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

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26 **Summary**

27 Since the separation of *Pseudallescheria boydii* and *P. apiosperma* in 2010, limited data on species-
28 specific susceptibility patterns of these and other species of *Pseudallescheria* and its anamorph
29 *Scedosporium* have been reported. This study presents antifungal susceptibility patterns of members
30 affiliated to both entities. Clinical and environmental isolates (n=332) from a wide range of sources
31 and origins were identified down to species level and tested according to CLSI M38-A2 against eight
32 antifungal compounds. While *P. apiosperma* (geometric mean MIC/MEC in µg/mL of 0.9, 2.4, 7.4,
33 16.2, 0.2, 0.8, 1.5, and 6.8, of voriconazole, posaconazole, isavuconazole, itraconazole, micafungin,
34 anidulafungin, caspofungin, and amphotericin B respectively) and *P. boydii* (geometric mean
35 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,
36 isavuconazole, itraconazole, micafungin, anidulafungin, caspofungin, and amphotericin B respectively)
37 had similar susceptibility patterns, those of *S. aurantiacum*, *S. prolificans*, and *S. dehoogii* were
38 different from each other. Voriconazole was the only drug with significant activity against *S.*
39 *aurantiacum* isolates. MIC distributions of all drugs except voriconazole did not show a normal
40 distribution and often showed two subpopulations, making a species-based prediction of antifungal
41 susceptibility difficult. Therefore antifungal susceptibility testing of all clinical isolates remains
42 essential for targeted antifungal therapy. Voriconazole was the only compound with low MIC values
43 (2 µg/mL) for *P. apiosperma* and *P. boydii*. Micafungin and posaconazole had reasonable activity
44 against the majority of *Scedosporium* strains.

45 **Keywords**

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

48

49 Introduction

50 *Scedosporium* species are involved in a wide range of human infections, especially in
51 immunocompromised patients^{22,26}. Cerebral abscesses are relatively frequent²⁴, reflecting the
52 neurotropic character of these fungi³. A typical disease entity of these fungi is the near-drowning
53 syndrome²⁴ when patients develop cerebral abscesses weeks to months after the inciting event¹⁶. In
54 cystic fibrosis patients, *Scedosporium* species are among the most common fungal colonizers of the
55 respiratory tract, but rarely become invasive^{4,42}. The increasing frequency and high mortality rates of
56 invasive infections caused by *Scedosporium* species necessitate the search for new treatment
57 strategies⁴⁸.

58 Recently species concepts in *Pseudallescheria* and *Scedosporium* have been narrowed as a result of
59 application of molecular phylogeny. The following species are now widely accepted in the scientific
60 community: *P. apiosperma* (anamorph: *S. apiospermum*), *S. aurantiacum*, *P. boydii* (*S. boydii*), *S.*
61 *dehoogii*, and *P. minutispora*^{9,11,12,42}. The differentiation of some smaller taxonomic entities such as *P.*
62 *angusta*, *P. desertorum*, *P. ellipsoidea*, *P. fusoidea*, and *S. deficiens*³³ are still under debate, but are
63 treated in this study as separate sibling species. A more distantly related *Scedosporium* species is *S.*
64 *prolificans*, which frequently is multi-drug resistant^{20,36}. Since the majority of *Scedosporium* isolates
65 display multiple antifungal resistance patterns^{7,13,34} the aim of the current study was to investigate
66 whether resistance patterns are species-specific and therefore identification down to species-level is
67 relevant for the choice of antifungal treatment. We tested eight systemic antifungal compounds against
68 a set of *Scedosporium/Pseudallescheria* isolates from a wide range of geographical origins and from
69 divergent environmental and clinical sources.

