

## Saprotrophic microscopic fungi and dermatophytes accompanying infections of the skin and nails of patients in the Moravian-Silesian Region (Czech Republic)

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Over a 19-month period, the spectrum of saprotrophic microscopic fungi isolated from 245 patients in the Moravian-Silesian Region (Czech Republic) was analysed. Saprotrophic microscopic fungi were isolated from nails (90 %) and skin (10 %). None was isolated from hair. The material was the most frequently positive for the presence of *Scopulariopsis brevicaulis* (32.6 %) followed by *Cladosporium sphaerospermum* (5.3 %), *Aspergillus versicolor* (4.0 %), *Geomyces pannorum* (4.0 %) and others. Dermatophytes and saprotrophic microscopic fungi were both studied within one year and represented 1110 isolates. Dermatophytes were isolated in most of the cases and represented 943 isolations (85 %). The saprotrophic microscopic fungus *Scopulariopsis brevicaulis* is a known causative agent of onychomycosis. In the evaluation including dermatophytes it ended in the 3rd position with 5.2 % of isolations behind *Trichophyton rubrum* (80 %) and *T. mentagrophytes* (8 %).

**Key words:** saprotrophic microscopic fungi, dermatophytes, superficial mycose, *Scopulariopsis brevicaulis*

Lysková P. (2007): Saprotrofní mikroskopické houby a dermatofyty doprovázející infekce kůže a nehtů u pacientů v Moravskoslezském kraji (Česká republika). – Czech Mycol. 59(1): 125–137.

Během 19 měsíců bylo studováno druhové spektrum saprotrofních mikroskopických hub izolovaných od 245 pacientů v Moravskoslezském kraji (Česká republika). Saprotrofní mikroskopické houby byly izolovány z nehtů (90 %) a kůže (10 %). Žádný saprotrofní mikromycet nebyl izolován z vlasů. Kultivace materiálu byla nejčastěji pozitivní na přítomnost *Scopulariopsis brevicaulis* (32,6 %), následovaly *Cladosporium sphaerospermum* (5,3 %), *Aspergillus versicolor* (4,0 %), *Geomyces pannorum* (4,0 %) a další. Během jednoho roku byly spolu se saprotrofními mikroskopickými houbami studovány dermatofyty a dohromady představovaly 1110 izolátů. Ve většině případů byly izolovány dermatofyty s 943 izolacemi (85 %). Saprotrofní mikromycet *Scopulariopsis brevicaulis* je známým původcem onychomýkóz. Druh se při vyhodnocení spolu s dermatofyty umístil na třetím místě s 5,2 % za *Trichophyton rubrum* (80 %) a *T. mentagrophytes* (8 %).

## INTRODUCTION

For many years saprotrophic microscopic fungi have been cultivated, for example, from nails but they have been considered either contaminants or commensals and, therefore, ignored in the etiology of nail disease. These organisms were thought to be secondary to dermatophytic infections (Greer 1995). Now it is clear that non-dermatophytic micromycetes isolated from dystrophic nails and from skin may be true pathogens (Greer 1995). Particularly in a body weakened or damaged by another illness they are more often reported as agents of mycotic infections (Vosmík and Skořepová 1995) and the spectrum of these agents has broadened (Rozkošná 2000, Volleková 2000).

The most common non-dermatophytic filamentous fungi reported in the literature as cases of fungal nail and skin infections were: *Scopulariopsis brevicaulis*, *Aspergillus* sp. div., *Fusarium* sp. div. (Summerbell et al. 1989, Nsanze et al. 1995, Kemna and Elewski 1996, Bokhari et al. 1999, Dobiášová et al. 2000, Torres-Rodríguez and López-Jodra 2000, Tosti et al. 2000, Malátová 2003).

Keratinolytic properties represent an important criterion in evoking the infectious process in superficial mycosis. Keratinolytic fungi are thought to be potentially pathogenic (Ali-Shtayeh and Jamous 2000).

To prove a real causal relationship it is necessary to fulfil certain criteria: (1) positive microscopic (or histological) preparation; (2) massive growth of identical saprotrophic fungi, i. e. in all test-tubes in three subsequent investigations; (3) growth in pure culture (Vosmík and Skořepová 1995).

