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In Vitro Interactions of Echinocandins with Triazoles against Multidrug-Resistant *Candida auris*

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ABSTRACT We determined the *in vitro* interactions between echinocandins and azoles against 10 multidrug-resistant *Candida auris* strains by use of a microdilution checkerboard technique. Our results suggest synergistic interactions between micafungin and voriconazole with fractional inhibitory concentration index (FICI) values of 0.15 to 0.5, and we observed indifferent interactions when micafungin was combined with fluconazole (FICI, 0.62 to 1.5). Combinations of caspofungin with fluconazole or voriconazole exhibited indifferent interactions. No antagonism was observed for any combination.

KEYWORDS *in vitro* interactions, azoles, echinocandins, *Candida auris*

Candidiasis infection caused by uncommon *Candida* species has increased in recent years, particularly among immunocompromised patients (1). In the *Metschnikowiaceae* clade, *Candida auris* causes various infections, ranging from superficial mucocutaneous candidiasis to severe bloodstream infections (2, 3). Remarkably, in recent years, multidrug-resistant *C. auris* has emerged in Asia, Africa, Europe, and the Americas, resulting in several cases of fungemia (3–14). Although European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the diagnosis and management of candidiasis recommend the use of azoles, polyenes, and echinocandins (15, 16), toxic effects of amphotericin B restrict its clinical application. In addition, resistance to azoles and echinocandins in *Candida* species has become a severe clinical challenge (17). Fungemia due to *C. auris* is associated with a high mortality rate and treatment failure, in addition to being potentially resistant to azoles, polyenes, and echinocandins (18–21). Thus, accurate identification of *C. auris* and *in vitro* antifungal susceptibility testing are highly recommended (22). Because of the limited available treatment choices and high rate of therapeutic failures, novel strategies are needed to improve patient outcomes (23). Combinations of echinocandins and azoles seem to be attractive treatment regimens, as both drug groups have different antifungal targets and modes of action. We therefore investigated the efficacy of echinocandins plus azoles against multidrug-resistant *C. auris* clinical isolates.

We studied 10 *C. auris* strains from patients with candidemia in tertiary care hospitals in Delhi, including fluconazole-resistant ($n = 10$) and micafungin-resistant ($n = 3$) isolates (according to non-species-specific *Candida* species breakpoints of >4

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TABLE 1 *In vitro* interactions of caspofungin with fluconazole and voriconazole against *Candida auris*

	CAS + FLU[b]				CAS + VRC[b]			
	MIC (μg/ml)				MIC (μg/ml)			
Strain no.	CAS	FLU	CAS/FLU	FICI/INT	CAS	VRC	CAS/VRC	FICI/INT
VPCI 482/P/13[a]	2	≥64	1/32	0.75/IND	2	2	1/0.5	0.75/IND
VPCI 1132/P/13[a]	2	32	1/8	0.75/IND	2	0.5	1/0.063	0.62/IND
VPCI 1133/P/13[a]	4	≥64	2/64	1/IND	4	1	2/0.25	0.75/IND
VPCI 265/P/14[a]	4	32	2/32	1.5/IND	4	8	2/0.25	0.75/IND
VPCI 1510/P/14[a]	0.5	32	0.5/32	2/IND	0.5	4	0.5/4	2/IND
VPCI 1514/P/14[a]	1	≥64	0.5/32	0.75/IND	1	0.5	1/0.25	1.5/IND
VPCI 266/P/14[a]	2	≥64	1/32	0.75/IND	2	0.5	1/0.25	1/IND
VPCI 267/P/14[a]	2	32	1/8	0.75/IND	2	0.5	2/0.063	0.62/IND
VPCI 487/P/14[a]	1	≥64	0.5/8	0.56/IND	1	1	0.5/0.125	0.62/IND
VPCI 518/P/14[a]	0.5	≥64	0.25/8	0.56/IND	0.5	1	0.25/0.25	0.75/IND

[a]Fluconazole-resistant isolates ($n = 10$).
[b]CAS, caspofungin; FLU, fluconazole; VRC, voriconazole; FICI, fractional inhibitory concentration index; IND, indifference; SYN, synergy; INT, interpretation.