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71 Materials and Methods

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73 **Isolates**

74 A total of 332 *Scedosporium* isolates of which 246 were of clinical origin, 82 of environmental origin,
75 and four isolates of unknown origin were included and came from the following continents Africa (n =
76 8), Asia (n= 33), Europe (n = 224), North America (n = 18), South America (n = 16), Oceania (n = 4), and
77 Antarctica (n = 1). The geographical origin of 28 strains was not traceable. As reference for molecular
78 species identification several type and ex-type strains were included: *P. angusta* (CBS 254.72 T), *P.*
79 *apiosperma* (CBS 117407 T), *S. aurantiacum* (CBS 116910 T), *P. boydii* (CBS 101.22 T), *S. dehoogii* (CBS
80 117406 T), *P. ellipsoidea* (CBS 418.73 T), *P. minutispora* (CBS 116911 T), and *S. prolificans* (CBS 114.90
81 T).

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83 **Amplified Fragment Length Polymorphism (AFLP)**

84 All isolates were identified down to species level based on the similarities of their AFLP profiles relative
85 to those of the included type and reference strains²⁵. The AFLP analysis was performed using
86 established procedures^{8,25,39}. Briefly, isolates were grown at 35 °C in the dark on Sabouraud's glucose
87 agar (SGA) until abundant sporulation (after approximately 14 – 18 days). Spores were collected using a
88 damp cotton swab and were disrupted using ceramic beads in a MagNA Lyser instrument (Roche
89 Diagnostics, Almere, the Netherlands). DNA was extracted using a MagNA Pure LC instrument (Roche
90 Diagnostics) in combination with the MagNA Pure LC DNA isolation kit III according to the instructions
91 of the manufacturer. AFLP was performed using the restriction enzymes MseI and HpyCH4IV (New
92 England Biolabs, Beverly, MA). The HpyCH4IV primer was labeled with fluorescein and contained one
93 selective T residue, the MseI primer contained four selective residues (TGAA). Amplification products
94 were analyzed on a MegaBACE 500 automated DNA analysis platform using standard procedures.

95 AFLP data were imported in BioNumerics v. 6.0 software (Applied Maths, Sint-Martens-Latem,
96 Belgium) and analyzed by UPGMA clustering using the Pearson correlation coefficient. The analysis was
97 restricted to DNA fragments in the range from 60 – 300 bp.

98

99 ***In vitro* susceptibility testing**

100 *In vitro* susceptibility testing was performed using broth microdilution for filamentous fungi, according
101 to CLSI document M38-A2⁵. The following antifungal drugs were used: amphotericin B (AMB, Bristol
102 Myers Squibb, Woerden, The Netherlands), anidulafungin (ANI, Pfizer Central Research, Sandwich,
103 Kent, United Kingdom), caspofungin (CAS, Merck Sharp & Dohme BV, Haarlem, The Netherlands),
104 isavuconazole (ISA, Basilea Pharmaceuticals, Basel, Switzerland[now Astellas]), itraconazole (ITC,
105 Janssen Cilag, Tilburg, The Netherlands), micafungin (MICA, Astellas Pharma Inc., Ibaraki, Japan),
106 posaconazole (POS, Schering-Plough Corp.[now Merck], Kenilworth, NJ, USA), and voriconazole (VRC,
107 Pfizer Central Research). All azoles and AMB were tested in concentrations ranging from 0.016 µg/mL
108 to 16 µg/mL, while all echinocandines were tested in concentrations ranging from 0.008 µg/mL to 8
109 µg/mL.

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

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115 **Statistical analyses.**

116 Geometric mean, minimal inhibitory concentration (MIC), and minimal effective concentration
117 (MEC) were calculated using Microsoft® Office Excel 2003 SP3. For geometric mean calculations, MIC
118 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

119 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
120 MIC₅₀/MEC₅₀ and MIC₉₀/MEC₉₀ data per antifungal and species were sorted in ascending order,
121 followed by median and 90th percentile determination. MIC/MEC distributions between clinical and
122 environmental isolates were compared using the Mann-Whitney-Wilcoxon test. A p-value of <0.05 was
123 considered to be statistically significant. The presence of cross-resistance was tested by analyzing the
124 MIC/MEC values of each pair of antifungal drugs by the Spearman rank correlation and was considered
125 statistically significant when p-values were lower than 0.01.