*Scopulariopsis brevicaulis* is the most frequent non-dermatophytic fungus causing infection of nails published in the literature. It is well established as an agent of onychomycosis (Sekhon and Garg 1986, Buchvald and Šimaljaková 1995, Greer 1995, Kemna and Elewski 1996, Křivanec 2000, Torres-Rodríguez and López-Jodra 2000, Tosti et al. 2000, Kuklová and Kučerová 2001). Sekhon and Garg (1986) also isolated *S. brevicaulis* from skin.

The article deals with the occurrence and representation of species of saprotrophic microscopic fungi in clinical material (skin, nails and hair) in patients of the Moravian-Silesian Region as possible causative agents of superficial mycosis during 19 months. It is based on the author's diploma thesis (Lysková 2004).

## MATERIAL AND METHODS

Material from patients of the Moravian-Silesian Region was collected in co-operation with the Institute of Public Health in Ostrava. Samples of clinical material (skin scales, nail fragments and hair) were taken from patients by doctors in the

region, were collected and brought to the Institute of Public Health in Ostrava. Skin scales, nail fragments and hair were inoculated in three test-tubes with Sabouraud glucose agar (SGA) with chloramphenicol, and with addition of cycloheximide (two test-tubes) or without addition of cycloheximide (Otčenášek et al. 1990) in the laboratory of clinical mycology. Fungal growth was examined after 3 weeks of incubation at a temperature of 26 °C. The identification of the fungi was based on the macroscopic and microscopic morphology of the colonies and, when necessary, subcultures were made on special agars.

Occurrence of both saprotrophic fungi and dermatophytes was studied during 2002 (Tab. 1). It was found that it would not be possible to carry out the study in the original extent because of the high number of dermatophyte isolates (especially *Trichophyton rubrum*), which could not be stored for technical reasons and because of difficult identification of saprotrophic fungi. A number of *T. rubrum* isolates, identified by workers in the mycological laboratory of the Institute of Public Health in Ostrava, was included into the study to demonstrate representation and proportion of fungi isolated during the year.

Dermatophytes were identified on SGA in the laboratory of clinical mycology at the Institute of Public Health in Ostrava during 2002 by the author with the exception of *Trichophyton rubrum*, as mentioned above.

The study deals with samples with positive growth of one species of saprotrophic microscopic fungus only, because positive growth of more than one species occurring in the original test-tube indicates contamination. Hence those saprotrophic fungi could not be considered as potential pathogens. Saprotrophic microscopic fungi were processed over 19 months from 1 January 2002 to 31 July 2003 (Tab. 1) at the Department of Botany, Charles University, Prague. Primary isolation of the fungi took place on SGA with chloramphenicol and cycloheximide, as mentioned above. One of the test-tubes did not contain cycloheximide to allow capture of saprotrophic fungi sensitive to cycloheximide. For identification of the saprotrophic fungi special media were used.

Isolated fungi were recorded along with patients data: sex, age and affected part of the body (skin, nails) when cultivation was positive for presence of saprotrophic fungus. Obtained data about the age of patients were divided into six age groups (Tab. 3) according to Foged and Nielsen (1982).

Data were evaluated statistically with CCA (canonical correspondence analysis).

## RESULTS

Saprotrophic microscopic fungi were isolated in 245 patients during the 19 months of the study (1 patient = 1 isolate). All cultivated fungi were primary isolated on SGA (as mentioned above), used as standard in medical mycology. Sev-

enty-two species of saprotrophic fungi were recorded (Tab. 2). Of the isolated fungi, the most common ones belonged to the anamorphic fungi. Fungi belonging to this group made up the major part of isolates with 226 isolations (92.2 %). Less often, representatives of the Zygomycota class Zygomycetes (4.5 %) and Ascomycota class Sordariomycetes (3.3 %) were isolated.

The most abundantly occurring saprotrophic micromycetes in the clinical material were *Scopulariopsis* spp. with 35.9 % (88 isolations) as shown in Tab. 2. The genus was represented by three species, the most common of which was *S. brevicaulis* (80 isolations). Another frequently represented genus was *Aspergillus* (12.7 %, 31 isolations) with 12 species, followed by *Penicillium* (10.2 %, 25 isolations) with twelve species. *Cladosporium* made up 9.8 % of isolations with 24 isolations and three species. *Geomyces pannorum*, the only representative of this genus represented 4.1 %. *Acremonium* (3.3 %) was represented by five species, of which three isolates were not successfully classified as species. The genus *Ulocladium* (2.9 %) was also represented by three species. *Alternaria alternata* was found six times. *Chrysosporium* was represented by four species identified in six samples.

