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In Vitro Activity of Isavuconazole and Comparators against Clinical Isolates of the *Mucorales* Order

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The *in vitro* activity of isavuconazole against *Mucorales* isolates measured by EUCAST E.Def 9.2 and CLSI M38-A2 methodologies was investigated in comparison with those of amphotericin B, posaconazole, and voriconazole. Seventy-two isolates were included: 12 of *Lichtheimia corymbifera*, 5 of *Lichtheimia ramosa*, 5 of group I and 9 of group II of *Mucor circinelloides*, 9 of *Rhizomucor pusillus*, 26 of *Rhizopus microsporus*, and 6 of *Rhizopus oryzae*. Species identification was confirmed by internal transcribed spacer (ITS) sequencing. EUCAST MICs were read on day 1 (EUCAST-d1) and day 2 (EUCAST-d2), and CLSI MICs were read on day 2 (CLSI-d2). Isavuconazole MIC_{50}s (range) (mg/liter) by EUCAST-d1, CLSI-d2, and EUCAST-d2 were 1 (0.125 to 16), 1 (0.125 to 2), and 4 (0.5 to >16), respectively, across all isolates. The similar values for comparator drugs were as follows: posaconazole, 0.25 (≤0.03 to >16), 0.25 (0.06 to >16), and 1 (0.06 to >16); amphotericin, 0.06 (≤0.03 to 0.5), 0.06 (≤0.03 to 0.25), and 0.125 (≤0.03 to 1); voriconazole, 16 (2 to >16), 8 (1 to >16), and >16 (8 to >16), respectively. Isavuconazole activity varied by species: *Lichtheimia corymbifera*, 1 (0.5 to 2), 1 (1 to 2), and 2 (1 to 4); *Lichtheimia ramosa*, 0.25 (0.125 to 0.5), 1 (0.5 to 2), and 2 (0.5 to 4); *Rhizomucor pusillus*, 0.5 (0.5 to 1), 1 (0.125 to 1), and 2 (1 to 2); *Rhizopus microsporus*, 1 (0.5 to 4), 0.5 (0.125 to 1), and 4 (1 to 8); and *Rhizopus oryzae*, 1 (0.5 to 4), 1 (0.125 to 2), and 4 (0.5 to 8), respectively, were more susceptible than *Mucor circinelloides*: group I, 8 (4 to 8), 4 (2 to 4), and 16 (2 to 16), respectively, and group II, 8 (1 to 16), 8 (1 to 8), and 16 (4 to >16), respectively. This was also observed for posaconazole. The essential agreement was best between EUCAST-d1 and CLSI-d2 (75% to 83%). Isavuconazole displayed *in vitro* activity against *Mucorales* isolates with the exception of *Mucor circinelloides*. The MICs were in general 1 to 3 steps higher than those for posaconazole. However, in the clinical setting this may be compensated for by the higher exposure at standard dosing.

Isavuconazole is a new broad-spectrum azole with activity against various yeasts and molds (1). It is administered as a water-soluble prodrug, isavuconazonium sulfate, which is available as cyclodextrin-free intravenous (i.v.) and oral (p.o.) formulations. Following administration, the prodrug is immediately and completely converted by plasma esterases to isavuconazole, which inhibits biosynthesis of ergosterol, an essential component of fungal membranes. Currently, amphotericin B and posaconazole are the only two compounds recommended for treatment of *Mucorales* infections in Europe, only amphotericin B is recommended for primary treatment, and only amphotericin B is licensed for treatment of these infections (2, 3). The clinical efficacy of isavuconazole against infections due to *Mucorales* species has been evaluated in a phase III study leading to its approval by the FDA for the primary treatment of mucormycosis (March 2015). An overall success rate of 31.4% (14.3% and 17.1%, complete and partial response, respectively) was reported at the end of treatment among 37 patients with *Mucorales* monoinfection (4). Twenty-one of these patients were matched and compared with patients from the FungiScope registry treated with an amphotericin B formulation (a third of the patients received conventional amphotericin B) (5). The median treatment duration was 108 days for isavuconazole and 18 days for amphotericin B, with approximately one-third of the patients receiving additional posaconazole therapy in the amphotericin B patient group. Overall survival rates on days 42 and 84 were similar (5).

The *in vitro* activity of isavuconazole has been studied using the EUCAST and CLSI methodologies against *Candida* and *Aspergillus*; however, data on *in vitro* activity against isolates of the *Mucorales* order are sparse and particularly so for EUCAST testing (6–9). The purpose of this study was to investigate and compare the *in vitro* activities against clinical isolates of the *Mucorales* order by the EUCAST and CLSI reference methodologies and to compare the activities with those of amphotericin B, posaconazole, and voriconazole. Such data are crucial for clarifying the correlation between *in vitro* and *in vivo* responses and for future development of epidemiological cutoff values (ECOFFs/ECVs) and clinical breakpoints.

