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Antifungal Susceptibility Patterns of Opportunistic Fungi in the Genera *Verruconis* and *Ochroconis*

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Species of *Verruconis* and species of *Ochroconis* are dematiaceous fungi generally found in the environment but having the ability to infect humans, dogs, cats, poultry, and fish. This study presents the antifungal susceptibility patterns of these fungi at the species level. Forty strains originating from clinical and environmental sources were phylogenetically identified at the species level by using sequences of the ribosomal DNA internal transcribed spacer (rDNA ITS). *In vitro* antifungal susceptibility testing was performed against eight antifungals, using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. The geometric mean MICs for amphotericin B (AMB), flucytosine (5FC), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), and posaconazole (POS) and minimum effective concentrations (MECs) for caspofungin (CAS) and anidulafungin (AFG) across the *Ochroconis* and *Verruconis* species were as follows, in increasing order. For *Verruconis* species, the values (μg/ml) were as follows: AFG, 0.04; POS, 0.25; ITC, 0.37; AMB, 0.50; CAS, 0.65; VRC, 0.96; 5FC, 10.45; and FLC, 47.25. For *Ochroconis* species, the values (μg/ml) were as follows: AFG, 0.06; POS, 0.11; CAS, 0.67; VRC, 2.76; ITC, 3.94; AMB, 5.68; 5FC, 34.48; and FLC, 61.33. Antifungal susceptibility of *Ochroconis* and *Verruconis* was linked with phylogenetic distance and thermotolerance. Echinocandins and POS showed the greatest *in vitro* activity, providing possible treatment options for *Ochroconis* and *Verruconis* infections.

Recently, by combined molecular phylogeny, morphology, and ecology, the taxonomy of the *Ochroconis* lineage was revised (1). Two genera were recognized: *Ochroconis* and *Verruconis*. Within melanized filamentous fungi, members of *Ochroconis* and *Verruconis* are morphologically exceptional by having sympodial conidiogenesis with rhexolytic conidial dehiscence (2). However, both genera are melanized, oligotrophic, and regularly encountered in indoor environments, in soil, or in heated habitats, and some species have the ability to cause superficial, cutaneous, and systemic infections in immunocompromised patients (3–6).

Verruconis species are thermophilic, with *Verruconis gallopava* occurring in hot environments, such as thermal soils, broiler house litter, hot springs, and self-heated waste (1). Pathology in *Verruconis* is restricted to *V. gallopava*, which is the main agent of human brain infections and is responsible for encephalitis in poultry and wild birds (7–15), dogs (16), and cats (17). In contrast, *Ochroconis* species are mesophilic saprobes, with an optimum growth temperature between 15 and 30°C and an inability to grow at 37°C, which occasionally infect cold-blooded vertebrates (1, 18). Only a single infection was noted in a warm-blooded animal, i.e., a subcutaneous lesion in a cat (19), while the first subcutaneous human infection due to *Ochroconis tshawytschae* was recently reported (20).

Despite significant medical and veterinary importance, little is known regarding the species-specific antifungal susceptibility profiles of *Verruconis* and *Ochroconis* species. The polyene agents exert their antifungal activity via binding to ergosterol in the fungal cell membrane. This disrupts cell permeability and results in rapid cell death. Flucytosine exerts antifungal activity via inhibition of both DNA synthesis and protein synthesis in the fungal cell. Azole agents exert their antifungal activity by blocking the demethylation of lanosterol, thereby inhibiting ergosterol synthesis. The mechanism of activity of the echinocandins is inhibition of the production of (1,3)-β-D-glucan, an essential component in the fungal cell wall (21). We therefore investigated the *in vitro* susceptibilities of a large collection of clinical and environmental isolates of thermophilic and mesophilic species to eight antifungal drugs.

(Some of these results were presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 10 to 13 September 2013.)

