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Species-Specific Antifungal Susceptibility Patterns of *Scedosporium* and *Pseudallescheria* Species

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Since the separation of *Pseudallescheria boydii* and *P. apiosperma* in 2010, limited data on species-specific susceptibility patterns of these and other species of *Pseudallescheria* and its anamorph *Scedosporium* have been reported. This study presents the antifungal susceptibility patterns of members affiliated with both entities. Clinical and environmental isolates ($n = 332$) from a wide range of sources and origins were identified down to species level and tested according to CLSI M38-A2 against eight antifungal compounds. Whereas *P. apiosperma* (geometric mean MIC/minimal effective concentration [MEC] values of 0.9, 2.4, 7.4, 16.2, 0.2, 0.8, 1.5, and 6.8 μg/ml for voriconazole, posaconazole, isavuconazole, itraconazole, micafungin, anidulafungin, caspofungin, and amphotericin B, respectively) and *P. boydii* (geometric mean MIC/MEC values of 0.7, 1.3, 5.7, 13.8, 0.5, 1.4, 2.3, and 11.8 μg/ml for voriconazole, posaconazole, isavuconazole, itraconazole, micafungin, anidulafungin, caspofungin, and amphotericin B, respectively) had similar susceptibility patterns, those for *S. aurantiacum*, *S. prolificans*, and *S. dehoogii* were different from each other. Voriconazole was the only drug with significant activity against *S. aurantiacum* isolates. The MIC distributions of all drugs except voriconazole did not show a normal distribution and often showed two subpopulations, making a species-based prediction of antifungal susceptibility difficult. Therefore, antifungal susceptibility testing of all clinical isolates remains essential for targeted antifungal therapy. Voriconazole was the only compound with low MIC values (MIC_{90} of ≤2 μg/ml) for *P. apiosperma* and *P. boydii*. Micafungin and posaconazole showed moderate activity against the majority of *Scedosporium* strains.

Scedosporium species are involved in a wide range of human infections, especially in immunocompromised patients (22, 26). Cerebral abscesses are relatively frequent (24), reflecting the neurotropic character of these fungi (3). A typical disease entity of these fungi is the near-drowning syndrome (24), when patients develop cerebral abscesses weeks to months after the inciting event (16). In cystic fibrosis patients, *Scedosporium* species are among the most common fungal colonizers of the respiratory tract but rarely become invasive (4, 41). The increasing frequency and high mortality rates of invasive infections caused by *Scedosporium* species necessitate the search for new treatment strategies (47).

Recently, species concepts in *Pseudallescheria* and *Scedosporium* have been narrowed as a result of application of molecular phylogeny. The following species are now widely accepted in the scientific community: *P. apiosperma* (anamorph: *S. apiospermum*), *S. aurantiacum*, *P. boydii* (*S. boydii*), *S. dehoogii*, and *P. minutispora* (9, 11, 12, 41). The differentiation of some smaller taxonomic entities, such as *P. angusta*, *P. desertorum*, *P. ellipsoidea*, *P. fusoidea*, and *S. deficiens* (33), is still under debate, but these species are treated here as separate sibling species. A more distantly related *Scedosporium* species is *S. prolificans*, which frequently is multidrug resistant (20, 36). Since the majority of *Scedosporium* isolates display multiple antifungal resistance patterns (7, 13, 34), the aim of the present study was to investigate whether resistance patterns are species specific and therefore whether identification down to species level is relevant for the choice of antifungal treatment. We tested eight systemic antifungal compounds against a set of *Scedosporium*/*Pseudallescheria* isolates from a wide range of geographical origins and from divergent environmental and clinical sources.

MATERIALS AND METHODS

Isolates. A total of 332 *Scedosporium* isolates were examined: 246 of clinical origin, 82 of environmental origin, and 4 of unknown origin were included and came from the continents of Africa ($n = 8$), Asia ($n = 33$), Europe ($n = 224$), North America ($n = 18$), South America ($n = 16$), Oceania ($n = 4$), and Antarctica ($n = 1$). The geographical origin of 28 strains was not traceable. As a reference for molecular species identification, several type and ex-type strains were included: *P. angusta* (CBS 254.72^T), *P. apiosperma* (CBS 117407^T), *S. aurantiacum* (CBS 116910^T), *P. boydii* (CBS 101.22^T), *S. dehoogii* (CBS 117406^T), *P. ellipsoidea* (CBS 418.73^T), *P. minutispora* (CBS 116911^T), and *S. prolificans* (CBS 114.90^T).

