The Frequency of Superficial Mycoses According to Agents Isolated During a Ten-Year Period (1999-2008) in Zagreb Area, Croatia

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SUMMARY Fungal infections involving the skin, hair and nails represent one of the most common mucocutaneous infections. Significant changes in the epidemiology, etiology and clinical pattern of mycotic infections have been observed during the last years. The aim of this retrospective study was to determine the incidence and the etiologic factors of superficial fungal infections in Zagreb area, Croatia, over a 10-year period (1999-2008). A total of 75828 samples obtained from 67 983 patients were analyzed. Dermatomycosis was verified by culture in 17410 (23%) samples obtained from 16086 patients. Female patients were more commonly affected than male (59% vs. 41%). Dermatophytes were responsible for 63% of all superficial fungal infections, followed by yeasts (36%) and molds (1%). *Trichophyton (T.) mentagrophytes* (both *var. interdigitalis* and *var. granulosa*) was the most frequent dermatophyte isolated in 58% of all samples, followed by *Microsporum (M). canis* (29%) and *T. rubrum* (10%). The most common clinical forms of dermatomycosis were onychomycosis (41%), tinea corporis (17%) and tinea pedis (12%). *Candida* spp. was mainly isolated from fingernail debris.

KEY WORDS: dermatomycosis, tinea, dermatophytes, Zagreb area

INTRODUCTION

Significant changes in the epidemiology, etiology and clinical pattern of mycotic infections have been observed during the last years (1). Fungal infections involving skin, hair and nails represent one of the most common mucocutaneous infections. Causative agents mostly include dermatophytes, yeast and nondermatophyte molds. The aim of this study was to identify the predominant causative organisms of superficial fungal infections in Zagreb area on a large number of patients and to assess the past and present trends accordingly, so that appropriate diagnostic and treatment strategies can be developed.

PATIENTS AND METHODS

This was a retrospective study of superficial mycosis carried out in the Reference Laboratory of Dermatological Mycology and Parasitology of the Ministry of Health and Social Welfare of the Republic of Croatia (Mycology Laboratory) at the...
University Department of Dermatology and Venereology, Zagreb University Hospital Center and University of Zagreb School of Medicine, Zagreb, Croatia, during a 10-year period, from January 1999 to December 2008. Croatia has about 4.5 million inhabitants, and Zagreb, its capital, about 1 million. A representative number of superficial mycosis patients converge at our Reference Laboratory, especially for diagnosis confirmation. Most samples were collected from patients referred to our laboratory and only a minority of samples were taken at other laboratories throughout Croatia and later sent to our Laboratory for a second opinion.

Data on age, sex and location of infection were recorded for each patient. The material taken from skin lesions and/or nails and involved hair was examined by direct microscopy with 20% KOH solution. The identification of the fungi was based on macroscopic morphology and microscopic characteristics of the colony on the modified Sabouraud medium. Statistical analysis was performed using the SPS system.

RESULTS

A total of 75828 samples were obtained from 68014 patients with clinically suspected dermatomycosis. Out of these samples, 17410 (23%) were positive. There were 16086 patients with culture proven dermatomycosis. A higher infection rate was observed in female patients (6535 male patients and 9551 female patients were affected). Dermatophytes were most frequently isolated and accounted for 63% (n=11071) of all positive samples. Yeasts were identified in 36% (n=6227) and non-dermatophytic molds in 1% (n=112) of samples (Table 1, Fig. 1). The most common clinical forms of dermatomycosis were onychomycosis (41%), tinea corporis (17%) and tinea pedis (12%).