MATERIALS AND METHODS

Fungal strains. Strains used in this study are listed in Table 1, with origin, identification number, and clinical data for each isolate. In total, 40 strains from clinical and environmental sources were used. Lyophilized fungal strains were obtained from the reference collection of the CBS-KNAW Fungal Biodiversity Centre (CBS, Utrecht, The Netherlands) and selected according to their historical pathogenicity. In addition, the representative type species of saprophytic strains were used for environmental isolates of both genera (Table 1). All isolates were cultured on malt extract agar (MEA) at 24°C for 14 days. Morphological identifications were confirmed

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TABLE 1 Isolation data for examined strains of *Ochroconis* and *Verruconis* spp.

CBS ID	Species	Other collection no.	GenBank accession no.	Source or origin	Geography (city, state, country)	Yr of isolation[a]	Reference(s)
CBS 125817	*V. calidifluminalis*	IFM 54739	AB385699	Hot spring effluent	Kanakawa, Hakone, Japan	2004	47
CBS 125818 (type strain)	*V. calidifluminalis*	IFM 54738	AB385698	Hot spring effluent	Kanakawa, Hakone, Japan	2004	47
CBS 118.91	*V. gallopava*	CDC B-4954	HQ667551	Human	Atlanta, GA, USA	1991	48
CBS 166.85	*V. gallopava*	dH 14821	HQ667554	Environment	France	1985	27
CBS 265.97	*V. gallopava*	dH 14836	HQ667555	Australorp chick	Brisbane, Queensland, Australia	1990	14
CBS 437.64 (type strain)	*V. gallopava*	ATCC 16027, CDC 45-492-62, MUCL 6683	HQ667553	Turkey (*Meleagris gallopavo*)	Bishopville, SC, USA	1964	7
CBS 547.81	*V. gallopava*		HQ667560	Environment	Christchurch, New Zealand	1981	27
CBS 862.95	*V. gallopava*	ATCC 60633, CDC B-4224		Human	South Carolina	1990	49
CBS 863.95	*V. gallopava*	CDC B-5637	HQ667548	Human, bronchial aspirate	USA	1996	27
CBS 865.95	*V. gallopava*	CDC B-4872	HQ667549	Human, mine worker, sputum	Johannesburg, South Africa	1989	27
CBS 866.95	*V. gallopava*	CDC B-4767		Human	Mobile, AL, USA	1996	27
CBS 867.95	*V. gallopava*	CDC B-4682	HQ667561	Human, sputum	Salisbury, MD, USA	1996	27
CBS 100437	*V. gallopava*	ATCC 48169, IMI 241149	HQ667556	Broiler chicken	Scotland, UK	1998	27
CBS 116660	*V. gallopava*	CDC B-5813	HQ667557	Human, bronchoalveolar lavage fluid	USA	ND	27
CBS 116646	*V. gallopava*	IMI 308437	HQ667559	Human, sputum	Western Australia		27
CBS 119640	*V. gallopava*	dH 14079, NCPF 7122		Human	Australia	ND	27
CBS 119641	*V. gallopava*	NCPF 2923, dH 4078	HQ667547	Human, sputum	UK	ND	27
CBS 119642	*V. gallopava*	dH 14077, NCPF 2221	HQ667550	Chick	ND	ND	27
CBS 119922	*V. gallopava*	dH 14073		Human, L3 puncture	The Netherlands		
CBS 120153	*V. gallopava*	dH13131, RKI 579/00		Human	Germany		
CBS 729.95 (type strain)	*O. mirabilis*	dH 14850	KF156029	Regulator of diver	Haarlem, The Netherlands	1995	50
CBS 124.65	*O. mirabilis*	MUCL 6479	HQ667532	Human	India	1965	
CBS 102468	*O. mirabilis*		HQ667533	Human	Nijmegen, The Netherlands	2000	
CBS 113948	*O. mirabilis*	dH 13215	HQ667530	Human	Haarlem, The Netherlands		
CBS 118685	*O. mirabilis*		HQ667529	Human, 8-year-old girl	Sweden		
CBS 123268	*O. mirabilis*	dH 17473	HQ667526	Human	Denmark		
CBS 124210	*O. mirabilis*	dH 17059	KF156028	Human	Denmark		
CBS 135920	*O. mirabilis*	dH 22275	KF156033	Human	Thailand	2011	
CBS 100438 (type strain)	*O. tshawytschae*	dH 10758, dH 14814, ATCC 9915	HQ667562	Fish (Chinook salmon)	USA	1946	51
CBS 129970	*O. tshawytschae*	CMCC(f)D.31a	JN974456	Human	Nanjing, China	2011	52
CBS 100486	*O. constricta*	NJM 9471	KF156026	Fish (devil stinger)	Kagoshima, Japan	1995	53
CBS 131913	*O. constricta*	dH22432	KF156025	Human	Thailand	2011	
CBS 211.53 (type strain)	*O. constricta*	ATCC 11419, DAOM 28282, IMI 051380, MUCL 9896	HQ667519	Soil	Ancaster, Ontario, Canada	1952	
CBS 135766	*Ochroconis* sp.	UIIII09		Fish	Sweden	2012	
CBS 475.80 (type strain)	*O. cordanae*	dH 14825	KF156022	Dead leaf (Palmae)	Colombia	1979	
CBS 116655 (type strain)	*O. humicola*	dH 13739, IMI 110131, UAMH 10241	HQ667521	Peat soil	Ontario, Canada	1962	54
CBS 510.71 (type strain)	*O. minima*	dH 14792, ATCC 22631, IMI 082933	HQ667522	Rhizosphere	Samaru, Zaria, Nigeria	1967	55
CBS 239.78 (type strain)	*O. gamsii*	dH 14835, CBS H-7440	KF156019	Plant leaf	Sri Lanka	1973	56
CBS 383.81 (type strain)	*O. verrucosa*	IMI 211655	KF156015	Soil	Kerala, Kolkata, India	1981	57
CBS 284.64 (type strain)	*O. anellii*	IHEM 4516, IMI 089069, MUCL 9473	FR832477	Stalactites	Bari, Italy	1962	58
CBS 131815 (type strain)	*O. lascauxensis*	CMFISB 1862, LX A1	FR832474	Black stains	Montignac, Lascaux Cave, France	2008	59