and ≥8 μg/ml for fluconazole- and echinocandin-resistant species, respectively) (Tables 1 and 2) (14). All isolates were previously identified by conventional and molecular methods, i.e., CHROMagar *Candida* medium (Difco, Becton Dickinson & Company, Baltimore, MD, USA), microscopic morphology on cornmeal agar (Difco Laboratories, Detroit, MI, USA) with 1% Tween 80, and sequencing of internal transcribed spacer ribosomal DNA (rDNA) and D1/D2 regions. In addition, the isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI Biotyper OC version 3.1; Bruker Daltonics, Bremen, Germany) (18). All strains were stored in 10% glycerol broth at −80°C at the Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, and were subcultured on Sabouraud dextrose agar (SDA) supplemented with 0.02% chloramphenicol at 35°C for 3 days to ensure purity and viability. All isolates were subcultured again on SDA before preparation of the inoculum. The interactions of caspofungin and micafungin with fluconazole or voriconazole were investigated by using a microdilution checkerboard method based on the CLSI reference technique with 96-well microtiter plates (24). Fluconazole (Pfizer, Groton, CT, USA), voriconazole (Pfizer), caspofungin (Merck), and micafungin (Astellas, Toyama, Japan) were dissolved in 100% dimethyl sulfoxide (DMSO). Drug dilutions were prepared to obtain four times the final concentration. Concentrations ranged from 8 to 0.016 μg/ml for caspofungin, 8 to 0.016 and 1 to 0.002 μg/ml for micafungin, 64 to 1 μg/ml for fluconazole, and 16 to 0.25 and 1 to 0.016

TABLE 2 *In vitro* interactions of micafungin with fluconazole and voriconazole against *Candida auris*

	MFG + FLU[c]				MFG + VRC[c]			
	MIC (μg/ml)				MIC (μg/ml)			
Strain no.	MFG	FLU	MFG/FLU	FICI/INT	MFG	VRC	MFG/VRC	FICI/INT
VPCI 482/P/13[a]	0.25	≥64	0.25/64	1.5/IND	0.25	2	0.016/0.5	0.31/SYN
VPCI 1132/P/13[a]	0.5	32	0.25/4	0.62/IND	0.5	0.5	0.016/0.125	0.28/SYN
VPCI 1133/P/13[a,b]	8	≥64	4/32	0.75/IND	8	1	2/0.25	0.5/SYN
VPCI 265/P/14[a]	0.5	32	0.5/8	1.25/IND	0.5	8	0.063/1	0.25/SYN
VPCI 1510/P/14[a]	0.125	32	0.063/8	0.75/IND	0.125	4	0.016/0.25	0.19/SYN
VPCI 1514/P/14[a,b]	8	≥64	8/16	1.12/IND	8	0.5	1/0.125	0.37/SYN
VPCI 266/P/14[a]	0.25	≥64	0.25/32	1.25/IND	0.25	0.5	0.008/0.125	0.28/SYN
VPCI 267/P/14[a,b]	8	32	8/8	1.25/IND	8	0.5	1/0.125	0.37/SYN
VPCI 487/P/14[a]	4	≥64	4/32	1.25/IND	4	1	0.5/0.125	0.25/SYN
VPCI 518/P/14[a]	0.5	≥64	0.25/64	1/IND	0.5	1	0.016/0.125	0.15/SYN

[a]Fluconazole-resistant isolates ($n = 10$).
[b]Micafungin-resistant isolates ($n = 3$).
[c]MFG, micafungin; FLU, fluconazole; VRC, voriconazole; FICI, fractional inhibitory concentration index; IND, indifference; SYN, synergy; INT, interpretation.

μg/ml for voriconazole. The concentration ranges of micafungin and voriconazole depended on the MIC results of each isolate. For two-dimensional microplate preparation, i.e., caspofungin plus fluconazole, caspofungin plus voriconazole, micafungin plus fluconazole, and micafungin plus voriconazole, 50 μl of each concentration of echinocandins (caspofungin and micafungin) was added to columns 1 through 11, and then 50 μl of azoles (fluconazole and voriconazole) was added to rows A through H, respectively. The wells of column 11 and the wells of row H contained 50 μl of RPMI medium containing 1% of the solvent. Row H and column 11 contained the echinocandins and azoles alone, respectively. Column 12 was the drug-free well that served as the growth control. The maximal final concentration of DMSO in the test wells was <1%. Trays were stored at −80°C until the day of testing. After the microtiter trays were defrosted, 100 μl of the inoculum was added to each well. Briefly, homogeneous suspensions were measured spectrophotometrically at 530 nm wavelength to a percentage transmission in the range of 75% to 77%. The final concentration of the stock inoculum suspensions of the isolates tested ranged from 1 to 3 × 10^3 CFU/ml, as determined by quantitative colony counts on Sabouraud glucose agar (Difco). Plates were incubated at 35°C and examined visually after 24 h to determine MIC values for the drugs alone and in combination. The MIC endpoints were determined with the aid of a reading mirror and were defined as the lowest concentration of drug that significantly reduced growth (≥50%) compared with the growth of a drug-free control. For calculations, high off-scale MICs were raised to the next $\log_2$ dilution step, while the low off-scale MICs were left unchanged (25). To assess the interactions of combinations of drugs, we calculated the fractional inhibitory concentration index (FICI). The FICI was defined as $FICI = FIC_A + FIC_B = (C_A/MIC_A) + (C_B/MIC_B)$, where MIC_A and MIC_B are the MICs of drugs A and B alone, and C_A and C_B are the concentrations of the drugs in combination, in all wells corresponding to an MIC. The interaction was considered synergistic when the FICI was ≤0.5, indifferent at >0.5 to ≤4.0, and antagonistic at >4 (24).