AFLP. All isolates were identified down to species level based on the similarities of their amplified fragment length polymorphism (AFLP) profiles relative to those of the included type and reference strains (25). AFLP analysis was performed according to established procedures (8, 25, 38). Briefly, isolates were grown at 35°C in the dark on Sabouraud glucose

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TABLE 1 *In vitro* antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species against eight antifungal compounds (AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA)

		MIC and MEC (μg/ml) and GM values															
		AMB				CAS				ANI				MICA			
Species	n^a	Range	MIC_{50}	MIC_{90}	GM	Range	MEC_{50}	MIC_{90}	GM	Range	MEC_{50}	MIC_{90}	GM	Range	MEC_{50}	MEC_{90}	GM
P. apiosperma	154	0.5–>16	8	>16	6.8	0.5–>8	1	8	1.5	0.125–>8	0.5	8	0.8	0.016–>8	0.125	4	0.2
P. boydii	60	0.5–>16	16	>16	11.8	1–>8	2	8	2.3	0.25–>8	1	8	1.4	0.062–>8	0.250	>8	0.5
S. prolificans	37	8–>16	>16	>16	28.6	2–>8	>8	>8	10.4	0.5–>8	4	>8	4.8	0.125–>8	>8	>8	7.9
S. dehoogii	22	2–>16	16	>16	12.8	1–>8	8	>8	7.5	1–>8	8	>8	8.3	0.125–>8	0.5	>8	1.1
S. aurantiacum	22	16–>16	>16	>16	28.2	2–>8	8	>8	6.8	1–>8	8	>8	7.5	1–>8	8	>8	6.8
P. ellipsoidea	16	4–>16	16	>16	16.0	1–2	1	2	1.2	0.125–>8	0.5	2	0.6	0.062–8	0.125	0.250	0.1
P. angusta	15	0.5–>16	8	16	6.9	1–>8	4	>8	4.5	2–>8	2	>8	2.3	0.062–>8	0.5	>8	0.9
P. minutispora	6	1–4	4	4	2.8	1–8	2	8	3.2	0.5–4	2	4	1.6	0.125–8	0.250	8	0.4

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agar until abundant sporulation had occurred (after approximately 14 to 18 days). The spores were collected using a damp cotton swab and were disrupted using ceramic beads in a MagNA Lyser instrument (Roche Diagnostics, Almere, Netherlands). DNA was extracted using a MagNA Pure LC instrument (Roche Diagnostics) in combination with the MagNA Pure LC DNA isolation kit III according to the instructions of the manufacturer. AFLP was performed with the restriction enzymes MseI and HpyCH4IV (New England Biolabs, Beverly, MA). The HpyCH4IV primer was labeled with fluorescein and contained one selective T residue, and the MseI primer contained four selective residues (TGAA). Amplification products were analyzed on a MegaBACE 500 automated DNA analysis platform according to standard procedures.

AFLP data were imported in BioNumerics v6.0 software (Applied Maths, Sint-Martens-Latem, Belgium) and analyzed by using UPGMA (unweighted pair-group method with arithmetic averages) clustering with the Pearson correlation coefficient. The analysis was restricted to DNA fragments in the range of 60 to 300 bp.

***In vitro* susceptibility testing.** *In vitro* susceptibility testing was performed using broth microdilution for filamentous fungi according to CLSI document M38-A2 (5). The following antifungal drugs were used: amphotericin B (AMB; Bristol Myers Squibb, Woerden, Netherlands), anidulafungin (ANI; Pfizer Central Research, Sandwich, Kent, United Kingdom), caspofungin (CAS; Merck Sharp & Dohme BV, Haarlem, Netherlands), isavuconazole (ISA; Basilea Pharmaceuticals [now Astellas], Basel, Switzerland), itraconazole (ITC; Janssen Cilag, Tilburg, Netherlands), micafungin (MICA; Astellas Pharma, Inc., Ibaraki, Japan), posaconazole (POS; Schering-Plough Corp. [now Merck], Kenilworth, NJ), and voriconazole (VRC; Pfizer Central Research). All azoles and AMB were tested in concentrations ranging from 0.016 to 16 μg/ml, while all echinocandins were tested in concentrations ranging from 0.008 to 8 μg/ml.

Candida parapsilosis ATCC 22019 and *C. krusei* ATCC 6258 served as quality control strains. The results were read after an incubation of 72 h at 37°C. The MICs for AMB, ITC, ISA, POS, and VRC were read visually, while minimal effective concentrations (MECs) for ANI, CAS, and MICA were read microscopically.

Statistical analyses. Geometric means (GM), MICs, and MECs were calculated using Microsoft Office Excel 2003 SP3. For GM calculations, MIC values of <0.016 mg/ml were set at 0.008 μg/ml, MIC values of >16 μg/ml were set at 32 μg/ml, MEC values of <0.008 μg/ml were set at 0.004 μg/ml, and MEC values of >8 μg/ml were set at 16 μg/ml. For MIC_{50}/MEC_{50} and MIC_{90}/MEC_{90}, the data per antifungal and species were sorted in ascending order, followed by the median and 90th percentile determinations. MIC/MEC distributions between clinical and environmental isolates were compared by using the Mann-Whitney-Wilcoxon test. A P value of <0.05 was considered statistically significant. The presence of cross-resistance was tested by analyzing the MIC/MEC values for each pair of antifungal drugs by the Spearman rank correlation and was considered statistically significant when P values were <0.01.

