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In Vitro Antifungal Activities of Isavuconazole (BAL4815), Voriconazole, and Fluconazole against 1,007 Isolates of Zygomycete, *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* Species▽

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Isavuconazole (BAL4815) is a promising novel broad-spectrum triazole in late-stage clinical development that has proven active in vitro against *Aspergillus* and *Candida* species. We compared the in vitro activities of this agent with those of voriconazole and fluconazole by the CLSI (formerly NCCLS) M38-A and M27-A2 procedures against a large collection of 1,007 relevant opportunistic fungi collected from 1986 to 2007: *Aspergillus* spp. ($n = 702$), *Candida* spp. ($n = 218$), Zygomycetes ($n = 45$), *Scedosporium* spp. ($n = 22$), and *Fusarium* spp. ($n = 20$). All *Candida* isolates were from patients with candidemia. For isavuconazole, these techniques were also compared with the Etest. Isavuconazole and voriconazole had MICs at which 50% and 90% of isolates were inhibited (MIC_{50} and MIC_{90}), respectively, of 1 and 1 μg/ml and 0.5 and 1 μg/ml against *Aspergillus* spp. and of 0.015 and 0.03 μg/ml and 0.25 and 0.125 μg/ml against *Candida* spp. (including fluconazole-resistant strains). The MIC_{50} partial and complete inhibition end points of isavuconazole and voriconazole against the non-*Aspergillus* molds were as follows: 1 and 2 μg/ml and 16 and >16 μg/ml against Zygomycetes; 1 and 4 μg/ml and 0.25 and 0.5 μg/ml against *Scedosporium apiospermum*; 4 to 16 and >16 μg/ml and 4 to 8 and 16 to >16 μg/ml (ranges) against *Scedosporium prolificans*; and 16 and 16 μg/ml and 4 and 4 μg/ml against *Fusarium* spp. Isavuconazole showed minimal fungicidal concentrations for 50% and 90% of the isolates of 1 and 1 μg/ml against *Aspergillus*, 16 and >16 μg/ml against *Candida*, and 4 and >16 μg/ml against Zygomycetes, respectively, and >16 μg/ml against the remaining molds. The Etest proved to be a suitable alternative method for determining the antifungal activities of isavuconazole against *Aspergillus* and *Candida*; the Etest results showed 96% and 93% agreement with the results of the CLSI M38-A and M27-A2 methods, respectively.

Invasive fungal infections (IFIs) are an important and increasing cause of morbidity and mortality in immunocompromised populations (6, 7). Despite correct antifungal treatment with available agents, the mortality rate of patients with IFIs remains extremely high.

Isavuconazole (formerly known as BAL4815) is a novel and promising broad-spectrum triazole in late-stage clinical development for the treatment of invasive aspergillosis. It is the active antifungal component of the water-soluble prodrug BAL8557, which can be administered intravenously and orally, and in preclinical studies it has demonstrated good pharmacokinetic parameters and low toxicity (11, 12).

Isavuconazole has proven active in vitro against *Aspergillus* and *Candida* spp. (13, 17). In an experimental neutropenic murine model of disseminated *Aspergillus flavus* infection, it showed high survival rates (19). However, in vitro studies with large numbers of *Aspergillus* and other mold isolates are necessary.

We compared the in vitro activities of isavuconazole with those of voriconazole and fluconazole against a large collection of clinically relevant opportunistic fungi. In addition, the results obtained by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) M38-A procedure, the CLSI M27-A2 procedure, and the Etest for isavuconazole were compared.

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MATERIALS AND METHODS

Organisms and source of samples. We analyzed a collection of 1,007 environmental and clinically opportunistic fungi collected from 1986 to 2007. The collection comprised 315 environmental mold isolates from outdoor air in the province of Madrid ($n = 257$), hospital air ($n = 47$), and other sources ($n = 11$), as well as 692 clinical isolates from 534 different patients in our hospital. The clinical sources of the isolates were abscesses/normally sterile fluids ($n = 23$), biopsy specimens ($n = 32$), wounds ($n = 40$), respiratory samples ($n = 339$), blood samples ($n = 222$), skin and soft tissue samples ($n = 19$), and other samples ($n = 17$).

