

In vitro antifungal susceptibility testing of *Scopulariopsis brevicaulis* strains using agar diffusion method[1]

Magdalena Skóra, Anna B. Macura

Department of Mycology, Chair of Microbiology, Jagiellonian University Medical College, 18 Czysta Street, 31-121 Kraków, Poland

Corresponding author: Magdalena Skóra; E-mail: magdalena.skora@uj.edu.pl

ABSTRACT. The genus *Scopulariopsis* is a common soil saprotroph and has been isolated from air, organic waste and also from plant, animal and human tissues. *Scopulariopsis* has mainly been associated in humans with superficial mycoses, but it has also been described as the cause of subcutaneous and invasive infections. The most common aetiological agent of infections in humans is *Scopulariopsis brevicaulis*. This species has been reported to be resistant *in vitro* to broad-spectrum antifungal agents available today. The aim of the study was to establish *in vitro* antifungal susceptibility of 35 *S. brevicaulis* strains against amphotericin B (AMB), flucytosine (FC), caspofungin (CAS), terbinafine (TER), ciclopirox (CIC), voriconazole (VOR), clotrimazole (CTR), miconazole (MCZ), econazole (ECO), ketoconazole (KET), itraconazole (ITR), and fluconazole (FLU). Antifungal susceptibility tests were evaluated by an agar diffusion method (Neo-Sensitabs, Rosco, Denmark). AMB, FC, CAS, ITR and FLU showed no antifungal activity against *S. brevicaulis*. TER, CIC, CTR, KET, VOR, ECO, and MCZ revealed inhibitory activity for *S. brevicaulis*, but it varied for each of the drugs. The best antifungal effect was observed for TER and CIC. All isolates had large inhibition zones for TER and CIC. CTR was also inhibitory for all tested *S. brevicaulis* isolates, but the diameters of inhibition zones were smaller than for TER and CIC. Nearly 89% isolates showed inhibition zones for KET and the mean diameter of the inhibition zone was comparable to CTR. The least antifungal activity exhibited VOR, ECO and MCZ. Because of the multiresistance of *S. brevicaulis*, infections due to this species may not respond to particular antifungal treatment and other therapeutic approaches should be considered e.g., combined therapy and/or surgery.

Key words: *Scopulariopsis brevicaulis*, antifungal susceptibility, Neo-Sensitabs

Introduction

The genus *Scopulariopsis* is widely spread in nature. It is a common soil saprotroph and has been isolated from air, organic waste and also from plant, animal and human tissues [1]. In immunocompetent patients *Scopulariopsis* has mainly been associated with superficial mycoses – predominatingly onychomycoses, rarely cutaneous infections. Usually it has been reported as secondary pathogen of the skin and nails, but it could also be a primary pathogen or a copathogen with dermatophyte [2,3]. *Scopulariopsis* has been described as a cause of subcutaneous, deep tissue and disseminated mycoses [1,4]. Invasive *Scopulariopsis* infections are relatively rare, but they have been increasingly reported during the last two decades, particularly in immunocompromised patients. *Scopulariopsis* has been found as a causative agent of pulmonary fungus ball, keratitis, endophthalmitis, sinusitis, otomycosis, endocarditis, cerebral phaeohyphomycosis and disseminated infections [5].

The genus *Scopulariopsis* includes more than 30 species, while *Scopulariopsis brevicaulis* is the most common aetiological agent of infections in humans [1,4,6]. Treatment of *S. brevicaulis* infections, both superficial and deep mycoses, is difficult because this species seems to be a multiresistant pathogen. It has been reported to be resistant *in vitro* to amphotericin B, flucytosine, terbinafine and azole compounds, but the information regarding the susceptibility of this species to antifungals is still sparse and somewhat contradictory [7,8].

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The aim of this study was to evaluate the *in vitro* antifungal activity of 12 drugs against clinical isolates and reference strains of *S. brevicaulis*.

Materials and methods

Strains

A total of 31 clinical isolates and 4 reference strains of *S. brevicaulis* (CBS 112377, CBS 119549, CBS 147.41, CBS 398.54) were evaluated. All clinical isolates were obtained from human nails or skin collected at the Department of Mycology, Chair of Microbiology, Jagiellonian University Medical College. Identification was based on the macroscopic and microscopic characteristics of the isolates in culture.

