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In Vitro Antifungal Activities of Isavuconazole and Comparators against Rare Yeast Pathogens[▽]

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We compared the *in vitro* activities of isavuconazole, posaconazole, voriconazole, and fluconazole against *Dipodascus capitatus* (*n* = 21), *Saccharomyces cerevisiae* (*n* = 20), *Rhodotorula mucilaginosa* (*n* = 18), and *Trichosporon* spp. (*n* = 15). The MIC_{50}s, MIC_{90}s, and MIC ranges (in μg/ml) obtained using the CLSI M27-A3 procedure were as follows: isavuconazole, 0.125, 0.5, and ≤0.015 to 2; posaconazole, 0.5, 2, and ≤0.015 to >16; voriconazole, 0.125, 2, and ≤0.015 to 8; and fluconazole, 4, >128, and ≤0.125 to >128. Isavuconazole showed potent activity against the isolates studied.

While most cases of fungemia are caused by *Candida* spp., the incidence of bloodstream and organ-specific infections due to other genera of yeasts is increasing (9, 15). Isavuconazole, an experimental triazole currently in phase III trials for treatment of fungemia, has potent *in vitro* activity against *Candida* and *Cryptococcus* isolates (5, 13). However, the *in vitro* activities of isavuconazole against non-*Candida* (and non-*Cryptococcus*) yeasts have been determined for relatively few other isolates, and in all cases only the Clinical and Laboratory Standards Institute (CLSI) M27-A3 broth microdilution method was used (14).

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We compared the *in vitro* activities of fluconazole, voriconazole, posaconazole, and isavuconazole against 74 rare yeast isolates recovered from blood and other clinical specimens: *Dipodascus capitatus* (*n* = 21), *Rhodotorula mucilaginosa* (*n* = 18), *Saccharomyces cerevisiae* (*n* = 20), and *Trichosporon* spp. (*n* = 15; *T. mucoides* [*n* = 8], *T. inkin* [*n* = 3], *T. jirovecii* [*n* = 2], *T. domesticum* [*n* = 1], and *T. asahii* [*n* = 1]). Isolates were identified by amplification and sequencing of the ITS1-5.8S-ITS2 rRNA genes (16). Yeasts were suspended in sterile distilled water and stored at −70°C. Prior to MIC testing, strains were revived and subcultured on potato dextrose agar (Tec-Laim S.A., Madrid, Spain) or Sabouraud dextrose agar (Francisco Soria Melguizo S.A., Madrid, Spain).

Antifungal susceptibility testing. Antifungal drugs were obtained as reagent-grade powders from their respective manufacturers (fluconazole, and voriconazole from Pfizer, Inc., New York, NY; posaconazole from Schering-Plough Corp., Kenilworth, NJ; isavuconazole from Basilea Pharmaceutica International Ltd., Basel, Switzerland).

MICs were obtained by broth microdilution according to CLSI guidelines (2). The concentration ranges of drug in microtiter plate wells were 0.015 to 16 μg/ml for isavuconazole, posaconazole, and voriconazole and 0.125 to 128 μg/ml for fluconazole. Inoculated trays were incubated at 35°C and examined visually after 48 h. The MIC was defined as the lowest drug concentration leading to a prominent (~50%) decrease in turbidity.

MICs also were determined using Etest strips spotted with posaconazole, voriconazole, and fluconazole (AB Biodisk, Solna, Sweden) and isavuconazole (donated by Basilea Pharmaceutica International Ltd.) according to the manufacturer's instructions. Yeast suspensions were streaked across the surface of 2% glucose-RPMI agar plates (Tec-Laim) using a cotton swab, and Etest strips were placed on the surface of agar plates. The MIC was defined as the lowest drug concentration at which the border of the elliptical inhibition zone intercepted the scale on the antifungal strip after 48 h of incubation at 35°C.

Because the Etest strips contain a continuous gradient of antifungal instead of the established 2-fold drug dilutions, the MIC endpoint obtained by the Etest was raised to the next 2-fold dilution matching the drug dilution on the scale used for the CLSI procedure. The CLSI and corrected (2-fold scale) Etest MICs obtained were converted to $\log_2$ MICs. Agreement between the Etest and the CLSI method was considered essential when the $\log_2$ MICs measured by

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TABLE 1. MICs for isavuconazole, posaconazole, voriconazole, and fluconazole against rare yeast pathogens obtained using the CLSI broth microdilution assay and the Etest

