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Molecular Characterization and *In Vitro* Antifungal Susceptibility Profile of *Schizophyllum commune*, an Emerging Basidiomycete in Bronchopulmonary Mycoses

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***Schizophyllum commune* ($n = 30$) showed lowest geometric mean MICs of isavuconazole (0.19 μg/ml), itraconazole (0.2 μg/ml), voriconazole (0.24 μg/ml), and amphotericin B (0.29 μg/ml) and high geometric mean MICs of fluconazole (19.39 μg/ml) and flucytosine (17.28 μg/ml). Five cases (of 8) of allergic bronchopulmonary mycosis that were treated with itraconazole had no recrudescence after 6 to 24 months of follow-up. One case each of invasive pulmonary mycosis and fungal ball were treated successfully with voriconazole and itraconazole.**

Schizophyllum commune is an emerging, filamentous pathogenic basidiomycete, causing an array of allergic and invasive clinical manifestations. Its definitive identification is problematic, as it grows in culture mostly as sterile cottony white colonies without sporulation (1, 2). This is particularly true of monokaryotic isolates, which, unlike the dikaryotic ones, are devoid of the characteristic spicules or clamp connections and thus cannot be identified by phenotypic methods and therefore require sequencing (1–3). Previously, we have reported *S. commune* as the etiologic agent in one case each of allergic bronchopulmonary mycosis (ABPM) and of pulmonary fungal ball and reviewed 76 cases with diverse clinical manifestations. *Schizophyllum commune* has a global distribution, causing respiratory infections in an overwhelming number (94.7%) of cases (4). As the majority of the respiratory infections reported to be due to *S. commune* are allergic bronchopulmonary and sinonasal, their therapy aims at suppression of the immune response and prevention/eradication of fungal colonization in the airways (5, 6). *Schizophyllum commune*-associated allergic rhinosinusitis usually runs a protracted course. Its surgical treatment alone is not adequate, and it must be combined with antifungal treatment (7). Furthermore, adjunctive antifungal therapy has been suggested to guard against the risk of dissemination or recurrence of the disease (8–11). Despite the increasing clinical importance of *S. commune*, information on its molecular identification and antifungal susceptibility profile is scanty and limited to a small number or individual isolates (4, 12). We report the *in vitro* antifungal susceptibility to azoles, amphotericin B, and flucytosine of a wide array of *S. commune* strains isolated from patients with sinonasal and bronchopulmonary mycoses and identified by the internal transcribed spacer (ITS) region of ribosomal DNA and D1/D2 of larger subunit (LSU) region sequencing. In addition, we discuss outcomes of antifungal treatment in 11 patients.

A total of 26 (0.5%) strains of *S. commune* were isolated from 4,662 clinical specimens from an equal number of patients with respiratory diseases who were residing in Northwestern India during January 2010 through September 2012. The clinical specimens included mucus plugs, sputum, bronchoalveolar lavage fluid, fine needle aspirate biopsy specimens, nasal washings, and sinus aspirates which were cultured on Sabouraud's glucose agar (SGA) plates with or without 10 μg/ml benomyl. Incorporation of benomyl in the medium facilitates selective isolation of basidiomycetes (13). The culture-positive specimens yielded multiple cottony white mold colonies after 7 to 9 days on SGA plates incubated at 28°C and 37°C. Slide cultures of all isolates on potato dextrose agar (PDA) after 4 weeks of incubation at 28°C showed hyaline, septate hyphae with clamp connections and spicules suggestive of a basidiomycete, but morphological identification was not possible (2). However, only 4 (15.3%) of the 26 isolates showed the development of fruiting body with fan-shaped basidiocarps on PDA at 28°C, after 4 to 5 weeks, with periodic exposure to light which was consistent with the presence of *S. commune*. Isolates were maintained on PDA slants and in normal saline suspensions at room temperature.