[a] ND, not determined.

by sequence-based analysis of the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) region, as described previously (1). Briefly, sequences were edited using the SeqMan tool of Lasergene software (DNASTar Inc., Madison, WI) and then aligned interactively using Ward's averaging in the BioNumerics package v. 4.61 (Applied Maths, Kortrijk, Belgium). The ITS sequences were finally aligned with the program MUSCLE (www.ebi.ac.uk/Tools/msa/muscle), and the aligned sequences were adjusted using BioEdit v. 7.0.5.2. The ITS data set was then analyzed by use of MEGA5 software (22), in which the Tamura three-parameter model with gamma distribution (T92+G) was searched as the best model. The maximum likelihood (ML) heuristic method with 1,000-replicate bootstrapping and the maximum parsimony (MP) method with 1,000-replicate bootstrapping were performed for tree reconstructions and phylogeny tests. To strongly confirm the analyses, the ML method with the approximate likelihood ratio test (aLRT) was also performed with PhyML (23). Trees were viewed and edited with TreeView v. 1.6.6, FigTree v. 1.1.2, and MEGA5.

***In vitro* antifungal susceptibility testing.** *In vitro* antifungal susceptibility testing was performed using a broth microdilution format against eight antifungal compounds according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (24). The final concentrations of the antifungal agents ranged from 0.016 to 16 μg/ml for amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), posaconazole (POS), caspofungin (CAS), and anidulafungin (AFG). Flucytosine (5FC) was assayed over a 2-fold concentration range from 0.064 to 64 μg/ml. Reading of results was performed using a reading mirror and a microtitration plate spectrophotometric reader (Anthos htIII; Anthos Labtec Instruments, Salzburg, Austria).

Ochroconis isolates were incubated at 25°C, and *Verruconis* isolates were incubated at 37°C. Agitation of plates was not used. The MICs of AMB, FLC, 5FC, ITC, VRC, and POS were determined visually with an inverted mirror by comparison of growth in the wells containing the drug and that of the drug-free control. The minimum effective concentrations (MECs) of CAS and AFG were read with a plate microscope (Olympus SZX9; Olympus Nederland, Zoeterwoude, The Netherlands) at a magnification of ×25 to ×50. The MEC was defined as the lowest concentration at which abnormal, short, and branched hyphal clusters were observed, in contrast to the long, unbranched hyphal elements that were seen in the growth control well.