MATERIALS AND METHODS

***Mucorales* isolates and species identification.** A total of 72 clinical *Mucorales* isolates were included. The isolates were obtained in 1998 to 2014 from samples or pure cultures referred to the mycology reference laboratory at the Statens Serum Institut, Denmark. Seventy isolates were confirmed to originate from nonsuperficial specimens, whereas no informa-

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TABLE 1 Overview of MIC ranges, MIC_{50} values, and proportions of *Mucorales* species isolates for which MICs fall within the wild-type MIC range for *A. fumigatus* when susceptibility is tested by EUCAST (E.Def 9.2) and CLSI (M38-A2) methodologies[e]

Antifungal compound and species (no. of isolates)	Visual reading result								
	EUCAST, day 1			EUCAST, day 2			CLSI, day 2		
	Range (mg/liter)	MIC_{50} (mg/liter)	% of MICs below *A. fumigatus* ECOFF[c]	Range (mg/liter)	MIC_{50} (mg/liter)	% of MICs below *A. fumigatus* ECOFF[c]	Range (mg/liter)	MIC_{50} (mg/liter)	% of MICs below *A. fumigatus* ECV[d]
Amphotericin B									
Lichtheimia corymbifera (12)	≤0.03 to 0.125	≤0.03	100	≤0.03 to 0.25	0.125	100	≤0.03 to 0.125	≤0.03	100
Lichtheimia ramosa (4[a]/5)	≤0.03	≤0.03	100	≤0.03 to 0.06	0.06	100	≤0.03	≤0.03	100
Mucor circinelloides									
Group I (4/5[b])	≤0.03 to 0.125	≤0.03	100	≤0.03 to 0.125	0.06	100	≤0.03	≤0.03	100
Group II (9)	≤0.03 to 0.125	0.06	100	0.06 to 0.25	0.125	100	≤0.03 to 0.125	0.06	100
Rhizomucor pusillus (8[a]/9)	≤0.03	≤0.03	100	≤0.03 to 0.25	0.06	100	≤0.03 to 0.25	≤0.03	100
Rhizopus microsporus (26)	0.06 to 0.5	0.125	100	0.25 to 1	0.5	100	≤0.03 to 0.25	0.125	100
Rhizopus oryzae (6)	0.125 to 0.5	0.25	100	0.5 to 1	0.5	100	≤0.03 to 0.25	0.06	100
Total (70/72[a,b])	≤0.03 to 0.5	0.06	100	≤0.03 to 1	0.125	100	≤0.03 to 0.25	0.06	100
Isavuconazole									
Lichtheimia corymbifera (12)	0.5 to 2	1	100	1 to 4	2	67	1 to 2	1	83
Lichtheimia ramosa (4[a]/5)	0.125 to 0.5	0.25	100	0.5 to 4	2	60	0.5 to 2	1	80
Mucor circinelloides									
Group I (4/5[b])	4 to 8	8	0	2 to 16	16	20	2 to 4	4	0
Group II (9)	1 to 16	8	11	4 to >16	16	0	1 to 8	8	11
Rhizomucor pusillus (8[a]/9)	0.5 to 1	0.5	100	1 to 2	2	100	0.125 to 1	1	100
Rhizopus microsporus (26)	0.5 to 4	1	92	1 to 8	4	35	0.125 to 1	0.5	100
Rhizopus oryzae (6)	0.5 to 4	1	83	0.5 to 8	4	33	0.125 to 2	1	83
Total (70/72[a,b])	0.125 to 16	1	77	0.5 to >16	4	44	0.125 to 2	1	77
Posaconazole									
Lichtheimia corymbifera (12)	0.06 to 0.25	0.125	100	0.125 to 0.5	0.25	75	0.125 to 0.5	0.25	100
Lichtheimia ramosa (4[a]/5)	≤0.03 to 0.125	≤0.03	100	0.06 to 0.5	0.5	40	0.06 to 0.5	0.25	100
Mucor circinelloides									
Group I (4/5[b])	0.25 to 1	0.5	40	0.5 to 8	1	0	0.5 to 1	0.5	75
Group II (9)	0.125 to >16	2	11	1 to >16	>16	0	0.125 to >16	2	11
Rhizomucor pusillus (8[a]/9)	≤0.03 to 0.125	0.06	100	0.125 to 0.5	0.25	78	0.06 to 0.25	0.125	100
Rhizopus microsporus (26)	0.25 to 1	0.5	12	0.5 to >16	2	0	0.06 to 0.5	0.25	100
Rhizopus oryzae (6)	0.25 to 2	0.5	50	0.25 to >16	0.5	17	0.125 to 0.5	0.5	100
Total (70/72[a,b])	≤0.03 to >16	0.25	47	0.06 to >16	1	26	0.06 to >16	0.25	87
Voriconazole									
Lichtheimia corymbifera (12)	4 to 16	16	0	>16	>16	0	16 to >16	16	0
Lichtheimia ramosa (4[a]/5)	2 to 16	8	0	16 to >16	>16	0	2 to >16	8	0
Mucor circinelloides									
Group I (4/5[b])	16 to >16	>16	0	8 to >16	>16	0	1 to >16	2	33
Group II (9)	4 to >16	>16	0	>16	>16	0	8 to >16	>16	0
Rhizomucor pusillus (8[a]/9)	4 to 8	8	0	16 to >16	16	0	1 to 16	8	11
Rhizopus microsporus (26)	4 to >16	8	0	16 to >16	>16	0	2 to 16	8	0
Rhizopus oryzae (6)	8 to 16	8	0	16 to >16	16	0	4 to 8	8	0
Total (70/72[a,b])	2 to >16	16	0	8 to >16	>16	0	1 to >16	8	3