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128 Results

129

130 Using established procedures, all isolates in this study were identified based on the similarities of their
131 AFLP fingerprints to those of the included type or ex-type strains²⁶. Out of a total of 332 strains, 154
132 were identified as *P. apiosperma* (124 clinical, 29 environmental, and one from unknown source), 60 as
133 *P. boydii* (44 clinical, 14 environmental, and two from unknown source), 37 as *S. prolificans*, 22 as *S.*
134 *aurantiacum*, 22 as *S. dehoogii*, 16 as *P. ellipsoidea*, 15 as *P. angusta*, and six as *P. minutispora*. Among
135 all clinical isolates (n = 246), the most prevalent species were *P. apiosperma* (n = 124), *P. boydii* (n = 44),
136 *S. prolificans* (n = 35), *S. aurantiacum* (n = 19), *P. ellipsoidea* (n = 11), *P. angusta* (n = 5), *S. dehoogii* (n =
137 6), and *P. minutispora* (n = 2). *Pseudallescheria apiosperma*, *P. boydii*, *S. aurantiacum*, *S. prolificans*,
138 and *P. ellipsoidea* (11 out of 16 strains) were mainly recovered from clinical specimens, whereas *P.*
139 *angusta* (10 out of 15 strains), *P. minutispora* (4 out of 6 strains), and *S. dehoogii* (16 out of 22 strains)
140 were mainly isolated from the environment.

141 Species-specific, *in vitro* MIC₅₀ and MEC₅₀ values, MIC₉₀ and MEC₉₀ values, ranges of MIC and
142 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 MIC 1.5 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 GMs/MIC/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 MIC₅₀ ≤ 1 µg/mL; MICA with MEC₅₀
152 ≤ 0.5 µg/mL), except *S. prolificans* (VRC with MIC₅₀ 16 µg/mL; MICA with MEC₅₀ > 8 µg/mL) and *S.*
153 *aurantiacum* (MICA with MEC₅₀ 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 (MIC₅₀ ≥ 4 µg/mL), ITC (MIC₅₀ > 16 µg/mL), and ISA (MIC₅₀ > 4 µg/mL) (Table 1). CAS had
157 MEC₅₀ and MEC₉₀ values suggesting reasonable *in vitro* activity against *P. ellipsoidea* strains only (MEC₅₀
158 1 µg/mL and MEC₉₀ 2 µg/mL). High MEC₅₀/MEC₉₀ values were obtained for ANI and *S. dehoogii* (MEC₅₀
159 8 µg/mL and MEC₉₀ > 8 µg/mL) and *S. aurantiacum* (MEC₅₀ 8 µg/mL and MEC₉₀ > 8 µg/mL). High MEC₅₀
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

167 for POS as well as for MICA between clinical and environmental strains of *P. apiosperma* (Mann-
168 Whitney-Wilcoxon test: $p = 0.0028$ and $p = 0.0495$, respectively) (Table 2, values marked with *). For
169 *P. boydii* no statistical significant differences between clinical and environmental strains were
170 detected for any of the tested compounds.

171 Within *P. apiosperma* and *P. boydii*, cross resistance between the different azoles was
172 observed, i.e. isolates in the higher MIC distribution of VOR were also within the higher MIC
173 distribution of POS. This was statistically evaluated using the Spearman correlation coefficient and
174 was found to be highly significant ($p < 0.0001$) (Table 3). Also for the echinocandins, a statistically
175 significant cross-resistance was observed (Table 3). No statistically significant cross-resistance was
176 observed between azoles and echinocandins (results not shown).

177 A major finding of this study was that almost none of tested compounds showed a normal
178 distribution of MIC/MEC values. Only VRC showed a normal distribution with *P. apiosperma* and *P.*
179 *boydii*. Especially, the MIC/MEC distributions of MICA, ITC, and POS clearly show the presence of two
180 different subpopulations with different susceptibilities. By analysing the MEC distribution of *P.*
181 *apiosperma* and *P. boydii* of MICA (Figs. 1 and 2) we observe one susceptible population with MEC
182 values lower than $1 \mu\text{g/mL}$ ($n = 139$ and $n = 45$, resp.) and a second subpopulation with MEC values of
183 $\geq 4 \mu\text{g/mL}$ (each $n = 15$). For POS a major partition of *P. apiosperma* and *P. boydii* population had MIC
184 values $\leq 4 \mu\text{g/mL}$ ($n = 94$ and $n = 47$, resp.), the other subpopulation was highly resistant with MIC ≥ 16
185 $\mu\text{g/mL}$ (Figs. 1 and 2). Major partitions of the *P. apiosperma* ($n = 124$) and *P. boydii* ($n = 46$)
186 population were found highly resistant for ITC with MIC values $\geq 16 \mu\text{g/mL}$, only a minority of the
187 populations had MIC values $\leq 8 \mu\text{g/mL}$ ($n = 30$ and $n = 14$, resp.).