Paecilomyces variotii (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality controls in all experiments. The ranges and geometric means (GM) of the MICs and MECs were determined for each species and drug after 48 to 168 h of incubation. Furthermore, the MIC_{50}s and MIC_{90}s for the isolates were calculated by use of the criteria for MIC determinations described above. The MIC_{50} and MIC_{90} values were calculated for those species with 10 or more isolates. If the MIC values of the replicates were different, the GM values of the replicates were used for comparison with other isolates. All experiments were performed in three independent replicates with each strain on different days.

Statistical analysis. Data analyses were performed by using GraphPad Prism, version 5.0, for Windows (GraphPad Software, San Diego, CA). MIC/MEC distributions between isolates were compared by using the Mann-Whitney-Wilcoxon test. Statistical significance was defined as having a P value of ≤ 0.05 (two-tailed).

RESULTS

All strains were identified to the species level by sequence-based analysis and tested against eight antifungal compounds. Two genera have been recognized based on molecular phylogeny, *viz.*, *Verruconis* and *Ochroconis*. In Fig. 1, data are summarized for relevant species, displaying their mutual phylogenetic distances, interspecies variability according to temperature tolerance, and antifungal susceptibility profiles per species. GM MICs, MIC ranges, and MIC_{50} and MIC_{90} distributions for eight antifungal agents are summarized in Table 2.

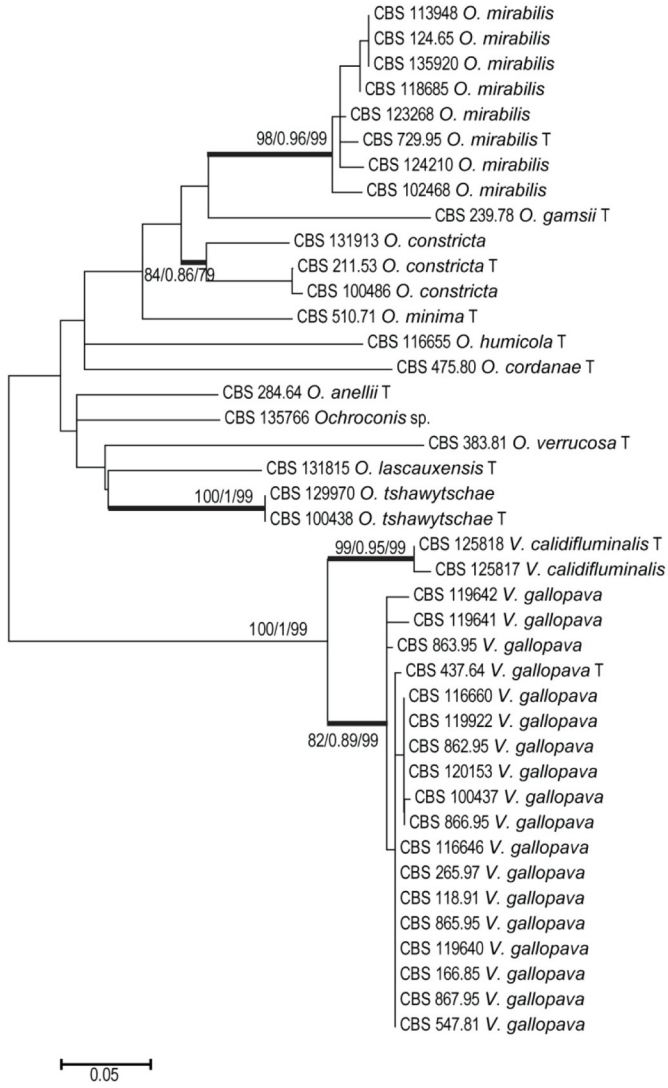
Overall, visual and spectrophotometric readings gave similar results for the MIC and MEC endpoints. The GM MICs for AMB, 5FC, FLC, ITC, VRC, and POS and the MEC values for CAS and AFG across the genera in this study are shown below, in increasing order. For *Verruconis*, the values (μg/ml) were as follows: AFG, 0.04; POS, 0.25; ITC, 0.37; AMB, 0.50; CAS, 0.65; VRC, 0.96; 5FC, 10.45; and FLC, 47.25. For *Ochroconis*, the values (μg/ml) were as follows: AFG, 0.06; POS, 0.11; CAS, 0.67; VRC, 2.76; ITC, 3.94; AMB, 5.68; 5FC, 34.48; and FLC, 61.33. The widest ranges were seen for FLC (range, 1 to ≥64 μg/ml) and 5FC (range, 0.5 to 64 μg/ml). The highest GM MICs were 47.25 μg/ml, for FLC, followed by 10.45 μg/ml, for 5FC. AMB MICs ranged from 0.125 to >16 μg/ml, and ITC had a MIC range of <0.016 to >16 μg/ml. POS exhibited potent activity against all strains, with MICs ranging from <0.0016 to 4 μg/ml, while the GM MIC of VRC (0.96 μg/ml) was 2 $\log_2$ dilution steps less potent than that of POS (0.25 μg/ml) against thermotolerant strains and 6 $\log_2$ dilution steps less active than in the *Ochroconis* species (2.76 μg/ml VRC versus 0.11 μg/ml POS). Notably, *Ochroconis* isolates had higher MICs of AMB, 5FC, FLC, ITC, and VRC than those for *Verruconis* strains. The two echinocandins showed susceptible profiles in their MECs. In most cases, AFG had a higher activity than that of CAS (AFG MEC of 0.04 μg/ml versus 0.65 μg/ml CAS against *Verruconis* strains, and AFG MEC of 0.06 μg/ml versus 0.67 μg/ml CAS against *Ochroconis* species). In addition, various susceptibility profiles were demonstrated within the genera. For *Ochroconis*, the triazole derivatives ITC and VRC and AMB offered significantly ($P \leq 0.05$) higher susceptible profiles for *O. mirabilis* than for the other species. However, 5FC and FLC were found to be less active against *V. gallopava* than against *V. calidifluminalis* ($P \leq 0.05$).