Antifungal susceptibility testing

Antifungal susceptibility tests were performed for 12 agents – amphotericin B (AMB), flucytosine (FC), caspofungin (CAS), terbinafine (TER), ciclopirox (CIC), voriconazole (VOR), clotrimazole (CTR), miconazole (MCZ), econazole (ECO), ketoconazole (KET), itraconazole (ITR), and fluconazole (FLU) using disc diffusion method in modified Shadomy medium (dextrose 10 g/l, asparagin 1.5 g/l, yeast extract 6.7 g/l, agar 15 g/l). Antifungal agents were obtained from the manufacturers as standardized tablets of 9 mm diameter (Neo-Sensitabs, Rosco Diagnostica, Denmark). Diffusible antifungal charge of tablets was 10 µg for AMB, 1 µg for FC, 5 µg for CAS, 30 µg for TER, 50 µg for CIC, 1 µg for VOR, 10 µg for CTR, 10 µg for MCZ, 10 µg for ECO, 15 µg for KET, 8 µg for ITR, 25 µg for FLU.

Fungal suspensions equivalent to a 1.0 MacFarland turbidity were prepared in sterile water from 7 to 14 days cultures grown on Czapek-Dox agar at 27°C. A 500 µl inoculum was spread over the surface of agar and plates were dried 30 min prior to placement of the antifungal tablets on the surface of the medium. After 3 days of incubation in reverse position at 27°C, the inhibition diameter areas around the tablets were measured.

Results

AMB, FC, CAS, ITR and FLU showed no antifungal activity against *S. brevicaulis* strains tested. Antifungal activity against *S. brevicaulis* isolates was revealed for TER, CIC, CTR, KET, VOR, ECO, and MCZ, but it very varied for each of drugs. Table 1 shows antifungal susceptibility tests results containing the diameters of the inhibition zones around the Neo-Sensitabs tablets obtained for each of *S. brevicaulis* isolate.

The best antifungal effect was observed for TER and CIC. All isolates have large inhibition zones for these antifungals – the mean diameter of the inhibition zones was 24.2 mm for TER and 24.3 mm for CIC.

CTR was also inhibitory for all tested *S. brevicaulis* isolates, but the diameter of inhibition zones was smaller than for TER and CIC with the mean diameter 13.3 mm. For 17 isolates (~49%) inside the inhibition area semi-inhibited colonies were observed.

Thirty one strains (~89%) showed inhibition zones for KET. The mean diameter of the inhibition zone was 13.4 mm, so it was comparable to CTR. Additionally, more isolates than for CTR – 27 isolates (~87%), showed inside the inhibition area partially inhibited colonies.

The least antifungal activity against *S. brevicaulis* isolates exhibited VOR, ECO and MCZ. Two isolates (~6%) revealed inhibition zones for VOR and ECO, and only one for MCZ. For VOR the growth inhibition around the tablet was not complete – inside the inhibition area there were semi-inhibited colonies. Also for ECO one isolate has partially inhibited colonies inside the inhibition area.

Discussion

Very little information exists about *in vitro* activity of antifungals against *S. brevicaulis*. Most studies have shown that *S. brevicaulis* is resistant *in vitro* to broad-spectrum antifungal agents including AMB, FC, TER, and azole compounds [8,9], but the results are sometimes contradictory, probably due to the variety of methods used.

In the previous antifungal susceptibility studies using microdilution methods *S. brevicaulis* usually exhibited high MICs values. Cuenca-Estrella et al. [10] revealed the resistance of *S. brevicaulis* to AMB, ITR, VOR, and ravuconazole (RAV) – $MICs_{90}$ for 29 isolates were >8 µg/ml for all antifungals tested. Cuenca-Estrella et al. [11] also showed that *S. brevicaulis* is resistant *in vitro* to posaconazole (POS) (MIC_{90} >8 µg/ml). Carrillo-Muńoz et al. [12] revealed high MICs for FLU (MIC_{90} >64 µg/ml) and VOR (MIC_{90}=16 µg/ml) against *S. brevicaulis* strains, however VOR had higher antifungal activity against this species. Aguilar et al. [13] testing 5 *S. brevicaulis* strains