Table 1. Fungal species isolated in Zagreb area between 1999 and 2008

<table>
<thead>
<tr>
<th>Species isolated</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. mentagrophytes</td>
<td>6473</td>
<td>37.18</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>6227</td>
<td>35.77</td>
</tr>
<tr>
<td>M. canis</td>
<td>3198</td>
<td>18.37</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>1135</td>
<td>6.52</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>112</td>
<td>0.64</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>99</td>
<td>0.57</td>
</tr>
<tr>
<td>E. floccosum</td>
<td>80</td>
<td>0.46</td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>62</td>
<td>0.36</td>
</tr>
<tr>
<td>T. violaceum</td>
<td>23</td>
<td>0.13</td>
</tr>
<tr>
<td>T. schoenleinii</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>17410</td>
<td>100</td>
</tr>
</tbody>
</table>

Onychomycosis

a) Fingernail onychomycosis

A total of 3458 isolates were obtained from infected fingernail debris. Candida spp. was the predominant isolate, accounting for 88% (n=3059) of positive cultures. The incidence of Candida infection increased constantly during the study period, from 238 isolates in 1999 to 326 isolates in 2008. Dermatophytes were responsible for 10% (n=320) of fingernail infection. Trichophyton (T.) mentagrophytes (85%, n=273) was the predominant dermatophyte followed by T. rubrum (11%, n=34), Microsporum (M.) canis (3%, n=10) and Microsporum (M.) gypseum (1%, n=3). The incidence of all dermatophyte species remained relatively
constant. The nondermatophyte mold *Aspergillus* spp. was isolated in 79 cases, accounting for 2% of fingernail onychomycosis. The total number of *Aspergillus* spp. isolates varied from year to year, ranging from 1 isolate in 1999 to 21 isolates in 2008 (Fig. 3).

b) Toenail onychomycosis
A total of 3455 isolates were obtained from toenails. In contrast to fingernail onychomycosis, which was predominately caused by *Candida* spp., dermatophytes accounted for most cases (80%, n=2775) of toenail onychomycosis. The incidence of toenail onychomycosis increased nearly 2-fold during the 10-year period, due to the upward trend in the incidence of *T. mentagrophytes* and *T. rubrum*. *Candida* spp. accounted for 19% (n=652) of all fungal isolates. *T. mentagrophytes* was the most frequently isolated dermatophyte, accounting for 85% (n=2360) of all toenail-derived dermatophyte isolates. *T. rubrum* ranged second, accounting for 14% (n=401) of dermatophyte isolates. There were sporadic cases of toenail onychomycosis caused by *M. canis* (n=7), *E. floccosum* (n=4), *T. violaceum* (n=2) and *M. gypseum* (n=1). The incidence of *T. mentagrophytes* was relatively constant till 2003 when a steep incidence increase of 55.49% was noted. A significant increase was recorded in the incidence of *T. rubrum*, from only 5 isolates in 1999 to 106 isolates in 2008. The nondermatophyte mold *Aspergillus* spp. was sporadically isolated, accounting for 1% (n=28) of all fungal isolates (Fig. 3).

In 221 nail samples that were referred from other laboratories data of the exact location (fingernail or toenail) were missing. In these samples, *Candida* spp. was the main isolate accounting for 67%, followed by dermatophytes (31%) and molds (2%) (Fig. 3).

Figure 3. Frequency of fungal isolates from nail debris.

**Figure 4.** Annual distribution of the most common fungal species isolated from samples obtained from the glabrous skin.

**Fungal infection of glabrous skin**
From different body sites involving the glabrous skin, 2879 isolates were obtained. Dermatophytes comprised the majority of isolates with the incidence of 90% (n=2600). *M. canis* was the main etiologic agent accounting for 47% (n=1230) of all dermatophytic isolates, followed by *T. mentagrophytes* (40%, n=1049) and *T. rubrum* (8%, n=217). *M. gyipseum*, *T. tonsurans*, *E. floccosum* and *T. violaceum* were isolated in 54, 25, 17 and 8 samples, respectively. The incidence of *M. canis* varied over years and a slight decrease was recorded. A steep decrease was observed in the incidence of *T. mentagrophytes*, from 128 cases in 1999 to 73 in 2008. The incidence of *T. rubrum* increased 6-fold during the study period, comprising 7 isolates in 1999 and increasing to 42 in 2008 (Fig. 4).