DISCUSSION

The genus *Ochroconis* was recently revised and currently contains 13 species (1). Species accepted within the lineage, within the order Venturiales, were keyed out on the basis of molecular phylogeny and phenotypic and physiologic characteristics. A new genus, *Verruconis*, was proposed for the neurotropic opportunist *Ochroconis gallopava* and its close relatives.

Notably, thermotolerance has a significant impact on the virulence potential of *Ochroconis* and *Verruconis* species, as shown previously in other melanized fungi. Species able to grow at temperatures of 37°C or above (e.g., *Cladophialophora bantiana*, *Exophiala dermatitidis*, and *Exophiala jeanselmei*) (25) may cause systemic or disseminated infections in mammals. The black yeast *Exophiala dermatitidis* has a maximum growth temperature of 42 to 45°C and has a natural habitat in association with birds and bats, which have a body temperature well above that of humans (26, 27). Mesophilic species with maximum growth temperatures of 27 to 33°C are restricted to cold-blooded vertebrates (25) or, occasionally, invertebrates (28, 29).

The availability of *in vitro* susceptibility profiles according to the latest taxonomic studies of *Ochroconis* and *Verruconis* species (1) is scant. Our study provides the first antifungal susceptibility data on a large set of clinical and environmental strains from a wide range of sources and origins. Our results indicate that thermotolerance has a significant impact on the antifungal susceptibility of *Ochroconis* and *Verruconis* species. Both thermotolerant and mesophilic species had susceptibility profiles with a uniform