188 Discussion

189

190 *Pseudallescheria boydii* and *P. apiosperma* strains have been isolated from clinical samples worldwide,
191 and both species can be regarded as environmental opportunistic fungi having similar spectra of clinical
192 manifestations. They are the most prevalent *Pseudallescheria* species⁴⁴, but published studies of *in*
193 *vitro* susceptibility profiles according to the latest taxonomical standards are rare²⁵. The two species
194 have similar susceptibility profiles, with the lowest MICs/MECs of VRC and MICA. However, *P.*
195 *apiosperma* was found more susceptible for POS than *P. boydii*. Moreover we found a statistically
196 significant difference between environmental and clinical *P. apiosperma* strains for POS and MICA,
197 clinical isolates of *P. apiosperma* had lower MICs of POS than environmental strains. The majority of the
198 *P. apiosperma* population had low POS MICs, therefore it might be possible that POS resistant strains
199 might be less virulent than susceptible strains, but to prove this hypothesis and to investigate this
200 further *in vivo* data in an animal model are needed. For MICA, the majority of the *P. apiosperma*
201 population exhibits low MECs. However, clinical strains have statistically significant higher MECs of
202 MICA than environmental strains. For *P. boydii* no statistically significant differences between clinical
203 and environmental strains were detected.

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

206 With normally distributed MIC/MEC values, if the MIC₅₀ or GM is known, one can reasonably
207 predict the MIC₉₀ and ECV values. Remarkable is that the MIC/MEC distribution of *P. boydii* and *P.*
208 *apiosperma* and all antifungal drugs except VRC do not show a normal distribution. Especially the
209 MIC/MEC distributions of *P. apiosperma* and *P. boydii* strains were bimodal for MICA, POS, and ITC and
210 showed signs of bimodality for AMB, CAS and ANI. The consequence of these distributions is that
211 susceptibilities of individual isolates are difficult to predict and susceptibility testing of clinical isolates
212 remains essential for targeted treatment. The subpopulations with the lower MIC/MEC values could be
213 the original susceptible wild-type populations, whereas the isolates with the higher MIC/MEC values

214 could have acquired antifungal resistance mechanisms. However, this presumptive explanation
215 requires further investigation.

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

219 *Scedosporium aurantiacum* showed high *in vitro* resistance to AMB and ITC, and all other
220 antifungal drugs tested, except VRC, showed poor activity. Our results are in concordance with those
221 of Gilgado *et al.*¹³ in that *S. aurantiacum* isolates are less susceptible to antifungal drugs than strains
222 of *P. apiosperma*. Therefore the differentiation of *S. aurantiacum* strains from other *Scedosporium*
223 species is also of interest for the choice of antifungal therapy. In contrast to the findings of Heath *et*
224 *al.*¹⁸ who reported a common trend in susceptibilities between *P. apiosperma* and *S. aurantiacum*
225 with good activity of ITC, our tested *S. aurantiacum* strains had high MICs of ITC (GM 19.3 µg/mL),
226 but low MICs of VRC (GM 0.6 µg/mL), which confirm data reported by Tintelnot *et al.*⁴³ and
227 Alastruey-Izquierdo *et al.*¹. Based on these data we judge VRC as the only antifungal compound with
228 promising *in vitro* activity against *S. aurantiacum*. Also clinically this antifungal drug was effective,
229 lowering mortality rates to 30.6 %^{1,18}. Kooijman *et al.*²³ reported *S. aurantiacum* osteomyelitis cured
230 by surgery and post-operative VRC therapy.

