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LETTER TO THE EDITOR

In Vitro Activities of Antifungal Combinations against Biofilms and Planktonic Forms of Clinical *Trichosporon asahii* Isolates

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Trichosporon species can cause biofilm-associated infections related to indwelling medical devices, especially intravenous catheters, and unacceptable mortality rates have been reported despite the administration of antifungal treatments (1). *Trichosporon asahii* can form biofilms with structured microbial communities *in vitro*, embedded within an extracellular matrix, with significantly increased resistance to antifungal compounds (2, 3), which might ultimately lead to clinical treatment failure. Antifungal combination may be an alternative therapy strategy for biofilm-related fungal infections (4). The synergistic effects of antifungal combinations against other fungal biofilms have been detected *in vitro*, such as amphotericin B-posaconazole for *Candida albicans* (5) and amphotericin B-caspofungin or voriconazole-caspofungin for *Aspergillus* spp. (6). The synergistic effects of antifungal combinations of voriconazole, amphotericin B, and caspofungin against planktonic *T. asahii* have been found *in vitro* (7). We evaluated the *in vitro* activity of the combinations of voriconazole-amphotericin B, voriconazole-caspofungin, and amphotericin B-caspofungin against 16 clinical isolates of *T. asahii* in biofilm and planktonic forms by a broth microdilution checkerboard method (5). *Trichosporon* biofilms were prepared according to the 96-well plate-based method (8). The effect of antifungal agents was determined by the 2,3-bis(2-methoxy-4-nitro-5-[(sulfenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT)-based colorimetric assay for both biofilms and planktonic cells (5, 8). The MIC and sessile MIC (SMIC) were determined as the lowest antifungal concentration (alone or in combination) that caused a 50% reduction in optical density for both biofilms and planktonic cells compared with the growth control (5, 6). The interaction was defined on the basis of the fractional inhibitory concentration indexes (FICIs) as follows: ≤0.5, synergy; >0.5 to 4, indifference; and >4.0, antagonism.

Under planktonic conditions, the amphotericin B-caspofungin combination showed the highest percentage of synergistic effects (81.25%; FICI, 0.125 to 0.5) (Table 1), as indicated by a previous *in vitro* study (7). Under biofilm conditions, the voriconazole-amphotericin B combination showed the highest percentage of synergistic effects (87.5%; FICI, 0.078 to 0.313), and the $SMIC_{90}$/SMIC ranges for these two drugs obviously decreased from >1,024/512 to >1,024 μg/ml to 64/4 to 128 μg/ml for voriconazole and from 1,024/32 to 1,024 μg/ml to 32/4 to 128 μg/ml for amphotericin B, respectively. The combinations of amphotericin B-caspofungin (93.75%) and voriconazole-caspofungin (81.25%) mainly yielded indifferent interactions, and no antagonistic interaction was observed in any of the combinations of either the biofilms or the planktonic forms of *T. asahii* isolates (Table 1).

Trichosporon now ranks as the second most common pathogen causing fungemia in patients with hematological malignant disease, mainly catheter-related bloodstream infections (CR-BSIs) (1, 9). For biofilm-related infections, catheter removal is recommended as an adjunctive strategy for the management of *Candida* CR-BSIs (4), which is also suggested for *Trichosporon* CR-BSIs

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TABLE 1 Interactions of voriconazole, amphotericin B, and caspofungin against biofilm and planktonic forms of 16 clinical isolates of *T. asahii*[a]