The spectrum of fungi from the clinical material was very broad (Tab. 2). The most often isolated representatives were the anamorphic fungi, of which the most commonly isolated was *Scopulariopsis brevicaulis* (32.6 %). In total, it was recorded in eighty cases, each time from nails. The second most common representative was *Cladosporium sphaerospermum* (5.3 %), followed by *Aspergillus versicolor* (4.0 %), *Geomyces pannorum* (4.0 %), *Cladosporium cladosporioides* (3.7 %), *Penicillium chrysogenum* (3.7 %) and others. The most common ascomycete was *Chaetomium globosum* (1.6 %). The most frequent zygomycete species were *Mucor circinelloides* f. *circinelloides* (1.2 %) and *Rhizopus stolonifer* (1.2 %).

One hundred and thirty-three cases involved material taken from men (54 %), and 112 samples taken from women (46 %). This division according to gender showed approximately equal distribution. The result was not statistically significant ( $p > 0.05$ ).

Distribution of the age of patients from whom a saprotrophic micromycete was cultivated is given in Tab. 3. It was cultivated more rarely in children, adolescents and adults up to 29 year old. From ages 30 to 44 the number of the patients started to increase. However, the number of persons markedly increased after the age of 45. The result was not statistically significant ( $p > 0.05$ ).

Saprotrophic microscopic fungi were the most commonly isolated from nails (90 %), namely in 221 samples (Tab. 4). The remaining 10 % of isolates were from skin. None of the saprotrophic fungi was isolated from hair. The result was statistically significant ( $p < 0.05$ ).

**Tab. 1.** Number of isolates of saprotrophic microscopic fungi and dermatophytes isolated during the study.

| Period                    | Saprotrophic microscopic fungi | Dermatophytes | Total number of isolates |
|---------------------------|--------------------------------|---------------|--------------------------|
| 1. 1. 2002 – 31. 12. 2002 | 167                            | 943           | 1110                     |
| 1. 1. 2003 – 31. 6. 2003  | 78                             | —             | 78                       |
| <b>Total</b>              | <b>245</b>                     | <b>943</b>    | <b>1188</b>              |

**Tab. 2.** Systematic classification of isolated saprotrophic fungi.

| Species                                                         | Number of isolates (frequency %) | Clinical material |
|-----------------------------------------------------------------|----------------------------------|-------------------|
| <b>Phylum: Zygomycota</b>                                       | <b>11 (4.5 %)</b>                |                   |
| <i>Absidia glauca</i> Hagem                                     | 1                                | nails             |
| <i>Mucor circinelloides</i> f. <i>circinelloides</i> Tiegh.     | 3                                | nails             |
| <i>Mucor plumbeus</i> Bonord.                                   | 1                                | nails             |
| <i>Mycocladius ramosus</i> (Lindt) J.H. Mirza                   | 1                                | nails             |
| <i>Rhizopus arrhizus</i> A. Fisch.                              | 2                                | nails             |
| <i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.                     | 3                                | skin, nails       |
| <b>Phylum: Ascomycota</b>                                       | <b>8 (3.3 %)</b>                 |                   |
| <i>Eurotium repens</i> de Bary                                  | 1                                | nails             |
| <i>Eurotium amstelodami</i> L. Mangin                           | 2                                | nails             |
| <i>Chaetomium globosum</i> Kunze                                | 4                                | nails             |
| <i>Sordaria fimicola</i> (Roberge ex Desm.) Ces. et De Not.     | 1                                | nails             |
| <b>Anamorphic fungi</b>                                         | <b>226 (92.2 %)</b>              |                   |
| <i>Acremonium</i> cf. <i>tsugae</i> W. Gams                     | 1                                | nails             |
| <i>Acremonium strictum</i> W. Gams                              | 4                                | skin, nails       |
| <i>Acremonium</i> sp. div.                                      | 3                                | nails             |
| <i>Alternaria alternata</i> (Fr.) Keissl.                       | 6                                | nails             |
| <i>Arthrimum arundinis</i> (Corda) Dyko et B. Sutton            | 1                                | nails             |
| <i>Arthrimum phaeospermum</i> (Corda) M.B. Ellis                | 1                                | nails             |
| <i>Aspergillus candidus</i> Link                                | 1                                | nails             |
| <i>Aspergillus carbonarius</i> (Bainier) Thom                   | 1                                | nails             |
| <i>Aspergillus fumigatus</i> Fresen.                            | 4                                | skin, nails       |
| <i>Aspergillus niger</i> Tiegh.                                 | 3                                | nails             |
| <i>Aspergillus ochraceus</i> G. Wilh.                           | 2                                | nails             |
| <i>Aspergillus sclerotiorum</i> G.A. Huber                      | 2                                | skin, nails       |
| <i>Aspergillus sydowii</i> (Bainier and Sartory) Thom et Church | 4                                | nails             |
| <i>Aspergillus terreus</i> Thom                                 | 1                                | nails             |
| <i>Aspergillus unguis</i> (Weill and L. Gaudin) Thom et Raper   | 1                                | nails             |
| <i>Aspergillus ustus</i> (Bainier) Thom and Church              | 1                                | nails             |
| <i>Aspergillus versicolor</i> (Vuill.) Tirab.                   | 10                               | nails             |
| <i>Aspergillus wentii</i> Wehmer                                | 1                                | nails             |
| <i>Aureobasidium pullulans</i> (de Bary) Arnaud                 | 1                                | nails             |