Isolate (n = 74)	Procedure	Isavuconazole MIC (μg/ml) MIC_{50}	Isavuconazole MIC (μg/ml) MIC_{90}	Isavuconazole MIC (μg/ml) Range	Isavuconazole P[a]	Posaconazole MIC (μg/ml) MIC_{50}	Posaconazole MIC (μg/ml) MIC_{90}	Posaconazole MIC (μg/ml) Range	Posaconazole P	Voriconazole MIC (μg/ml) MIC_{50}	Voriconazole MIC (μg/ml) MIC_{90}	Voriconazole MIC (μg/ml) Range	Voriconazole P	Fluconazole MIC (μg/ml) MIC_{50}	Fluconazole MIC (μg/ml) MIC_{90}	Fluconazole MIC (μg/ml) Range	Fluconazole P
D. capitatus[b]	CLSI	0.06	0.5	≤0.015–0.5	NS[c]	0.125	0.5	≤0.015–1	NS	0.06	0.25	≤0.015–0.5	0.003	4	16	0.25–32	0.001
	Etest	0.125	1	≤0.015–1		0.06	0.5	≤0.015–1		0.03	0.06	≤0.015–0.06		1	4	≤0.125–8	
R. mucilaginosa[d]	CLSI	0.5	2	0.125–2	0.001	1	8	0.25–>16	<0.001	2	8	0.5–8	<0.001	>128	>128	>128	NS
	Etest	1	4	0.25–4		>16	>16	>16		>16	>16	8–>16		>128	>128	>128	
S. cerevisiae[e]	CLSI	0.03	0.5	≤0.015–1	0.016	1	2	0.06–2	0.001	0.125	0.25	0.03–0.5	0.001	4	16	1–32	NS
	Etest	0.125	0.5	0.03–2		2	>16	≤0.015–>16		0.03	0.125	≤0.015–0.125		4	64	0.5–>128	
Trichosporon spp.[f]	CLSI	0.06	0.5	≤0.015–0.5	NS	0.125	0.25	0.03–0.25	NS	0.06	0.25	≤0.015–0.25	0.005	2	8	≤0.125–8	NS
	Etest	0.06	0.125	≤0.015–0.125		0.125	0.5	≤0.015–0.5		0.03	0.125	≤0.015–0.125		1	4	0.03–4	
Overall	CLSI	0.125	0.5	≤0.015–2	0.001	0.5	2	≤0.015–>16	<0.001	0.125	2	≤0.015–8	NS	4	>128	≤0.125–>128	NS
	Etest	0.125	2	≤0.015–4		0.5	>16	≤0.015–>16		0.06	>16	≤0.015–>16		2	>128	≤0.125–>128	

[a] Differences in the antifungal susceptibilities obtained by CLSI and Etest procedures reached statistical significance for P values of <0.05.
[b] MICs for 1 *D. capitatus* isolate were read after 3 days (CLSI) or 4 days (Etest) of incubation due to poor fungal growth.
[c] NS, nonsignificant.
[d] MICs for 2 *R. mucilaginosa* isolates were read after 7 days (CLSI and Etest) due to poor fungal growth.
[e] MICs for 5 *S. cerevisiae* isolates were read after 5 days (CLSI) due to poor fungal growth.
[f] MICs for 3 *Trichosporon* isolates were read after 3 days (CLSI) or 4 days (Etest) due to poor fungal growth.

each method were within ±2 or fewer 2-fold dilutions of each other (4, 8).

In addition, strains and antifungal agents were compared to calculate categorical agreement using the CLSI M27-A3 breakpoints, as follows: voriconazole (≤1 μg/ml, susceptible; 2 μg/ml, susceptible/dose dependent; ≥4 μg/ml, resistant); fluconazole (≤8 μg/ml, susceptible; 16 to 32 μg/ml, susceptible/dose dependent; ≥64 μg/ml, resistant) (10).

The quality control strains *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were tested to ensure proper performance of the assay. All MIC results with these strains were within the recommended CLSI limits.

Values for the MIC_{50}, MIC_{90}, and MIC range (μg/ml) of each antifungal toward the different yeast genera are presented in Table 1. Isavuconazole, posaconazole, and voriconazole had reasonably low MICs for most of the strains examined, whereas the MICs for fluconazole tended to be at least several $\log_2$ dilution steps higher. Posaconazole and voriconazole showed comparable MIC_{50}s and MIC_{90}s, which were higher than those of isavuconazole (especially in the case of *S. cerevisiae* and *R. mucilaginosa*). Isavuconazole was the only azole that showed partial antifungal activity against *R. mucilaginosa*.

With few exceptions, the MIC_{90}s obtained using the Etest were generally higher than those obtained by CLSI broth microdilution. Irrespective of the technique, the MIC_{90}s for the four azoles were lower for *D. capitatus* and *Trichosporon* spp. than for *S. cerevisiae* and *R. mucilaginosa*.

CLSI M27-A3 and Etest results were compared for each antifungal agent and species as shown in Table 2. The essential agreement between the two methods was moderate and ranged from 52.7% (posaconazole) to 83.8% (fluconazole). According to the breakpoints adopted for *Candida* spp., the complete cohort of yeasts was classified according to the results of each test for fluconazole and voriconazole (Table 3). Resistance to voriconazole was found in *R. mucilaginosa* (22.2%) and *Trichosporon* spp. All strains of *R. mucilaginosa* were fluconazole resistant.