Source	Geometric Mean MIC/MEC (mg/L)								Opt temp (°C)
	AMB	5FC	FLC	ITC	VRC	POS	CAS	AFG	
Human	12.62	21	64	10.09	5.75	0.21	0.75	0.054	25
Human									
Human									
Human									
Human									
Reg. diver									
Human									
Human									
Plant	1	64	64	0.25	2	0.031	0.5	0.031	
Human	1.33	64	64	0.06	0.67	0.02	0.35	0.031	
Soil									
Fish									
Rhizosphere	1	8	64	0.063	0.5	0.016	0.5	0.031	
Peat soil	1	64	64	0.125	1	0.063	1	0.125	
Dead leaf	1	16	64	0.25	2	0.016	0.5	0.031	
Stalactites	1	4	64	0.031	0.5	0.031	0.5	0.031	
Fish	4	64	64	0.25	0.25	0.031	0.5	0.031	
Soil	0.25	16	64	0.25	2	0.031	0.25	0.016	
Black stains	1	64	64	0.25	1	0.063	0.25	0.031	
Human	2	32.06	36	0.13	0.31	0.125	1.5	0.187	
Fish									
Hot spring	0.094	0.75	2.5	0.016	0.094	0.016	0.75	0.047	37
Hot spring									
Human	0.54	11.53	52.22	0.40	1.06	0.28	0.64	0.04	
Human									
Human									
Turkey									
Human									
Human									
Human									
Human									
Chicken									
Human									
Human									
Chicken									
Human									
Human									
Human									
ND									
Human									
ND									

FIG 1 MEGA5 maximum likelihood tree created from ITS sequences of *Ochroconis* and *Verruconis* isolates. The geometric mean susceptibility profiles of eight antifungals against each species have been incorporated into the figure. Numbers on branches are percent bootstrap values obtained from ML, aLRT, and MP analyses. Type strains are highlighted by a "T." ND, not determined.

pattern of low MICs for POS, AFG, and CAS. VRC, AMB, and ITC showed efficacy against *Verruconis* species, with 1-, 3-, and 4-$\log_2$ less susceptibility, respectively, than *Ochroconis* species. The majority of strains demonstrated high MICs for 5FC and FLC, indicating poor activity of these drugs against the pathogens. Both echinocandins were found to have potent *in vitro* activity against *Ochroconis* species. This matches previously reported data for CAS, with a MEC of 0.25 μg/ml against *Ochroconis tshawytschae* (20) and 0.03 to 1 μg/ml against *Verruconis gallopava* (30, 31). This is in contrast with previously published data on most melanized fungi, which appear to be tolerant to echinocandins, probably due to the presence of melanin, which prevents penetration of antifungals into fungal cells (32). Nevertheless, *O. mirabilis* demonstrated less susceptibility to ITC, VRC, and AMB ($P \leq 0.05$) than the other *Ochroconis* species, and *V. calidifluminalis* was more susceptible to 5FC and FLC than *V. gallopava* ($P \leq 0.05$).

Given that the echinocandins and the triazole POS showed the highest *in vitro* activity against thermotolerant and mesophilic species, a possible treatment option for *Ochroconis* and *Verruconis* infections in both warm-blooded (human) and cold-blooded animals may be provided. The triazole POS is an expanded-spectrum triazole with fungicidal activity against a wide spectrum of molds, including *Aspergillus* species and members of the Mucorales, as well as enhanced activity against *Candida* and other yeasts (33). The echinocandins represent the newest class of antifungals that exhibit fungicidal activity against many *Candida* species, making this drug class a desirable alternative to the azole agents, which exhibit only static activity against yeasts. Because mammalian cells have no cell wall, the echinocandins have very few adverse effects in humans (33).

In general, the divergent antifungal profiles of the *Verruconis* and *Ochroconis* genera and the interspecies variability observed

TABLE 2 Geometric mean MICs, MIC ranges, MIC_{50}s, and MIC_{90}s obtained by susceptibility testing of antimycotic agents against *Ochroconis* and *Verruconis* spp.