[a] EUCAST MICs for one *Lichtheimia ramosa* isolate and one *Rhizomucor pusillus* isolate could not be evaluated on day 1 due to insufficient growth.
[b] CLSI MICs for one *Mucor circinelloides* group I isolate could not be evaluated due to no growth on day 2.
[c] Amphotericin B, 1 mg/liter; posaconazole, 0.25 mg/liter; isavuconazole, 2 mg/liter; voriconazole, 1 mg/liter (13, 15–17).
[d] Amphotericin B, 2 mg/liter; posaconazole, 0.5 mg/liter; isavuconazole, 1 mg/liter; voriconazole, 1 mg/liter (18, 19).
[e] MIC ranges and MIC_{50} values for *Mucorales* species isolates were determined by EUCAST (E.Def 9.2) and CLSI (M38-A2) methodologies.

tion regarding sample site was available for two samples. All isolates underwent confirmatory molecular species identification by internal transcribed spacer (ITS) DNA sequencing using the universal fungal primers (ITS1, TCGTAGGTGAACCTGCGG, and ITS4, TCCTCCGCTT ATTGATATGC [10]) and the online pairwise sequence alignment tool available through the webpage for Centraalbureau voor Schimmelcultures (http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx). The molecularly confirmed species distribution was as follows: 12 of *Lichtheimia corymbifera*, 5 of *Lichtheimia ramosa*, 5 of *Mucor circinelloides* group I (>99.8% match to CBS strains 106.10 and 195.68), 9 of *Mucor circinelloides* group II (>99.8% match to CBS strains 542.80 and 416.77), 9 of *Rhizomucor pusillus*, 26 of *Rhizopus microsporus*, and 6 of *Rhizopus oryzae* (see Fig. S1 in the supplemental material).

Susceptibility testing. Susceptibility testing was performed using the EUCAST E.Def 9.2 and the CLSI M38-A2 methodologies (11, 12). All isolates were cultured twice on Sabouraud dextrose agar (SSI Diagnostika, Hillerød, Denmark) before susceptibility testing to ensure viability. Stock solutions (5,000 mg/liter) in dimethyl sulfoxide (DMSO) and manufacturers were as indicated: DMSO, Sigma-Aldrich, Vallensbæk Strand, Denmark (catalog no. D8779); isavuconazole, Astellas Pharma Inc., Tokyo, Japan; amphotericin B, Sigma-Aldrich; posaconazole, Merck, Ballerup, Denmark; voriconazole, Pfizer A/S, Ballerup, Denmark. The drug concentration range studied was 0.03 to 16 mg/liter for all compounds. For both methods, plates were made in one batch, immediately frozen (−80°C), and used as soon as thawed. Inoculated plates were incubated at 35°C and read visually (blinded to the species identity) at days

