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In Vitro Activities of Approved and Investigational Antifungal Agents against 44 Clinical Isolates of Basidiomycetous Fungi

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The in vitro activity of amphotericin B, fluconazole, flucytosine, itraconazole, voriconazole, and posaconazole was evaluated against 44 clinical isolates of filamentous basidiomycetous fungi. No statistically significant differences were noted between *Schizophyllum commune* ($n = 5$), *Coprinus* species ($n = 8$), *Bjerkandera adusta* ($n = 14$), and sterile, uncharacterized basidiomycetes ($n = 17$).

The spectrum of documented infections reported for basidiomycetes, namely *Schizophyllum commune* and *Coprinus* species, include endocarditis, meningitis, sinusitis, ulcerative lesions of the hard palate, fungal ball of the lung, allergic bronchopulmonary conditions, bronchial mucoid impaction, and chronic respiratory disease. Mycoses have occurred in both immunocompetent and immunocompromised hosts (1, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15).

Although filamentous basidiomycetes fungi are being increasingly recognized, their definitive identification is problematic, with many isolates remaining sterile in culture (12).

In addition to the difficulty surrounding identification, assessment of pathogenicity is equally uncertain. Despite these obstacles, clinicians frequently request information regarding potential therapeutic regimens. We therefore sought to determine the in vitro activity of various approved and investigational antifungal compounds against this group of fungi.

Testing was accomplished in a head-to-head format on 44 clinical isolates submitted to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio. Isolates tested and their presumptive identifications were as follows: *S. commune* ($n = 5$), *Coprinus* species ($n = 8$), *Bjerkandera adusta* ($n = 14$), and sterile, uncharacterized basidiomycetes ($n = 17$). Strains are identified in Table 1. While the clinical significance of each isolate was difficult to determine, several isolates were recovered from profoundly immunocompromised patients whose underlying conditions support invasion by normally nonvirulent fungi.

Isolates were maintained in water suspensions at room temperature until testing. The antifungal agents used were provided as standard powders of known potency. Serial twofold dilutions of each antifungal agent were prepared to give the following final drug concentrations: 0.03 to 16 μg/ml for amphotericin B (AMB); 0.125 to 64 μg/ml for fluconazole (FLC),

TABLE 1. Basidiomycete isolates used in this study

Organism	Accession no.	Source	Geographic location	Clinical diagnosis
S. commune	96-1252	Bronchial washing	Houston	
	97-126	Frontal sinus tissue	Houston	Sinusitis
	98-148	Ethmoid tissue	Houston	Sinusitis
	98-653	Tissue maxillary sinus	Minneapolis	Sinusitis
	99-1789	Sinus	Omaha	
B. adusta	98-260	Sputum	Houston	
	98-316	Bronchial washing	Houston	
	98-484	Bronchial washing	Houston	
	98-1020	Pleural fluid	Nashville	
	98-1351	Skin	Oakland	Dermatitis
	98-2020	Sputum	Scarborough	
	98-2163	Bronchial washing	Missoula	
	99-1014	Sputum	Houston	
	99-1069	Bronchial washing	Houston	
	99-1510	Bronchial washing	Danville	Carcinoma of the lung
	99-1622	Lung tissue	Hershey	
	99-1644	Bronchial washing	Ohio	
	99-1944	Bronchial washing	Jacksonville	Pulmonary nodule
	99-1974	Bronchial washing	Baton Rouge	Carcinoma of the lung
Coprinus species	98-1898	Bronchial washing	Jacksonville	
	98-1950	Bronchial washing	Madison	
	98-1953	Bronchial washing	Jacksonville	
	98-2056	Sputum	Madison	
	98-2080	Sputum	San Antonio	Leukemia
	99-998	Bronchial washing	San Antonio	
	99-1360	Sphenoid tissue	Denver	Sinusitis
	99-1546	Bronchial washing	Cleveland	
Unidentified basidiomycetes	98-1259	Bronchial washing	Middletown	
	98-1914	Bronchial washing	Middletown	Carcinoma of the lung
	98-1976	Sputum	Seattle	
	98-2001	Bronchial washing	San Antonio	Chest mass
	98-2041	Bronchial washing	Jacksonville	
	98-2060	Bronchial washing	San Antonio	
	99-968	Bronchial washing	Seattle	
	99-1181	Bronchial washing	Jacksonville	
	99-1631	Bronchial washing	Great Falls	Pneumonia
	99-1633	Lung tissue	Houston	Autopsy
	99-1651	Bronchial washing	San Antonio	
	99-1907	Bronchial washing	Harrisburg	Chest mass
	99-1769	Sputum	Missoula	
	99-1770	Bronchial washing	Missoula	
	99-1844	Bronchial washing	San Antonio	
	99-1858	Pulmonary nodule	Boston	Transplant recipient
	99-1945	Bronchial washing	Jacksonville	Hemoptysis

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TABLE 2. In vitro antifungal susceptibility