Table 1. The results of antifungal susceptibility testing of 35 *S. brevicaulis* strains using disc diffusion method

No. of *S. brevicaulis* strain	Antifungal agents inhibition zone diameter [mm]											
	AMB	FC	CAS	TER	CIC	VOR	CTR	MCZ	ECO	KET	ITR	FLU
CBS 112377	0	0	0	26	26	0	14	0	0	13	0	0
CBS 119549	0	0	0	25	24	0	12	0	0	12	0	0
CBS 147.41	0	0	0	25	22	0	12	0	0	15	0	0
CBS 398.54	0	0	0	25	23	0	13	0	0	0	0	0
06/1174	0	0	0	25	24	12	17	0	0	17	0	0
06/610	0	0	0	23	23	0	13	0	0	12	0	0
06/777	0	0	0	22	25	0	12	0	0	11	0	0
07/1187	0	0	0	27	19	0	13	0	0	12	0	0
07/392	0	0	0	19	23	0	12	0	0	12	0	0
07/507	0	0	0	27	23	0	14	0	0	17	0	0
07/521	0	0	0	25	28	0	12	0	0	11	0	0
07/701	0	0	0	23	25	17	18	15	22	24	0	0
08/1184	0	0	0	25	27	0	15	0	11	13	0	0
08/1323	0	0	0	25	25	0	14	0	0	14	0	0
08/1356	0	0	0	22	23	0	14	0	0	12	0	0
08/479	0	0	0	27	25	0	15	0	0	15	0	0
08/585	0	0	0	26	21	0	13	0	0	12	0	0
08/699	0	0	0	22	24	0	11	0	0	11	0	0
08/958	0	0	0	25	24	0	11	0	0	13	0	0
08/D1	0	0	0	27	24	0	12	0	0	13	0	0
08/D2	0	0	0	22	23	0	12	0	0	0	0	0
09/1194	0	0	0	21	22	0	11	0	0	11	0	0
09/1316	0	0	0	26	19	0	16	0	0	14	0	0
09/205	0	0	0	28	26	0	13	0	0	0	0	0
09/2184	0	0	0	24	25	0	12	0	0	13	0	0
09/456	0	0	0	20	27	0	16	0	0	13	0	0
09/600	0	0	0	27	23	0	15	0	0	15	0	0
09/x	0	0	0	19	25	0	13	0	0	12	0	0
10/1161	0	0	0	19	27	0	11	0	0	12	0	0
10/1313	0	0	0	24	28	0	13	0	0	13	0	0
10/820	0	0	0	23	24	0	14	0	0	13	0	0
10/874	0	0	0	28	26	0	16	0	0	15	0	0
10/976	0	0	0	23	26	0	12	0	0	13	0	0
HIV 112/09	0	0	0	25	29	0	12	0	0	0	0	0
HIV 115/10	0	0	0	27	23	0	14	0	0	13	0	0
Range	–	–	–	19–28	19–29	–	11–17	–	–	0–24	–	–
Arithmetic mean ± SD	–	–	–	24.2 ± 2.6	24.3 ± 2.3	–	13.3 ± 1.8	–	–	13.4 ± 2.5	–	–

SD – standard deviation

found very high MICs for FC (MIC >128 µg/ml), FLU (MIC >64 µg/ml) and ITR (MIC >16 µg/ml). Tested strains exhibited lower MICs for AMB, KET and MCZ – MICs ranges were 1->16 µg/ml for AMB and KET, and 4->16 µg/ml for MCZ [13]. Carrillo-Muńoz et al. [14] showed that TER has greater antifungal activity against *S. brevicaulis* than ITR – geometric mean of MICs were 1.38 µg/ml and 16 µg/ml, respectively. Different results for TER susceptibility testing obtained Garcia-Effron group [15]. Twenty one strains of *S. brevicaulis* included in their study exhibited high MIC values for TER with geometric mean 12.3 µg/ml [15]. Cuenca-Estrella et al. [16] in the antifungal susceptibility tests of 32 clinical *S. brevicaulis* isolates stated also very high MICs for TER, as well as for AMB, FC, VOR, and ITR – geometric means of MICs were 14.4 µg/ml, 13 µg/ml, >64 µg/ml, 25.4 µg/ml, and >8 µg/ml, respectively. However AMB, VOR and TER

showed better activity *in vitro* than FC and ITR [16].

