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In Vitro Activities of Eight Antifungal Drugs against a Global Collection of Genotyped *Exserohilum* Isolates

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The *in vitro* susceptibilities of 24 worldwide *Exserohilum* isolates belonging to 10 species from human and environmental sources were determined for eight antifungal drugs. The strains were characterized by internal transcribed spacer (ITS) sequencing and amplified fragment length polymorphism fingerprinting. Posaconazole had the lowest geometric mean MIC (0.16 μg/ml), followed by micafungin (0.21 μg/ml), amphotericin B (0.24 μg/ml), itraconazole (0.33 μg/ml), voriconazole (0.8 μg/ml), caspofungin (1.05 μg/ml), isavuconazole (1.38 μg/ml), and fluconazole (15.6 μg/ml).

The anamorphic genus *Exserohilum* (teleomorph *Setosphaeria*, family *Pleosporaceae*, order *Pleosporales*) comprises approximately 35 species, which are common saprobic fungi on plant debris worldwide (1). Until recently, *Exserohilum* species were considered to be rare opportunistic pathogens reported sporadically from cases of keratitis, cutaneous and subcutaneous phaeohyphomycosis, and invasive infections, especially in immunosuppressed patients (2, 3). However, at the end of 2012, the meningitis outbreak in the United States due to *Exserohilum* demonstrated this fungus to be an agent of severe life-threatening infections (4, 5). Although several fungi were implicated in the outbreak, the vast majority of infections were caused by *Exserohilum rostratum*, which was traced to contaminated steroid injections as the source (4–8). Previously, *Exserohilum rostratum*, *Exserohilum longirostratum*, and *Exserohilum mcginnisii* were reported as opportunistic human pathogens. However, molecular studies have demonstrated that these species are conspecific, with *E. rostratum* being the accepted species (1).

The pathobiology of this melanized mold, including infections in the immunocompromised host, is not clearly understood. A recent report (9) suggested that methylprednisolone-induced suppression of phagocytosis by polymorphonuclear leukocytes was responsible for the rapid development of an *E. rostratum* outbreak in the United States. The therapeutic approach to combat this pathogen has not yet been established; therefore, knowledge regarding its pathogenic potential and antifungal susceptibility is of paramount importance (8, 10, 11). The large majority of patients in the recent outbreak were administered voriconazole with or without amphotericin B; this was based on a small case series, personal experience, and the favorable pharmacokinetic profile of voriconazole with regard to cerebral infections (10). Clinical outcomes with voriconazole seemed to be satisfactory, although a recent case showed failure after 4.5 months of voriconazole therapy, with excellent trough levels (12); therefore, its upfront use has been challenged (13, 14). *In vitro* antifungal susceptibility (AFS) data for *Exserohilum* species are scarce. Three studies reported AFS data on *E. rostratum* (1, 4, 15). Given the fact that *E. rostratum* is a coincidental opportunist, there is no *a priori* reason to believe that this will be the only *Exserohilum* species with this ability. Therefore, we aimed to study the potential activity of isavuconazole, a new triazole recently approved by the FDA for the treatment of invasive aspergillosis and mucormycosis, against *E. rostratum* ($n = 10$) and 9 other species of *Exserohilum* ($n = 14$) made available by the Centraalbureau Schimmelcultures (CBS)-KNAW Fungal Biodiversity Centre (Utrecht, The Netherlands) and the Vallabhbhai Patel Chest Institute (Delhi, India) (Table 1).

(Portions of this work were presented previously at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO [16].)

The *Exserohilum* isolates were analyzed by amplified fragment length polymorphism (AFLP) genotyping (see Fig. S1 in the supplemental material), as described previously (17, 18), and subjected to sequencing of the internal transcribed spacer (ITS) region (see Fig. S2 in the supplemental material). The MICs of amphotericin B (Bristol Myers Squibb, Woerden, The Netherlands), fluconazole, voriconazole (Pfizer Central Research, Sandwich, Kent, United Kingdom), itraconazole (Janssen Cilag, Tilburg, The Netherlands), posaconazole (Merck, Whitehouse Station, NJ), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), caspofungin (Merck), and micafungin (Astellas, Toyama, Japan) were determined using the microdilution method, in accordance with the guidelines of the CLSI document M38-A2 (19).