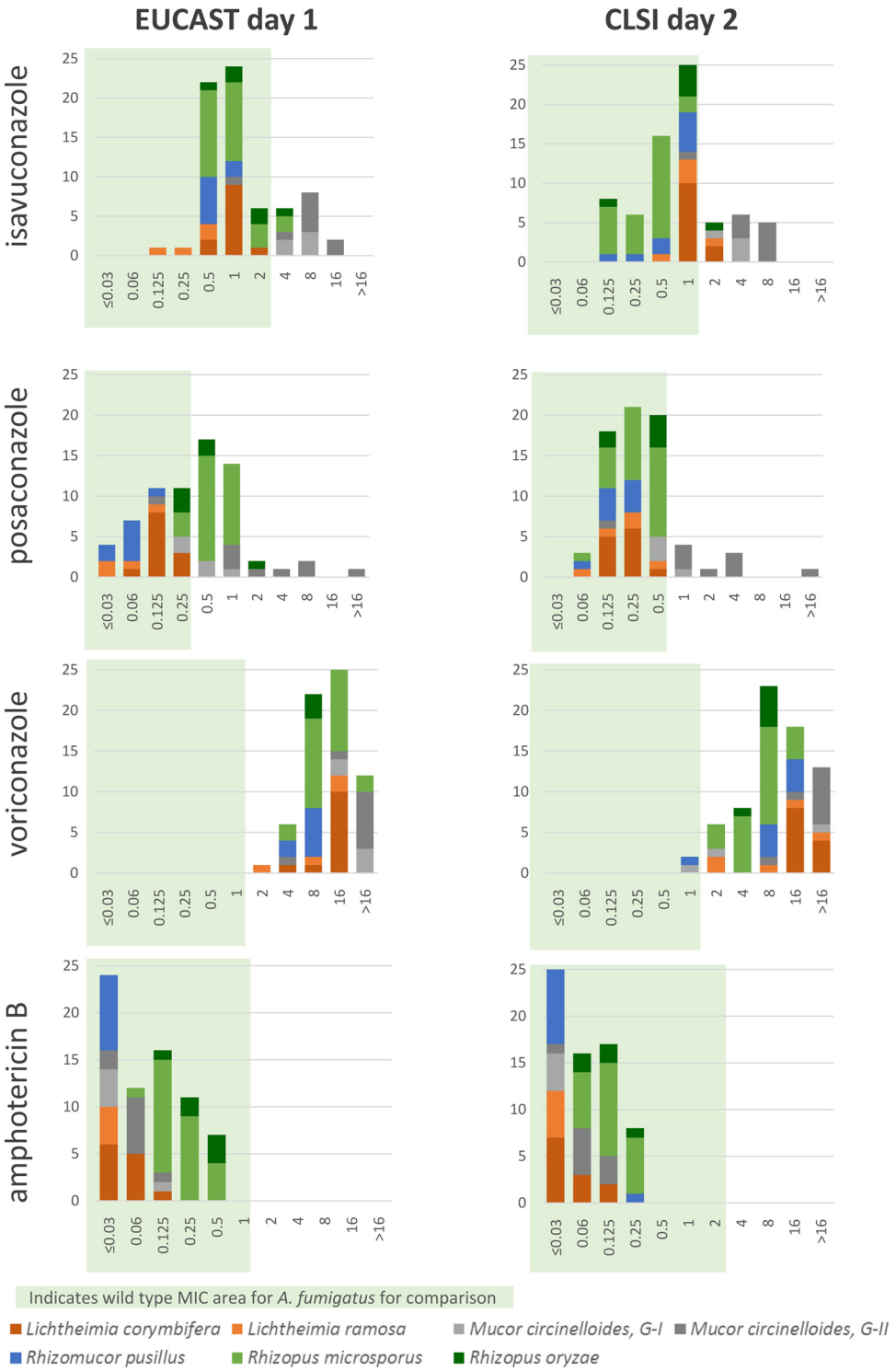

FIG 1 Isavuconazole, posaconazole, voriconazole, and amphotericin B MICs against various *Mucorales* species determined by EUCAST (endpoint reading after 1 day of incubation) and CLSI (endpoint reading after 2 days of incubation) methodologies. MIC ranges for wild-type *A. fumigatus* are shown as shaded areas for comparison. *x* axes, MIC (milligrams per liter); *y* axes, number of isolates.

1 (EUCAST-d1) and 2 (EUCAST-d2) for the EUCAST methodology and only at day 2 (CLSI-d2) for the CLSI plates as growth was insufficient at day 1. The MIC was the lowest drug concentration that prevented any discernible growth (100%) as defined in the reference methodologies. The ATCC 6258 strain of *Candida krusei* was included as a control strain. Amphotericin B, isavuconazole, posaconazole, and voriconazole MIC ranges were as follows with the reference quality control (QC) ranges in parentheses (all values in milligrams per liter):

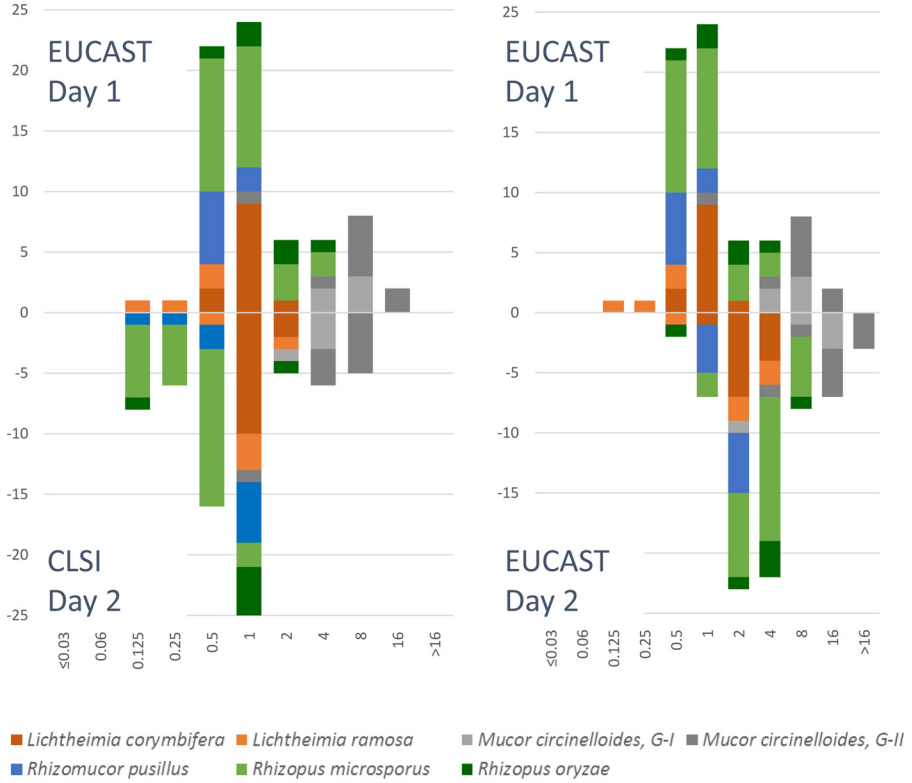

FIG 2 Comparison between isavuconazole MICs obtained by the EUCAST E.Def 9.2 (above the *x* axis) and CLSI M38 (below the *x* axis) methods (left diagram) and between EUCAST day 1 (above the *x* axis) and day 2 (below the *x* axis) readings (right diagram).