|                                                              |                    |             |
|--------------------------------------------------------------|--------------------|-------------|
| <i>Beauveria bassiana</i> (Bals.-Criv.) Vuill.               | 2                  | nails       |
| <i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries  | 9                  | skin, nails |
| <i>Cladosporium herbarum</i> (Pers.) Link                    | 2                  | nails       |
| <i>Cladosporium sphaerospermum</i> Penz.                     | 13                 | nails       |
| <i>Fusarium oxysporum</i> Schldtl.                           | 2                  | skin, nails |
| <i>Fusarium solani</i> (Mart.) Sacc.                         | 1                  | skin        |
| <i>Geomyces pannorum</i> (Link) Sigler et J.W. Carmich.      | 10                 | skin, nails |
| <i>Humicola fuscoatra</i> Traaen                             | 1                  | nails       |
| <i>Chrysosporium keratinophilum</i> D. Frey ex J.W. Carmich. | 2                  | nails       |
| <i>Chrysosporium queenslandicum</i> Apinis et R.G. Rees      | 2                  | nails       |
| <i>Chrysosporium sulfureum</i> (Fiedl.) Oorschot et Samson   | 1                  | nails       |
| <i>Chrysosporium tropicum</i> J.W. Carmich.                  | 1                  | nails       |
| <i>Malbranchea pulchella</i> Sacc. and Penz.                 | 1                  | skin        |
| <i>Myriodontium keratinophilum</i> Samson et Polon.          | 1                  | nails       |
| <i>Oidiodendron cerealis</i> (Thüm.) G.L. Barron             | 1                  | nails       |
| <i>Paecilomyces variotii</i> Bainier                         | 1                  | nails       |
| <i>Penicillium aurantiogriseum</i> Dierckx                   | 2                  | nails       |
| <i>Penicillium camemberti</i> Thom                           | 1                  | nails       |
| <i>Penicillium</i> cf. <i>digitatum</i> (Pers.) Sacc.        | 2                  | skin, nails |
| <i>Penicillium</i> cf. <i>hirsutum</i> Dierckx               | 1                  | nails       |
| <i>Penicillium citrinum</i> Thom                             | 2                  | nails       |
| <i>Penicillium hirsutum</i> Dierckx                          | 1                  | nails       |
| <i>Penicillium chrysogenum</i> Thom                          | 9                  | nails       |
| <i>Penicillium italicum</i> Stoll                            | 3                  | skin, nails |
| <i>Penicillium piceum</i> Raper et Fennell                   | 1                  | nails       |
| <i>Penicillium polonicum</i> K.M. Zalessky                   | 1                  | nails       |
| <i>Penicillium verrucosum</i> Dierckx                        | 1                  | nails       |
| <i>Penicillium viridicatum</i> Westling                      | 1                  | nails       |
| <i>Phialophora heteromorpha</i> (Nannf.) C.J.K. Wang         | 1                  | skin        |
| cf. <i>Phialophora</i> sp.                                   | 1                  | nails       |
| <i>Sagenomella diversispora</i> (J.F.H. Beyma) W. Gams       | 1                  | nails       |
| <i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier            | 80                 | nails       |
| <i>Scopulariopsis brumptii</i> Salv.-Duval                   | 6                  | skin, nails |
| <i>Scopulariopsis candida</i> (Guég.) Vuill.                 | 2                  | nails       |
| <i>Stachybotrys atra</i> Corda                               | 2                  | nails       |
| <i>Tolyposcladium inflatum</i> W. Gams                       | 1                  | skin        |
| <i>Trichoderma harzianum</i> Rifai                           | 1                  | nails       |
| <i>Trichothecium roseum</i> (Pers.) Link                     | 1                  | nails       |
| <i>Ulocladium atrum</i> Preuss                               | 1                  | skin        |
| <i>Ulocladium botrytis</i> Preuss                            | 3                  | skin, nails |
| <i>Ulocladium chartarum</i> (Preuss) E.G. Simmons            | 3                  | nails       |
| <b>Total</b>                                                 | <b>245 (100 %)</b> |             |

