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Antifungal Susceptibilities of the Species of the *Pseudallescheria boydii* Complex▽†

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Eighty-four isolates belonging to eight species that constitute the *Pseudallescheria boydii* complex were tested against 11 antifungal agents by using the microdilution method. There were significant differences among the species, with *Scedosporium aurantiacum* being the most resistant. In general, voriconazole was the most active drug, followed by posaconazole.

In the last few decades, *Pseudallescheria boydii* sensu lato has been emerging as an important human pathogen, particularly in immunocompromised hosts (8). The optimal treatment for these infections is unknown, and the mortality rate is very high despite aggressive antifungal treatment (8). It has been repeatedly demonstrated that *P. boydii* sensu lato has low in vitro (6) and in vivo (3, 4, 9) susceptibilities to traditional antifungal drugs. However, the new triazoles, such as voriconazole (VRC), ravuconazole (RVC), and posaconazole (PSC), have shown some in vitro activities against this fungus (5). VRC has also shown efficacy both in animal models (3, 4) and in the clinical setting (1, 13). However, not all the strains of *P. boydii* tested responded equally to VRC. For instance, Capilla and Guarro (3) demonstrated that one strain that showed a VRC MIC of 0.5 to 1 μg/ml was susceptible to this drug in a guinea pig model, while another strain with a VRC MIC of 8 μg/ml was resistant. Similarly, some human infections have responded to treatment with this drug (1) and others have not (15). This could be explained by the fact that *P. boydii* does not represent a single species but instead is a complex comprising at least six known species (*P. boydii*, *Pseudallescheria angusta*, *Pseudallescheria ellipsoidea*, *Pseudallescheria fusoidea*, *Pseudallescheria minutispora*, and *Scedosporium aurantiacum*) and two cryptic species represented by clades 3 and 4 as described by Gilgado et al. (7). Since the antifungal susceptibilities of these species are unknown, we have evaluated the in vitro activities of 11 drugs against strains representing all of them.

Eighty-four isolates were tested (Table 1). The isolates were stored in slant cultures of potato dextrose agar (Difco Laboratories, Detroit, Mich.) covered with paraffin oil, subcultured on potato dextrose agar plates, and incubated at 30°C for 5 to 6 days. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were included as quality controls. Antifungal agents were obtained as pure powders. Amphotericin B (AMB) (USP, Rockville, MD), itraconazole (ITC) and ketoconazole (KTC) (Janssen Pharmaceutica, Beerse, Belgium), albaconazole (J. Uriach & Cía, Barcelona, Spain), VRC (Pfizer Inc., Madrid, Spain), PSC (Schering-Plough Ltd., Hertfordshire, United Kingdom), RVC (Bristol-Myers Squibb Company, New Brunswick, NJ), and terbinafine (Novartis, Basel, Switzerland) were diluted in dimethyl sulfoxide (Panreac Química S.A., Barcelona, Spain), and micafungin (MFG) (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), flucytosine (5FC) (Sigma-Aldrich Corp., St. Louis, MO), and fluconazole (FLC) (Pfizer Inc., Madrid, Spain) were diluted in sterile distilled water. Microplates were prepared as described in the NCCLS M38-A document (11). Final drug concentrations ranged from 32 to 0.06 μg/ml for MFG, from 64 to 0.12 μg/ml for FLC and 5FC, and from 16 to 0.03 μg/ml for the other drugs. The microplates were incubated at 35°C and read at 48 h. The MIC endpoints for the triazoles and AMB were defined as the lowest concentrations that produced complete inhibition of growth, and those for FLC, KTC, 5FC, and MFG were defined as the lowest concentrations that produced 50% growth inhibition. Approximately 80% of the tests were repeated, and the results showed the same tendencies (data not shown). However, when the results did not coincide, the test was repeated and the mode of the three MIC values was considered.

Results are shown in Table 1. VRC was the most active drug, showing a total geometric mean (GM) MIC of 0.61 μg/ml. *S. aurantiacum* was the species that was most resistant to this drug (GM MIC of 1.48 μg/ml). PSC was the second most active drug, with a total GM MIC of 0.89 μg/ml, although this drug was not active against species such as *S. aurantiacum* (GM MIC of 3.62 μg/ml) and *P. fusoidea* (GM MIC of 2 μg/ml). The activity of PSC was more variable than that of VRC and depended on the species tested.

AMB was not active against any of the isolates tested, but important differences were noticed between the MICs for the two species more commonly involved in human infections (7; F. Gilgado, J. Cano, J. Gené, and J. Guarro, Abstr. 16th Congr. Int. Soc. Hum. Anim. Mycol., abstr. P-0750, 2006). Thus, against the isolates of clade 4, this drug had a GM MIC of 4.33, whereas against *P. boydii*, it was 14.92 μg/ml. 5FC MICs were always >64 μg/ml.