RESULTS

Using established procedures, all isolates in the present study were identified based on the similarities of their AFLP fingerprints to those of the included type or ex-type strains (26). Of a total of 332 strains, 154 were identified as *P. apiosperma* (124 clinical, 29 environmental, and 1 from an unknown source), 60 were identified as *P. boydii* (44 clinical, 14 environmental, and 2 from an unknown source), 37 were identified as *S. prolificans*, 22 were identified as *S. aurantiacum*, 22 were identified as *S. dehoogii*, 16 were identified as *P. ellipsoidea*, 15 were identified as *P. angusta*, and 6 were identified as *P. minutispora*. Among all clinical isolates (n = 246), the identified species were *P. apiosperma* (n = 124), *P. boydii* (n = 44), *S. prolificans* (n = 35), *S. aurantiacum* (n = 19), *P. ellipsoidea* (n = 11), *P. angusta* (n = 5), *S. dehoogii* (n = 6), and *P.*

TABLE 2 MIC/MEC value comparison for clinical versus environmental isolates of *P. apiosperma* and *P. boydii* for all tested antifungal compounds (AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA)

		MIC and MEC (μg/ml)[b] and GM values															
		AMB				CAS				ANI				MICA			
Species	n^a	Range	MIC_{50}	MIC_{90}	GM	Range	MEC_{50}	MEC_{90}	GM	Range	MEC_{50}	MEC_{90}	GM	Range	MEC_{50}	MEC_{90}	GM
P. apiosperma	124*	0.5–>16	8	>16	6.5	0.5–>8	1	8	1.6	0.125–>8	0.5	8	0.9	0.006–>8	0.125	4	**0.2
	29†	1–>16	16	>16	9.0	1–>8	1	2	1.2	0.125–8	0.5	4	0.6	0.031–>0.5	0.125	0.5	**0.1
P. boydii	44*	0.5–>16	16	>16	11.3	1–>8	2	8	2.1	0.25–>8	1	4	1.3	0.062–>8	0.25	8	0.4
	14†	2–>16	16	>16	13.1	1–>8	2	>8	3.1	0.5–>8	2	8	1.8	0.062–>8	0.25	>8	1.2

(Continued on next page)

TABLE 1 (Continued)

MIC and MEC (μg/ml) and GM values															
ITC				VRC				POS				ISA			
Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM
0.25–>16	>16	>16	16.2	0.25–8	1	2	0.9	0.25–>16	1	>16	2.4	1–>16	8	16	7.4
0.125–>16	>16	>16	13.8	0.125–2	1	1	0.7	0.125–>16	1	4	1.3	0.5–>16	8	16	5.7
>16–>16	>16	>16	32.0	4–>16	16	>16	15.4	>16–>16	>16	>16	32.0	8–>16	>16	>16	25.6
0.5–>16	>16	>16	16.0	0.5–>16	1	8	1.5	0.5–>16	1	>16	3.4	2–>16	8	>16	8.0
1–>16	>16	>16	19.3	0.5–1	0.5	1	0.6	1–>16	1	>16	2.7	4–16	8	16	6.8
2–>16	>16	>16	22.6	0.5–4	1	2	0.9	0.5–>16	1	>16	3.1	2–>16	8	>16	8.4
0.25–>16	>16	>16	8.3	0.25–2	0.5	2	0.6	0.25–>16	1	>16	1.4	1–>16	4	16	4.5
0.5–>16	>16	>16	16.0	0.25–2	0.5	2	0.8	0.5–>16	1	>16	1.6	2–16	8	16	6.3

[a] *n*, Number of isolates.

minutispora ($n = 2$). *Pseudallescheria apiosperma*, *P. boydii*, *S. aurantiacum*, *S. prolificans*, and *P. ellipsoidea* (11 of 16 strains) were mainly recovered from clinical specimens, whereas *P. angusta* (10 of 15 strains), *P. minutispora* (4 of 6 strains), and *S. dehoogii* (16 of 22 strains) were mainly isolated from the environment.