The isolates studied belonged to *Aspergillus* spp. ($n = 702$), *Candida* spp. ($n = 218$), Zygomycetes ($n = 45$), *Scedosporium* spp. ($n = 22$), and *Fusarium* spp. ($n = 20$). All *Candida* isolates were from patients with candidemia. Mold isolates were from the environment or from patients who had IFIs or were colonized.

All strains were cultured in Sabouraud dextrose agar, sheep blood agar, or Bactec medium and were identified by conventional morphological, chromogenic, and/or biochemical procedures. They were stored as spore or yeast suspensions in a solution of sterile distilled water at −70°C. To ensure viability and purity, each isolate was subcultured on potato dextrose agar or Sabouraud dextrose agar before being tested.

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TABLE 1. In vitro activities of voriconazole and isavuconazole against *Aspergillus* spp. by the CLSI M38-A procedure[a]

Isolate group (*n*)	Voriconazole MIC				Isavuconazole activity							
					MIC				MFC			
	GM	90%	50%	Range	GM	90%	50%	Range	GM	90%	50%	Range
A. fumigatus (602)	0.477	1	0.5	0.125–2	0.822	1	1	0.125–4	0.834	1	1	0.125–4
A. flavus (34)	0.652	1	1	0.125–2	0.752	1	1	0.25–2	0.799	1	1	0.5–2
A. niger (32)	0.771	1	1	0.25–2	1.189	2	1	0.25–4	1.215	2	1	0.25–4
A. terreus (25)	0.624	1	1	0.25–1	0.660	1	1	0.125–1	0.753	1	1	0.25–2
Other *Aspergillus* spp.[b] (9)				0.125–8				0.25–4				0.25–16
Environmental strains (302)	0.542	1	0.5	0.125–8	0.859	1	1	0.25–4	0.893	1	1	0.25–16
Clinical strains (400)	0.427	1	0.5	0.125–2	0.827	1	0.5	0.125–2	0.818	1	1	0.125–2
Overall (702)	0.473	1	0.5	0.125–8	0.827	1	1	0.125–4	0.850	1	1	0.125–16

[a] Activities are given for the most clinically relevant species and overall. All MICs and MFCs are expressed in micrograms per milliliter. GM, geometric mean. For the two azoles against *Aspergillus* spp., the MIC end point was defined as the lowest concentration that produced complete inhibition of growth (MIC-0) after 48 h of incubation.

[b] Due to the limited number of strains, antifungal activity is expressed only as a range of concentrations.

Antifungal agents. The antifungal drugs used in the study and obtained as reagent-grade powders from their respective manufacturers were isavuconazole (BAL4815) (Basilea Pharmaceutica, Basel, Switzerland), voriconazole (Pfizer Pharmaceutical Group, New York, NY), and fluconazole (Pfizer Pharmaceutical Group). The activities of isavuconazole and voriconazole were determined against all isolates and that of fluconazole only against *Candida* spp. Antifungal activities were determined using the CLSI M38-A and M27-A2 broth microdilution methods for molds and *Candida* spp., respectively. We also studied the antifungal activity of isavuconazole using the Etest.

CLSI M38-A and M27-A2 microdilution procedures. Stock solutions of isavuconazole, voriconazole, and fluconazole were prepared in dimethyl sulfoxide (Sigma, Madrid, Spain). Trays containing a 0.1-ml aliquot of the appropriate drug solution (2 × final concentration) in each well were first subjected to quality control and then sealed and stored at −70°C until use. The final concentrations of drug in the wells ranged from 0.015 μg/ml to 16 μg/ml for isavuconazole and voriconazole and from 0.125 μg/ml to 128 μg/ml for fluconazole.