In the present study we investigated the antifungal susceptibility pattern of 31 clinical isolates and 4 reference strains of *S. brevicaulis*. We used Neo-Sensitabs agar diffusion method, which had been previously applied for this species by Carillo-Muńoz et al. [7,17,18] and Hryncewicz-Gwóźdź et al. [19]. The difference between our method and method applied by aforementioned researchers was the interpretation of the inhibition zones diameter, which in theirs studies allowed to classify strains as susceptible, susceptible-dose dependent/intermediate and resistant. The second difference was in the type of medium used. We and Carillo-Muńoz et al. [7,17,18] used modified Shadomy agar in contrast to Hryncewicz-Gwóźdź et al. [19], who applied Sabouraud agar.

In our research AMB, FC, CAS, ITR, and FLU showed no antifungal activity against *S. brevicaulis*. Hryncewicz-Gwóźdź et al. [19] similarly to us revealed for all *S. brevicaulis* strains resistance to AMB and FLU, but only 3 strains of 16 tested (~19%) were resistant to ITR, 8 strains (50%) exhibited sensitivity to ITR and 5 (~30%) were intermediate. Carillo-Muńoz et al. [7,17,18] also found *S. brevicaulis* resistance to AMB, FLU, and ITR – 80% of strains were resistant to AMB, 95% to FLU and 80% to ITR.

The present study showed antifungal activity of TER, CIC, CTR, KET, VOR, ECO, and MCZ against *S. brevicaulis* isolates. The excellent anti-*S. brevicaulis* effect for all isolates tested was observed for TER and CIC. CTR showed inhibitory property for all isolates, but its antifungal activity was definitely smaller than for TER and CIC. KET had also good antifungal activity against *S. brevicaulis* because nearly 89% isolates tested showed inhibition zones for this drug and its antifungal effect was comparable to CTR. Hryncewicz-Gwóźdź et al. [19] also revealed very good efficacy of TER and CIC – all strains tested were sensitive to those antifungals. They found high percentage of intermediate strains (~88%) for CTR, one strain was sensitive and one was resistant [19]. They revealed different results for KET – 15 strains (~94%) were resistant to KET and only one strain was intermediate [19]. Carillo-Muńoz et al. [17,18] reported similar results for TER – 80% of strains were susceptible, 15% intermediate and 5% resistant. They also showed antifungal activity against *S. brevicaulis* for CTZ and KET, and the inhibitory effect was better for CTZ than for KET [17,18]. Respectively, 30%, 60%, 10% and 5%, 55%, 40% of isolates were sensitive, intermediate and resistant to CTZ and KET [17,18].

In our study the lowest anti-*S. brevicaulis* activity was observed for MCZ, VOR, and ECO. Only 2 isolates revealed inhibition zones for VOR and ECO, and only one for MCZ. Similarly Hryncewicz-Gwóźdź et al. [19] showed high percentage of resistant strains to MCZ (~88%). In this study only 2 strains (~13%) were intermediate [19]. Carillo-Muńoz et al. [18] reported 5% of strains susceptible to MCZ and 5% intermediate. For ECO the percentages of susceptible, intermediate and resistant strains were 15%, 60% and 25% [18]. As for VOR Carillo-Muńoz et al. [7] revealed resistance for 90% of isolates and 10% were susceptible [7].

Conclusions

In general, our results confirm other authors' data that *S. brevicaulis* is resistant to broad spectrum antifungal agents available today. Because of the multiresistance of *S. brevicaulis*, infections due to this species may not respond to particular antifungal treatment and other therapeutic approaches should be considered e.g., combined therapy and/or surgery. Promising are results obtained by Cuenca-Estrella et al. [8], which has indicated that some combinations of antifungal agents (POS plus TER, VOR plus TER, ITR plus TER, AMB plus CAS, POS plus CAS, VOR plus CAS) exhibit *in vitro* synergy against *S. brevicaulis*.