From the gluteal and genital region 890 isolates were obtained. The incidence of *Candida* spp. was high, accounting for 97% of all isolates. The incidence of dermatophytes (*T. mentagrophytes* and *T. rubrum*) remained low (3%) (Fig. 4).

**Fungal infection of the groin**
A total of 792 isolates were obtained from the groin. *Candida* spp. was isolated in 44% (n=349) of cases and its incidence stayed relatively constant during the study period. Dermatophytes accounted for 56% (n=443) of isolates, with *T. mentagrophytes* being the major dermatophytic pathogen (65%, n=289), followed by *T. rubrum* (28%, n=126) and *E. floccosum* (4%, n=19). *M. canis* was isolated in 8 samples and *M. gyipseum* in 1 sample.

**Tinea capitis**
*Tinea capitis* was culture-proven in 1767 samples. *M. canis* ranked first among the causative
agents of tinea capitis (92%, n=1617), *T. mentagrophytes* and *M. gypseum* representing 6% (n=104) and 1% (n=18) of isolates, respectively. *T. tonsurans* was isolated in 14 hair samples. Anthropophilic *T. violaceum*, *T. rubrum* and *T. schoenleinii* were isolated in 8, 5 and 1 patient, respectively (Fig. 5).

**Fungal infection of the foot**

Fungal infection of the foot caused by dermatophytes, was confirmed by culture in 1774 samples. Regarding the etiologic agents, the most common dermatophyte responsible for 84% (n=1495) of all tinea pedis infections was *T. mentagrophytes*, followed by *T. rubrum* (13%, n=239) and *E. floccosum* (2%, n=33). *M. canis* was isolated in 6 samples and *T. tonsurans* in 1 sample. *Candida* spp. was isolated in 17% (n=367) of foot-derived samples.

**Fungal infection of the hand**

A total of 874 isolates were obtained from the hand. Dermatophytes were the major pathogen accounting for 65% (n=570) of all fungal isolates, with *T. mentagrophytes* being the most commonly isolated dermatophytic pathogen (77%, n=437). *T. rubrum* and *M. canis* ranked second and third, accounting for 13% (n=75) and 7% (n=41) of dermatophytic isolates, respectively. *M. gypseum*, *E. floccosum* and *T. tonsurans* were isolated in 10, 5 and 2 cases, respectively. *Candida* spp. accounted for 35% (n=304) of all fungal isolates.

**Fungal infection of the glabrous skin of the face**

Regarding the glabrous skin of the face, 867 isolates were obtained. Dermatophytes accounted for 76% (n=662) of isolates, with *T. mentagrophytes* being the major dermatophytic isolate (51%, n=338), followed by *M. canis* (40%, n=264) and *T. rubrum* (4%, n=25). *Candida* spp. was isolated in the remaining 24% (n=205) of samples. The overall incidence of tinea faciei declined by 32% due to a decrease in the incidence of both yeast and dermatophytes.

**Tinea barbae**

From beard and mustache, 66 isolates were obtained. *T. mentagrophytes* was the major pathogen accounting for 68% (n=45) of all dermatophytic isolates. *M. canis* ranked second, accounting for 22% (n=14) of dermatophytic isolates, followed by *T. tonsurans* (6%, n=4) and *T. rubrum* (3%).

**DISCUSSION**

During the last decade, changes in fungal spectrum were noted (1). In Central and Northern Europe, since the fifties of the last century, *T. rubrum* is the most common dermatophyte, accounting for 80%-90% of strains, mainly due to the increased incidence of patients with toenail onychomycosis and tinea pedis (2). In Mediterranean countries, an increase of *M. canis* infections has been recorded during the past decades and *M. canis* has become one of the major pathogens, especially in tinea capitis infections, also causing the kerion type which has previously been “reserved” for *Trichophyton* spp. only (2,3). Zoophilic dermatophytes such as *T. verrucosum* and *M. canis* are the most prevalent species in southern Europe and Arabic countries (2). During the study period, dermatophytes remained the most common fungal pathogens with the exception of finger onychomycosis and mycosis of the genital and perigenital region, which were mainly due to *Candida* spp. An overall increase in the incidence of fingernail onychomycosis was due to the upward trend in the incidence of *Candida* spp. infection.