In some works (5, 17), drugs like ITC and RVC showed good in vitro activities, but in our study, they showed poor activities against most of the strains tested. This could be explained by the fact that we have tested a greater number of isolates than

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TABLE 1. Activities of conventional and new antifungal drugs against 84 isolates belonging to species of the *P. boydii* complex

Drug against indicated fungus or clade (no. of isolates tested)[a]	MIC (μg/ml)[b]			
	Range	GM	50%	90%
S. aurantiacum (7)				
AMB	16–>16	28.98	>16	>16
FLC	16–64	32	32	64
ITC	2–>16	19.5	>16	>16
VRC	0.5–>16	1.48	1	>16
RVC	4–>16	9.75	8	>16
PSC	1–>16	3.62	2	>16
ABC	4–>16	5.38	4	>16
MFG	>32	>32	>32	>32
TBF	>16	>16	>16	>16
KTC	1–4	2.2	2	4
P. minutispora (4)				
AMB	2–>16	8	4	>16
FLC	4–64	20.15	32	64
ITC	1–8	2.37	2	8
VRC	0.12–1	0.41	0.5	1
RVC	0.5–4	2	2	4
PSC	0.12–1	0.49	1	1
ABC	0.5–4	1.68	2	4
MFG	>32	>32	>32	>32
TBF	>16	>16	>16	>16
KTC	0.12–1	0.39	0.5	1
Clade 3[c] (5)				
AMB	4–>16	18.37	>16	>16
FLC	4–64	16	32	32
ITC	1–>16	9.18	>16	>16
VRC	0.25–1	0.5	0.5	1
RVC	2–16	8	16	16
PSC	0.5–1	0.87	1	1
ABC	0.5–8	2.29	2	8
MFG	>32	>32	>32	>32
TBF	>16	>16	>16	>16
KTC	1	1	1	1
Clade 4[c] (26)				
AMB	1–>16	4.33	4	8
FLC	2–>64	19.80	16	64
ITC	0.5–>16	1.89	2	>16
VRC	0.12–2	0.48	0.5	1
RVC	0.25–8	2.54	4	8
PSC	0.5–2	0.87	1	1
ABC	0.5–8	2.34	2	8
MFG	32–64	60.67	>32	>32
TBF	>16	>16	>16	>16
KTC	0.25–2	0.56	0.5	1
P. boydii (30)				
AMB	2–>16	16.87	>16	>16
FLC	0.5–>64	16.46	16	64
ITC	0.5–>16	2.41	1	>16
VRC	0.5–2	0.68	0.5	1
RVC	0.5–16	3.11	2	4
PSC	0.12–1	0.66	0.5	1
ABC	0.25–8	2.28	2	4
MFG	1–64	29.34	>32	>32
TBF	>16	>16	>16	>16
KTC	0.12–2	0.53	0.5	1
P. angusta (4)				
AMB	16–>16	26.9	>16	>16
FLC	32–64	40.31	32	64
ITC	1–>16	6.72	8	>16
VRC	0.5–1	0.7	0.5	1
RVC	4–8	5.65	4	8
PSC	1–2	1.58	2	2
ABC	2–4	3.36	4	4
MFG	8–64	32	>32	>32
TBF	>16	>16	>16	>16
KTC	1–2	1.58	2	2
P. fusoidea (2)				
AMB	>16	>16	>16	>16
FLC	64	64	64	64
ITC	2–>16	8	2	>16
VRC	0.5	0.5	0.5	0.5
RVC	2–8	4	2	8
PSC	2	2	2	2
ABC	2–4	2.82	2	4
MFG	>32	>32	>32	>32
TBF	16–32	22.62	16	>16
KTC	1–2	1.41	2	2
P. ellipsoidea (6)				
AMB	2–>16	14.25	>16	>16
FLC	2–64	10.55	16	64
ITC	0.5–>16	1.74	1	>16
VRC	0.5–1	0.56	0.5	1
RVC	0.5–>16	2.82	0.5	>16
PSC	0.25–2	0.57	0.5	2
ABC	0.5–8	1.78	1	8
MFG	0.5–64	16	>32	>32
TBF	2–32	20.15	>16	>16
KTC	0.12–1	0.41	0.25	1
Total (84)				
AMB	2–>16	11.31	16	>16
FLC	0.5–>64	19.29	32	64
ITC	0.5–>16	3.1	2	>16
VRC	0.12–>16	0.61	0.5	1
RVC	0.25–>16	3.44	2	16
PSC	0.12–>16	0.89	1	2
ABC	0.25–>16	2.46	4	8
MFG	0.5–64	43.18	>32	>32
TBF	2–32	29.59	>16	>16
KTC	0.12–4	0.70	1	2

[a] ABC, albaconazole; TBF, terbinafine.
[b] 50%, MIC at which 50% of the isolates were inhibited; 90%, MIC at which 90% of the isolates were inhibited.
[c] Clades 3 and 4 were described by Gilgado et al. (7).

previous studies. The poor activity of ITC agrees with the failure of this drug to resolve some clinical cases (9, 13). The treatment of *Pseudallescheria* infections is often challenging and complex. This study confirms VRC as the recommended treatment for scedosporiosis (8, 12), since it is the drug that showed the lowest MIC against the eight species tested. PSC has also shown good activity, but there is little clinical experience with this drug. Therapy with this drug resolved completely a brain abscess in a leukemia patient (10). Since MFG showed poor activities against all the species tested in our study, candins, in general, would be expected to have low levels of activity against *Pseudallescheria* species. However, Yustes and

Guarro (18) demonstrated that the combination of MFG with AMB has potential for the treatment of scedosporiosis.

The application of genealogical concordance phylogenetic species recognition, which is an operational method based on the analysis of multigene sequences, on numerous pathogenic fungi (16) revealed the existence of numerous cryptic species. As with the *P. boydii* complex, among other important pathogenic fungi, such as *Candida albicans* or *Aspergillus fumigatus*, several cryptic species with different antifungal susceptibilities have been detected (2, 14).

In conclusion, this study demonstrates that the proper identification of the species of the *P. boydii* complex involved in a given infection could be important for appropriate treatment. For instance, if the species causing the infection is *S. aurantiacum*, it is likely that the response to the treatment with VRC would be poorer than if the species was *P. boydii*.

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