EUCAST-d1, 0.5 (0.125 to 1), ≤0.03 (not established but 0.015 to 0.125 in the work of Howard et al. [13]), ≤0.03 to 0.06 (0.015 to 0.06), and 0.125 to 0.25 (0.03 to 0.25) (14); EUCAST-d2 (no reference ranges established for the day 2 reading), 0.5, ≤0.03 to 0.06, ≤0.03, and 0.25; and CLSI-d2, 0.25 to 0.5 (1 to 4), ≤0.03 to 0.06 (not established), 0.125 (0.125 to 1), and 0.25 to 0.5 (0.125 to 1) (12).

Clinical breakpoints have not been defined for *Mucorales* isolates. However, as amphotericin B, isavuconazole, posaconazole, and voriconazole have documented clinical efficacy against wild-type *Aspergillus fumigatus*, we hypothesized that these four agents may also have clinical efficacy against *Mucorales* isolates for which MICs were within the MIC range for wild-type *A. fumigatus*. Hence, isolates were classified as potentially susceptible (pot-S) when the MIC was below the defined epidemiological cutoff values for *A. fumigatus*: amphotericin B, 1 mg/liter for the EUCAST and 2 mg/liter for the CLSI method; posaconazole, 0.25 mg/liter for the EUCAST and 0.5 mg/liter for the CLSI method; isavuconazole, 2 mg/liter for the EUCAST and 1 mg/liter for the CLSI method; and voriconazole, 1 mg/liter for both methods (13, 15–19).

Comparison between EUCAST and CLSI. The percent essential agreement (±1 2-fold dilution) between the EUCAST and the CLSI methods was calculated for each species. The median and range of 2-fold dilution differences between the two methods were also calculated. In order to calculate the exact differences between the methods, off-scale MICs (≤0.03 and >16 mg/liter) were excluded from this analysis.

The categorical agreement between the two methods was calculated as percentage of isolates classified as pot-S or non-pot-S by both methods. Finally, categorical agreement was also calculated for posaconazole using 1 mg/liter as the MIC cutoff value, recognizing the notable difference in posaconazole susceptibility between *Mucor circinelloides* and the other species and the fact that a cutoff value at 0.25 mg/liter bisected the combined posaconazole MIC distribution of non-*Mucor circinelloides* species.

RESULTS

The *in vitro* activity of isavuconazole was species dependent. The EUCAST-d1 isavuconazole MIC ranges were 0.125 to 4 mg/liter across *Lichtheimia corymbifera*, *Lichtheimia ramosa*, *Rhizomucor pusillus*, *Rhizopus microsporus*, and *Rhizopus oryzae* but somewhat higher against *Mucor circinelloides* groups I and II (1 to 16 mg/liter) (Table 1; Fig. 1). Similarly, the CLSI-d2 isavuconazole MIC ranges were 1 to 8 mg/liter for *Mucor circinelloides* groups I and II in comparison with 0.125 to 2 mg/liter for the other species. Overall, the isavuconazole MIC_{50} across all species was 1 mg/liter for both EUCAST-d1 and CLSI-d2 and with almost identical MIC ranges (0.125 to 16 mg/liter for EUCAST-d1 and 0.125 to 8 mg/liter for CLSI-d2). Reading the EUCAST plates after 2 days of incubation elevated the MICs 1 to 2 steps but did not change the overall species-dependent susceptibility pattern (Table 1 and Fig. 2).

The overall MIC_{50}s for amphotericin B and posaconazole were 0.06 and 0.25 mg/liter, respectively, when determined by either the EUCAST-d1 or the CLSI-d2 method and again with almost identical MIC ranges (Table 1). The *in vitro* activity of posaconazole varied by species, with *Mucor circinelloides* group II being the least susceptible species by both methods (MIC_{50}, 2 mg/liter; range, 0.125 to >16 mg/liter). In comparison, the amphotericin B *in vitro* activity was more uniform with species-specific MIC_{50}s between ≤0.03 and 0.25 mg/liter when determined by EUCAST-d1, ≤0.03 to 0.125 mg/liter by CLSI-d2, and 0.06 to 0.5 mg/liter by EUCAST-d2. Finally, the MIC ranges for voriconazole were 2 to >16, 1 to >16, and 8 to >16 mg/liter obtained by EUCAST-d1,

TABLE 2 Comparison between EUCAST and CLSI methods for antifungal susceptibility testing of *Mucorales*[d]