*T. mentagrophytes* was the major etiologic pathogen isolated from toenail debris, face and beard samples, feet and hands. Variations in the incidence were noted over years, ranging from 53% to 63% of all dermatophytic isolates. *M. canis* remained the main etiologic agent of tinea corporis and tinea capitis, especially in children. An overall decrease was observed in the incidence of *M. canis*, from 37% to 22%.

During the study period, a significant increase in the incidence of *T. rubrum* infection was recorded, ranging from 5% of all dermatophytic isolates in 1999 to 22% in 2008, mainly due to the increase of *T. rubrum* caused toenail onychomycosis, tinea corporis and tinea cruris.
The incidence of other dermatophytes such as M. gypseum, T. tonsurans, T. violaceum and E. floccosum remained low.

Aspergillus spp. was mainly isolated from fingernail and toenail debris. These results should be interpreted carefully as Aspergillus might be a contaminant rather than the true etiologic agent.

**Onychomycosis**

In our study, onychomycosis was the main clinical type encountered and it accounted for 41% of all fungal infections. According to literature data, onychomycosis represents up to 30% of superficial mycotic infections diagnosed (4). Analysis of fingernail and toenail onychomycosis showed an inverse relationship between dermatophytes and yeasts. Whereas Candida spp. was the main etiologic agent isolated from fingernails, dermatophytes prevailed in toenails in 80% of isolates. Among dermatophytes, T. mentagrophytes was the main isolate, accounting for 68% of toenail and 8% of fingernail onychomycosis. In Western, some parts of Central Europe and USA, T. rubrum is the dermatophytic pathogen most frequently isolated from toenail debris (4-8). In the USA and Southern Europe, a high prevalence of Candida spp. was noted in fingernail isolates (6,9). In geographical regions with warm climate, Candida spp. might be regarded as an etiologic agent (and not only as a contaminant), while findings of nondermatophytic filamentous fungi must be analyzed critically (2,9,10).

**Fungal infection of glabrous skin**

Fungal infection of glabrous skin ranked second in the incidence and accounted for 17% of all superficial fungal infections. The most frequently isolated fungal species were M. canis (42%), T. mentagrophytes (36%) and Candida spp. (10%). Results of the studies from our neighboring countries and some other Mediterranean countries demonstrated M. canis to be the major pathogen causing tinea corporis (11-13). In the USA, T. rubrum is the major pathogen responsible for tinea corporis (2,14).

**Fungal infection of the groin**

The incidence of fungal infection of the groin was 4%. Candida spp. was the major pathogen isolated in 44% of samples, followed by T. mentagrophytes (36%) and T. rubrum (16%). A remarkable decline of 69% was noted in the incidence of T. mentagrophytes. A 6-fold increase in the incidence of T. rubrum was recorded. Accordingly, in 2007 and 2008, T. rubrum was the major dermatophytic pathogen accounting for 57% and 70% of dermatophytic isolates, respectively. Other studies showed T. rubrum to be the major dermatophytic pathogen of tinea cruris (5-7,15).

**Tinea capitis**

The etiology of tinea capitis varies according to geographical and climatic regions. With the introduction of griseofulvin in 1958, M. audouinii and T. schoenleinii, once the main pathogens, have been practically eradicated in developed countries (16-18). In Mediterranean countries as well as in Slovenia, Bosnia and Herzegovina, Austria, Hungary, Germany and Poland, M. canis remained the main causative agent (3,11,14,19-22). In North America and in the UK and Ireland, a significant increase in the incidence of T. tonsurans has been recorded, achieving almost exclusive proportions in tinea capitis infections (up to 95.8%) (3,6,23,24). Due to migration of the population, an increase of tinea capitis caused by anthropophilic dermatophytes has been reported in European metropolises like Madrid, Paris, Rotterdam, Stockholm and London (3,25). Most cases of tinea capitis in these regions are recorded in children immigrants from African countries.

