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In Vitro Antifungal Susceptibilities of *Scopulariopsis* Isolates

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MICs and minimum fungicidal concentrations of amphotericin B, miconazole, itraconazole, ketoconazole, fluconazole, and flucytosine against 17 isolates of *Scopulariopsis* spp. were determined by a broth microdilution method. All the isolates were resistant to itraconazole, fluconazole, and flucytosine, and amphotericin B, miconazole, and ketoconazole MICs were low for only a few.

Scopulariopsis is a large anamorphic genus comprising mainly soil species which are frequently isolated from food, paper, and other materials and occur also as laboratory contaminants. *Scopulariopsis* species are among the most common nondermatophytic fungi that cause onychomycosis, and they are also responsible, although less frequently, for deep-tissue infections (4, 13, 17). In approximately 90% of reported invasive infections caused by *Scopulariopsis* there were some well-defined predisposing factors, the most frequent being AIDS, organ transplantation, corticosteroid therapy, peritoneal dialysis, surgery, cardiac diseases, and trauma, etc. Eight species of *Scopulariopsis* have been reported as producing infections in humans, five of them causing only onychomycosis. They are *Scopulariopsis acremonium*, *S. asperula*, *S. flava*, *S. fusca*, *S. koningii*, *S. brevicaulis*, *S. brumptii*, and *S. candida. S. brevicaulis* is the species most frequently associated with invasive infections (7). Some species of the ascomycete genus *Microascus*, which comprises teleomorphs (sexual states) of *Scopulariopsis*, have also been cited as pathogenic to humans. They are *Microascus cinereus*, *M. cirrosus*, and *M. manginii* (7).

The majority of clinical cases have been treated with amphotericin B, although many other antifungals have also been used. Very little information exists about the in vitro activity of antifungals against *Scopulariopsis*. In the majority of reports, only one strain was tested and details about the technique used are generally scarce (1, 2, 4, 5, 8, 9, 11, 13, 14, 20, 21). In this study, in vitro antifungal susceptibilities of clinical and environmental isolates of *Scopulariopsis* spp. were evaluated by the broth microdilution method, broadly following the National Committee for Clinical Laboratory Standards' guidelines (standard M38-P).

Broth microdilution method. The method used was detailed previously (18). A total of 17 isolates of *Scopulariopsis* spp. were tested (Table 1). Antifungal agents included amphotericin B (E.R. Squibb & Sons, Barcelona, Spain), flucytosine (Hoffmann-La Roche, Basel, Switzerland), fluconazole (Pfizer, Madrid, Spain), ketoconazole (Roig-Farma, Barcelona, Spain), miconazole (Roig-Farma), and itraconazole (Janssen Pharmaceutica, Beerse, Belgium). Fungizone and Diflucan, the commercial intravenous preparations of amphotericin B and fluconazole, respectively, were used as stock solutions. The inoculum was 1×10^4 to 5×10^4 conidia per ml. The concentrations of the test drug were 0.03 to 16 μg/ml for amphotericin B, miconazole, itraconazole, and ketoconazole; 0.125 to 64 μg/ml for fluconazole; and 0.25 to 128 μg/ml for flucytosine. The temperature of incubation was of 30°C, and MIC readings were made after 48 and 72 h. The amphotericin B MIC was defined as the lowest drug concentration with which there was a complete absence of growth. MICs of the azoles and flucytosine were defined as the lowest drug concentration that gave only slight growth, corresponding to approximately 25% of the growth of the control. The minimum fungicidal concentrations (MFC) were obtained by placing 10 μl from each well which showed inhibition onto oatmeal plates. Fungal colonies were counted after incubation for 48 h at 30°C. The MFC was defined as the lowest drug concentration at which one colony or less was visible on the agar plate. To compare the MIC readings at 48 and 72 h, differences of no more than 1 dilution (one well) were used to calculate the percentage of agreement. The kappa test was used to calculate the degree of agreement. A kappa value (κ) greater than 0.75 was taken to represent excellent agreement, values below 0.40 were taken to represent poor agreement, and values between 0.40 and 0.75 were taken to represent fair to good agreement.

Table 1 shows that only some of the strains, belonging to different species, displayed moderate susceptibility to amphotericin B, miconazole, and ketoconazole. All of them were clearly resistant to itraconazole, fluconazole, and flucytosine. *S. carbonaria* was the species for which MICs and MFCs were generally the lowest. *S. brevicaulis* showed very variable results. Amphotericin B MICs were relatively low for two of the five isolates, miconazole MICs were low for three, and ketoconazole MICs were low for four, although MICs were not lower than 1 μg/ml in any case. The MIC ranges were generally wider and lower than the ranges of the MFCs. Excellent agreement between the MICs at 48 and 72 h was shown for itraconazole ($\kappa = 1$), fluconazole ($\kappa = 1$), flucytosine ($\kappa = 1$), and miconazole ($\kappa = 0.76$). Good agreement was shown for ketoconazole ($\kappa = 0.64$), and poor agreement was shown for amphotericin B ($\kappa = 0.28$).