Comparison and species	No. of strains	% essential agreement, median (range) 2-fold difference				% categorical agreement			
		AMB	POSA	ISA	VORI	AMB	POSA[a]	ISA	VORI
EUCAST day 1 vs CLSI									
Lichtheimia corymbifera	12	100, 0 (−1 to 1)	100, −0.5 (−1 to 0)	100, 0 (−1 to 1)	88, 0 (−2 to 0)	100	100/100	83	100
Lichtheimia ramosa	4	100, 0 (0–0)	75, −1 (−3 to 1)	75, −1 (−3 to 1)	75, 0.5 (0–2)	100	100/100	100	100
Mucor circinelloides									
Group I	4	100, 0 (0–0)	100, 0 (−1 to 0)	75, 1 (0–2)	0, 3[b]	100	50/75	100	67
Group II	9	100, 0 (−1 to 1)	43, 0 (−2 to 3)	100, 0 (0–1)	100, −1[b]	100	100/67	100	100
Rhizomucor pusillus	8	100, 0 (0–0)	63, −1 (−2 to 1)	88, 0 (−1 to 2)	88, −0.5 (−1 to 2)	100	100/100	100	88
Rhizopus microsporus	26	73, 1 (−1 to 4)	73, 1 (0–2)	54, 1 (0–3)	75, 1 (−1 to 3)	100	12/100	92	100
Rhizopus oryzae	6	17, 2 (1–2)	83, 0.5 (−1 to 2)	67, 0.5 (0–2)	100, 1 (0–1)	100	50/83	67	100
All	70	83, 0 (−1 to 4)	76, 0 (−3 to 3)	75, 1 (−3 to 3)	81, 0 (−2 to 3)	100	59/93	91	97
EUCAST day 2 vs CLSI									
Lichtheimia corymbifera	12	58, 1 (0–2)	100, 1 (−1 to 1)	75, 1 (0–2)	ND[c]	100	75/100	67	100
Lichtheimia ramosa	4	100, 1 (0–1)	80, 0 (0–2)	80, 1 (0–2)	0, 3[b]	100	40/100	80	100
Mucor circinelloides									
Group I	4	75, 1 (0–2)	50, 2 (0–3)	25, 2 (1–3)	ND	100	25/75	100	67
Group II	9	89, 1 (0–2)	50 (0 and 3[b])	67, 1 (1–2)	ND	100	89/89	89	100
Rhizomucor pusillus	8	100, 1 (0–1)	89, 1 (0–2)	67, 1 (0–3)	86, 1 (0–4)	100	78/100	100	89
Rhizopus microsporus	26	35, 2 (0–5)	16, 3 (1–5)	4, 3 (1–6)	33, 2 (0–3)	100	0/35	35	100
Rhizopus oryzae	6	0, 3 (2–4)	80, 1 (0–2)	17, 2 (1–2)	80, 1 (1–2)	100	17/83	50	100
All	70	58, 1 (0–5)	61, 1 (−1 to 5)	38, 2 (0–6)	59, 1 (0–4)	100	39/72	63	97

[a] Categorical agreement using the cutoffs of 0.25 mg/liter and 1 mg/liter.
[b] Fewer than 3 isolates with on-scale MICs. The 2-fold dilution differences are presented for each isolate.
[c] ND, not determined because of off-scale MICs.
[d] Abbreviations: AMB, amphotericin B; POSA, posaconazole; ISA, isavuconazole; VORI, voriconazole.

CLSI-d2, and EUCAST-d2 reading, respectively, with MIC_{50}s of 8 to >16 mg/liter.

Based on the hypothesis that *Mucorales* isolates could be regarded as potentially susceptible (pot-S) when the MIC was within the wild-type MIC range for *A. fumigatus*, the proportion of such (pot-S) isolates was calculated (Table 1). All isolates were pot-S to amphotericin B independently of which susceptibility test was used, but only 0 to 3% were classified as pot-S to voriconazole. For isavuconazole, 77% of the isolates were pot-S by EUCAST-d1 and CLSI-d2 testing with significant variation between the species, e.g., 0 and 11% were pot-S for *Mucor circinelloides* groups I and II, respectively, but 80 to 100% were pot-S for the other species. For posaconazole, 47% and 87% were pot-S by EUCAST-d1 and CLSI-d2 testing, respectively, including all *Lichtheimia* and *Rhizomucor pusillus* isolates but only 11% of *Mucor circinelloides* group II isolates. For the other species (*Mucor circinelloides* group I, *Rhizopus microsporus*, and *Rhizopus oryzae*), more were classified as pot-S by the CLSI-d2 (75 to 100%) than by the EUCAST-d1 (12 to 50%) methodology.

The best essential agreement was found between the CLSI-d2 method and the EUCAST-d1 method, with overall essential agreement ranging from 75% for isavuconazole to 83% for amphotericin B. The median (range) 2-fold dilution differences were 0 (−3 to 4) (Table 2). The essential agreement was highest for *Lichtheimia corymbifera* (100% across amphotericin B, posaconazole, and isavuconazole and 7/8, 88%, for voriconazole) and lowest for amphotericin B against *Rhizopus oryzae* (1/6, 17%), isavuconazole against *Rhizopus microsporus* (14/26, 54%), and posaconazole against *Mucor circinelloides* group II (3/7, 43%) and *Rhizomucor pusillus* (5/8, 63%). The essential agreement between the CLSI-d2 method and the EUCAST-d2 method was 38% to 61% and lowest for isavuconazole.