E. rostratum isolates ($n = 8$) clustered together in AFLP, along with 2 isolates that were previously identified by conventional methods as *E. mcginnisii* (CBS 325.87^T and CBS 120308), confirming their synonymy (see Fig. S1 in the supplemental material) (1). Also, two isolates each of *Exserohilum prolatum* (CBS 128058

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TABLE 1 Origin, source, and MIC/MEC data of all *Exserohilum* isolates tested

Species	Country of origin	CBS no./source	GenBank accession no.	MIC/MEC (μg/ml) of[a]:							
				AMB	ISA	POS	ITC	VRC	FLU	CAS[b]	MFG[b]
E. rostratum (*E. mcginnisii*)	USA	CBS 120308/clinical	KT265236	0.125	2	0.063	0.25	0.5	32	1	0.125
		CBS 325.87^T/clinical	KT265237	0.25	4	0.125	0.25	1	16	2	0.5
E. rostratum	Canada	CBS 112815/clinical	KT265238	1	1	0.125	0.5	1	>64	4	8
		CBS 128063/clinical	KT265239	0.25	8	0.25	0.5	2	32	1	<0.008
		CBS 128061/clinical	KT265240	0.063	1	0.125	0.125	0.5	32	0.5	<0.008
		CBS 128060/clinical	KT265245	0.125	2	0.125	0.25	1	8	0.5	0.25
		CBS 131565/clinical	KT265242	0.25	4	0.125	0.25	1	16	1	0.125
		CBS 128062/clinical	KT265247	0.5	4	0.25	0.5	2	32	2	0.25
	India	CBS 134641/clinical	KT265248	0.5	8	0.25	0.5	2	32	0.5	0.25
		CBS 134640/clinical	KT265241	0.125	4	0.5	0.25	2	32	1	0.25
E. gedarefense	Sudan	CBS 504.90/environment	KT265243	0.25	1	0.063	0.063	1	8	1	0.5
		CBS 297.80^T/environment	KT265244	0.25	1	0.125	0.5	2	16	1	0.063
E. neoregeliae IM201-D	Japan	CBS 132832^T/environment	KT265254	0.25	2	0.125	0.25	1	16	1	0.125
E. neoregeliae IM201-E		CBS 132833/environment	KT265255	0.125	2	0.125	0.125	0.5	32	1	0.063
E. pedicellatum	USA	CBS 322.64/environment	KT265258	0.25	0.5	0.125	0.063	0.25	1	1	0.25
	Turkey	CBS 375.76/environment	KT265259	0.5	0.25	0.125	0.25	0.5	2	0.5	0.125
E. protrudens	Australia	CBS 132710^T/environment	KT265256	0.125	2	0.5	0.25	2	8	2	0.5
E. fusiforme		CBS 132709^T/environment	KT265257	0.031	0.125	0.031	0.031	0.063	1	1	0.25
E. antillanum	Cuba	CBS 412.93^T/environment	KT265246	1	4	0.25	0.25	2	>64	4	8
E. curvatum	Venezuela	CBS 505.90^T/environment	KT265252	0.25	2	1	>16	2	32	1	2
E. prolatum	Unknown	CBS 128058/unknown	KT265249	1	1	0.125	0.25	0.5	>64	2	4
	Unknown	CBS 128059/unknown	KT265250	0.25	1	0.25	0.5	1	8	4	1
E. holmii	Unknown	CBS 128053/unknown	KT265253	0.25	1	1	>16	1	64	1	0.125
Exserohilum sp.	Unknown	CBS 128064/unknown	KT265251	0.25	8	2	>16	4	>64	1	0.125

[a] AMB, amphotericin B; ISA, isavuconazole; POS, posaconazole; ITC, itraconazole; VRC, voriconazole; FLU, fluconazole; CAS, caspofungin; MFG, micafungin.
[b] For echinocandins, MECs were defined as lowest drug concentrations that allowed the growth of small, rounded, and degenerated hyphae *vis-à-vis* the growth in the control well.

