AAC Accepts, published online ahead of print on 30 January 2012
Antimicrob. Agents Chemother. doi:10.1128/AAC.05910-11

Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species

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Summary

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 antifungal susceptibility patterns of members affiliated to 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. While *P. apiosperma* (geometric mean MIC/MEC in µg/mL of 0.9, 2.4, 7.4, 16.2, 0.2, 0.8, 1.5, and 6.8, of voriconazole, posaconazole, isavuconazole, itraconazole, micafungin, anidulafungin, caspofungin,and amphotericin B respectively) and *P. boydii* (geometric mean MIC/MEC in µg/mL of 0.7, 1.3, 5.7, 13.8, 0.5, 1.4, 2.3, and 11.8, of voriconazole, posaconazole, isavuconazole, itraconazole, micafungin, anidulafungin, caspofungin,and amphotericin B respectively) had similar susceptibility patterns, those of *S. aurantiacum*, *S. prolificans,* and *S. dehoogii* were different from each other. Voriconazole was the only drug with significant activity against *S. aurantiacum* isolates. 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 (2 µg/mL) for *P. apiosperma* and *P. boydii*. Micafungin and posaconazole had reasonable activity against the majority of *Scedosporium* strains.

Keywords

Voriconazole, micafungin, posaconazole, isavuconazole, itraconazole, caspofungin, amphotericin B, anidulafungin

Introduction

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 [4,42]. The increasing frequency and high mortality rates of invasive infections caused by *Scedosporium* species necessitate the search for new treatment strategies[48].

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* [9,11,12,42]*.* The differentiation of some smaller taxonomic entities such as *P. angusta, P. desertorum, P. ellipsoidea, P. fusoidea,* and *S. deficiens* [33] are still under debate, but are treated in this study as separate sibling species. A more distantly related *Scedosporium* species is *S. prolificans*, which frequently is multi-drug resistant [20,36]. Since the majority of *Scedosporium* isolates display multiple antifungal resistance patterns [7,13,34] the aim of the current study was to investigate whether resistance patterns are species-specific and therefore 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 of which 246 were of clinical origin, 82 of environmental origin, and four isolates of unknown origin were included and came from the following continents 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 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).

Amplified Fragment Length Polymorphism (AFLP)

All isolates were identified down to species level based on the similarities of their AFLP profiles relative to those of the included type and reference strains [25] . The AFLP analysis was performed using established procedures [8,25,39]. Briefly, isolates were grown at 35 °C in the dark on Sabouraud's glucose agar (SGA) until abundant sporulation (after approximately 14 – 18 days). Spores were collected using a damp cotton swab and were disrupted using ceramic beads in a MagNA Lyser instrument (Roche Diagnostics, Almere, the 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 using the restriction enzymes MseI and HpyCH4IV (New England Biolabs, Beverly, MA). The HpyCH4IV primer was labeled with fluorescein and contained one selective T residue, the MseI primer contained four selective residues (TGAA). Amplification products were analyzed on a MegaBACE 500 automated DNA analysis platform using standard procedures.

AFLP data were imported in BioNumerics v. 6.0 software (Applied Maths, Sint-Martens-Latem, Belgium) and analyzed by UPGMA clustering using the Pearson correlation coefficient. The analysis was restricted to DNA fragments in the range from 60 – 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, The Netherlands), anidulafungin (ANI, Pfizer Central Research, Sandwich, Kent, United Kingdom), caspofungin (CAS, Merck Sharp & Dohme BV, Haarlem, The Netherlands), isavuconazole (ISA, Basilea Pharmaceuticals, Basel, Switzerland[now Astellas]), itraconazole (ITC, Janssen Cilag, Tilburg, The Netherlands), micafungin (MICA, Astellas Pharma Inc., Ibaraki, Japan), posaconazole (POS, Schering-Plough Corp.[now Merck], Kenilworth, NJ, USA), and voriconazole (VRC, Pfizer Central Research). All azoles and AMB were tested in concentrations ranging from 0.016 µg/mL to 16 µg/mL, while all echinocandines were tested in concentrations ranging from 0.008 µg/mL to 8 µg/mL.