231 Here we report the first *in vitro* antifungal susceptibility data of *S. dehoogii*. We found that
232 second to *S. prolificans*, *S. dehoogii* has the highest MIC values of VRC (up to > 16 µg/mL). The species
233 is considered to be environmental, but in our dataset were three clinical isolates: one from CF-
234 sputum and two from cutaneous infections. The absence of published case reports suggests that the
235 virulence of this species is low, although in a murine model *S. dehoogii* and *S. aurantiacum* were
236 found to be the most virulent *Scedosporium* species¹⁰. Another possible explanation for the absence

237 of *S. dehoogii* clinical cases might be that the species is not distinguishable from other *Scedosporium*
238 species or *Pseudallescheria* by morphological characteristics and can therefore be easily misidentified
239 ²⁴.

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

245 We found that *S. prolificans* isolates were resistant to AMB, ITC, POS, and ISA. This matches
246 with reports on *S. prolificans* being resistant to all systemically active antifungals, including the new
247 echinocandins and azoles ^{29,37}. This species differs from other *Scedosporium* species by having also high
248 MICs of VRC (GM MIC of 15.4 µg/mL). All echinocandins were found to have moderate *in vitro* activity,
249 at least against few strains of *S. prolificans*. Concordance of *in vitro* resistance profiles and *in vivo*
250 outcome has also been reported ¹⁴. *In vitro* combinations of AMB and VRC, AMB and MICA, and VRC
251 and MICA were all indifferent, while the triple combination of MICA, AMB and VRC showed synergistic
252 activity against *S. prolificans* in a murine model ³⁴. The highest rates of synergy were with combinations
253 of azoles and echinocandins, while no antagonism was found ⁷ suggesting combination antifungal
254 therapy may be more effective than monotherapy. Successful VRC and terbinafin (TRB) combination
255 therapy in an immunocompromised patient with a brain infection was reported by Bhat *et al.* ².
256 Meletiadis *et al.* ^{30,31} reported synergy of TRB and ITC against *S. prolificans*. Osteomyelitis due to *S.*
257 *prolificans* was cured in an immunocompetent patient with a combination of VRC and CAS ^{2,40}.
258 Successful combination treatment with VRC and TRB without surgical intervention was reported by
259 Gosbell *et al.* ¹⁴. In immunocompromised patients *S. prolificans* infection represents a life-threatening

260 disease⁴³ and reports with a positive clinical outcome are rare. Successful therapy with aggressive
261 surgical debridement plus combination therapy with VRC and TRB was achieved in a bone marrow
262 recipient²¹. Combination of surgery and antifungal therapy repeatedly proved to be favorable^{21,41,41},
263 especially with recovery of the immune system⁶.

264 VRC is well tolerated by most patients, including children⁴⁷ and remains the most effective
265 drug against *P/S* (except *S. prolificans*), followed by MICA and POS. Due to the promising *in vitro* activity
266 of MICA against most *Scedosporium* species, this drug represents a potential alternative compound for
267 the treatment of *Scedosporium* infections, especially in combination with VRC or POS. MICA exerts
268 antifungal activity via inhibition of (1,3)- β -D-glucan synthase and by subsequently disturbing fungal cell
269 wall synthesis. This activity may enhance the action of other, less active antifungals, such as AMB or ITC
270 and would be a further reason to combine MICA with azoles in future *in vitro* and *in vivo* investigations.
271 Cuenca-Estrellas *et al.*⁷ reported the highest *in vitro* synergistic effects of azole and echinocandin
272 combinations. AMB alone inhibited *Scedosporium* strains poorly, but synergistic effects have been
273 shown with *in vitro* combination of AMB with various azoles^{7,46}. VRC treatment of *S. prolificans*
274 infections showed a 40 % clinical response despite a MIC₅₀ of 4 mg/mL⁴⁵. At present VRC is the only
275 licensed antifungal agent for the treatment of *Scedosporium* infections in Europe. Pharmacokinetic
276 studies showed that VRC is well-distributed through the body including eyes and brain tissue^{17,28,38}. A
277 concentration of 1 μ g/mL of VRC in serum is achievable³². In contrast, MICA was present only in low
278 levels in the brain, indicating limited penetration into the nervous system²⁷. Hope *et al.*¹⁹ detected
279 only insignificant amounts of MICA in cerebrospinal fluid, while drug penetration into the various CNS
280 compartments was not statistically different in infected and non-infected rabbits. Groll *et al.*¹⁵ found a
281 linear disposition outside nervous tissue with dosages of 0.5 to 2 μ g /kg. MICA concentration in rabbit
282 lungs was 2.26 μ g/g to 11.76 μ g/g, in liver 2.05 to 8.82 μ g/g, in spleen 1.87 to 9.05 μ g/g and in kidney
283 1.4 to 6.12 μ g/g, while concentrations in brain tissue ranged between 0.08 and 0.18 μ g/g. Therefore