Organism (*n*) and drug	MIC or MEC (mg/liter)[a] Range	50%	90%	Geometric mean
All thermotolerant strains (*Verruconis* spp.) (*n* = 20)				
Amphotericin B	0.125–4	0.25	0.5	0.50
Flucytosine	0.5–64	4	32	10.45
Fluconazole	1–>64	64	>64	47.25
Itraconazole	<0.016–4	0.125	0.5	0.37
Voriconazole	0.063–2	1	2	0.96
Posaconazole	<0.016–4	0.031	0.125	0.25
Caspofungin	0.25–1	0.5	1	0.65
Anidulafungin	0.016–0.125	0.031	0.063	0.04
All mesophilic strains (*Ochroconis* spp.) (*n* = 20)				
Amphotericin B	0.25->16	2	>16	5.68
Flucytosine	0.125->64	16	>64	34.48
Fluconazole	8->64	64	>64	61.33
Itraconazole	<0.016–>16	0.25	>16	3.94
Voriconazole	0.125–8	2	8	2.76
Posaconazole	<0.016–0.25	0.063	0.25	0.11
Caspofungin	0.063–2	0.5	1	0.67
Anidulafungin	0.016–0.25	0.031	0.125	0.06
V. gallopava (*n* = 18)				
Amphotericin B	0.125–4	0.25	0.5	0.54
Flucytosine	0.5–64	4	32	11.53
Fluconazole	4->64	64	>64	52.22
Itraconazole	0.016–4	0.125	0.5	0.40
Voriconazole	0.5–2	1	2	1.06
Posaconazole	<0.016–4	0.031	0.125	0.28
Caspofungin	0.25–1	0.5	1	0.64
Anidulafungin	0.016–0.125	0.031	0.063	0.04
V. calidifluminalis (*n* = 2)				
Amphotericin B	0.063–0.125	NC	NC	0.094
Flucytosine	0.5–1	NC	NC	0.75
Fluconazole	1–4	NC	NC	2.5
Itraconazole	≤0.16	NC	NC	0.016
Voriconazole	0.063–0.125	NC	NC	0,.094
Posaconazole	≤0.16	NC	NC	0.016
Caspofungin	0.5–1	NC	NC	0.75
Anidulafungin	0.031–0.063	NC	NC	0.047
O. mirabilis (*n* = 8)				
Amphotericin B	1->16	NC	NC	12.625
Flucytosine	8–64	NC	NC	21
Fluconazole	≥64	NC	NC	64
Itraconazole	0.25–>64	NC	NC	10.09375
Voriconazole	2–8	NC	NC	5.75
Posaconazole	0.063–0.25	NC	NC	0.211
Caspofungin	0.5–1	NC	NC	0.75
Anidulafungin	0.031–0.125	NC	NC	0.05475
O. tshawytschae (*n* = 2)				
Amphotericin B	0.125–>64	NC	NC	2
Flucytosine	8–>64	NC	NC	32.0625
Fluconazole	0.016–0.25	NC	NC	36
Itraconazole	0.125–0.5	NC	NC	0.133
Voriconazole	<0.016–0.125	NC	NC	0.3125
Posaconazole	1–2	NC	NC	0.125
Caspofungin	0.125–0.25	NC	NC	1.5
Anidulafungin	0.031–0.125	NC	NC	0.1875
O. constricta (*n* = 3)				
Amphotericin B	1–2	NC	NC	1.33
Flucytosine	>64	NC	NC	64.00
Fluconazole	64–> 64	NC	NC	64.00
Itraconazole	0.063	NC	NC	0.06
Voriconazole	0.5–1	NC	NC	0.67
Posaconazole	0.016	NC	NC	0.02
Caspofungin	0.063–0.5	NC	NC	0.35
Anidulafungin	0.031	NC	NC	0.03
Ochroconis sp. (*n* = 1)				
Amphotericin B	4	NC	NC	NC
Flucytosine	64	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	0.25	NC	NC	NC
Posaconazole	0.031	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Anidulafungin	0.016	NC	NC	NC
O. cordanae (*n* = 1)				
Amphotericin B	1	NC	NC	NC
Flucytosine	16	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	2	NC	NC	NC
Posaconazole	0.016	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Anidulafungin	0.031	NC	NC	NC
O. humicola (*n* = 1)				
Amphotericin B	1	NC	NC	NC
Flucytosine	64	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.125	NC	NC	NC
Voriconazole	1	NC	NC	NC
Posaconazole	0.063	NC	NC	NC
Caspofungin	1	NC	NC	NC
Anidulafungin	0.125	NC	NC	NC
O. minima (*n* = 1)				
Amphotericin B	1	NC	NC	NC
Flucytosine	8	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.063	NC	NC	NC
Voriconazole	0.5	NC	NC	NC
Posaconazole	0.016	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Anidulafungin	0.031	NC	NC	NC
O. gamsii (*n* = 1)				
Amphotericin B	1	NC	NC	NC