Identification of all the *S. commune* isolates was done by sequencing ITS and LSU regions as described previously (4, 14). ITS sequences (GenBank accession numbers KC414792 to KC414814) of 23 *S. commune* isolates showed 99% homology to *S. commune* sequences with accession numbers FJ372689.1, AB369910.1, and JF19817.1 and LSU sequences (Genbank accession numbers KC414815 to KC414840) of all 26 isolates showed 99% homology with accession numbers HM595605.1, GQ254661.1, and AB428351.1 in GenBank. ITS sequences of 3 isolates were nonreadable. All of the 26 isolates were deposited in the CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands.

Antifungal susceptibility testing of the 26 clinical isolates and 4 reference strains (CBS10320, CBS333.85, CBS579.83, and CBS109426) of *S. commune* was performed using a slightly mod-

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TABLE 1 *In vitro* profiles of susceptibility of 26 clinical and 4 reference isolates of *Schizophyllum commune* to various antifungals

Parameter	Value(s) (μg/ml)[a]					
	AMB	FLU	ITC	VRC	ISA	FC
GM	0.29	19.39	0.20	0.24	0.19	17.28
MIC_{50}	0.5	16	0.125	0.25	0.125	32
MIC_{90}	1	64	1	0.5	0.5	64
Range	0.03–2	2–64	0.03–8	0.06–2	0.015–2	2–64

[a] AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VRC, voriconazole; ISA, isavuconazole; FC, flucytosine.

ified version of the CLSI-M38-A2 method (15). The modifications included growth of isolates on PDA for 2 weeks at 28°C and a higher working inoculum of 2.5×10^4 to 5.0×10^4 hyphal fragments/spores per ml. Microtiter plates were incubated at 35°C for 96 h. MIC endpoints were read visually. The quality control strain data were in the recommended ranges, and the reproducibility of the *in vitro* results was assessed by determining MICs for all strains twice on two different days. The results of the *in vitro* susceptibility assessments are presented in Table 1. The isolates had low geometric mean (GM) MICs of azoles and amphotericin B (0.29 μg/ml) but high MICs of fluconazole (19.39 μg/ml) and flucytosine (17.28 μg/ml).

Clinical evaluation revealed that 12 (46%) of the cases had ABPM, 3 (11.5%) each had pulmonary fungal ball and invasive pulmonary mycosis, and 2 (7.6%) had allergic fungal sinusitis. Of the remaining 6 patients, 3 had pulmonary tuberculosis, including 2 with cavitation and 1 with interstitial lung disease who was on long-term systemic steroids, and 3 had chronic obstructive pulmonary diseases (COPD) with bronchiectasis. Therapeutic outcome was available for 11 of the 26 patients. Of these, 8 patients with ABPM, and 1 each with invasive pulmonary mycosis and fungal ball, received itraconazole at 200 mg (twice a day [BID]) for 3 to 6 months and 1 with a case of invasive pulmonary mycosis received voriconazole at 200 mg (BID) for 4 months.

This report provides information on the *in vitro* antifungal susceptibility of a large collection of *S. commune* strains isolated from patients with respiratory diseases. Information previously available was collected in a sporadic manner and mostly restricted to individual isolates, except one report by González et al. (12), who investigated the susceptibility of 5 strains (Table 2) (4, 7, 12, 16–19). They showed that itraconazole had the lowest GM MICs followed by posaconazole, amphotericin B, and voriconazole (12). Notably, isavuconazole, a newer azole, had not been tested previously for its activity against *S. commune*. Our isolates had variable MICs of fluconazole, ranging between 2 and 64 μg/ml. This kind of variation in susceptibility to fluconazole was previously reported with single isolates of *S. commune* which had MICs of 12.5 μg/ml (17) and 4 μg/ml (18).