References

[1] Bochenek M., Witalis J., Macura A.B. 2008. The occurrence and pathogenicity of the genus *Scopulariopsis*. *Mikologia Lekarska* 15: 104-108.

[2] Stefanato C.M., Verdolini R. 2009. Histopathologic evidence of the nondermatophytic mould *Scopulariopsis brevicaulis* masking the presence of dermatophytes in a toenail infection. *Journal of Cutaneous Pathology* 36 Suppl 1: 8-12.

[3] Bonifaz A., Cruz-Aguilar P., Ponce R.M. 2007. Onychomycosis by molds. Report of 78 cases. *European Journal of Dermatology* 17: 70-72.

[4] Petanović M., Tomić Paradzik M., Kristof Z., Cvitković A., Topolovac Z. 2010. *Scopulariopsis brevicaulis* as the cause of dermatomycosis. *Acta Dermatovenerologica Croatica* 18: 8-13.

[5] Salmon A., Debourgogne A., Vasbien M., Clément L., Collomb J., Plénat F., Bordigoni P., Machouart M. 2010. Disseminated *Scopulariopsis brevicaulis* infection in an allogeneic stem cell recipient: case

report and review of the literature. *Clinical Microbiology and Infection* 16: 508-512.
[6] Moreno G., Arenas R. 2010. Other fungi causing onychomycosis. *Clinics in Dermatology* 28: 160-163.
[7] Carillo-Muńoz A.J., Cárdenes C.D., Carrillo-Orive B., Rodríguez V., Del Valle O., Casals J.B., Ezkurra P.A., Quindós G. 2005. *In vitro* antifungal activity of voriconazole against dermatophytes and superficial isolates of *Scopulariopsis brevicaulis*. *Revista Iberoamericana de Micología* 22: 110-113.
[8] Cuenca-Estrella M., Gomez-Lopez A., Buitrago M.J., Mellado E., Garcia-Effron G., Rodriguez-Tudela J.L. 2006. *In vitro* activities of 10 combinations of antifungal agents against the multiresistant pathogen *Scopulariopsis brevicaulis*. *Antimicrobial Agents and Chemotherapy* 50: 2248-2250.
[9] Chung W.K., Sung H., Kim M.N., Lee M.W., Shin J.H., Choi J.H., Moon K.C., Koh J.K. 2009. Treatment-resistant *Scopulariopsis brevicaulis* infection after filler injection. *Acta Dermatovenereologica* 89: 636-638.
[10] Cuenca-Estrella M., Gomez-Lopez A., Mellado E., Garcia-Effron G., Monzon A., Rodriguez-Tudela J.L. 2005. *In vitro* activity of ravuconazole against 923 clinical isolates of nondermatophyte filamentous fungi. *Antimicrobial Agents and Chemotherapy* 49: 5136-5138.
[11] Cuenca-Estrella M., Gomez-Lopez A., Mellado E., Buitrago M.J., Monzon A., Rodriguez-Tudela J.L. 2006. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. *Antimicrobial Agents and Chemotherapy* 50: 917-921.
[12] Carrillo-Muńoz A.J., Giusiano G., Guarro J., Quindós G., Guardia C., del Valle O., Rodríguez V., Estivill D., Cárdenes C.D. 2007. *In vitro* activity of voriconazole against dermatophytes, *Scopulariopsis brevicaulis* and other opportunistic fungi as agents of onychomycosis. *International Journal of Antimicrobial Agents* 30: 157-161.
[13] Aguilar C., Pujol I., Guarro J. 1999. *In vitro* antifungal susceptibilities of *Scopulariopsis* isolates. *Antimicrobial Agents and Chemotherapy* 43: 1520-1522.
[14] Carrillo-Muńoz A.J., Giusiano G., Cárdenes D., Hernández-Molina J.M., Eraso E., Quindós G., Guardia C., del Valle O., Tur-Tur C., Guarro J. 2008. Terbinafine susceptibility patterns for onychomycosis-causative dermatophytes and *Scopulariopsis brevicaulis*. *International Journal of Antimicrobial Agents* 31: 540-543.
[15] Garcia-Effron G., Gomez-Lopez A., Mellado E., Monzon A., Rodriguez-Tudela J.L., Cuenca-Estrella M. 2004. *In vitro* activity of terbinafine against medically important non-dermatophyte species of filamentous fungi. *The Journal of Antimicrobial Chemotherapy* 53: 1086-1089.
[16] Cuenca-Estrella M., Gomez-Lopez A., Mellado E., Buitrago M.J., Monzón A., Rodriguez-Tudela J.L. 2003. *Scopulariopsis brevicaulis*, a fungal pathogen resistant to broad-spectrum antifungal agents. *Antimicrobial Agents and Chemotherapy* 47: 2339-2341.
[17] Carrillo-Muńoz A.J., Santos P., del Valle O., Casals J.B., Quindós G. 2004. Is amphotericin B active against dermatophytes and *Scopulariopsis brevicaulis*? *Revista Española de Quimioterapia* 17: 244-249.
[18] Carrillo-Muńoz A.J., Guglietta A., Palacín C., Casals J., del Valle O., Guardiŕ C., Rodríguez V., Quindós G. 2004. *In vitro* antifungal activity of sertaconazole compared with nine other drugs against 250 clinical isolates of dermatophytes and *Scopulariopsis brevicaulis*. *Chemotherapy* 50: 308-313.
[19] Hryncewicz-Gwóźdź A., Plomer-Niezgoda E., Baran E., Walów B., Czarnecka A. 2008. *In vitro* antifungal susceptibilities and enzymatic activities of *Scopulariopsis brevicaulis* isolates. *Mikologia Lekarska* 15: 209-212.

