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In Vitro Activities of a Wide Panel of Antifungal Drugs against Various *Scopulariopsis* and *Microascus* Species

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The *in vitro* activities of 11 antifungal drugs against 68 *Scopulariopsis* and *Microascus* strains were investigated. Amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole, miconazole, posaconazole, voriconazole, and ciclopirox showed no or poor antifungal effect. The best activities were exhibited by terbinafine and caspofungin, where the MIC and MEC (minimal effective concentration) ranges were 0.0313 to >16 μg/ml and 0.125 to 16 μg/ml, respectively. The MIC and MEC modes were both 1 μg/ml for terbinafine and caspofungin; the MIC_{50} and MEC_{50} were 1 μg/ml for both drugs, whereas the MIC_{90} and MEC_{90} were 4 μg/ml and 16 μg/ml, respectively.

The genera *Scopulariopsis* and *Microascus* include opportunistic fungal pathogens of humans. Taxonomically, they belong to the family Microascaceae within the class Sordariomycetes (Ascomycota). *Scopulariopsis* species are best known as the causative agents of onychomycoses, i.e., less common skin, subcutaneous, and deep tissue infections. They have been implicated in, for example, keratitis (1), sinusitis (2), bronchitis (3), endocarditis (4), meningitis (5), pulmonary infection (6), and disseminated mycoses (7). Infections due to *Microascus* species are locally invasive, involving organs such as the lungs (8), brain (9), and endocardium (10), or disseminated (11). The prognosis in invasive infections is poor, and many of the reported cases have ended in death. Therapeutic difficulties have been associated with patients' underlying disease, lack of clear guidelines for treatment, and resistance of the fungi to antimycotics (12–22). Data on the *in vitro* antifungal susceptibility of *Scopulariopsis* and *Microascus* are scant and relate almost exclusively to *Scopulariopsis brevicaulis*, which has been clinically the most frequently isolated species. Most of these studies have indicated that *S. brevicaulis* exhibited a multidrug-resistant phenotype (12–22). To the best of our knowledge, there have been only three published studies reporting on drug susceptibility results also for other than *S. brevicaulis* species (12, 13, 21). The purpose of this study was to evaluate the *in vitro* activities of 11 antifungal drugs against various *Scopulariopsis* and *Microascus* species, including rare species, which were not tested before.

A total of 68 fungal strains were evaluated: 23 *Microascus* and 45 *Scopulariopsis* strains, representing 10 and 16 species, respectively. All strains were purchased from the Centraalbureau voor Schimmelcultures (CBS) culture collection (Utrecht, The Netherlands). The list of strains tested is presented in Table S1 in the supplemental material. Fungal inocula were prepared from 14-day-old cultures in Czapek yeast agar using the method described previously (20). The following antifungal drugs were used in the study: 5-fluorocytosine (5FC), amphotericin B (AMB), caspofungin (CFG), ciclopirox (CPX), fluconazole (FLC), itraconazole (ITC), ketoconazole (KTC), miconazole (MCZ), posaconazole (POS), terbinafine (TRB), and voriconazole (VRC) (Sigma-Aldrich). Drug susceptibility assay was performed with the broth microdilution method outlined in Clinical and Laboratory Standards Institute (CLSI) document M38-A2 (23), with some modifications (20). Briefly, the final fungal inoculum densities used in the study were about 0.3×10^4 CFU/ml, and the incubation temperature of the microdilution trays was 27°C. The assay was validated by using *Aspergillus flavus* (ATCC 204304) as a quality control strain. The plates were examined on the first day that sufficient growth of fungi was present in the growth control well but not earlier than 48 h. *A. flavus* was examined after 48 h. The visual readings of MICs (for all drugs except CFG) and minimal effective concentrations (MECs) (for CFG) were made. MIC values were defined as the lowest drug concentrations corresponding to 100% growth inhibition (AMB, ITC, MCZ, POS, VRC), ≥50% reduction in growth (5FC, FLC, KTC), or ≥80% reduction in growth (CPX, TRB) compared to the growth in the growth control well (23) (we established the endpoint for MCZ). The MECs were defined as the lowest drug concentrations leading to the growth of small, rounded, compact hyphal forms in comparison to growth in the growth control well (23).