Currently, experience with antifungal therapy in cases of respiratory mycoses due to *S. commune* is limited. Among the 29 globally reported cases of *S. commune* infection who had received antifungal therapy, there were 11 cases each with ABPM, 9 with sinusitis, 4 with bronchial mucoid impaction, and 1 case each with brain abscess, ulcer of the palate, fungal ball, bronchopneumonia, and pulmonary nodule (4, 7–10, 16–37). Of the 18 patients treated with itraconazole, all of them responded favorably without recurrence (4, 8–10, 19, 20, 24–31, 33–35). These included eight cases with ABPM, seven with sinusitis, and three with mucoid impaction and a solitary case of fungal ball. Likewise, of the eight cases of ABPM treated with oral itraconazole therapy in this study, five showed no recrudescence of symptoms after 6 to 18 months of follow-up. This includes three patients who are under further follow-up, and they are still in remission. It is noteworthy that a low dose of itraconazole prevented relapse of ABPM upon environmental reexposure of the patient (27). Also, a solitary case of fungal ball with hemoptysis in our study was successfully managed with itraconazole administered at 200 mg (BID) for 4 months. The patient's hemoptysis stopped, and he has been asymptomatic for the last 9 months.

Cases of invasive disease due to *S. commune* have been treated with amphotericin B, itraconazole, and fluconazole, singly or in combination, with variable success (2, 7, 16, 36). Of the 3 patients with suspected invasive pulmonary diseases in the present report, one presented with a lung mass in the right upper lobe and improved with 4 months of voriconazole. In the remaining two patients, outcome of therapy could not be assessed, as one patient with a right parahilar mass succumbed to sudden hemoptysis after 2 weeks of itraconazole therapy and a second patient with diffuse nodular shadows died shortly after initiation of amphotericin B therapy at 1 mg/kg/day. Restrepo et al. (36) reported a case of invasive palatal ulceration treated with amphotericin B for 3 months which resulted in complete regression. Also, a case of

TABLE 2 Overview of published reports on *in vitro* antifungal susceptibility testing of *Schizophyllum commune* isolates[a]

No. of isolates tested	Source of isolates	MIC (μg/ml) of antifungal(s)	Yr	Reference
1	Maxillary sinus drainage	AMB, <0.025	1992	7
1	Resected lung	AMB, <0.03; FLU, 8	1996	16
1	Mucoid plugs	ITC, 0.125; AMB, 0.39; FLU, 12.5	2000	17
5	Bronchial washing, frontal sinus tissue, ethmoid tissue, maxillary sinus tissue, sinus tissue	AMB, 0.5–0.5; FLU, 8–16; ITC, 0.06–0.125; VRC, 0.5–1; POS, 0.25–0.5; FC, 8–16	2001	12
1	Bronchial aspirate	FLU, 4	2008	18
1	Maxillary sinus drainage	AMB, 0.023; FLU, >256; ITC, 0.25; VRC, 0.06	2010	19
2	Mucoid plugs, sputum	AMB, 0.5–1; ITC, <0.06–0.125; VRC, 0.5–1; POS, <0.015–0.125; ISA, 0.125–0.25	2012	4
30	Sputum, mucoid plugs, bronchial aspirates, BAL, FNAB	AMB, 0.03–2; FLU, 2–64; ITC, 0.03–8; VRC, 0.06–2; ISA, <0.015–2; FC, 2–64	2012	Present study

[a] BAL, bronchoalveolar lavage; FNAB, fine needle aspirate biopsy; AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; FC, flucytosine; ISA, isavuconazole.

brain abscess in a patient who received amphotericin B for over 36 days is on the record. Although his lesions regressed, he died due to respiratory failure (16).

Molecular techniques are required for definitive identification of this emerging basidiomycete. Kamei et al. (38) reported that 58% of the *S. commune* isolates were monokaryotic and therefore unidentifiable by morphological methods. In the present study, all of the 26 isolates were confirmed by LSU sequencing but only 4 showed formation of basidiocarps. Therefore, gene sequencing is required for accurate identification to determine the etiology of the disease. Although the role of amphotericin B in invasive *S. commune* disease is well established, our study highlights the utility of itraconazole and voriconazole in managing chronic respiratory diseases.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this work are available under GenBank accession numbers KC414792 to KC414840.

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