Species-specific *in vitro* MIC_{50} and MEC_{50} values, MIC_{90} and MEC_{90} values, ranges of MICs and MECs, and GM MICs/MECs were sorted by antifungal compound are listed in Table 1. *Pseudallescheria apiosperma* isolates had the lowest GM values for MICA, followed by ANI and VRC. *Pseudallescheria boydii* strains had the lowest GM values for MICA, followed by VRC and POS. Strains of *S. aurantiacum* had only low GM values for VRC and the lowest GM values for S. *dehoogii* were found with MICA (GM MEC = 1.1 μg/ml) and VRC (GM MIC = 1.5 μg/ml). Strains of *P. minutispora* had the lowest GMs for MICA (GM MEC = 0.4 μg/ml) and VRC (GM MIC = 0.8 μg/ml). The majority of *S. prolificans* strains showed the highest GM MICs/MECs (in μg/ml) of all the tested antifungal drugs (AMB, 28.6; CAS, 10.4; ANI, 4.8; MICA, 7.9; ITC, 32; VRC, 15.4; POS, 32; ISA, 25.6); only a few strains had low MECs for ANI (0.25 μg/ml) and MICA (0.125 μg/ml) (Table 1). Judged by the GM values, VRC and/or MICA showed reasonable *in vitro* activity against all *Pseudallescheria/Scedosporium* species (VRC with an MIC_{50} of $\leq$1 μg/ml and MICA with an MEC_{50} of $\leq$0.5 μg/ml), except for *S. prolificans* (VRC with an MIC_{50} of 16 μg/ml and MICA with an MEC_{50} of >8 μg/ml) and *S. aurantiacum* (MICA with an MEC_{50} of 8 μg/ml).

The species-specific MIC and MEC values for all *Pseudallescheria* and *Scedosporium* species are listed in Table 1. All *Scedosporium* and *Pseudallescheria* species were found to have high MIC/MEC values of AMB ($MIC_{50} \geq 4$ μg/ml), ITC ($MIC_{50} > 16$ μg/ml), and ISA ($MIC_{50} > 4$ μg/ml) (Table 1). CAS had MEC_{50} and MEC_{90} values that suggested reasonable *in vitro* activity against *P. ellipsoidea* strains only ($MEC_{50} = 1$ μg/ml and $MEC_{90} = 2$ μg/ml). High MEC_{50}/MEC_{90} values were obtained for ANI and *S. dehoogii* ($MEC_{50} = 8$ μg/ml and $MEC_{90} > 8$ μg/ml) and *S. aurantiacum* ($MEC_{50} = 8$ μg/ml and $MEC_{90} > 8$ μg/ml). High MEC_{50} values were found for MICA and *S. prolificans* and *S. aurantiacum* only. Limited *in vitro* activity of VRC was found only for the species *S. prolificans* and *S. dehoogii.* POS and VRC are the most promising drugs against all *Pseudallescheria* and *Scedosporium* species other than *S. prolificans* and MICA against *Pseudallescheria* and *Scedosporium* species other than *S. prolificans* and *S. aurantiacum* (Table 1).

We evaluated whether the MIC/MEC values correlated with the origins of the isolates (clinical versus environmental). The MIC and MEC values of clinical isolates and environmental isolates of *P. apiosperma* and *P. boydii* are listed in Table 2. A statistically significant difference in susceptibility was observed for POS, as well as for MICA, between clinical and environmental strains of *P. apiosperma* (Mann-Whitney-Wilcoxon test [$P = 0.0028$ and $P = 0.0495$, respectively]) (Table 2, values marked with asterisks). For *P. boydii* no statistical significant differences between clinical and environmental strains were detected for any of the tested compounds.

Within *P. apiosperma* and *P. boydii*, cross-resistance between the different azoles was observed, i.e., isolates in the higher MIC distribution of VOR were also within the higher MIC distribution of POS. This was statistically evaluated using the Spearman correlation coefficient and was found to be highly significant ($P < 0.0001$) (Table 3). Moreover, for the echinocandins, a statistically

TABLE 2 (Continued)

MIC and MEC (μg/ml)[b] and GM values															
ITC				VRC				POS				ISA			
Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM
0.25–>16	>16	>16	15.3	0.25–>8	1	2	0.9	0.25–>16	1	>16	**2.0	1–>16	8	16	7.1
0.5–>16	>16	>16	20.3	0.25–4	1	2	1.0	0.25–>16	2	>16	**5.1	1.00–>16	8	16	9.0
0.125–>16	>16	>16	11.8	0.125–2	0.5	2	0.7	0.125–>16	1	>16	1.5	0.50–>16	8	16	5.8
4.0–>16	>16	>16	27.6	0.5–1	1	1	0.8	0.5–2	1	2	1.1	2–16	8	8	5.9

[a] *, The number of isolates obtained from clinicalspecimens; †, the number of isolates obtained from environmental samples.
[b] **, GM MIC/MEC exhibiting a statistically significant difference between clinical and environmental isolates ($P \leq 0.05$).