Although amphotericin B is the drug most frequently used, the correct treatment for invasive *Scopulariopsis* infections is still unclear. The success rate of this drug is only about 40%. On six occasions amphotericin B was used alone (11, 12, 15, 17, 22, 23), but on only three of them did the patients make good progress. One patient was first treated with different doses of amphotericin B without success, but the change to a liposomal preparation of the same polyene compound resolved the infection (10). In another five patients, amphotericin B was used in combination with other antifungal drugs (ketoconazole for one patient, miconazole for another, and itraconazole for another three) (4, 9, 15, 16, 19). Combined with itraconazole,

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TABLE 1. Antifungal susceptibilities of 17 isolates of *Scopulariopsis* spp.[a]

Species and strain[b]	AMB		MCZ		ITC		KTC		FLC		5FC	
	MIC[c]	MFC[c]	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
S. acremonium IMI 250097	1	>16	2	>16	>16	>16	1	>16	>64	>64	>128	>128
S. brevicaulis FMR 3503	1	>16	4	>16	>16	>16	1	>16	>64	>64	>128	>128
S. brevicaulis UTHSC 95.1589	4	>16	8	>16	>16	>16	8	>16	>64	>64	>128	>128
S. brevicaulis UTHSC 95.1265	>16	>16	>16	>16	>16	>16	>16	>16	>64	>64	>128	>128
S. brevicaulis UTHSC 95.402	>16	>16	>16	>16	>16	>16	8	>16	>64	>64	>128	>128
S. brevicaulis UTHSC 95.313	>16	>16	8	>16	>16	>16	8	>16	>64	>64	>128	>128
S. brumptii FMR 3280	>16	>16	4	>16	>16	>16	8	8	>64	>64	>128	>128
S. candida FMR 3377	2	>16	4	>16	>16	>16	2	>16	>64	>64	>128	>128
S. carbonaria FMR 3724	1	1	4	8	>16	>16	4	8	>64	>64	>128	>128
S. carbonaria FMR 4051	1	1	4	4	>16	>16	8	8	>64	>64	>128	>128
S. chartarum FMR 3997	4	>16	4	>16	>16	>16	2	>16	>64	>64	>128	>128
S. koningii CBS 268.61	2	>16	4	>16	>16	>16	4	>16	>64	>64	>128	>128
S. koningii CBS 208.61	1	>16	2	>16	>16	>16	2	>16	>64	>64	>128	>128
S. fusca CBS 334.53	>16	>16	4	16	>16	>16	>16	>16	>64	>64	>128	>128
Scopulariopsis sp. strain FMR 4012	2	>16	2	>16	>16	>16	1	>16	>64	>64	>128	>128
Scopulariopsis sp. strain FMR 4459	0.5	>16	4	>16	>16	>16	8	>16	>64	>64	>128	>128
Scopulariopsis sp. strain FMR 4469	8	>16	8	>16	>16	>16	4	>16	>64	>64	>128	>128

[a] Abbreviations of drugs: AMB, amphotericin B; MCZ, miconazole; ITC, itraconazole; KTC, ketoconazole; FLC, fluconazole; 5FC, flucytosine.
[b] IMI, International Mycological Institute (Egham, United Kingdom); FMR, Faculty of Medicine, Reus (Reus, Spain); UTHSC, The University of Texas Health Science Center at San Antonio (San Antonio, Tex.); CBS, Centraalbureau voor Schimmelcultures (Baarn, The Netherlands).
[c] In micrograms per milliliter.

amphotericin B showed good results on three occasions. Itraconazole was also used alone for one patient with ulcerous cheilitis (1) and one with plantar infection (5). The first patient relapsed after a certain degree of improvement, but the second was cured completely after the failure of ketoconazole and fluconazole. Ketoconazole was effective for the treatment of psoriasiform plaques in a patient with human immunodeficiency virus (6), and miconazole cured a mycotic corneal ulcer which had developed after herpetic keratitis (2). Flucytosine was used for only one patient with mycetoma, in whom it was not effective (3).

Results obtained by authors who have tested the in vitro antifungal susceptibilities of *Scopulariopsis* spp. are generally contradictory, probably due to the variety of methods used. However, in only six articles were there details of the method employed (5, 9, 11, 14, 20, 21). In four of the studies the agar dilution method was used, a disc diffusion method was used in another, and a broth dilution method was used in the sixth. Regli et al. (20) carried out the widest study, testing 38 strains of *S. brevicaulis* against 10 antifungal drugs. They compared three solid media (Sabouraud dextrose agar, yeast malt agar, and Casitone agar) and concluded that *S. brevicaulis* was highly resistant to griseofulvine, tolnaftate, amphotericin B, and flucytosine and moderately sensitive to miconazole and ketoconazole. More data about the in vitro antifungal susceptibilities of clinical isolates of *Scopulariopsis* have appeared in some reports of human infections caused by these fungi (1, 2, 4, 5, 9, 11, 13, 14, 21). Amphotericin B was defined as ineffective every time it was tested (seven times). MICs of fluconazole and flucytosine were low for only one of four and one of six isolates, respectively. Miconazole and ketoconazole showed high MICs for half of the assayed isolates (2 of 4 in each case). Itraconazole, being active in three of the five tests, was the antifungal drug that showed the lowest MIC in these clinical reports, and another isolate was defined as relatively sensitive. This contrasts with our results, where itraconazole MICs for none of the 17 strains could be considered as indicating activity.

In general, our data confirm the high in vitro resistance of *Scopulariopsis* spp. reported by other authors. More data are needed to draw more valid conclusions about the susceptibility of *Scopulariopsis* both in vitro and in vivo and to determine whether in vitro testing is more reliable with conidia than with hyphae (8). Every clinical case report would therefore need to include data on in vitro antifungal susceptibility testing, in which the method used, the MICs, the treatment, and the clinical outcome would be described in detail. However, the data available make us pessimistic about an effective treatment for these infections, although the relative success shown by itraconazole in some clinical cases merits special attention. Experimental studies to test the effectiveness of this drug alone and/or in combination with others are required to confirm the sparse clinical data that exist. The finding of new compounds active against this refractory fungus constitutes a priority challenge to modern medicine.

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