and CBS 128059 [no type strain]) and *Exserohilum antillanum* (CBS 412.93^T) clustered with *E. rostratum*; *E. antillanum* can thus be regarded as another synonym of *E. rostratum*. Notably, in the ITS phylogenetic tree, *E. antillanum* also could not be discriminated from *E. rostratum* (see Fig. S2 in the supplemental material). *E. rostratum* isolates exhibited somewhat variable banding patterns, suggesting genotypic diversity in this complex. Two isolates of *Exserohilum pedicellatum*, neither of which was a type strain, clustered together (see Fig. S1). Further, two isolates for each of the species *Exserohilum gedarefense* (CBS 297.80^T) and *Exserohilum neoregeliae* (CBS 132832^T) and a solitary isolate each of *Exserohilum fusiforme* (CBS 132709^T) and *Exserohilum protrudens* (CBS 132710^T), all type strains, represented four different species. Overall, ITS sequencing could not discriminate *E. rostratum*, *E. gedarefense*, *E. antillanum*, and *E. mcginnisii*. Also, similar but not identical AFLP profiles were observed for *Exserohilum holmii* (no type strain) and *Exserohilum curvatum* (CBS 505.90^T) representing two different species, which was in conformity with ITS phylogeny.

The MIC ranges, MIC_{50}s/MIC_{90}s, and geometric mean MICs of all isolates are presented in Table 2, along with data from the three previously published studies for comparison (1, 4, 15). Table 1 summarizes the origins and sources of the isolates. They originated from the United States ($n = 3$), Australia ($n = 2$), Canada ($n = 6$), Cuba ($n = 1$), India ($n = 2$), Japan ($n = 2$), Sudan ($n = 2$), Turkey ($n = 1$), and Venezuela ($n = 1$). Of these, 10 were environmental and 10 clinical isolates, whereas 4 isolates were of unknown origin and source. Posaconazole, itraconazole, and amphotericin B had the lowest MIC_{90}s across all *Exserohilum* isolates, whereas fluconazole had no activity. The MIC ranges of 10 *E. rostratum* isolates (inclusive of *E. mcginnisii*) for isavuconazole spread over 2 to 3 twofold dilutions (1 to 8 μg/ml) and were similar to those of posaconazole (0.06 to 0.5 μg/ml), voriconazole (0.5 to 2 μg/ml), itraconazole (0.125 to 0.5 μg/ml), caspofungin (0.5 to 4 μg/ml), amphotericin B (0.06 to 1 μg/ml), and micafungin (0.008 to 8 μg/ml). The geometric mean (GM) MIC of isavuconazole for *E. rostratum* was 3 μg/ml, compared to 1.15 μg/ml for voriconazole and 0.16 μg/ml for posaconazole. Notably, the geometric mean (GM) MICs of isavuconazole for all *Exserohilum* spp. (1.38 μg/ml) were lower than that of *E. rostratum* (3.03 μg/ml). In the present study, isavuconazole had the lowest *in vitro* activity among azoles, which was in conformity with the data of the U.S. meningitis outbreak caused by *E. rostratum* strains (4). Further, the *E. rostratum* outbreak strains exhibited equivalent susceptibility against itraconazole, posaconazole, amphotericin B, and to a lesser extent, voriconazole (4). Notably, in the present study, for all *Exserohilum* species, the GM minimum effective concentration (MEC) of caspofungin (1.18 μg/ml) was 3-fold higher than that of micafungin (0.36 μg/ml). Further, in contrast to previous findings with *Candida*, all tested isolates had reproducible MECs for caspofungin when performed on two occasions.