TABLE 3 Evaluation of cross-resistance between the different azoles (ITC, ISA, VRC, and POS) and different echinocandins (CAS, ANI, and MICA) for *P. apiosperma* and *P. boydii* using the Spearman rank coefficient[a]

Species	Azoles					Echinocandins			
		ITC	VRC	POS	ISA		CAS	ANI	MICA
P. apiosperma	ITC	1	0.37*	0.52*	0.44*	CAS	1	0.73*	0.66*
	VRC		1	0.70*	0.72*	ANI		1	0.78*
	POS			1	0.76*	MICA			1
	ISA				1				
P. boydii	ITC	1	0.64*	0.58*	0.63*	CAS	1	0.86*	0.86*
	VRC		1	0.67*	0.77*	ANI		1	0.90*
	POS			1	0.72*	MICA			1
	ISA				1				

[a] A P value of <0.01 was considered statistically significant. *, $P < 0.0001$.

significant cross-resistance was also observed ($P < 0.0001$) (Table 3). No statistically significant cross-resistance was observed between azoles and echinocandins (results not shown).

A major finding of the present study was that almost none of tested compounds showed a normal distribution of MIC/MEC values. Only VRC showed a normal distribution with *P. apiosperma* and *P. boydii*. In particular, the MIC/MEC distributions of MICA, ITC, and POS clearly show the presence of two different subpopulations with different susceptibilities. By analyzing the MEC distribution of *P. apiosperma* and *P. boydii* of MICA (Fig. 1 and 2), we observed one susceptible population with MEC values lower than 1 μg/ml ($n = 139$ and $n = 45$, respectively) and a second subpopulation with MEC values of $\geq$4 μg/ml (each $n = 15$). For POS, a major partition of *P. apiosperma* and *P. boydii* population had MIC values of $\leq$4 μg/ml ($n = 94$ and $n = 47$, respectively); the other subpopulation was highly resistant with MIC $\geq$ 16 μg/ml) (Fig. 1 and 2). Major partitions of the *P. apiosperma* ($n = 124$) and *P. boydii* ($n = 46$) populations were found to be highly resistant to ITC, with MIC values $\geq$16 μg/ml, whereas only a minority of the populations had MIC values of $\leq$8 μg/ml ($n = 30$ and $n = 14$, respectively).

DISCUSSION

Pseudallescheria boydii and *P. apiosperma* strains have been isolated from clinical samples worldwide, and both species are regarded as environmental opportunistic fungi with similar spectra of clinical manifestations. They are the most prevalent *Pseudallescheria* species (43), but published studies of *in vitro* susceptibility profiles according to the latest taxonomical standards are rare (25). The two species have similar susceptibility profiles, with the lowest MICs/MECs of VRC and MICA. However, *P. apiosperma* was found to be more susceptible to POS than was *P. boydii*. Although we found a statistically significant difference between environmental and clinical *P. apiosperma* strains for POS and MICA, clinical isolates of *P. apiosperma* had lower MICs of POS than environmental strains. The majority of the *P. apiosperma* population had low POS MICs; therefore, it might be possible that POS-resistant strains might be less virulent than susceptible strains; however, to prove this hypothesis and to investigate this further, *in vivo* data in an animal model are needed. For MICA, the majority of the *P. apiosperma* population exhibits low MECs. However, clinical strains have statistically significant higher MECs of MICA than do environmental strains. For *P. boydii* no statistically significant differences between clinical and environmental strains were detected.

For strains of *P. apiosperma* and *P. boydii*, cross-resistance was observed between azoles as well as between the echinocandins. Similar findings have been described before for *Aspergillus fumigatus* (35).

With normally distributed MIC/MEC values, if the MIC_{50} or GM is known, one can reasonably predict the MIC_{90} and epidemiological cutoff values (ECV). It is remarkable that the MIC/MEC distributions of *P. boydii* and *P. apiosperma* and all antifungal drugs except for VRC do not show a normal distribution. In particular, the MIC/MEC distributions of *P. apiosperma* and *P. boydii* strains were bimodal for MICA, POS, and ITC and showed signs of bimodality for AMB, CAS, and ANI. The consequence of these distributions is that the susceptibilities of individual isolates are difficult to predict and thus susceptibility testing of clinical isolates remains essential for targeted treatment. The subpopulations with the lower MIC/MEC values could be the original susceptible wild-type populations, whereas the isolates with the higher MIC/MEC values could have acquired antifungal resistance mechanisms. However, this presumptive explanation requires further investigation.

Since *Scedosporium* species do not have a normal MIC/MEC distribution, prediction of antifungal susceptibility of a single strain is difficult, but the various species have at least different tendencies of susceptibilities for the various antifungal compounds.