284 MICA represents a potential alternative drug for disseminated *Pseudallescheria* infections¹⁵. In case of
285 brain involvement MICA may be used in combination with VRC.

286 Even though ISA showed very good *in vitro* activity against a number of *Aspergillus* spp.,
287 *Candida* spp. and less common fungal pathogens⁴⁹, the *in vitro* activity against *Pseudallescheria* and
288 *Scedosporium* spp. was poor (MIC₅₀ ≥ 4 µg/mL; MIC₉₀ ≥ 16 µg/mL) showing a potential therapeutic gap
289 towards infections caused by these fungi.

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

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299

300 **Declaration of interest**

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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).

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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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469 TABLE 3. Evaluation of cross-resistance between the different azoles (ITC, ISA, VRC, and POS) and the
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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473 FIGURE 1. Minimal inhibitory concentration (MIC) and minimal effective concentration (MEC)
474 distribution of *P. apiosperma* and the antifungal compounds AMB, CAS, ITC, ISA, VRC, ANI, POS, and
475 MICA.

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477 FIGURE 2. Minimal inhibitory concentration (MIC) and minimal effective concentration (MEC)
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)

Species	n	AMB			CAS			ANI			MICA						
		Range	MIC ₅₀	MIC ₉₀	GM	Range	MEC ₅₀	MIC ₉₀	GM	Range	MEC ₅₀	MIC ₉₀	GM				
<i>P. apiosperma</i>	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
<i>P. boydii</i>	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
<i>S. prolificans</i>	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
<i>S. dehoogii</i>	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
<i>S. aurantiacum</i>	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
<i>P. ellipsoidea</i>	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
<i>P. angusta</i>	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
<i>P. minutispora</i>	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

Species	n	ITC			VRC			POS			ISA						
		Range	MIC ₅₀	MIC ₉₀	GM	Range	MIC ₅₀	MIC ₉₀	GM	Range	MIC ₅₀	MIC ₉₀	GM				
<i>P. apiosperma</i>	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
<i>P. boydii</i>	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
<i>S. prolificans</i>	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
<i>S. dehoogii</i>	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
<i>S. aurantiacum</i>	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
<i>P. ellipsoidea</i>	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
<i>P. angusta</i>	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
<i>P. minutispora</i>	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.

	n	AMB			CAS			ANI			MICA						
		Range	MIC ₅₀	MIC ₉₀	GM	Range	MEC ₅₀	MEC ₉₀	GM	Range	MEC ₅₀	MEC ₉₀	GM				
<i>P. apiosperma</i> ^{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
<i>P. apiosperma</i> ^{em}	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
<i>P. boydii</i> ^{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
<i>P. boydii</i> ^{em}	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

	n	ITC			VRC			POS			ISA						
		Range	MIC ₅₀	MIC ₉₀	GM	Range	MIC ₅₀	MIC ₉₀	GM	Range	MIC ₅₀	MIC ₉₀	GM				
<i>P. apiosperma</i> ^{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
<i>P. apiosperma</i> ^{em}	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
<i>P. boydii</i> ^{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
<i>P. boydii</i> ^{em}	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^{em}, number of strains from environmental samples; n /unknown, number of unknown origin/source; *MIC/MEC distribution statistically significant difference (p ≤ 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.

<i>P. apiosperma</i>								
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				

<i>P. boydii</i>								
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