Candida parapsilosis ATCC 22019 and *C. krusei* ATCC 6258 served as quality control strains. Results were read after an incubation of 72 h at 37 °C. Minimal inhibitory concentration (MIC) for AMB, ITC, ISA, POS, and VRC were read visually, while minimal effective concentration (MEC) for ANI, CAS, and MICA were read microscopically.

Statistical analyses.

Geometric mean, minimal inhibitory concentration (MIC), and minimal effective concentration (MEC) were calculated using Microsoft® Office Excel 2003 SP3. For geometric mean calculations, MIC values <0.016 mg/mL were set as 0.008 µg/mL and MIC values >16 µg/mL were set to 32 µg/mL, MEC

values <0.008 µg/mL was set at 0.004 µg/mL and MEC values >8 µg/mL were set to 16 µg/mL. For MIC_{50}/MEC_{50} and MIC_{90}/MEC_{90} data per antifungal and species were sorted in ascending order, followed by median and 90^{th} percentile determination. MIC/MEC distributions between clinical and environmental isolates were compared using the Mann-Whitney-Wilcoxon test. A p-value of <0.05 was considered to be statistically significant. The presence of cross-resistance was tested by analyzing the MIC/MEC values of each pair of antifungal drugs by the Spearman rank correlation and was considered statistically significant when p-values were lower than 0.01.

Results

Using established procedures, all isolates in this study were identified based on the similarities of their AFLP fingerprints to those of the included type or ex-type strains [26]. Out of a total of 332 strains, 154 were identified as *P. apiosperma* (124 clinical, 29 environmental, and one from unknown source), 60 as *P. boydii* (44 clinical, 14 environmental, and two from unknown source), 37 as *S. prolificans*, 22 as *S. aurantiacum,* 22 *as S. dehoogii ,* 16 as *P. ellipsoidea,* 15 as *P. angusta,* and six as *P. minutispora*. Among all clinical isolates (n = 246), the most prevalent 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. minutispora* (n = 2). . *Pseudallescheria apiosperma, P. boydii, S. aurantiacum, S. prolificans,* and *P. ellipsoidea* (11 out of 16 strains) were mainly recovered from clinical specimens, whereas *P. angusta* (10 out of 15 strains), *P. minutispora* (4 out of 6 strains), and *S. dehoogii* (16 out 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 MIC and MEC, and geometric mean (GM) MICs/MECs sorted by antifungal compound are listed in Table 1.

143 *Pseudallescheria apiosperma* isolates had the lowest GM values of MICA, followed by ANI and VRC.
144 *Pseudallescheria boydii* strains had the lowest GM of MICA, followed by VRC, and POS. Strains of *S.*
145 *aurantiacum* had only low GM values of VRC and the lowest GM values for S*. dehoogii* were found with
146 MICA (GM MEC 1.1 µg/mL) and VRC (GM MIC1.5 µg/mL). Strains of *P. minutispora* had the lowest GMs
147 for MICA (GM MEC 0.4 µg/mL) and VRC (GM MIC 0.8 µg/mL). The majority of *S. prolificans* strains
148 showed the highest GMsMIC/MEC (µg/mL) of all tested antifungal drugs (AMB 28.6, CAS 10.4, ANI 4.8,
149 MICA 7.9, ITC 32, VRC 15.4, POS 32, ISA 25.6); only a few strains had low MECs of ANI (0.25 µg/mL)and
150 MICA (0.125 µg/mL) (Table 1). Judged by GM values VRC and/or MICA showed reasonable *in vitro*
151 activity against all *Pseudallescheria/Scedosporium* species (VRC with MIC_{50} ≤ 1 µg/mL; MICA with MEC_{50}
152 ≤ 0.5 µg/mL), except *S. prolificans* (VRC with MIC_{50} 16 µg/mL; MICA with MEC_{50} > 8 µg/mL) and *S.*
153 *aurantiacum* (MICA with MEC_{50} 8 µg/mL).