S. aurantiacum showed high *in vitro* resistance to AMB and ITC, and all other antifungal drugs tested, except VRC, showed poor activity. Our results are in concordance with those of Gilgado et al. (13) in that *S. aurantiacum* isolates are less susceptible to antifungal drugs than strains of *P. apiosperma*. Therefore, the differentiation of *S. aurantiacum* strains from other *Scedosporium* species is also of interest for the choice of antifungal therapy. In contrast to the findings of Heath et al. (18), who reported a common trend in susceptibilities between *P. apiosperma* and *S. aurantiacum* with good ITC activity, with our tested *S. aurantiacum* strains, the MICs of ITC (GM = 19.3 μg/ml) were high, but MICs of VRC were low (GM = 0.6 μg/ml), which confirms the data reported by Tintelnot et al. (42) and Alastruey-Izquierdo et al. (1). Based on these data, we judge VRC as the only antifungal compound with promising *in vitro* activity against *S. aurantiacum*. This antifungal drug was also clinically effective, lowering mortal-

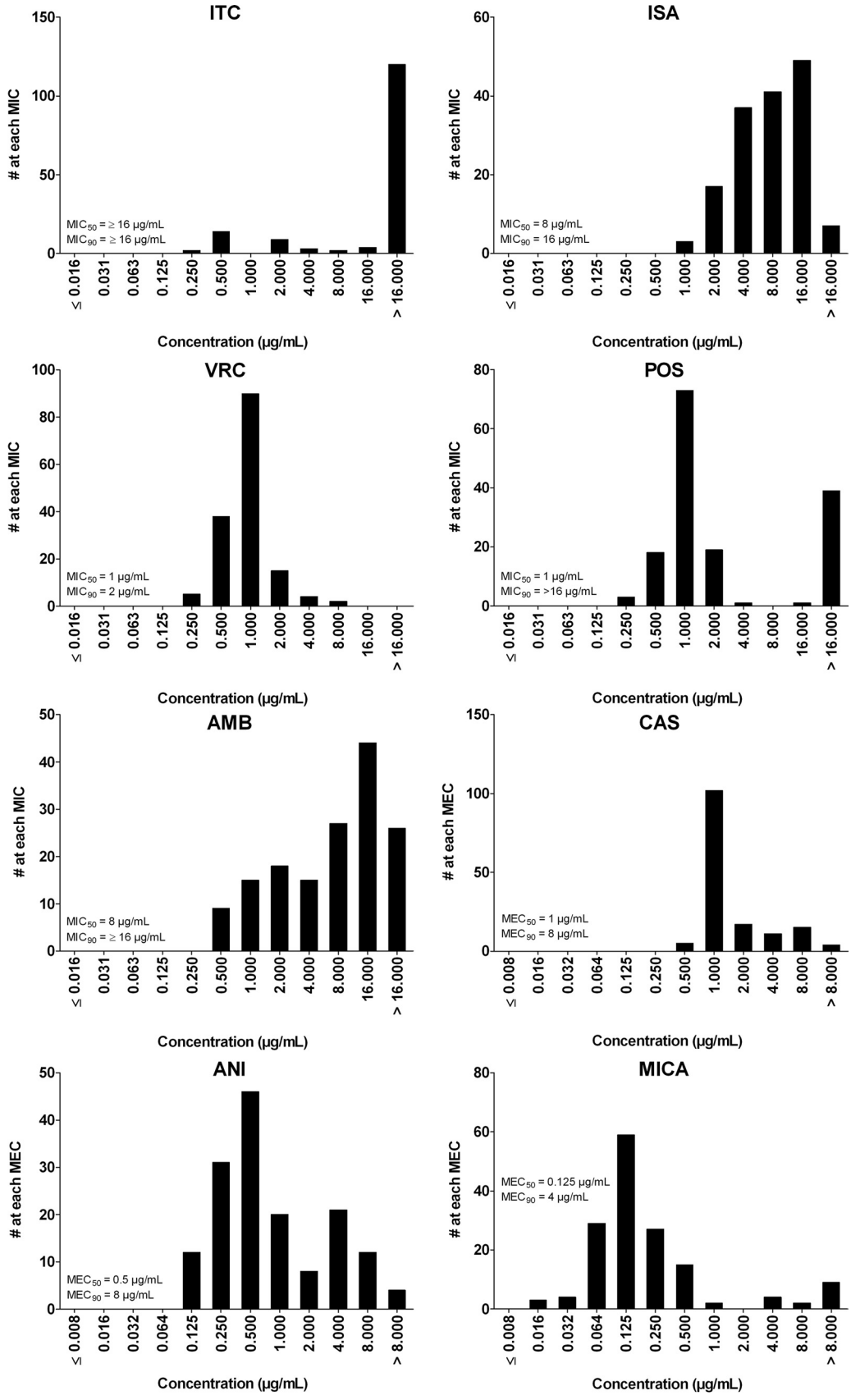

FIG 1 MIC and MEC distribution of *P. apiosperma* and the antifungal compounds AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA.

