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Molecular Identification and *In Vitro* Response to Antifungal Drugs of Clinical Isolates of *Exserohilum*

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Exserohilum **is an agent of human and animal mycoses. Although classification has been based on a few subtle morphological differences, three species of clinical interest have been traditionally accepted. In this study, by using a multigene sequence analysis, we have demonstrated that *Exserohilum longirostratum* and *E. mcginnisii* are probable synonyms of *E. rostratum*. The isolates tested were mainly from the nasal region. Antifungal susceptibility testing demonstrated high activity of the eight agents tested against this fungus.**

The anamorphic genus *Exserohilum* (teleomorph *Setosphaeria*, family *Pleosporaceae*, order *Pleosporales*) is comprised of approximately 35 species, which are common saprobic fungi on plant debris, although some cause infections in plants, animals, and humans (1, 9, 10, 23, 24). Although these fungi have a broad geographical distribution, human infections occur predominantly in tropical and subtropical regions, affecting mainly immunocompetent patients (1). *Exserohilum* causes mainly allergic sinusitis, keratitis, and, less frequently, endocarditis, endophthalmitis, peritonitis, and invasive infections affecting brain, bones, lungs, and urinary tract (7, 21, 22). Three species, *E. rostratum*, *E. longirostratum*, and *E. mcginnisii*, have been reported as opportunistic pathogens for humans (1, 3, 19, 22). These species are characterized by quick growth, forming dark colonies, geniculate conidiophores, and ellipsoid to fusiform, straight to curved, multidistoseptate conidia with a protruding hilum. Such species can be mainly differentiated by the conidial morphology (7, 17).

To assess the incidence of *Exserohilum* species in clinical samples, we have identified morphologically and molecularly a set of isolates from different clinical specimens, which were sent to a reference laboratory during a period of 7 years (2003 to 2009) from different regions of the United States for identification and/or antifungal susceptibility testing.

A total of 34 clinical isolates, presumably belonging to *Exserohilum* spp., was received in the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio, and some type or reference strains were included in the present study. The majority of the isolates were from the nasal region (47%), followed by cutaneous and subcutaneous infections (20.5%) and ocular infections (14.7%). The remaining 11.6% of the isolates were from abscesses, blood, bronchoalveolar lavage fluid, and lumbar disc, while 6.2% were of unknown origin (Table 1). The fungi were cultured on potato carrot agar (PCA) and oatmeal agar (OA).

The internal transcribed spacer (ITS) region and the 28S ribosomal DNA (rDNA) (D1/D2), actin (ACT), and elongation factor 1-alpha (*EF*-1α) genes were amplified and sequenced following previously described protocols (4, 18, 25).

The *in vitro* activity of eight antifungal agents (amphotericin B, itraconazole, posaconazole, voriconazole, anidulafungin, caspofungin, micafungin, and terbinafine) against the isolates tested was evaluated according to reference guidelines (6). *Paecilomyces variotii* ATCC MYA-3630 was used as a quality control strain.

All the isolates examined displayed the typical features of the genus *Exserohilum* (24). Thirty-two isolates showed straight or slightly curved conidia, which were ellipsoidal to fusiform or rostrate, with smooth to finely roughened walls and brown to olivaceous brown coloring, measuring 25 to 91 by 9 to 22 μm, with 4 to 9 distosepta and dark bands at both ends, and were identified as *E. rostratum*. Two isolates were identified as *E. longirostratum*, due to the presence of markedly larger conidia (up to 228 by 12 to 19 μm), which had 6 to 16 distosepta and were centrally curved (7, 24). The type strain of *E. mcginnisii* was examined and showed straight, cylindrical, or slightly clavate conidia, which were smooth-walled and brown, measuring 44 to 76 by 11 to 18 μm, with 4 to 8 distosepta and without dark bands at both ends (7, 17).

With the primers used, we were able to amplify and sequence 392 to 411, 317 to 318, 464, and 609 bp of the ITS, ACT, D1/D2, and *EF*-1α loci, respectively. Sequences of the four regions obtained from the 37 isolates (34 clinical isolates and 3 type or reference strains) included in the study were analyzed phylogenetically. Comparison of such sequences unequivocally proved that all the isolates tested belonged to a single species (Table 2), which demonstrated that *E. longirostratum* and *E. mcginnissii* are synonyms of *E. rostratum*.