All the inoculated microdilution trays were incubated at 35°C and read macroscopically at 24 h (for Zygomycetes) and 48 h (for the remaining molds and *Candida* spp.). According to the CLSI procedures, the MIC end point for isavuconazole and voriconazole with *Aspergillus* spp. was defined as the lowest concentration that produced complete inhibition of growth (MIC-0). For *Fusarium* spp., *Scedosporium* spp., and Zygomycetes, we also calculated MIC-1, defined as the lowest concentration that produced slight growth or approximately 25% that of the growth control (1). The MIC end point for azoles and *Candida* spp. was defined as the lowest concentration at which a prominent decrease in turbidity, corresponding to approximately 50% inhibition of growth, was observed (MIC-2) after 48 h of incubation (2).

We also calculated the minimum fungicidal concentration (MFC) for isavuconazole. For each isolate, 100 μl was removed from all wells with no visible fungal growth. Each aliquot was spot inoculated onto Sabouraud dextrose agar plates; the liquid was allowed to soak into the agar; and the plate was streaked. The plates were incubated at 35°C and read after 24 h (with confirmation after 48 h). The MFC was defined as the lowest concentration that killed 99% of spores or yeast cells present in the original inoculum in each well (50 or fewer colonies of molds and 2 colonies of yeasts), according to previous studies (17–19).

Etest method. Etest strips (AB Biodisk, Solna, Sweden) of isavuconazole were supplied by Basilea Pharmaceutica. The inocula of spores and yeast cells were the same as those used for the microdilution procedure before the 50-fold dilution. A swab was dipped into the cell suspensions and streaked across the surfaces of RPMI agar plates with 2% glucose. The plates were incubated at 35°C and read at 24 h (for Zygomycetes) and 48 h (for the remaining molds and *Candida* spp.).

The MIC was determined by the Etest in accordance with the manufacturer's instructions. It was defined as the lowest drug concentration at which the border of the elliptical inhibition zone intercepted the scale on the antifungal strip.

Quality control. Quality control was ensured by testing the following strains: *A. flavus* ATCC 204304, *Aspergillus fumigatus* ATCC 204305, *Candida krusei* ATCC 6258, and *Candida parapsilosis* ATCC 22019. All results were within the recommended CLSI limits.

Data analysis. The activities of isavuconazole, voriconazole, and fluconazole were expressed as geometric means, MICs at which 90% and 50% of isolates were inhibited (MIC_{90} and MIC_{50}, respectively), MFCs at which 90% and 50% of spores or yeast cells were killed (MFC_{90} and MFC_{50}, respectively), and ranges of MICs and MFCs.

For *Aspergillus* spp. and the remaining molds, no breakpoints for the new triazoles have been established. For *Candida* spp., the classification of the strains for voriconazole was as follows: susceptible, breakpoint of ≤1 μg/ml; susceptible–dose dependent, 2 μg/ml; resistant, ≥4 μg/ml (9). The classification for fluconazole was as follows: susceptible, breakpoint of ≤8 μg/ml; susceptible–dose dependent, 16 to 32 μg/ml; resistant, ≥64 μg/ml.

Because the Etest strips contain a continuous gradient of isavuconazole instead of the established twofold drug dilutions, the MIC end point obtained by the Etest was raised to the next twofold dilution concentration matching the drug dilution on the scale used for the CLSI procedure. The CLSI and corrected Etest MIC distributions for isavuconazole against each clinical isolate were converted to log MICs. We compared the results of the Etest at 48 h of incubation with the CLSI results. MIC end point discrepancies of no more than ±2-fold dilutions were used to calculate the percentage of agreement between the two methods according to previous studies (4, 8).

RESULTS

Antifungal activity. For all 1,007 strains, the in vitro activity of each antifungal drug, expressed as the geometric mean, MIC_{90} and MIC_{50}, range, and MFC_{90} and MFC_{50} (in micrograms per milliliter) is shown in Tables 1 to 4.