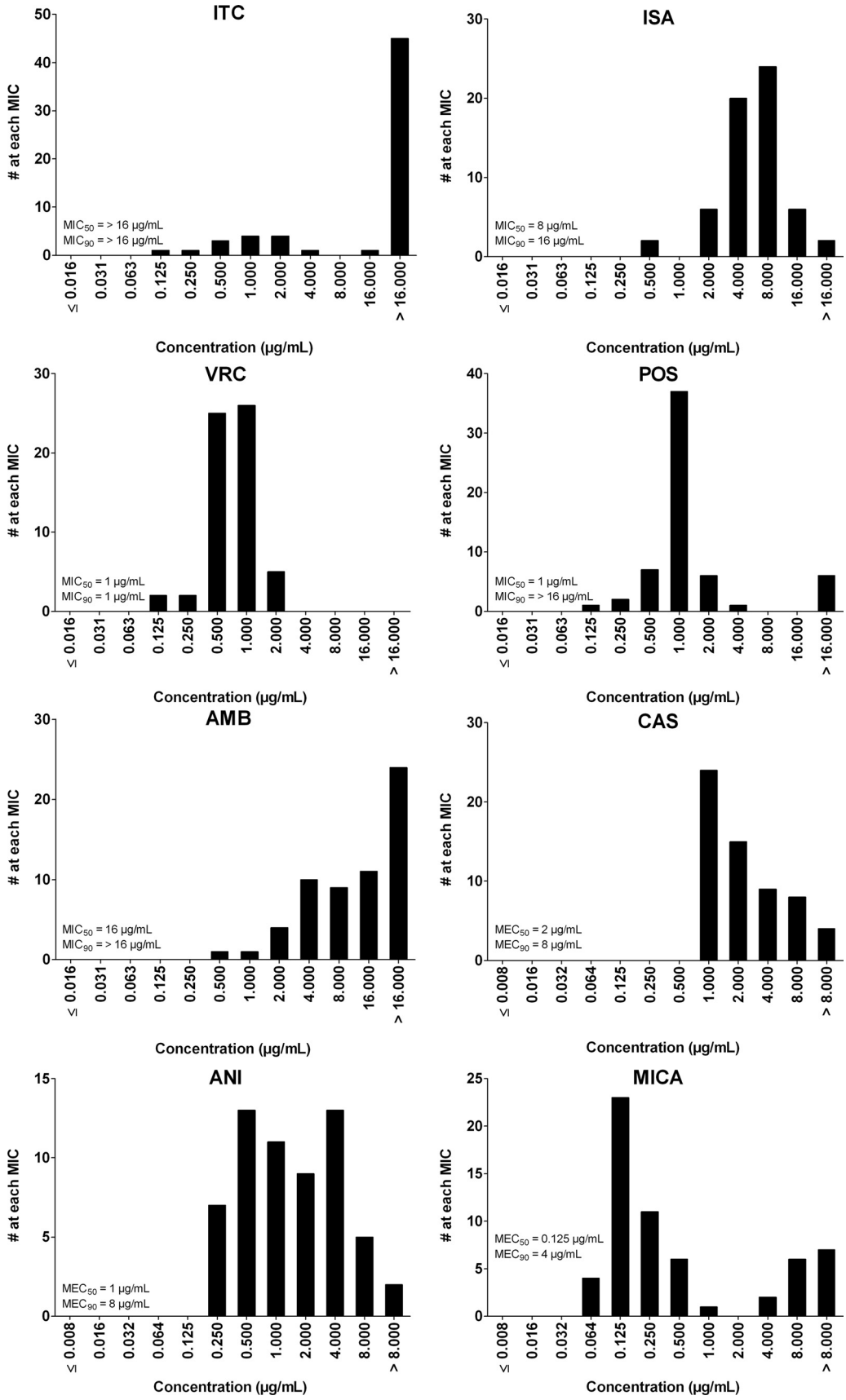

FIG 2 MIC and MEC distribution of *P. boydii* and the antifungal compounds AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA.

ity rates to 30.6% (1, 18). Kooijman et al. (23) reported that *S. aurantiacum* osteomyelitis was cured by surgery and postoperative VRC therapy.

We report here the first *in vitro* antifungal susceptibility data of *S. dehoogii*. We found that after only *S. prolificans*, *S. dehoogii* is associated with the highest VRC MIC values (up to >16 μg/ml). The species is considered to be environmental, but in our data set there were three clinical isolates: one from cystic fibrosis (CF) sputum and two from cutaneous infections. The absence of published case reports suggests that the virulence of this species is low, although in a murine model *S. dehoogii* and *S. aurantiacum* were found to be the most virulent *Scedosporium* species (10). Another possible explanation for the absence of *S. dehoogii* clinical cases might be that the species is not distinguishable from other *Scedosporium* species or *Pseudallescheria* by morphological characteristics and can therefore be easily misidentified (24).

As far as we are aware, no clinical cases of infection due to *P. minutispora* strains have been reported. Two *P. minutispora* strains in this collection were isolated from sputum. Our data differ from those of Gilgado et al. (13) with regard to susceptibility to MICA. The *P. minutispora* profile shows a similar trend as for *P. apiosperma* and *P. boydii*, but since we tested only six isolates, it is difficult to generalize.