From the total number of 245 isolates, *S. brevicaulis* represented the most often isolated species. It was isolated 80 times in total, which represents 1/3 of all isolated saprotrophic micromycetes. *S. brevicaulis* was each time isolated from pathologically changed nails. Based on the data obtained from patients of the Moravian-Silesian Region, in 61 % of cases men were affected (49 cases) and in 39 % women (31 cases). Children, adolescents and adults up to 29 years of age were very rarely among the persons affected by *S. brevicaulis* (Tab. 5). In persons between 30 and 44 years old a moderate increase of the illness appeared. Incidence of the illness markedly increased after the age of 45.

Occurrence of saprotrophic microscopic fungi was observed along with an incidence of dermatophytes during 2002 (Tab. 1). In total 1110 microscopic fungi were recorded from clinical material during the year. The most frequently isolated fungi were dermatophytes, which represented 943 isolates (85 %). The number of isolates equals the number of patients. Saprotrophic microscopic fungi were isolated during this period in 167 cases (15 %).

Since the role of most saprotrophic fungi in the etiology of superficial mycosis is debatable, only the occurrence of the dermatophytes and *S. brevicaulis* were further compared (Tab. 6). Dermatophytes along with *S. brevicaulis* represented 994 isolations in total in 2002. Of those, 943 isolations represented dermatophytes (95 %). *S. brevicaulis* made up the remaining 5 % with 51 isolations. The five percent occurrence of this species represented a relatively high incidence of onychomycosis. In comparison with other species of dermatophytes evoking superficial mycosis, *S. brevicaulis* was even ended in the 3rd position behind *Trichophyton rubrum* and *T. mentagrophytes*.

## DISCUSSION

### Isolated saprotrophic microscopic fungi

Saprotrophic micromycetes from skin and nails made up a total of 245 isolations representing 72 species (Tab. 2). Isolated fungi could either really have caused the disease or commensals, spores accidentally being caught on the body of a patient and contaminants coming from the laboratory.

Of 72 recorded species, 33 were mentioned in the literature as agents causing superficial mycosis. These are: *Acremonium strictum*, *Alternaria alternata*, *Arthrimum phaeospermum*, *Aspergillus candidus*, *A. fumigatus*, *A. niger*, *A. sclerotiorum*, *A. sydowii*, *A. terreus*, *A. unguis*, *A. ustus*, *A. versicolor*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Fusarium oxysporum*, *F. solani*, *Geomyces pannorum*, *Chaetomium globosum*, *Chryso-sporium keratinophilum*, *C. queenslandicum*, *C. tropicum*, *Mucor circinelloides* f.

*circinelloides*, *Oidiodendron cerealis*, *Paecilomyces variotii*, *Penicillium chrysogenum*, *P. piceum*, *Rhizopus arrhizus*, *Scopulariopsis brevicaulis*, *S. brumptii*, *S. candida* and *Ulocladium chartarum* (Jesenská 1994, Jesenská 1998, de Hoog et al. 2000, Otčenášek et al. 2000, Anadolu et al. 2001).

Some of the detected species were presented in the literature as agents of other human diseases, or possibly of animals, which suggests their ability to evoke the infection process: *Aspergillus ochraceus* (Bassiouny et al. 1982), *Beauveria bassiana* (Sachs et al. 1985), *Malbranchea pulchella* (Benda and Corey 1994), *Myriodontium keratinophilum* (Maran et al. 1985), *Penicillium aurantiogriseum* (syn. *P. cyclopium*) (Bengoa et al. 1994), *P. citrinum* (Mok et al. 1997), *Rhizopus stolonifer* (Ferry and Abedi 1983) and *Trichoderma harzianum* (Guarro et al. 1999).