Organism (*n*) and drug	MIC (μg/ml)[a]			
	GM	Range	MIC_{50}	MIC_{90}
S. commune (5)				
AMB	0.5	0.5–0.5	0.5	0.5
5FC	9.18	8–16	8	8
FLC	10.55	8–16	8	16
ITC	0.06	0.06–0.125	0.06	0.06
VRC	0.57	0.5–1	0.5	0.5
PSC	0.43	0.25–0.5	0.5	0.5
B. adusta (14)				
AMB	0.43	0.25–0.5	0.5	0.5
5FC	11.31	8–16	8	16
FLC	11.31	8–16	8	16
ITC	0.11	0.06–0.25	0.125	0.125
VRC	0.43	0.25–0.5	0.5	0.5
PSC	0.52	0.25–1	0.5	0.5
Coprinus species (8)				
AMB	0.45	0.25–0.5	0.5	0.5
5FC	14.67	8–32	16	32
FLC	13.45	8–32	16	32
ITC	0.08	0.03–0.125	0.125	0.125
VRC	0.45	0.25–0.5	0.5	0.5
PSC	0.42	0.125–0.5	0.5	0.5
Unidentified basidiomycetes (17)				
AMB	0.31	0.25–0.5	0.25	0.5
5FC	8.33	4–16	8	16
FLC	8.88	4–16	8	16
ITC	0.08	0.06–0.125	0.06	0.125
VRC	0.54	0.5–1	0.5	0.5
PSC	0.33	0.125–0.5	0.25	0.5

[a] Abbreviations: GM, geometric mean; MIC_{50} and MIC_{90}, MIC inhibiting 50 or 90% of the isolates.

voriconazole (VRC), and flucytosine (5FC); and 0.015 to 8 μg/ml for itraconazole (ITC) and posaconazole (PSC). A 0.1-ml aliquot of the twofold serial dilutions was dispensed into a sterile plastic snap-cap tube (12 by 75 mm) that was then maintained at −70°C until needed. AMB was tested in antibiotic medium 3 (Difco, Detroit, Mich.), and other agents were tested in RPMI 1640 (Angus, Niagara Falls, N.Y.). Isolates were tested using the National Committee for Clinical Laboratory Standards macrobroth dilution method M38-P (reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi, proposed standard). Briefly, isolates were grown on potato flakes agar prepared in-house (8) for 7 days at 30°C. Inocula were standardized spectrophotometrically to 80% transmittance at 530 nm. Suspensions were further diluted 1:10 in media for a final concentration of approximately 1×10^4 CFU/ml. Previously prepared aliquots of frozen drugs containing 0.1 ml of drug were allowed to thaw and were inoculated with 0.9 ml of the suspension. Tubes were incubated at room temperature, and the 72- to 96-h MICs were defined as the drug concentration of the first tube that yielded a score of 0 (optically clear) for AMB and a score of 2 (reduction in turbidity of ≥80% compared to the drug-free control tube) for FLC, VRC, 5FC, ITC, and PSC. The University of Texas Health Science Center *Paecilomyces* control strain 90-459 was included with all testing.

Table 2 summarizes the in vitro antifungal susceptibility data for the 44 strains tested. The 96-hour geometric mean, range, and MICs inhibiting 50 and 90% of the isolates for AMB, ITC, VRC, and PSC were consistently low. For 5FC and FLC, the values were somewhat higher although still within normally achievable concentrations in serum. The data were evaluated by the Sidak multiple comparisons *t* test with a one-way analysis of variance. Results indicated no statistically significant differences between the organisms evaluated.

Previous in vitro susceptibility data for basidiomycetes have been limited to small numbers of strains or individual isolates. The *S. commune* isolate described by Rihs et al. (7) with dissemination to the brain required MICs of <0.03 and 8 μg/ml for AMB and FLC, respectively. ITC could not be tested due to failure of the isolate to grow in the test medium. Gené et al. (2) studied a total of 12 environmental and clinical strains of *Coprinus cinereus*, *Hormographiella aspergillata*, and *Hormographiella verticillata*. All strains were susceptible to miconazole, ITC, and ketoconazole with MICs ranging from 0.6 to 5.0 μg/ml, 0.07 to 0.6 μg/ml, and 0.2 to 1.6 μg/ml, respectively. They were resistant to fluconazole (20 to >80 μg/ml) and 5FC (322 to >322 μg/ml), while their susceptibility to AMB was variable (≤0.07 to 4.6 μg/ml). All strains of *H. verticillata* appeared susceptible to AMB; however, four of seven strains of *C. cinereus* displayed resistance (2.3 to 4.6 μg/ml). Verweij et al. (15) reported a case of fatal pneumonia due to *H. aspergillata* in a patient receiving intensive cytotoxic treatment. The results of in vitro susceptibility testing by agar dilution and broth macrodilution of the isolate showed low MICs for AMB and high MICs for ITC (8 to 32 mg/liter), suggesting in vitro resistance to that agent. The MIC for FLC was also high, at >64 mg/liter. No correlation between in vitro susceptibility data and therapeutic response was noted, as AMB-associated toxicity required changing to an ITC regimen, with the patient expiring 9 days later.

In vitro susceptibility data for approved and investigational antifungal agents are presented. Additional studies may further elucidate the correlation between in vitro data and clinical efficacy in mycoses caused by basidiomycetous fungi.

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