Ocena *in vitro* lekowrażliwości szczepów *Scopulariopsis brevicaulis* metodą dyfuzji w agarze

M. Skóra, A.B. Macura

Grzyby z rodzaju *Scopulariopsis* powszechnie występują w glebie jako organizmy saprotroficzne. Izolowane są także z powietrza, odpadów organicznych oraz z tkanek roślinnych, zwierzęcych i ludzkich. Zazwyczaj u ludzi powodują grzybice powierzchniowe, ale opisywano także przypadki zakażeń podskórnych i inwazyjnych. Najczęstszym czynnikiem etiologicznym zakażeń u ludzi jest *Scopulariopsis brevicaulis*. Gatunek ten jest uważany za oporny *in vitro* na wiele dostępnych dzisiaj leków przeciwgrzybiczych.

Celem pracy była ocena w warunkach *in vitro* wrażliwości 35 szczepów *S. brevicaulis* na amfoterycynę B (AMB), 5-fluorocytozynę (FC), kaspofunginę (CAS), terbinafinę (TER), cyklopiroks (CIC), worikonazol (VOR), klotrimazol (CTR), mikonazol (MCZ), ekonazol (ECO), ketokonazol (KET), itrakonazol (ITR) i flukonazol (FLU). Badanie lekowrażliwości wykonano metodą dyfuzji w agarze (Neo-Sensitabs, Rosco, Dania).

AMB, FC, CAS, ITR i FLU nie wykazywały aktywności przeciwgrzybiczej w stosunku do badanych szczepów *S. brevicaulis*. Właściwości prze-

ciwgrzybicze stwierdzono dla TER, CIC, CTR, KET, VOR, ECO i MCZ, przy czym aktywność przeciwgrzybicza była różna w zależności od leku. Największy efekt przeciwgrzybiczy wykazano dla TER i CIC. Wszystkie szczepy posiadały duże strefy zahamowania wzrostu dla powyższych leków. Aktywność w stosunku do wszystkich badanych szczepów *S. brevicaulis* wykazywał również CTR, przy czym średnice stref zahamowania wzrostu były mniejsze niż dla TER i CIC. Prawie 89% badanych izolatów miało strefy zahamowania wzrostu dla KET, a średnia średnica stref zahamowania wzrostu była porównywalna do CTR. Najniższą aktywność przeciwgrzybiczą wykazywały VOR, ECO i MCZ.

W związku z opornością na wiele leków *S. brevicaulis*, zakażenia wywołane przez ten gatunek mogą nie odpowiadać na poszczególne leki przeciwgrzybicze i może być konieczne zastosowanie innych rozwiązań terapeutycznych np. terapii skojarzonej i/lub interwencji chirurgicznej.

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