Saprotrophic micromycetes presented in Lysková (2004) were isolated only from samples of nails (90 %) and skin (10 %). None of the micromycetes were isolated from the hair. It seems likely that hair is not a suitable substrate for microscopic fungi. From the results it follows that the most suitable substrate is nails. Primarily they could be micromycetes present there as harmless saprotrophs finding a suitable refuge especially in dirt under the nails changing from harmless saprotrophs into opportunity pathogens if they have optimal conditions. These were isolated from the skin less often. Dry and uncovered skin most likely does not represent optimal conditions for fungi that demand moisture.

Most of the authors mention onychomycosis caused by the species *Scopulariopsis brevicaulis*, *Aspergillus* sp. div., *Fusarium* sp. div. and *Acremonium* sp. div. (Summerbell et al. 1989, Kemna and Elewski 1996, Bokhari et al. 1999, Gugnani 2000, Torres-Rodríguez and López-Jodra 2000, Tosti et al. 2000). Cases caused by saprotrophic microscopic fungi must be supported by meeting criteria demonstrating the real causal relationship mentioned above. Applying these criteria is relatively unlikely, knowing that in dermatomycology it is necessary to inoculate each sample in nine test-tubes to achieve growth of the fungi in at least one of them (Vosmík and Skořepová 1995). Another element that complicated meeting of all criteria was the presence of cycloheximide in the media. Authors who studied the impact of media containing cycloheximide on the growth of some saprotrophic fungi found that the use of media containing cycloheximide inhibits identification of many saprotrophic fungi that may be potential pathogens (Fragner and Belšan 1974a, Onsberg 1979, Summerbell et al. 1989).

The most often encountered species from nails was *Scopulariopsis brevicaulis*, which represented a third (32.6 %) of all identified saprotrophic fungi. It is often mentioned in the literature as an agent of nail disease (Fragner and Belšan 1974a, Fragner and Belšan 1974b, Sekhon and Garg 1986, Buchvald and Šimaljaková 1995, Greer 1995, Kemna and Elewski 1996, Křivanec 2000, Tosti et al. 2000, Torres-Rodríguez and López-Jodra 2000, Kuklová and Kučerová 2001).



**Tab. 3.** Number of isolates of saprotrophic microscopic fungi per age groups.

| Age group            | 1    | 2     | 3     | 4     | 5     | 6   |
|----------------------|------|-------|-------|-------|-------|-----|
| Number of isolations | 6    | 12    | 33    | 120   | 62    | 12  |
| Age                  | 0–14 | 15–29 | 30–44 | 45–59 | 60–74 | >75 |

**Tab. 4.** Number of isolates of saprotrophic microscopic fungi from skin and nails.

|                      | Affected part |       |
|----------------------|---------------|-------|
|                      | Skin          | Nails |
| Number of isolations | 24            | 221   |
| Frequency (%)        | 10            | 90    |

**Tab. 5.** Number of isolates of *Scopulariopsis brevicaulis* in different age groups.

| Age group            | 1 | 2 | 3  | 4  | 5  | 6 |
|----------------------|---|---|----|----|----|---|
| Number of isolations | 1 | 1 | 13 | 41 | 19 | 5 |

**Tab. 6.** Frequency of dermatophytes and *Scopulariopsis brevicaulis* (2002).

| Microscopic fungus                 | Number of isolates | Frequency (%) |
|------------------------------------|--------------------|---------------|
| <i>Trichophyton rubrum</i>         | 795                | 80            |
| <i>Trichophyton mentagrophytes</i> | 80                 | 8             |
| <i>Scopulariopsis brevicaulis</i>  | 51                 | 5.2           |
| <i>Trichophyton interdigitale</i>  | 47                 | 4.7           |
| <i>Microsporum canis</i>           | 10                 | 1             |
| <i>Epidermophyton floccosum</i>    | 8                  | 0.8           |
| <i>Microsporum gypseum</i>         | 3                  | 0.3           |
| <b>Total</b>                       | 994                | 100           |

In the evaluation of all acquired data from patients in the Moravian-Silesian Region, saprotrophic micromycetes were isolated almost with the same frequency in men (54 %) and in women (46 %). The number of persons that were positive in cultivation for saprotrophic micromycetes gradually increased with age. The highest number of micromycetes was recorded in persons aged 45 to 59. As some of these fungi did not represent real cases of disease, the results probably reflect a relative distribution in various age groups of patients who were sent to the doctor with suspicion of superficial mycosis. This presumption would also support the almost equal representation of both sexes.