Regarding the published reviews of the therapeutic outcomes of cases of sinusitis and cutaneous infections with *Exserohilum*, successful outcomes with amphotericin B and more recently with itraconazole and voriconazole have been reported (2, 11, 20). The expert panel for the U.S. outbreak recommended voriconazole for treatment of meningitis in patients with less severe disease and the lipid formulation of amphotericin B for those with severe extraneural disease or refractory infections (10). The present study showed low amphotericin B MICs against *E. rostratum*, while vori-

TABLE 2 Comparison of present and previously published *in vitro* antifungal susceptibility profiles of *Exserohilum* species and *E. rostratum*, according to CLSI M38-A2

Species tested (*n*)	MIC parameter	MIC (μg/ml) of[a]: AMB	ISA	POS	ITC	VRC	FLU	CAS[b]	MFG[b]	AFG[b]	Reference (yr)
All *Exserohilum* spp. (24)	GM	0.24	1.38	0.19	0.38	0.97	17.4	1.18	0.36		Present study (2015)
	MIC_{50}	0.25	1	0.125	0.25	1	32	1	0.25		
	MIC_{90}	1	4	1	16	2	64	4	8		
	Range	0.03 to 1	0.125 to 8	0.03 to 2	0.03 to 16	0.06 to 2	1 to >64	0.5 to 4	<0.008 to 8		
E. rostratum (10)	GM	0.23	3.03	0.16	0.30	1.14	25.9	1.07	0.16		
	MIC_{50}	0.25	4	0.125	0.25	1	32	1	0.25		
	MIC_{90}	1	8	0.5	0.5	2	64	4	8		
	Range	0.06 to 1	1 to 8	0.06 to 0.5	0.125 to 0.5	0.5 to 2	8 to 64	0.5 to 4	0.008 to 8		
Exserohilum spp. other than *E. rostratum* (14)	GM	0.24	1.16	0.21	0.45	0.86	13.1	1.28	0.37		
	MIC_{50}	0.25	1	0.125	0.25	1	16	1	0.25		
	MIC_{90}	1	8	2	16	4	64	4	8		
	Range	0.03 to 1	0.125 to 8	0.03 to 2	0.03 to 16	0.06 to 4	1 to 64	0.5 to 4	0.06 to 8		
E. rostratum (6)	Range	2 to 4		0.5 to 1	1 to 2	2		4			15 (2014)
E. rostratum (50)	MIC_{50}	0.25	4	0.5	0.5	1					4 (2013)
	MIC_{90}	0.5	4	1	1	2					
	Range	0.03 to 2	2 to 4	0.25 to 1	0.25 to 4	1 to 2					
E. rostratum (34)	GM	0.02		0.03	0.02	0.1		0.06	0.27	0.06	1 (2012)
	MIC_{90}	0.03		0.03	0.03	0.25		0.125	0.05	0.125	
	Range	<0.03 to 0.125		<0.03 to 0.125	<0.03 to 0.125	<0.03 to 1		<0.03 to >16	<0.03 to >16	<0.03 to 1	

[a] AMB, amphotericin B; ISA, isavuconazole; POS, posaconazole; ITC, itraconazole; VRC, voriconazole; FLU, fluconazole; CAS, caspofungin; MFG, micafungin; AFG, anidulafungin.
[b] For echinocandins, MECs were defined as lowest drug concentrations that allowed the growth of small, rounded, and degenerated hyphae *vis-à-vis* the growth in the control well.

conazole had geometric mean MICs of >1 μg/ml. In contrast, da Cunha et al. (1) reported low geometric mean MICs of voriconazole (0.1 μg/ml) for 34 *E. rostratum* isolates originating from the United States. The different susceptibilities to voriconazole in the present study could be attributed to strains tested from various geographical locations. Recently, using whole-genome analysis, significant genomic variability has been reported among *E. rostratum* strains unrelated to the outbreak strains (21).

Although the clinical relevance of MIC data for *Exserohilum* has not been established, we conclude that amphotericin B, itraconazole, and posaconazole are the most active drugs *in vitro*, exhibiting MICs of <1 μg/ml, with both isavuconazole and voriconazole showing MICs below achievable serum trough levels.

The accession numbers for the *Exserohilum* isolates tested in this study are listed in Table 1.

Nucleotide sequence accession numbers. The ITS nucleotide sequences of all 24 *Exserohilum* species have been deposited in GenBank under accession numbers KT265236 and KT265259 (Table 1).

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