Appropriate first-line therapy for fungemia caused by *D. capitatus*, *S. cerevisiae*, *Trichosporon* spp., or *Rhodotorula* has not been defined, mainly due to the low number of cases reported; however, diminished susceptibility toward the most commonly used antifungal agents (amphotericin B and fluconazole) has been observed (3, 7, 11). Isavuconazole has been shown to have good *in vitro* activity against *Aspergillus*, *Candida*, and *Cryptococcus* spp. (5, 6, 12). Although the number of strains in the present survey is limited, the results corroborate previous reports (14) that isavuconazole is likely to be effective against infections caused by *D. capitatus*, *S. cerevisiae*, *Trichosporon* spp., or *Rhodotorula*.

Agreement between the MIC results obtained by the two methods for the four triazoles tested was <83%. The principal discrepancies were observed for *R. mucilaginosa* (posaconazole and voriconazole). A definitive comparison between broth microdilution and Etest strips for these rare yeasts will require much larger numbers of clinical isolates.

TABLE 2. Comparison between CLSI M27-A3 and Etest procedures[a]

Drug and organism	% of strains								
	≥−3	−2	−1	0	+1	+2	≥+3	Within ±1 log[b]	Within ±2 logs[c]
Isavuconazole									
Dipodascus capitatus	9.5	14.3	14.3	9.5	19	14.3	19.1	42.8	71.4
Rhodotorula mucilaginosa			5.6	16.7	33.3	22.2	22.2	55.6	78.8
Saccharomyces cerevisiae			15	15	25	15	30	55	70
Trichosporon spp.	6.7	20	40	20	6.7	6.7		66.7	93.4
Overall	4.1	8.1	17.6	14.9	20.3	14.9	20.4	52.8	75.8
Posaconazole									
Dipodascus capitatus	14.3	19	19	9.5	14.3	4.8	19.1	42.8	66.6
Rhodotorula mucilaginosa				6.2			93.8	6.2	6.2
Saccharomyces cerevisiae	5		5	5	30	10	45	40	50
Trichosporon spp.	6.7	6.7	33.3	26.7	6.7	20		66.7	93.8
Overall	6.8	6.8	13.5	10.8	13.5	8.1	40.6	37.8	52.7
Voriconazole									
Dipodascus capitatus	28.6	23.8	14.3	4.8	14.3	9.5	4.8	33.4	66.7
Rhodotorula mucilaginosa					5.6	5.6	88.8	5.6	11.2
Saccharomyces cerevisiae	5	20	50	20	5			75	95
Trichosporon spp.	6.7	20	46.7	20		6.7		66.7	93.4
Overall	10.8	16.2	27	10.8	6.8	5.4	23.1	44.6	66.2
Fluconazole									
Dipodascus capitatus	19	19	33.3	9.5	19			61.8	80.8
Rhodotorula mucilaginosa				100				100	100
Saccharomyces cerevisiae	5	5	10	30	25	15	10	65	85
Trichosporon spp.	20	20	6.7	26.7	13.3		13.4	46.7	66.7
Overall	10.8	10.8	13.5	40.5	14.9	4.1	5.5	68.9	83.8

[a] Percentages of strains for which the MICs for triazoles differed by ±1, ±2, and ≥(±3) $\log_2$ dilution steps are shown.
[b] Percentage of strains with ±1-dilution differences from the results with the CLSI method.
[c] Percentage of strains with ±2-dilution differences from the results with the CLSI method.

TABLE 3. Percentages of isolates included in each category (susceptible, susceptible/dose dependent, or resistant) by each procedure (CLSI and Etest) for the MICs of voriconazole and fluconazole

Species	Procedure	% of MICs in each category[a]					
		Voriconazole			Fluconazole		
		S	SDD	R	S	SDD	R
D. capitatus	CLSI	100	0	0	85.7	14.3	0
	Etest	100	0	0	100	0	0
R. mucilaginosa	CLSI	33.3	44.4	22.2	0	0	100
	Etest	0	0	100	0	0	100
S. cerevisiae	CLSI	100	0	0	80	20	0
	Etest	100	0	0	80	5	15
Trichosporon spp.	CLSI	83.8	10.8	5.4	66.2	9.5	24.3
	Etest	75.7	0	24.3	70.3	1.4	28.4
Total	CLSI	83.8	10.8	5.4	66.2	9.5	24.3
	Etest	75.7	0	24.3	70.3	1.4	28.4

[a] Percentages of CLSI and Etest MICs that were within the *Candida* breakpoints chosen for fluconazole (MICs of ≤8 μg/ml, susceptible [S]; MICs of 16 to 32 μg/ml, susceptible/dose dependent [SDD]; MICs of ≥64 μg/ml, resistant [R]) and voriconazole (MICs of ≤1 μg/ml, S; MICs of 2 μg/ml, SDD; MICs of ≥4 μg/ml, R) (2, 10).

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