The overall categorical agreement between the CLSI-d2 method and the EUCAST-d1 method ranged from 91% for isavuconazole, 93% for posaconazole (with the 1 mg/liter cutoff), to 100% for amphotericin B. For isavuconazole, the lowest categorical agreement was found for *Rhizopus oryzae* (4/6, 67%). For posaconazole, the categorical agreement between EUCAST-d1 and CLSI-d2 was calculated using the *A. fumigatus* ECOFF of 0.25 mg/liter as well as 1 mg/liter to avoid bisecting the non-*Mucor circinelloides* MIC distributions. The agreement was highest using the 0.25-mg/liter cutoff for *Mucor circinelloides* overall (85% versus 69%) and *Mucor circinelloides* group II in particular (100% versus 67%) but using 1 mg/liter for *Rhizopus microsporus* (12% versus 100%), *Rhizopus oryzae* (50% versus 83%), and *Mucor circinelloides* group I (50% versus 75%).

Finally, the pharmacokinetic characteristics of isavuconazole in comparison with those for the other mold-active azoles were compared (Table 3) (20–27). The isavuconazole minimum concentration of drug in serum (C_{min}) (3.91 mg/liter) and area under the concentration-time curve (AUC) (97.9 mg · h/liter) were 6- to 3-fold higher than the similar parameters for posaconazole oral solution (0.64 mg/liter and 17.2 mg · h/liter, respectively) and i.v. formulation (1.07 mg/liter and 34.3 mg · h/liter, respectively) (Table 3).

DISCUSSION

Overall, the MICs correlated with the well-accepted clinical antifungal spectrum associated with efficacy and failure. Thus, the MICs for voriconazole, which has no clinical efficacy against *Mucorales* infections, were high and above the MIC range correlated with clinical efficacy for *A. fumigatus* (15, 17, 19). In contrast, amphotericin B MICs fell in the MIC range that for other mold and yeast species normally would predict susceptibility (15, 16, 18,

TABLE 3 Human pharmacokinetic data for the mold-active azole compounds[c]

Drug	Route or form of administration	Patient group (reference)	Dosage	Day(s) when steady state reached	Bioavailability[d]	Mean C_{max}, mg/liter[d]	Mean C_{min}, mg/liter[d]	Mean C_{av}, mg/liter[d]	Mean total body CL/*F* (liters/h)[d]	Mean $t_{1/2}$ (h)[d]	Mean AUC_{24} (mg · h/liter)[d]	Fraction unbound (%)	Mean *V*/*F* (liters/kg)[d]
Isavuconazole	Oral or i.v.	Patients with IA ($n = 222$) (20)	200 mg TID on days 1–2, 200 mg QD	14	0.98–1		3.91 (49)		2.4 (44)	100 (50–150)	97.9 (58)	1	>5 (44)
Posaconazole	Oral suspension	Febrile neutropenic patients or patients with refractory invasive fungal diseases ($n = 23$) (21)	400 mg BID	7–10	0.54–0.75	0.85 (82)	0.64 (98)	0.72 (86) [6.70–2,256]	76.1 (78) [14.9–256]	31.7 (42) [12.4–67.3]	17.2 (86) [3.1–53.6]	2	44 (84) [5.8–187]
	Gastroresistant tablet (day 8)	Neutropenic patients receiving cytotoxic chemotherapy for AML or MDS ($n = 32$) (22)	300 mg BID on day 1, 300 mg QD	7–10	0.54–0.75	1.96 (33)	[0.343–2.55]	1.46 (38)			35 (41) [11.8–62.3]		
	i.v. (day 14)	Neutropenic patients receiving cytotoxic chemotherapy for AML or MDS ($n = 19$) (23)	300 mg BID on day 1, 300 mg QD		1	2.61 (39)	1.07 (50)	1.43 (42)			34.3 (42)		
Voriconazole	Oral	Adult patients with IA ($n = 43$) (24)	400 mg BID on day 1, 200 mg BID	5–7	0.82 (15)	3.57 (48.5)[a]	0.83 (197)		11.52 (73)[a]	11.31 (87.3)[a]	36 (119)		2.6 (96)
		Adult patients with proven or probable IA on combination therapy with anidulafungin ($n = 454$) (25)	6 mg/kg of body wt BID on day 1, 4 mg/kg BID for 7 days, 300 mg BID	5–7	0.64 (24)		2.04 (54)		5.30 (11)		66 (45)	45–55[b]	2.38 (15–26)
	i.v.	Adult patients with IA ($n = 43$) (24)	6 mg/kg BID on day 1, 4 mg/kg BID		1		2.54 (231)				90.4 (168)	45–55[b]	2.6 (96)
		Adult patients with proven or probable IA on combination therapy with anidulafungin ($n = 454$) (25)	6 mg/kg BID on day 1, 4 mg/kg BID		1		3.10 (52)		5.30 (11)		102 (43)		2.38 (15–26)

[a] Data obtained from reference 26.