The results of drug susceptibility profiling in terms of species groups are summarized in Table 1 (for susceptibility profiles of individual strains, see Table S1 in the supplemental material). 5FC and FLC were inactive against all strains tested, as was ITC, except in the case of one *S. parva* strain. Other azoles, with the exception of KTC, and AMB had similar low activities, and only single strains showed low MIC values. These findings are consistent with previous studies performed on *S. brevicaulis* alone and together

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TABLE 1 MIC/MEC individual values and MIC/MEC ranges obtained for *Microascus* and *Scopulariopsis* species groups

Species name (no. of strains tested)	MIC/MEC parameter	MIC[a] (μg/ml)										MEC[a] (μg/ml)
		AMB	CPX	TRB	5FC	FLC	ITC	KTC	MCZ	POS	VRC	CFG
Microascus albonigrescens (2)	Individual values	8[b]	>16	0.5	>64	>64	>16	0.5[b]	1, >16	>16	8, >16	0.5, 8
M. caviariformis (1)	Individual value	1	>16	2	>64	>64	>16	0.5	4	>16	16	0.25
Microascus cinereus (3)	Range	>16	16–>16	1–2	>64	>64	>16	16[b]	4–>16	>16	16–>16	1–8
M. cirrosus (3)	Range	16–>16	8–>16	1[b]	>64	>64	>16	0.5–8	2–>16	>16[b]	2–16	1[b]
M. longirostris (3)	Range	2–>16	4–>16[b]	1–0.5[b]	>64	>64	>16	0.5–2	16–>16	>16	8–>16	0.5–1
M. manginii (4)	Range	16–>16[b]	>16	0.25–4	>64	>64	>16	4–8[b]	>16	>16	16–>16	0.5–16
M. nidicola (1)	Individual value	4	1	1	>64	>64	8	0.5	ND[c]	>16	4	ND
M. pyramidus (1)	Individual value	ND	>16	8	>64	>64	>16	ND	16	>16	4	1
M. senegalensis (2)	Individual values	16, >16	>16	1, >16	>64	>64	>16	1, 4	16, >16	0.5, >16	8, >16	8
M. trigonosporus (3)	Range	2–>16[b]	16–>16	1–2	>64	>64	>16	4–16[b]	>16	>16	>16	0.5–2
Scopulariopsis acremonium (3)	Range	4–>16	8–>16	0.5–2	>64	>64	>16	0.25–0.5	4–16	1–>16	2–8	0.125–0.25
S. asperula (3)	Range	2–8	>16	1	>64	>64	>16	8	>16	>16	16	1–2
S. brevicaulis (8)	Range	8–>16	>16	0.5–4	>64	>64	>16	4–16	>16	>16	>16	0.25–16
S. brumptii (4)	Range	>16[b]	4–>16[b]	0.0313–4	>64	>64	>16	8–16[b]	8–>16	>16	4–>16	0.25–8
S. canadensis (1)	Individual value	8	>16	0.5	>64	>64	>16	0.5	8	>16	8	8
S. carbonaria (3)	Range	4–>16	>16	0.0313–8	>64	>64	>16	0.5–8	8–>16	1–>16	1–>16	0.25–8[b]
S. chartarum (3)	Range	>16	>16	0.0625–4	>64	>64	>16	8	16–>16	>16	16–>16	0.25–16
S. coprophila (1)	Individual value	0.5	16	0.0313	>64	>64	>16	4	4	ND	16	0.25
S. croci (1)	Individual value	>16	>16	1	>64	>64	>16	2	>16	>16	16	8
S. flava (3)	Range	16–>16	>16	1–8	>64	>64	>16	1–8	>16	>16	16–>16	0.25–1
S. fusca (3)	Range	2–8	>16	1–2	>64	>64	>16	16	>16	>16	>16	0.5–1
S. gracilis (2)	Individual values	>16	>16	1	>64	>64	>16	8, 16	>16	>16	>16	0.5, 8
S. humicola (3)	Range	8–>16	2–>16	0.5–1	>64	>64	>16	2–4	8–>16	>16	8–>16	0.5–16[b]
S. koningii (3)	Range	4–>16	>16	1–8	>64	>64	>16	2–16	>16	>16	>16	0.5–16
S. murina (2)	Individual values	>16	>16	1	>64	>64	>16	2, 4	>16	>16	>16	1, 16
S. parva (2)	Individual values	0.25, 4	>16	0.0313[b]	>64	>64	0.25, >16	0.125, 2	0.25, 16	0.0313, 2	1, 16	0.125, 8