(Continued on following page)

TABLE 2 (Continued)

Organism (*n*) and drug	MIC or MEC (mg/liter)[a]			
	Range	50%	90%	Geometric mean
Flucytosine	64	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	2	NC	NC	NC
Posaconazole	0.031	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Anidulafungin	0.031	NC	NC	NC
O. verrucosa ($n = 1$)				
Amphotericin B	0.25	NC	NC	NC
Flucytosine	16	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	2	NC	NC	NC
Posaconazole	0.031	NC	NC	NC
Caspofungin	0.25	NC	NC	NC
Anidulafungin	0.016	NC	NC	NC
O. anellii ($n = 1$)				
Amphotericin B	1	NC	NC	NC
Flucytosine	4	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.031	NC	NC	NC
Voriconazole	0.5	NC	NC	NC
Posaconazole	0.031	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Anidulafungin	0.031	NC	NC	NC
O. lascauxensis ($n = 1$)				
Amphotericin B	1	NC	NC	NC
Flucytosine	64	NC	NC	NC
Fluconazole	64	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	1	NC	NC	NC
Posaconazole	0.063	NC	NC	NC
Caspofungin	0.25	NC	NC	NC
Anidulafungin	0.031	NC	NC	NC

[a] The MIC_{50} and MIC_{90} values were calculated for those species with 10 or more isolates. NC, not calculated, because <10 strains per species were available for testing.

for *O. mirabilis* and *V. calidifluminalis* clearly suggest that routine *in vitro* susceptibility testing can be useful for obtaining reliable information on treatment options. Until now, there have been no guidelines for optimal antifungal regimens for *Ochroconis* and *Verruconis* species. Although various efficacies have been documented (34), several studies suggest that POS and ITC may provide optimal therapies for *Ochroconis* infection, followed by AMB and VRC, and that 5FC and FLC are the least effective drugs (6, 30, 31, 35, 36), which is in agreement with the *in vitro* results of the present study.

Treatment of *Verruconis* infections with VRC is supported by *in vitro* results, and it proved to be active in a chronic granulomatous disease (CGD) patient (34). VRC has an optimal oral bioavailability and penetration to the blood-brain barrier, indicating its use for cerebral infections. In some cases of *V. gallopava* infections, AMB was also used successfully in empirical antifungal therapy (37). However, further studies are required to establish the optimal treatment. In addition, as recommended for other fungal infections, supportive management strategies, such as surgical excision of lesions, are recommended whenever feasible (34). Early diagnosis and treatment are also mandatory in order to avoid dissemination to the brain, which carries a very poor prognosis (20).

In conclusion, although there are no clinically defined breakpoints for *Verruconis* and *Ochroconis* species and the lack of interpretative breakpoints makes MICs difficult to interpret, antifungal susceptibility testing can be helpful in guiding clinical management of patients with these infections. Based on the data presented in the current study, POS and echinocandins were the antimycotics with the best overall activity, having broad-spectrum activity against both thermotolerant and mesophilic species. The apparently good penetration of POS into the central nervous system (CNS), with the MIC falling well below the serum levels achievable with standard dosing regimens (38), combined with excellent *in vitro* data (39) and activity in animal models (40–43), supports the use of POS for difficult-to-treat disseminated brain infections. In the clinical setting, POS has been used successfully in cases of cerebral and disseminated phaeohyphomycosis (44, 45). In addition, POS and VRC are routinely recommended for treatment, prophylaxis, and salvage therapy of life-threatening fungal infections, such as *Aspergillus* diseases. POS also has a label indication for the treatment of less common infections, including chromoblastomycosis, mycetoma, and coccidioidomycosis. Therefore, standard dosing regimens and provisional target concentrations used for the prevention or treatment of invasive fungal infections (46) might be optimal tentative suggestions for *Verruconis* and *Ochroconis* infections.

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