### **Keratinophilic and keratinolytic properties of saprotrophic fungi**

Most of the species isolated in Lysková (2004) were already earlier mentioned as agents of human disease, or of animal disease. The possible evoking of disease in skin and nails (or hair) by microscopic fungi lies in its ability to colonise or use keratin or compounds of its disintegration. Based on data in the literature (Bahuguna and Kushwara 1989, Ali-Shtayeh and Jamous 2000, Marchisio 2000, Marchisio et al. 2000) most of the species isolated in Lysková (2004) would support that presumption. An important aspect is the intraspecific variability of the ability to colonise and disintegrate keratin (Marchisio 2000).

Species in which keratinophilic or keratinolytic properties has so far not been demonstrated with the highest probability could not be real causative agents of disease. Those species could present contaminants or commensals already be present in clinical samples after poor cleaning of the lesion or get into a test-tube from the surrounding environment by the inoculation of material.

Of the species isolated in Lysková (2004), the following species and genera, with respect to their keratinophilic or keratinolytic properties, would be chosen as possible agents of disease: *Acremonium* sp. div., *Alternaria alternata*, *Aspergillus fumigatus*, *A. sclerotiorum*, *A. sydowii*, *A. terreus*, *A. unguis*, *A. versicolor*, *Aureobasidium pullulans*, *Beauveria bassiana*, *Fusarium oxysporum*, *F. solani*, *Geomyces pannorum*, *Chaetomium globosum*, *Chrysosporium keratinophilum*, *C. queenslandicum*, *C. tropicum*, *Malbranchea pulchella*, *Myriodontium keratinophilum*, *Rhizopus stolonifer*, *Scopulariopsis brevicaulis*, *S. brumptii* and *S. candida*.

### **Dermatophytes and saprotrophic microscopic fungi isolated in 2002**

One thousand one hundred and ten isolates of dermatophytes and saprotrophic microscopic fungi were isolated from clinical material in 2002 (Tab. 1). The predominant part was represented by dermatophytes (943 isolations, 85 %). Saprotrophic microscopic fungi represented the remaining 15 % (167 isolations). Some authors (Kemna and Elewski 1996, Tosti et al. 2000) mention that attacking nails by non-dermatophytic micromycetes is not common, with frequencies differing from 1.45 % to 17.6 %. It is necessary to emphasise that the 15 % representation of saprotrophic fungi discovered in Lysková (2004) comprises contaminants as well as possible causative agents of disease (potential pathogens). Therefore, the presented relative representation of real causative agents of disease will be considerably lower. Nielsen (1983) states that proper washing of skin lesions with ethanolether reduces the isolation of saprotrophic fungi by about 17 % in comparison with cycloheximide added to SGA.

## Dermatophytes and *Scopulariopsis brevicaulis* isolated in 2002

*Scopulariopsis brevicaulis* is a saprotrophic microscopic fungus well known as a true cause of onychomycosis. Its evaluation together with dermatophytes (Tab. 6) in Lysková (2004) detected a 5 % representation of the species in 2002. The species was found to be the third most common cause of superficial mycosis. The representation of *S. brevicaulis* differs markedly from different authors (Sekhon and Garg 1986, Kemna and Elewski 1996, Bokhari et al. 1999, Křivanec 2000, Kuklová and Kučerová 2001, Křivanec 2003). Vosmík and Skořepová (1995) mention a maximum representation of 3 % as an agent of nail diseases caused by the species. *S. brevicaulis* exceeded the 3 % limit in Lysková (2004).

## Mixed fungal infections

One isolate was isolated from each patient. Mixed fungal infections were not recorded. A possible explanation is, that one fungus could overgrow or suppress the growth of another one. A number of studies report different values of incidence of mixed infections. This variation reflects the difficulty of proving the etiology of such infections (Greer 1995).

Species recorded in Lysková (2004) represent the basic spectrum of saprotrophic micromycetes isolated from skin and nails of patients in the Moravian-Silesian Region. It is necessary to provide good conditions for growth of fungi to meet all criteria for demonstrating the causal relationship between saprotrophic microscopic fungi and superficial mycosis.

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