The results for the tested drugs alone and in combination against 10 *C. auris* strains are summarized in Tables 1 and 2. The MIC ranges of drugs alone against the strains were 32 to ≥64 μg/ml for fluconazole, 0.5 to 8 μg/ml for voriconazole, 0.5 to 4 μg/ml for caspofungin, and 0.125 to 8 μg/ml for micafungin. Based on findings with the checkerboard microdilution assay, when caspofungin was combined with fluconazole, the MIC ranges for caspofungin and fluconazole decreased to 0.25 to 2 μg/ml and 8 to 64 μg/ml, respectively; the combination exhibited indifferent activity against all 10 strains (FICI, 0.56 to 2). When caspofungin was combined with voriconazole, the MIC ranges for caspofungin and voriconazole decreased to 0.25 to 2 μg/ml and 0.063 to 4 μg/ml, respectively, and demonstrated indifferent activity against all strains (FICI, 0.62 to 2) (Table 1). When micafungin was combined with fluconazole, the MIC ranges of micafungin and fluconazole were reduced to 0.063 to 8 μg/ml and 4 to 64 μg/ml, respectively; indifference was also observed (FICI, 0.62 to 1.5) (Table 2). Synergistic effects of micafungin with voriconazole were shown against the 10 multidrug-resistant *C. auris* isolates (FICI, 0.15 to 0.5); the MIC ranges of micafungin and voriconazole were reduced to 0.008 to 2 μg/ml and 0.125 to 1 μg/ml, respectively (Table 2). Overall, no antagonistic effects were observed for any combination.

In this study, we used the checkerboard microdilution method to analyze drug-drug interactions of echinocandins with azoles against multidrug-resistant *C. auris*. The emergence of new species and antifungal resistance has raised the issue of using alternative therapeutic strategies. Evidence to support treatment choices for multidrug-resistant *C. auris* disease is rare. Except for one study (20), *in vitro* antifungal profiles are relatively scarce and based on low numbers of test isolates (14, 19, 21). The *in vivo* efficacy of antifungal therapy against *C. auris* is undetermined, and *in vitro* data from different sources are inadequate. Use of echinocandins is the recommended treatment for patients with potent activity, an excellent safety profile, and favorable pharmacokinetics (26–28), but unsuccessful treatment of *C. auris* infections with fluconazole, voriconazole, amphotericin B, caspofungin, and anidulafungin has been reported (6).

On the other hand, micafungin is used for prophylaxis and treatment with a broad spectrum of activity in both neutropenic and nonneutropenic patients (15, 29). Concordant with other reports (30–32), micafungin activity was shown to be as effective as caspofungin *in vitro* against *Candida glabrata* isolates with and without *fks* mutations. Micafungin was also effective *in vivo* for decreasing the fungal burden in mice infected with *C. glabrata* with *fks* mutations. It seems that lower concentrations of drugs cause fewer side effects and improve the treatment outcomes. We have shown that interaction between micafungin and voriconazole exhibited synergistic activity against multidrug-resistant *C. auris* strains, suggesting that the combination may be considered for patients with candidiasis. However, *in vivo* studies with suitable animal models of *C. auris* infection are needed to confirm the *in vitro* results presented here. Clearly, more research is indicated to explore clinical management. In conclusion, the combination of micafungin and voriconazole exhibited synergistic activity against multidrug-resistant *C. auris*, suggesting that this is an alternative approach to overcome antifungal drug resistance. However, use of this combination therapy *in vivo* and determination of the underlying mechanism of this synergistic action need further study.

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We alone are responsible for the content and writing of the paper.

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