Isolate no.	SMIC (μg/ml)									MIC (μg/ml)								
	VRZ	AMB	CAS	VRZ/AMB	FICI	SMIC (μg/ml) for VRZ/CAS	FICI	SMIC (μg/ml) for AMB/CAS	FICI	VRZ	AMB	CAS	VRZ/AMB	FICI	MIC (μg/ml) for VRZ/CAS	FICI	MIC (μg/ml) for AMB/CAS	FICI
1	512	128	64	4/16	0.133	2/16	0.254	2/32	0.516	0.0625	2	16	0.0313/0.5	0.75	0.0313/0.25	0.516	0.5/4	0.5
2	512	256	64	4/64	0.258	4/32	0.508	0.5/64	1.002	0.0625	8	32	0.0625/4	1.5	0.0313/2	0.625	2/1	0.281
3	>1,024	512	32	16/32	0.078	8/16	0.508	0.5/32	1.001	0.125	8	16	0.0313/1	0.375	0.0313/0.5	0.281	0.5/2	0.188
4	>1,024	512	64	8/32	0.07	2/32	0.502	0.5/64	1.001	0.125	4	32	0.0625/4	1.5	0.0625/0.5	0.516	0.125/4	0.156
5	>1,024	512	64	64/4	0.07	2/32	0.502	4/32	0.508	0.0625	4	32	0.0157/4	1.35	0.0625/1	1.031	0.5/1	0.156
6	>1,024	32	32	32/16	0.531	1/32	1.001	0.5/32	1.016	0.0625	4	16	0.0313/1	0.75	0.0313/1	0.563	0.25/8	0.625
7	>1,024	256	128	8/128	0.508	4/32	0.254	1/64	1.004	0.125	2	16	0.125/2	2	0.0313/0.25	0.375	0.5/2	0.375
8	>1,024	128	64	4/16	0.129	2/32	0.502	4/32	0.531	0.0625	1	8	0.0625/0.5	1.5	0.0625/0.5	1.063	0.0625/2	0.313
9	1,024	256	64	4/32	0.129	4/32	0.504	1/64	1.004	0.0313	2	32	0.0313/0.25	1.25	0.0157/4	0.625	0.25/1	0.156
10	>1,024	1,024	64	128/8	0.133	2/32	0.502	1/32	0.501	0.0313	2	32	0.0313/1	1	0.0625/2	1.125	1/8	0.75
11	>1,024	1,024	32	64/16	0.078	0.5/32	1	0.5/32	0.5	0.0625	4	16	0.0313/4	2	0.0313/0.25	1.016	0.5/2	0.25
12	>1,024	1,024	64	32/128	0.156	1/32	0.501	1/64	1.001	0.0625	4	16	0.0313/1	1.25	0.0313/0.5	1.031	0.5/4	0.375
13	>1,024	128	64	16/16	0.281	2/16	0.252	2/16	0.266	0.0313	8	16	0.0313/2	0.75	0.0625/0.25	1.016	1/1	0.188
14	>1,024	1,024	64	128/32	0.156	16/32	0.516	2/64	1.002	0.0625	8	32	0.0625/8	2	0.0313/1	0.531	0.5/2	0.125
15	1,024	64	64	16/8	0.141	0.5/16	0.25	0.5/64	1.008	0.0313	8	32	0.0313/2	1.25	0.0313/1	1.031	0.25/8	0.281
16	>1,024	128	32	64/32	0.313	2/32	1.001	1/32	1.008	0.0625	4	16	0.0313/1	0.75	0.0157/0.5	0.281	2/2	0.625

[a] VRZ, voriconazole; AMB, amphotericin B; CAS, caspofungin. SMIC, sessile MIC, defined as the concentration that causes a 50% reduction in optical density of the biofilms compared with the optical density of the untreated biofilm formed by the same isolates; MIC, the concentration causing a 50% reduction in optical density of the planktonic cells compared with the optical density of the untreated cells of the same isolates; FICI, fractional inhibitory concentration index: ≤0.5, synergy; >0.5 to 4, indifference; >4.0, antagonism.

when feasible, because the SMICs of single common antifungals against *T. asahii* have been demonstrated to be very high (2, 3), and even the decreased SMICs of the voriconazole-amphotericin B combination are still higher than the highest plasma drug concentrations safely used in clinical practice. For patients needing catheter salvage due to limited venous access or catheter reinsertion or for those with thrombocytopenia or some other coagulopathy, the use of a single antifungal agent as a lock solution (0.33 to 5 mg/ml for amphotericin B or 3.33 mg/ml for caspofungin) in antifungal lock therapy has been utilized in the management of *Candida* CR-BSIs (10). However, a single antifungal agent (caspofungin or amphotericin B) may be not suitable for use as a lock solution for *T. asahii* CR-BSIs because the paradoxical growth of *T. asahii* biofilms was observed at high doses (512 to 1,024 μg/ml) of caspofungin in our study, and the SMICs indicated the lower activity of amphotericin B against *T. asahii* biofilms (32 to 1,024 μg/ml) than *Candida albicans* biofilms (2 to 4 μg/ml) (5). The synergistic effect of the voriconazole-amphotericin B combination against *T. asahii* biofilms was achieved along with a significant decrease in the SMICs of voriconazole (up to 256-fold) and amphotericin B (up to 128-fold) when used in combination. Thus, the synergistic voriconazole-amphotericin B combination may be an option as a lock solution for *T. asahii* CR-BSIs, especially for patients who are catheter dependent or have risks associated with catheter removal.

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The authors declare they have no competing interests.

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