We found that *S. prolificans* isolates were resistant to AMB, ITC, POS, and ISA. This matches with reports on *S. prolificans* being resistant to all systemically active antifungals, including the new echinocandins and azoles (29, 36). This species differs from other *Scedosporium* species in that the VRC MICs are also high (GM MIC of 15.4 μg/ml). All echinocandins were found to have moderate *in vitro* activity, at least against few strains of *S. prolificans*. A concordance among *in vitro* resistance profiles and *in vivo* outcome has also been reported (14). *In vitro* combinations of AMB and VRC, AMB and MICA, and VRC and MICA were all indifferent, whereas the triple combination of MICA, AMB, and VRC showed synergistic activity against *S. prolificans* in a murine model (34). The highest rates of synergy were with combinations of azoles and echinocandins, whereas no antagonism was found (7), suggesting that combination antifungal therapy may be more effective than monotherapy. Successful VRC and terbinafin (TRB) combination therapy in an immunocompromised patient with a brain infection was reported by Bhat et al. (2). Meletiadis et al. (30, 31) reported synergy of TRB and ITC against *S. prolificans*. Osteomyelitis due to *S. prolificans* was cured in an immunocompetent patient with a combination of VRC and CAS (2, 39). Successful combination treatment with VRC and TRB without surgical intervention was reported by Gosbell et al. (14). In immunocompromised patients, *S. prolificans* infection represents a life-threatening disease (42), and reports with a positive clinical outcome are rare. Successful therapy with aggressive surgical debridement plus combination therapy with VRC and TRB was achieved in a bone marrow recipient (21). A combination of surgery and antifungal therapy repeatedly proved to be favorable (21, 40), especially with recovery of the immune system (6).

VRC is well tolerated by most patients, including children (46), and remains the most effective drug against *Pseudallescheria* and *Scedosporium* species (except *S. prolificans*), followed by MICA and POS. Due to the promising *in vitro* activity of MICA against most *Scedosporium* species, this drug represents a potential alternative compound for the treatment of *Scedosporium* infections, especially in combination with VRC or POS. MICA exerts antifungal activity via inhibition of (1,3)-β-D-glucan synthase and by subsequently disturbing fungal cell wall synthesis. This activity may enhance the action of other, less active antifungals, such as AMB or ITC, and would be a further reason to combine MICA with azoles in future *in vitro* and *in vivo* investigations. Cuenca-Estrella et al. (7) reported the highest *in vitro* synergistic effects of azole and echinocandin combinations. AMB alone inhibited *Scedosporium* strains poorly, but synergistic effects have been shown with *in vitro* combination of AMB with various azoles (7, 45). VRC treatment of *S. prolificans* infections showed a 40% clinical response despite an MIC_{50} of 4 mg/ml (44). At present, VRC is the only licensed antifungal agent for the treatment of *Scedosporium* infections in Europe. Pharmacokinetic studies showed that VRC is well distributed through the body, including the eyes and brain tissue (17, 28, 37). A concentration of 1 μg of VRC/ml in serum is achievable (32). In contrast, MICA was present only in low levels in the brain, indicating limited penetration into the nervous system (27). Hope et al. (19) detected only insignificant amounts of MICA in cerebrospinal fluid, while drug penetration into the various central nervous system compartments was not statistically different in infected and uninfected rabbits. Groll et al. (15) found a linear disposition outside nervous tissue, with dosages of 0.5 to 2 μg/kg. The MICA concentrations were 2.26 to 11.76 μg/g in rabbit lungs, 2.05 to 8.82 μg/g in rabbit livers, 1.87 to 9.05 μg/g in rabbit spleens, and 1.4 to 6.12 μg/g in rabbit kidneys, while the concentrations in brain tissue ranged between 0.08 and 0.18 μg/g. Therefore, MICA represents a potential alternative drug for disseminated *Pseudallescheria* infections (15). In cases of brain involvement, MICA may be used in combination with VRC.

Even though ISA showed very good *in vitro* activity against a number of *Aspergillus* spp., *Candida* spp., and less common fungal pathogens (48), the *in vitro* activity against *Pseudallescheria* and *Scedosporium* spp. was poor ($MIC_{50} \geq 4$ μg/ml; $MIC_{90} \geq 16$ μg/ml), showing a potential therapeutic gap toward infections caused by these fungi.

In conclusion, in addition to VRC as monotherapy, the potential use of MICA and POS also should be taken into account as other possible combination therapeutic options for the therapy of infections due to *P. apiosperma* and *P. boydii*, preferably combining an azole with an echinocandin, such as POS/MICA and VRC/MICA. The antifungal profiles of *P. apiosperma* and *P. boydii* were found to be very similar, except that *P. apiosperma* was less susceptible to POS. The antifungal profiles of *S. aurantiacum*, *S. dehoogii*, and *S. prolificans* varied from those of *P. boydii* and *P. apiosperma* and from each other. Due to the bimodal MIC/MEC distribution, the prediction of the antifungal susceptibility of individual strains remains difficult.

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