Table 1 shows the activities of isavuconazole and voriconazole against the *Aspergillus* sp. isolates. Both drugs showed MIC_{90}s of 1 μg/ml against *Aspergillus* spp. Although there were no significant differences in the activity of isavuconazole between different species of *Aspergillus*, isavuconazole tended to show slightly higher MICs (onefold dilution) against *A. niger*. Isavuconazole and voriconazole showed antifungal activity against *A. terreus*, a well-known amphotericin B-resistant species. Only for one *A. nidulans* strain was the voriconazole MIC 8 μg/ml, but the isavuconazole MIC for that strain was 1 μg/ml. For the remaining strains of the most clinically relevant *Aspergillus* species, voriconazole and isavuconazole MICs were ≤4 μg/ml. Isavuconazole also presented an equivalent MFC_{90} and MIC_{90} against *Aspergillus* spp.

TABLE 2. In vitro activities of voriconazole and isavuconazole against *Fusarium* spp., *Scedosporium* spp., and Zygomycetes by the CLSI M38-A procedure[a]

Isolate group (*n*)	Voriconazole MIC-0, MIC-1				Isavuconazole activity						
					MIC-0, MIC-1				MFC		
	GM	90%	50%	Range	GM	90%	50%	Range	90%	50%	Range
Zygomycetes[b]											
Mucor (21)		>16, >16	>16, 16	2–>16, 2–>16		16, 8	2, 1	1–>16, 0.25–16	>16	8	2–>16
Rhizopus (12)		>16, >16	8, 8	8–>16, 4–>16		16, 4	1, 0.5	1–16, 0.5–4	>16	4	1–>16
Absidia (6)				16–>16, 4–>16				1–16, 0.5–8			4–>16
Cunninghamella (4)				16–>16, 8–16				2–16, 0.5–8			16–>16
Syncephalastrum (2)				4–>16, 2–16				0.25–8, 0.125–4			1–16
Overall (45)		>16, >16	>16, 16	2–>16, 2–>16	3.364, 1.506	16, 8	2, 1	0.25–>16, 0.125–16	>16	4	1–>16
S. apiospermum (16)		1, 0.5	0.5, 0.25	0.25–8, 0.125–4		4, 2	4, 1	0.5–8, 0.06–8	>16	8	2–>16
S. prolificans (6)				16–>16, 4–8				>16, 4–16			>16
Overall (22)	1.438, 0.571	>16, 8	0.5, 0.25	0.25–>16, 0.125–8	5.040, 1.257	>16, 8	4, 1	0.5–>16, 0.06–16	>16	>16	2–>16
Fusarium spp. (20)	7.727, 3.605	16, 8	4, 4	1–>16, 1–8	16.564, 11.314	>16, >16	16, 16	1–>16, 2–>16	>16	>16	4–>16

[a] Activities are given for the most clinically relevant genera of Zygomycetes and overall. All values are expressed as micrograms per milliliter. GM, geometric mean. For the two azoles against *Fusarium* spp., *Scedosporium* spp., and Zygomycetes, the MIC end point was defined as the lowest concentration that produced complete inhibition of growth (MIC-0). We also calculated MIC-1, defined as the lowest concentration that produced slight growth or approximately 25% of that of the growth control.

[b] MICs for Zygomycetes were read after 24 h of incubation.

Table 2 shows the activities of voriconazole and isavuconazole against the non-*Aspergillus* molds. Zygomycetes showed poor susceptibility to voriconazole, with a MIC_{90} of ≥16 μg/ml, independently of the MIC end point chosen (MIC-0 or MIC-1). In contrast, isavuconazole presented a limited antifungal effect against Zygomycetes, especially when the end point used was MIC-1 (MIC_{90} and MIC_{50}, 8 μg/ml and 1 μg/ml, respectively). The two species of *Scedosporium* tested showed marked differences in susceptibility: whereas voriconazole and isavuconazole showed poor activity against *Scedosporium prolificans*, *Scedosporium apiospermum* showed more susceptibility to both agents, with a MIC_{90} very similar to those for *Aspergillus* spp. when MIC-1 was the end point. The MFC_{90} of isavuconazole against *S. apiospermum* was dramatically higher than that observed against *Aspergillus* spp. For *Fusarium* spp., which are also resistant to several antifungals, the geometric mean MIC of voriconazole (7.72 μg/ml) was lower than that of isavuconazole (16.56 μg/ml).