154 Species-specific MIC and MEC values of all *Pseudallescheria/Scedosporium* (*P/S*) species are
155 listed in Table 1. All *Scedosporium* and *Pseudallescheria* species were found to have high MIC/MEC
156 values of AMB (MIC_{50} ≥ 4 µg/mL), ITC (MIC_{50} > 16 µg/mL), and ISA (MIC_{50} > 4 µg/mL) (Table 1). CAS had
157 MEC_{50} and MEC_{90} values suggesting reasonable *in vitro* activity against *P. ellipsoidea* strains only (MEC_{50}
158 1µg/mL and MEC_{90} 2 µg/mL). High MEC_{50}/MEC_{90} values were obtained for ANI and *S. dehoogii* (MEC_{50}
159 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}
160 values were found for MICA and *S. prolificans* and *S. aurantiacum* only. Limited *in vitro* activity of VRC
161 was found only for the species *S. prolificans* and *S. dehoogii.* POS and VRC are the most promising drugs
162 against all *P/S* species other than *S. prolificans* and MICA against *P/S* species other than *S. prolificans*
163 and *S. aurantiacum* (Table 1).

164 We evaluated if the MIC/MEC values correlated with the origin of isolates (clinical vs.
165 environmental). MIC and MEC values of clinical isolates and environmental isolates of *P. apiosperma*
166 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 *). 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). Also for the echinocandins, a statistically significant cross-resistance was observed (Table 3). No statistically significant cross-resistance was observed between azoles and echinocandins (results not shown).

A major finding of this 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*. Especially, the MIC/MEC distributions of MICA, ITC, and POS clearly show the presence of two different subpopulations with different susceptibilities. By analysing the MEC distribution of *P. apiosperma* and *P. boydii* of MICA (Figs. 1 and 2) we observe one susceptible population with MEC values lower than 1 µg/mL (n = 139 and n = 45, resp.) and a second subpopulation with MEC values of ≥ 4 µg/mL (each n = 15). For POS a major partition of *P. apiosperma* and *P. boydii* population had MIC values ≤ 4 µg/mL (n= 94 and n =47, resp.), the other subpopulation was highly resistant with MIC ≥ 16 µg/mL) (Figs. 1 and 2). Major partitions of the *P. apiosperma* (n =124) and *P. boydii* (n = 46) population were found highly resistant for ITC with MIC values ≥ 16 µg/mL, only a minority of the populations had MIC values ≤ 8 µg/mL (n = 30 and n = 14, resp.).

Discussion

Pseudallescheria boydii and *P. apiosperma* strains have been isolated from clinical samples worldwide, and both species can be regarded as environmental opportunistic fungi having similar spectra of clinical manifestations. They are the most prevalent *Pseudallescheria* species [44], 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 more susceptible for POS than *P. boydii*. Moreover 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, but 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 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 ECV values. Remarkable is that the MIC/MEC distribution of *P. boydii* and *P. apiosperma* and all antifungal drugs except VRC do not show a normal distribution. Especially 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 susceptibilities of individual isolates are difficult to predict and 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.

As *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.

Scedosporium 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 activity of ITC, our tested *S. aurantiacum* strains had high MICs of ITC (GM 19.3 µg/mL), but low MICS of VRC (GM 0.6 µg/mL), which confirm data reported by Tintelnot *et al*. [43] 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*. Also clinically this antifungal drug was effective, lowering mortality rates to 30.6 % [1,18]. Kooijman *et al*. [23] reported *S. aurantiacum* osteomyelitis cured by surgery and post-operative VRC therapy.

Here we report the first *in vitro* antifungal susceptibility data of *S. dehoogii*. We found that second to *S. prolificans, S. dehoogii* has the highest MIC values of VRC (up to > 16 µg/mL). The species is considered to be environmental, but in our dataset were three clinical isolates: one from 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] in 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 [29,37]. This species differs from other *Scedosporium* species by having also high MICs of VRC (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.* Concordance of *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, while 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, while no antagonism was found [7] suggesting 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 [2,40]. 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 [43] 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]. Combination of surgery and antifungal therapy repeatedly proved to be favorable [21,41,41], especially with recovery of the immune system[6].