The epidemiological situation regarding causative agents of tinea capitis infection in Croatia is somewhat similar to that recorded in our neighboring countries, with M. canis being the most prevalent dermatophyte (11,19,22). The anthropophilic T. tonsurans, the first ranking pathogen in the USA (3) and the UK, has been recorded sporadically over the past 10 years and is still exclusively found among high contact sports practitioners and members of their families. T. violaceum, endemic in African countries, was isolated in a couple of cases, reflecting the low immigration rate from endemic countries. The incidence of geophilic M. gypseum remained low during the study period and showed no significant variations, apart of the fact that M. gypseum appeared as the causative agent of the kerion type of tinea capitis in a limited number of cases. We have no consistent explanation for this phenomenon. There was one case of scalp ringworm caused by T. schoenleinii in a young immigrant woman from Janjevo (outside Croatia).

During the study period, the incidence of tinea capitis declined from 215 cases in 1999 to 127 cases in 2008, due to a decrease in the frequency of M. canis infection. We hope that this presents the real epidemiological situation. Further studies...
are required to establish whether this declining trend will continue in the future.

**Fungal infection of the foot**

In our study, fungal infection of the foot ranked third in the prevalence and accounted for 12% of all fungal infections. The incidence of each major dermatophytic pathogen (*T. mentagrophytes*, *T. rubrum* and *E. floccosum*) remained relatively constant, with slight variations from year to year. A decrease in the incidence (of 57.14%) of *Candida* spp. was noted, from 56 (26%) cases in 1999 to 24 (12%) cases in 2008.

The incidence of tinea pedis in developed countries is increasing, possibly due to the frequent use of athletic footwear by both men and women (26). In northern and Central Europe, USA and Mexico, *T. rubrum* is the predominant causative agent of tinea pedis (1,6,7,11,13). In a study from Caligari, Italy, *T. mentagrophytes* was the main etiologic factor causing tinea pedis, with *T. rubrum* ranking second (27).

It is considered that these results obtained in our study do not entirely reflect the true epidemiological situation. It is believed that a considerable number of patients (especially male) affected by tinea pedis do not consider their infection serious and do not seek medical help, whereas some patients are treated by general practitioners without etiologic evidence.

**Fungal infection of the hand**

The incidence of fungal infection of the hand was 5%. *T. mentagrophytes* was once again the major pathogen accounting for 50% of all fungal isolates, followed by *Candida* spp. (35%) and *T. rubrum* (9%). Variation in the incidence of all pathogens was observed, without any specific regularity that could be followed over years. Other studies showed *T. rubrum* to be the main causative agent of tinea manus (5-7,11,15).

**Fungal infection of the face**

Ringworm of the glabrous skin of the face accounted for 5% of all fungal infections. An overall decrease in the incidence of tinea faciei was noted due to a decline in the incidence of all three major fungal pathogens (*T. mentagrophytes*, *M. canis* and *Candida* spp.). Some cases of tinea incognita due to *T. mentagrophytes* were recorded in our study. In a study from Ljubljana, Slovenia, *M. canis* was the major pathogen, followed by *T. rubrum* and *T. mentagrophytes* (11).

**CONCLUSION**

The prevalence of superficial mycotic infections has risen worldwide over the last decades, making them one of the most frequently encountered forms of infection. The etiologic agents and the predominant site of infection differ depending on geographical region, as well as on environmental and cultural factors. Although each geographical region favors its infection pattern, the spectrum of fungi is not static. Due to tourism, international sports activities and migration of the population, new fungal species have been imported and disseminated. Our findings confirm the changing pattern of etiologic agents, as well as changes in the predominant site of infection with the passage of time. There should be greater awareness of the dynamic nature of the superficial mycotic infections, so that the true etiology would not be missed and the appropriate preventive measures would be taken accordingly.

References

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