Table 3 shows the activities of voriconazole, isavuconazole, and fluconazole against *Candida* spp. Isavuconazole MIC_{90}s were low for fluconazole-susceptible isolates of *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. For the fluconazole-resistant species *C. krusei*, isavuconazole MICs were low, although the number of strains included was limited. The MIC_{90}s of the three azole derivatives for *C. glabrata*, regarded as a fluconazole-susceptible–dose-dependent species, were higher than those for the other *Candida* species evaluated.

Of all the *Candida* strains studied, 186 (85.32%) were susceptible to fluconazole, 22 (10.01%) were susceptible–dose dependent (including the 5 strains of the inherently fluconazole resistant species *C. krusei*), and 10 (4.59%) were resistant (6 *C. albicans* and 4 *C. glabrata* strains). No strains were resistant to voriconazole, and only for one strain of *C. glabrata* was the isavuconazole MIC 2 μg/ml. Two strains (1%) of *C. glabrata* were susceptible–dose dependent to voriconazole (with isavuconazole MICs of 1 μg/ml and 2 μg/ml, respectively). These two strains were fluconazole resistant (MIC, 128 μg/ml). Overall, the isavuconazole MIC for 99.5% of the *Candida* isolates was ≤1 μg/ml.

Table 4 shows the activities of isavuconazole determined by the Etest. For *Candida* and *Aspergillus* spp., the MIC_{90} obtained by the Etest tended to be slightly lower than that ob-

TABLE 3. In vitro activities of voriconazole, isavuconazole, and fluconazole against *Candida* spp. by the CLSI M27-A2 procedure[a]

Species (no. of isolates)	Voriconazole MIC			Isavuconazole activity						Fluconazole MIC		
				MIC			MFC					
	90%	50%	Range	90%	50%	Range	90%	50%	Range	90%	50%	Range
C. albicans (70)	0.06	0.03	<0.015–1	<0.015	0.03	<0.015–2	>16	>16	0.03–>16	16	0.25	0.125–>128
C. parapsilosis (84)	0.06	<0.015	<0.015–0.5	0.03	<0.015	<0.015–0.125	16	4	0.03–>16	1	0.5	0.25–8
C. glabrata (37)	1	0.125	<0.015–2	1	0.25	<0.015–2	>16	16	0.03–>16	32	16	0.25–128
C. tropicalis (15)	0.06	0.03	<0.015–0.125	0.06	<0.015	<0.015–0.125	>16	>16	2–>16	2	0.25	0.25–8
Other *Candida* spp. (7)			<0.015–0.006			<0.015–0.125			0.003–>16			0.25–2
C. krusei (5)			0.125–0.25			0.125–0.25			0.5–4			16–64
Overall (21)	0.125	0.03	<0.015–2	0.25	0.015	<0.015–2	>16	16	0.03–>16	16	0.5	0.125–>128

[a] Activities are given for the most clinically relevant species of *Candida* and overall. All values are expressed in micrograms per milliliter. For azoles and *Candida*, the MIC end point was defined as the lowest concentration at which a prominent decrease in turbidity, corresponding to approximately 50% inhibition of growth, was observed (MIC-2) after 48 h of incubation.