[a] AMB, amphotericin B; CPX, ciclopirox; TRB, terbinafine; 5FC, 5-fluorocytosine; FLC, fluconazole; ITC, itraconazole; KTC, ketoconazole; MCZ, miconazole; POS, posaconazole; VRC, voriconazole; MEC, minimal effective concentration; CFG, caspofungin.

[b] The results were not obtained for some strains because of poor or no fungal growth in the wells (i.e., with and/or without antifungal drug).

[c] ND, not determined.

with other *Scopulariopsis* and *Microascus* species (12–22). In our study, CPX had no activity, even against *S. brevicaulis*, for which good or moderate antifungal effect was previously demonstrated (19, 20, 24–26).

KTC had greater activity than other azoles (MIC range, 0.125 to 16 μg/ml; MIC mode, 8 μg/ml; MIC_{50}, 4 μg/ml; MIC_{90}, 16 μg/ml). The lowest MICs were recorded for *S. acremonium* and *S. parva* strains. Aguilar et al. (13) showed that different *Scopulariopsis* species reach MICs for KTC in the range of 1 to ≥16 μg/ml. Low KTC MICs were demonstrated for single *S. acremonium*, *S. brevicaulis*, *S. chartarum*, *S. koningii* (MICs, 1 μg/ml), and *S. candida* (MIC, 2 μg/ml) strains (13).

The highest antifungal activities were seen for TRB and CFG, whose MIC and MEC ranges, MIC/MEC modes, and MIC_{50}/MEC_{50}, and MIC_{90}/MEC_{90} values amounted to 0.0313 to >16, 1, 1, and 4 μg/ml and 0.125 to 16, 1, 1, and 16 μg/ml, respectively. Species most sensitive to TRB were *S. brumptii*, *S. chartarum*, *S. coprophila*, and *S. parva*. The lowest CFG MECs were observed for *S. acremonium*, *S. flava*, and *S. parva*. The TRB MIC values obtained were consistent with available data; the MICs for *S. brevicaulis* ranged from 0.01 to >16 μg/ml (14, 16, 20), whereas in the study by Sandoval-Denis et al. (12), who included other *Scopulariopsis* and *Microascus* species, the MIC for TRB ranged from 0.5 to 4 μg/ml. Studies on the efficacy of CFG against *Scopulariopsis* and *Microascus* are scarce and therefore quite ambiguous. Cuenca-Estrella et al. (16), when testing *S. brevicaulis*, established CFG MEC values in the range of 4 to ≥16 μg/ml. Similar results were obtained by Sandoval-Denis et al. (12) for *S. brevicaulis* and other *Scopulariopsis* and *Microascus* species (MEC range, 1 to 16 μg/ml). However, Odero et al. (21) demonstrated no CFG activity (MEC, ≥8 μg/ml) upon testing *S. acremonium*, *S. brevicaulis*, *S. brumptii*, *S. candida*, *S. flava*, *S. fusca*, and *S. koningii*.

In conclusion, our results indicate a high level of drug resistance among *Scopulariopsis* and *Microascus* species. Only TRB and CFG showed some *in vitro* efficacy against these fungi and thus may be successfully used for the treatment of their infections. CPX and azoles are by far the most ineffective agents, and AMB has limited activity. Some potency has been observed for amorolfine and other echinocandins (12, 25–27). However, the data are limited, and further studies are required to decisively determine the utility of these drugs against *Scopulariopsis* and *Microascus* fungi.

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