In general, all the antifungal drugs tested showed relatively low MICs against *Exserohilum* isolates, with only a few exceptions for echinocandins. Caspofungin and micafungin showed relatively high MICs against seven isolates and anidulafungin against one (Table 3).

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TABLE 1 Clinical isolates and type and reference strains of *Exserohilum* spp. included in the study[a]

Species	Isolate	Origin	GenBank accession no.			
			ITS	**D1/D2**	**ACT**	**EF-1α**
E. longirostratum	UTHSC 03-3090	Skin BX, Maryland	HE664036	HE664028	HE664076	HE664085
E. longirostratum	UTHSC 05-424	Intervertebral disc, Louisiana	HE664039	HE664030	HE664078	HE664083
E. longirostratum	IP 1229.80	Heart valve prosthesis, Martinique	HE664033	HE664025	HE664072	HE664080
E. mcginnisii	CBS 325.87(T)	Nasal polyp, Arizona	HE664035	HE664027	HE664074	HE664082
E. rostratum	UTHSC 03-1932	Maxillary sinus, Texas	HE664063			
E. rostratum	UTHSC 03-3639	Arm tissue, Colorado	HE664046			
E. rostratum	UTHSC 04-1248	Nasal wash, Texas	HE664038			
E. rostratum	UTHSC 04-2744	Sphenoid sinus, Texas	HE664058			
E. rostratum	UTHSC 04-2416	Sinus, Texas	HE664068	HE664029	HE664077	HE664086
E. rostratum	UTHSC 04-2629	Sinus, Texas	HE664066			
E. rostratum	UTHSC 04-3327	Middle turbinate tissue, Texas	HE664047			
E. rostratum	UTHSC 05-3456	Sinus, Texas	HE664052			
E. rostratum	UTHSC 06-2113	Cornea, Texas	HE664040			
E. rostratum	UTHSC 06-1857	Eye, California	HE664042			
E. rostratum	UTHSC 06-2618	Nasal (frog), Massachusetts	HE664043			
E. rostratum	UTHSC 06-3237	Great toe, Texas	HE664045			
E.rostratum	UTHSC 06-3226	Eye, Texas	HE664060			
E. rostratum	UTHSC 06-530	Ethmoid sinus, South Carolina	HE664064			
E. rostratum	UTHSC 07-263	Sinus, Minnesota	HE664048			
E. rostratum	UTHSC 07-1310	Unknown, Texas	HE664057			
E. rostratum	UTHSC 07-1292	Shin skin, Texas	HE664037			
E. rostratum	UTHSC 07-3092	Nose wound, Arkansas	HE664059			
E. rostratum	UTHSC 07-1498	Unknown, Texas	HE664041			
E. rostratum	UTHSC 07-622	Blood, Texas	HE664049			
E. rostratum	UTHSC 08-3261	Wound, Utah	HE664053			
E. rostratum	UTHSC 08-655	Elbow, Texas	HE664054			
E. rostratum	UTHSC 08-2771	Cornea, Utah	HE664050			
E. rostratum	UTHSC 08-922	Nasal BX, Florida	HE664065	HE664032	HE664075	
E. rostratum	UTHSC 08-3638	Sinus, Utah	HE664061			
E. rostratum	UTHSC 08-2940	Inferior turbinate tissue, Texas	HE664055			
E. rostratum	UTHSC 09-2018	Abscess, South Carolina	HE664069	HE664031	HE664079	HE661084
E. rostratum	UTHSC 09-131	Maxillary sinus, Montana	HE664062			
E. rostratum	UTHSC 09-718	Maxillary sinus, Texas	HE664056			
E. rostratum	UTHSC 09-1259	Eye, Georgia	HE664051			
E. rostratum	UTHSC 09-1211	Inferior turbinate tissue, Texas	HE664067			
E. rostratum	UTHSC 09-109	Bronchial wash, Minnesota	HE664044			
E. rostratum	CBS 467.75(T)	Soil, India	HE664034	HE664026	HE664073	HE664081

[a] UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; IP, Institut Pasteur, Paris, France; (T), type strain; BX, biopsy.

Some studies have clearly demonstrated the ability of *Exserohilum* to cause humans infections, although they are mainly single case reports where the fungal identification was based exclusively on morphological criteria (7). This is the first study in which a large panel of *Exserohilum* clinical isolates from different anatomical sites has been identified using phenotypic and genetic criteria. The anatomical sites from where the fungi were isolated agree with those reported in previous studies (8, 15, 20, 21).