TABLE 4. In vitro activities of isavuconazole (BAL4815) against all fungi, determined by the Etest[a]

Genus or species (no. of isolates)	Isavuconazole MIC		
	90%	50%	Range
Aspergillus spp.			
A. fumigatus (602)	0.5	0.25	0.047–4
A. flavus (34)	0.5	0.38	0.016–0.5
A. niger (32)	0.75	0.5	0.094–1
A. terreus (25)	0.5	0.38	0.094–0.5
Other (9)			0.02–1.5
Overall (702)	0.5	0.25	0.002–4
Zygomycetes			
Mucor (21)	>32	2	0.19–>32
Rhizopus (12)	>32	1	0.5–>32
Absidia (6)			0.25–>32
Cunninghamella (4)			0.38–>32
Syncephalastrum (2)			0.25–0.5
Overall (45)	>32	1	0.19–>32
Scedosporium spp.			
S. prolificans (6)			>32
S. apiospermum (16)	>32	>32	1–>32
Overall (22)	>32	>32	1–>32
Fusarium spp. (20)	>32	>32	0.38–>32
Candida spp.			
C. albicans[b] (69)	0.015	0.004	0.002–0.125
C. parapsilosis (84)	0.023	0.004	<0.002–0.125
C. glabrata (37)	0.38	0.094	0.006–6
C. tropicalis (15)	0.125	0.015	0.003–0.25
Other (7)			0.004–0.16
C. krusei (5)			0.012–0.38
Overall (217)	0.125	0.006	<0.002–6

[a] All values are expressed in micrograms per milliliter.
[b] One strain of *Candida albicans* did not grow on RPMI agar plates.

TABLE 5. Number of strains for which the MICs of isavuconazole (BAL4815) by Etest differed by ± 1, ±2, and >±3 log dilutions from those obtained by the CLSI M38-A and M27-A2 procedures

Genus or species (no. of isolates)	No. of isolates for which the Etest MICs differed by the following no. of log dilutions:							% Within ±1 $\log_2$ dilution[a]	% Within ±2 $\log_2$ dilutions[b]
	≥−3	−2	−1	0	+1[d]	+2	≥+3		
Aspergillus spp.									
A. fumigatus (602)				69	261	255	17	54.82	97.18
A. flavus (34)			1	5	18	8	2	70.59	94.12
A. niger (32)				1	22	7	2	71.87	93.75
A. terreus (25)				8	12	5		80.00	100
Other (9)				1	2	4	2	33.33	77.78
Overall (702)			1	84	315	279	23	56.98	96.73
Zygomycetes									
Mucor (21)	3	1	7	8		1	1	71.43	80.95
Rhizopus (12)	3		2	6	1			75.00	75.00
Absidia (6)			1	3	1	1		83.33	100
Cunninghamella (4)				2	1	1		75.00	100
Syncephalastrum (2)						2		0	100
Overall (45)	6	1	10	19	3	5	1	71.11	84.44
Scedosporium spp.									
S. prolificans (6)				6				100	100
S. apiospermum (16)	13	2	1					6.25	18.75
Overall (22)	13	2		6	1			31.82	40.91
Fusarium spp. (20)	1	4	4	9		1	1	65.00	85.00
Candida spp.									
C. albicans (69)[c]	2	2	3	47	10	4	1	85.51	94.20
C. parapsilosis (84)	3	3	13	63	1	1		91.67	96.43
C. glabrata (37)		1	5	14	5	8	4	62.00	89.12
C. tropicalis (15)	2	1	2	6	3	1		73.33	86.67
Other (7)	1			6				85.71	85.71
C. krusei (5)			2	1	1		1	80.00	80.00
Overall (217)	7	7	25	138	19	13	7	84.33	93.09

[a] Percentage of strains included between dilutions −1 and +1.
[b] Percentage of strains included between dilutions −2 and +2.
[c] One strain of *C. albicans* did not grow on RPMI agar.
[d] MIC by the E-test 1 dilution step lower.

tained by the CLSI procedure (approximately a onefold dilution). In contrast, the MICs of isavuconazole for Zygomycetes by the Etest were higher than those obtained by broth microdilution. *S. prolificans* and *Fusarium* spp. also proved to be less susceptible to isavuconazole by this method. However, wide discrepancies were found for *S. apiospermum*: the Etest value and CLSI MIC_{90} were >32 μg/ml and 4 μg/ml, respectively. For *Candida* spp., the two procedures yielded very similar activities for all the species evaluated.

