fluconazole-resistant infections, especially due to non-*albicans Candida* species [24]. *C. parapsilosis* is one of the common non-*albicans Candida* species able to form biofilms on catheters and other medical devices and can be transmitted horizontally in healthcare facilities. It is a big threat for patients in intensive care units and a known cause of candidaemia in susceptible paediatric patients [25–28]. Nosocomial outbreaks due to this species have been reported in different geographical regions [13,29–31]. Azole-resistance has been reported in *C. parapsilosis*, especially among cases of invasive infections and those previously treated with fluconazole [18,32,33]. This resistance can also be acquired during systemic antifungal therapy [34,35]. Although echinocandins have been recommended as first-line treatment for invasive candidiasis, reports have shown that *C. parapsilosis* has higher MICs than other *Candida* species to echinocandins [20,36]. An alternative method for treatment of resistant cases is the use of a combination of two drugs with different mechanisms of action [21], which needs *in vitro* and animal model studies prior to being used in a clinical setting.

In the present study, the antifungal activity of triazoles (itraconazole, fluconazole) combined with echinocandins (anidulafungin, micafungin) was determined on fluconazole-resistant *C. parapsilosis* complex isolates. The results showed synergism for all combinations. Antifungal interactions may be different depending on the antifungal classes. In a recent study Chassot et al. investigated the combinations of amphotericin B, fluconazole, voriconazole and flucytosine against echinocandin-susceptible and echinocandin-resistant *C. parapsilosis sensu stricto* strains and, unlike the current results, most of the interactions were indifferent [37]. Variations of *in vitro* interactions can also occur and members within an antifungal class do not necessarily interact in the same way. Combinations of posaconazole with either caspofungin or anidulafungin and voriconazole with anidulafungin were found to be indifferent, while voriconazole with caspofungin resulted in an antagonistic interaction against *C. parapsilosis sensu stricto* isolates [38]. Moreover, voriconazole in combination with micafungin exhibited a synergistic interaction against the emerging pathogen *C. auris*, while the interaction of voriconazole with caspofungin and fluconazole with either caspofungin or anidulafungin was indifferent [39]. Although inter-species variations have been reported for the results of antifungal combinations [40], no difference was noted in the current results obtained for *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*. This might be due to the close relatedness of these species, which belong to the same phylogenetic complex. However, this finding is based on a limited number of isolates and because there are no other data on the combination of antifungal drugs against *C. orthopsilosis* and *C. metapsilosis*, future studies in this area may change our understanding.

In conclusion, the combination of fluconazole or itraconazole with either anidulafungin or micafungin showed a synergistic interaction against the *C. parapsilosis* species complex, especially against isolates with elevated MIC values. The use of these combinations in clinical practice and the clinical relevancy of these *in vitro* results should be further explored.

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Competing interests

The authors declare no conflicts of interest regarding this study.

Ethical approval

Ethical Approval Number: IR.TUMS.IKHC.REC.1397.213.

Authors' contributions

SK: designed and supervised the study; AA, AM, AI, MG and SM: performed the experiments and drafted the manuscript, SR, SJH, HB and FA: performed data analysis; ED and JFM: critical review of the manuscript. All authors approved the final version of the manuscript.

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