Exserohilum species are mainly identified by the conidial morphology seen when growing in the natural substratum (24). *In vitro* identification is more difficult, the conidia tending to be smaller and the isolates losing the ability to sporulate (17, 24). In this study, all the isolates grew well in the media used, although they sporulated better on PCA than on OA. However, the conidia never reached the maximum length described for any of those species in the natural substrate.

Previous taxonomic and molecular studies suggested that *E. rostratum* and *E. longirostratum* could be the same species (7, 13, 14). The present study proved through a multilocus analysis that all isolates, including the type strain of *E. mcginnisii* and reference strains of *E. longirostratum*, showed a high homology with the type strain of *E. rostratum*, indicating that all are probable conspecific species. However, further studies sequencing additional genes are required to demonstrate such synonymy. *In vitro* antifungal susceptibility data for *Exserohilum* spp. are very variable; however, they are scarce and, in general, are from studies performed before the standardization of the procedures for antifungal susceptibility testing for molds (2, 5, 11, 12, 16). The high *in vitro* activity of all the antifungals tested here against *Exserohilum* is remarkable, although it is unknown how this can be translated to clinical practice. Data on the clinical treatment of infections by *Exserohilum* are also very scarce, but recent reviews of cases of sinusitis and cutaneous infections by these fungi report successful outcomes with amphotericin B and more recently with itraconazole and voriconazole (8, 15).

TABLE 3 Results of *in vitro* antifungal susceptibility testing for 34 clinical isolates of *Exserohilum rostratum*[a]

Antifungal agent	MIC or MEC (μg/ml) at 48 h		MIC_{90} (μg/ml) at 48 h	MIC or MEC (μg/ml) at 72 h		MIC_{90} (μg/ml) at 72 h
	GM range			GM range		
Amphotericin B	0.02	<0.03 to 0.125	0.03	0.02	<0.03 to 0.125	0.03
Anidulafungin	0.06	<0.03 to 1	0.125	0.10	<0.03 to >16	0.25
Caspofungin	0.06	<0.03 to >16	0.125	0.21	<0.03 to >16	2
Itraconazole	0.02	<0.03 to 0.125	0.03	0.02	<0.03 to 0.125	0.03
Micafungin	0.27	<0.03 to >16	0.05	0.41	0.03 to >16	0.5
Posaconazole	0.03	<0.03 to 0.125	0.03	0.03	<0.03 to 0.5	0.06
Terbinafine	0.02	<0.03 to 0.03	0.03	0.03	<0.03 to 0.25	0.06
Voriconazole	0.10	<0.03 to 1	0.25	0.14	0.03 to 1	0.25

[a] GM, geometric mean.

TABLE 2 Percentages of similarity among type and reference strains of the three clinically relevant *Exserohilum* species[a]

Strain	% identity to indicated strain by:											
	ITS			D1/D2 (28′S)			ACT			EF-1α		
	IP 1229.80 *E. longirostratum*	CBS 325.87(T) *E. mcginnisii*	CBS 467.75(T) *E. rostratum*	IP 1229.80 *E. longirostratum*	CBS 325.87(T) *E. mcginnisii*	CBS 467.75(T) *E. rostratum*	IP 1229.80 *E. longirostratum*	CBS 325.87(T) *E. mcginnisii*	CBS 467.75(T) *E. rostratum*	IP 1229.80 *E. longirostratum*	CBS 325.87(T) *E. mcginnisii*	CBS 467.75(T) *E. rostratum*
UTHSC 04-2416 *E. rostratum*	99.7	99.7	99.5	100	100	100	98.8	98.8	99	99.1	99.2	99.2
UTHSC 09-2018 *E. rostratum*	99.7	99.7	99.5	100	100	100	99.8	99.8	99.2	98.9	99.1	99.1
UTHSC 05-424 *E. longirostratum*	99.7	99.7	99.5	100	100	100	99	99	98.4	98.9	98.7	98.7
IP 1229.80 *E. longirostratum*		100	99.8		100	100		99.6	99		99.9	99.9
CBS 325.87(T) *E. mcginnisii*			99.8			100			99			99.7
CBS 467.75(T) *E. rostratum*												

[a] (T), type strain.

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