[b] Data obtained from reference 27.

[c] Abbreviations: IA, invasive aspergillosis; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; TID, three times daily; QD, once daily; BID, twice daily; C_{max}, maximum concentration of drug in serum; C_{min}, minimum concentration of drug in serum; C_{av}, average concentration of drug in serum; CL, clearance; *F*, bioavailability; $t_{1/2}$, half-life; AUC_{24}, area under the concentration-time curve at 24 h; *V*, volume of distribution.

[d] Pharmacokinetic data in parentheses are percent coefficients of variation; data in brackets are ranges.

28). This observation is somewhat reassuring as susceptibility testing of molds is challenging and the correlation with clinical outcome is often debated.

By EUCAST and CLSI susceptibility testing, isavuconazole MICs for the *Mucorales* isolates were similar to those found for *Aspergillus* species, with the exception of *Mucor circinelloides*, which was notably less susceptible than the other species across all methods and endpoints (13, 29). Accordingly, 83 to 100% of the isolates were classified as pot-S using EUCAST-d1 and CLSI-d2 across all isolates except *Mucor circinelloides*. This observation suggests species-specific differential clinical efficacy against the clinically relevant *Mucorales* species. A similar pattern was found for posaconazole, which was also found to be less active against *Mucor circinelloides* and against *Mucor circinelloides* group II in particular, and even for voriconazole, the MICs against *Mucor circinelloides* were the highest ones. However, in addition to this species-specific differential activity, some additional and method-dependent differential activity was noted. For example, *Rhizopus microsporus* was clearly less susceptible than *Lichtheimia* species and *Rhizomucor pusillus* to posaconazole when susceptibility was tested by the EUCAST method but not when tested by the CLSI method. Similarly, *Rhizopus* spp. were more susceptible to isavuconazole than *Lichtheimia* spp. when tested by the CLSI method but not when tested by the EUCAST method. The clinical impact of these observations, if any, remains to be understood, but they clearly demonstrate that clinical breakpoints have to be species as well as method specific in order to provide the same categorization of isolates as susceptible or resistant. Whereas no species-specific *in vivo* outcome data have been published for infections due to *Mucor* species isolates, *in vivo* data suggest isavuconazole efficacy against isolates of *Rhizopus*. Thus, a successful clinical outcome of rhinocerebral mucormycosis by a *Rhizopus oryzae* isolate with a MIC of 1 mg/liter has been reported after isavuconazole salvage therapy with trough plasma levels maintained at 1.3 to 3.24 mg/liter (30). Moreover, preclinical studies showed that high doses of isavuconazole were as effective as high-dose liposomal amphotericin B against experimental mucormycosis by a *Rhizopus delemar* isolate with a MIC of 0.125 mg/liter (31).

The isavuconazole MIC_{50}s across the isolates were 2 dilution steps higher than those for posaconazole and 4 steps higher than those for amphotericin B. Direct comparisons of MICs across compounds are, however, not meaningful because bioavailability and pharmacokinetic and pharmacodynamics parameters associated with clinical efficacy are different among compounds and drug classes (Table 3) (20–27). For the azole drugs, outcome is best predicted by the AUC/MIC ratio. Noticeably, the AUC for isavuconazole is 4 to 6 times higher than that for posaconazole, which may compensate for the 2-dilution-step-lower MIC and explain the clinical efficacy observed in the clinical trial despite higher MICs (Table 3). Some support for this hypothesis was further derived from the observations made when adopting the ECOFF/ECVs for these four agents for *A. fumigatus* as potential breakpoints for susceptibility. Thus, all isolates were rightfully classified as susceptible to amphotericin B and virtually none were classified as susceptible to voriconazole, and interestingly, more isolates were classified as potentially susceptible to isavuconazole than to posaconazole independently of which susceptibility testing method was used. Therefore, this study provides some *in vitro* support for the assumption that isavuconazole may be an appropriate choice for most *Mucorales* species with the exception of *Mucor circinelloides*.

The EUCAST susceptibility plates were read on day 1, whereas the CLSI plates were read on day 2 due to a lack of visible growth after the first day of incubation. This difference is most likely explained by the 10-fold-lower inoculum used for the CLSI method and the 10-fold-lower glucose concentration, test conditions which are associated with lower growth rates for *Candida* species. When the reading of the EUCAST plates was repeated on day 2, the MICs rose approximately 2 dilutions for the three azoles and 1 dilution for amphotericin B, leading to a marked decrease in the categorical agreement with CLSI-d2 results for isavuconazole and posaconazole. Similarly, MICs reported in the literature for day 2 readings are in general higher than the EUCAST-d1 MICs presented here (6, 7). It is a well-known phenomenon that MICs rise with extended time of incubation and also that MIC endpoints may vary considerably across methods and endpoint criteria in general and also specifically for isavuconazole and *Mucorales* (32). Hence, standardization is key and future clinical breakpoints should be specific for the method, species, and incubation time used.

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