Agreement between the CLSI M38-A procedure, the CLSI M27-A2 procedure, and the Etest for isavuconazole. To determine the levels of agreement between the "gold standard" procedures (CLSI M38-A and CLSI M27-A2) and the Etest, we calculated the percentage of strains for which the isavuconazole MICs by Etest differed by ± 1, ±2, and >±3 dilutions from those obtained by the gold standard. These results are summarized in Table 5. For *Aspergillus* spp., the correlation was very good, with an overall agreement above 96% (±2 dilutions) for all clinically relevant species. The correlation for Zygomycetes was moderate but variable between the different genera of the group. Isavuconazole showed poor antifungal activity against *S. prolificans* by both methods (agreement, 100% [±2 dilutions]), although there was a high percentage of disagreement for *S. apiospermum*. Isavuconazole showed poor activity against *Fusarium* spp. by both procedures. For *Candida* spp., the Etest correlated very well with the CLSI M27-A2 procedure (±2 dilutions), especially for *C. albicans* and *C. parapsilosis*, the most common etiological agents of candidemia.

We detected only one strain of *C. glabrata* for which the isavuconazole MIC was 2 μg/ml by the CLSI M27-A2 procedure but 6 μg/ml by the Etest.

DISCUSSION

Our study showed that isavuconazole was active against *Aspergillus* and *Candida* spp. and that there were high levels of agreement between both CLSI procedures and the Etest.

Previous series have shown good in vitro activities of voriconazole against *Aspergillus* spp, but the data for isavuconazole are still limited (3, 5, 10, 15, 17). Isavuconazole and voriconazole proved to be active against *Aspergillus* spp. in the present study. In addition, the MFC of isavuconazole was the same as or only onefold higher than the MIC, as reported in recent series (17). Isavuconazole also demonstrated substantial activity against *A. terreus*, a well-known amphotericin B-resistant species (14, 16). No *Aspergillus* strains were resistant to voriconazole, but other reports have shown that isavuconazole had in vitro activity against itraconazole-resistant strains (17).

Zygomycetes are known to be resistant to voriconazole in vitro and in vivo. Due to the limited number of drugs active against Zygomycetes and other rare but multiresistant molds, we decided to show the activity of isavuconazole using MIC-0

and MIC-1 as end points. Isavuconazole presented a limited antifungal effect against Zygomycetes.

The differences in the in vitro activities of isavuconazole against *S. prolificans* and *S. apiospermum* are similar to those reported for posaconazole (10). *S. apiospermum* is susceptible to the new triazoles. The activities of isavuconazole and voriconazole were also limited for *Fusarium* spp. However, due to the limited number of non-*Aspergillus* mold isolates included, other confirmatory studies are necessary.

Isavuconazole presented good antifungal activity against *Candida* strains isolated from patients with candidemia, including species inherently resistant to fluconazole (*C. krusei*) and those with the ability to develop such resistance (*C. glabrata*). In a recent study, Seifert et al. reported similar in vitro activities of isavuconazole against *Candida* spp. (13), indicating that antifungal resistance to the new triazoles is infrequent.

The Etest has the potential to become a good alternative to the CLSI M38-A and M27-A2 procedures for determining the antifungal activities of isavuconazole against *Aspergillus* and *Candida* spp., with levels of agreement above 96% and 93%, respectively. We found only one strain of *C. glabrata* with a MIC of 2 μg/ml by the CLSI procedure and 6 μg/ml by the Etest. In the absence of specific breakpoints, the Etest showed the ability to detect the strain of *Candida* with the highest isavuconazole microdilution MIC included in the study. One shortcoming of our study, however, is the limited number of *Aspergillus* and *Candida* strains with reduced susceptibility to isavuconazole. Susceptibility testing for the limited number of molds other than *Aspergillus* species makes it difficult to reach other conclusions.

In summary, isavuconazole and voriconazole showed good antifungal activities against *Candida* spp., including fluconazole-resistant strains, and *Aspergillus* spp. The activity of isavuconazole against the non-*Aspergillus* mold isolates should be investigated in a series with a larger number of isolates.

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