VRC is well tolerated by most patients, including children [47] and remains the most effective drug against *P/S* (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-Estrellas *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 [7,46]. VRC treatment of *S. prolificans* infections showed a 40 % clinical response despite a MIC_{50} of 4 mg/mL [45]. 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 eyes and brain tissue [17,28,38]. A concentration of 1 µg/mL of VRC 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 CNS compartments was not statistically different in infected and non-infected rabbits. Groll *et al.* [15] found a linear disposition outside nervous tissue with dosages of 0.5 to 2 µg /kg. MICA concentration in rabbit lungs was 2.26 µg/g to 11.76 µg/g, in liver 2.05 to 8.82 µg/g, in spleen 1.87 to 9.05 µg/g and in kidney 1.4 to 6.12 µg/g, while 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 case 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[49], 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 towards infections caused by these fungi.

In conclusion, beside VRC as monotherapy, also MICA and POS should be taken into account as other potential 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 *P. apiosperma* being less susceptible towards 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, prediction of the antifungal susceptibility of individual strains remains difficult.

Acknowledgements

We thank E. Geertsen and C. Bens for expert technical assistance.

Declaration of interest

P.E. Verweij has received research grants from Pfizer, Gilead, Basilea, Merck, Biorad and Schering-Plough. J. F. Meis has received research grants from Astellas, Merck, Basilea and Schering-Plough, is a consultant to Astellas, Basilea and Merck and has received speaker's fees from Merck, Pfizer, Schering-Plough and Janssen Pharmaceutica. C.H. Klaassen received a research grant from Pfizer.

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459 Legends to the tables and figures.
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463 TABLE 1. *In vitro* antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species against
464 eight antifungal compounds (AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA).
465
466 TABLE 2. MIC/MEC value comparison for clinical versus environmental isolates of *P. apiosperma* and *P.*
467 *boydii* for all tested antifungal compounds (AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA).
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470 different echinocandins (CAS, ANI, and MICA) for *P. apiosperma* and *P. boydii* using the Spearman rank
471 coefficient, a p-value of <0.01 was considered to be statistically significant.
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474 distribution of *P. apiosperma* and the antifungal compounds AMB, CAS, ITC, ISA, VRC, ANI, POS, and
475 MICA.
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478 distribution of *P. boydii* and the antifungal compounds AMB, CAS, ITC, ISA, VRC, ANI, POS, and MICA.

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)

		AMB				CAS				ANI				MICA			
Species	**n**	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

		ITC				VRC				POS				ISA			
Species	**n**	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM
P. apiosperma	154	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
P. boydii	60	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
S. prolificans	37	>16 - > 16	> 16	> 16	32.0	4 - > 16	16	> 16	15.4	> 16 - > 16	> 16	> 16	32.0	8 - > 16	> 16	> 16	25.6
S. dehoogii	22	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
S. aurantiacum	22	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
P. ellipsoidea	16	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
P. angusta	15	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
P. minutispora	6	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

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)

TABLE 2. MIC/MEC value comparison for clinical *versus* environmental isolates of *P. apiosperma* and *P. boydii* isolates.

		AMB				CAS				ANI				MICA			
	n	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 [cl]	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
P. apiosperma [en]	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 [cl]	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
P. boydii [en]	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

		ITC				VRC				POS				ISA			
	n	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM
P. apiosperma [cl]	124	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
P. apiosperma [en]	29	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
P. boydii [cl]	44	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
P. boydii [en]	14	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

n^{cl},number of strains from clinical specimens; n^{en}, number of strains from environmental samples; n^{-}/unknown, number of unknown origin/source;*MIC/MEC distribution statistically significant difference ($p \leq 0.05$);

TABLE 3. Evaluation of cross-resistance between the different azoles (ITC, ISA, VRC, and POS) and the different echinocandins (CAS, ANI, and MICA) for *P. apiosperma* and *P. boydii* using the Spearman rank coefficient, a p-value of <0.01 was considered to be statistically significant.

P. apiosperma

	Azoles					Echinocandins		
	ITC	VRC	POS	ISA		CAS	ANI	MICA
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

	Azoles					Echinocandins		
	ITC	VRC	POS	ISA		CAS	ANI	MICA
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				

*p < 0.0001