Causative Agents of Superficial Mycoses in Outpatients Attending Cerrahpaşa Medical Faculty Hospital, in İstanbul, Turkey (01 April 2010 – 01 June 2014)

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Abstract

**Background:** Superficial fungal infections are among the world’s most common diseases and the distribution of etiological agents varies in different countries and geographic areas.

**Aims:** The aim of this study was to determine the frequency of etiological agents of superficial mycoses encountered in outpatients attended to Dermatology Department of Cerrahpasa Medical Faculty, Istanbul.

**Materials and methods:** Clinical samples were collected from 2125 patients over a period of four years and examined by direct microscopy and culture.

**Result:** Isolated fungi were identified by classical mycology methods. Pathogen fungi (n= 643) were detected in 623 of the patients. Of the isolates were 206 (32.0%) Candida spp, 308 (47.9%) dermatophytes, 3 (0.5%) Malassezia spp and 126 (19.6%) other keratinophilic fungi, 18 (2.8%) Fusarium, 106 (16.5%) Trichosporon spp, 2 (0.3%) Phoma spp. Two different significant fungi were cultured from samples of 20 (3.2%) patients. T. rubrum was the most frequent isolate (n=135, 21.0%) and toenail onychomycosis was the most common type of infection (n=294, 47.2%).

**Conclusion:** The most common agents isolated were Trichophyton species, being Candida spp the second prevalent. Non dermatophyte molds were cultured as agents of onychomycosis. Epidemiological surveys will be a useful tool for the awareness of emerging species and infection control.

Introduction

Superficial fungal infections of the skin, nails and hair are among the most common infections in the world. Many epidemiological studies have investigated the prevalence of etiological agents of superficial mycoses in different parts of the world. The distribution of etiologic agents varies in different countries and populations depending on several factors such as climate (temperature and humidity), heavy exposure, contact with animals, age, gender, life style, local socio-economic conditions and cultural practices [1, 2]. This study was undertaken to investigate the epidemiology and prevailing agents of superficial mycoses in outpatients attending dermato-
logy department of a university hospital, in Istanbul, Turkey, in a 4-year period.

**Materials and Methods**

Skin scales and scapings, nail and hair specimens of patients referred by the department of dermatology with suspected dermatomycosis were collected and examined in our laboratory over a 2-year period. Detailed history were taken from patients and samples were collected before antifungal treatment started. Skin and nail surfaces were disinfected by 70% ethanol and specimens were collected from the edge of the lesions with a sterile surgical blade and approximately 5 to 10 hair roots were pulled out with sterile epilator forceps. Nail fragments were collected with the aid of a sterile scissors from the deepest part of the nail and as close as possible to the healthy nail. All samples were placed in labelled sterile Petri dishes and processed freshly. Clinical samples were examined by direct microscopy and culture.

Part of each specimen was mounted in aqueous solution of 10% and 30% (w/v) potassium hydroxide (skin and nail samples respectively) and examined microscopically under x10 and x40 magnifications after 5 minutes (hair samples), or 30 minutes (skin samples) or two hours (nail samples) for the presence of mycelium, arthrospores and/or yeast cells and their distribution pattern in hair (ectothrix, endodtrix or favic type).

All samples were cultured irrespective of the negative or positive examination result. Finely divided pieces from each sample were cultured on three Sabouraud dextrose agar (SDA, Difco, Detroit, MI, USA) slants with gentamycin (0.04 mg/ml) and one SDA with gentamycin and cycloheximide (0.05 mg/ml) and incubated 3 weeks at 25°C except one with gentamycin which was incubated at 37°C, before discarding as negative. Cultures were examined twice in a week for any evidence of growth. Growing colonies were examined macroscopically and microscopically to determine purity and to select potential causative agents. Fungi grown were identified using conventional techniques based on morphological and biochemical criteria. Methylene blue stained preparations of yeast-like colonies were prepared and examined under x100 magnification for the presence of blastoconidia, pseudohyphae, true hyphae and arthroconidia. Germ tube test and chlamydospore formation test was performed for differentiating *Candida albicans* from non-albicans species.
Table 1. Distribution of Isolated Significant Fungi (n= 643) to Samples Collected (n=2125) and Body Sites (01 April 2010 - 01 June 2014)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Anatomical site</th>
<th>Candida spp (n= 206, 32.0% )</th>
<th>Dermatophytes (n=308, 47.9% )</th>
<th>Other keratinophylic fungi (n=126, 19.6% )</th>
<th>Total number of isolates per anatomical site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1C. a</td>
<td>2C. s</td>
<td>3T. r. 4T. m. 5T. t. 6T. v. 7T. v. 8T. s.</td>
<td>9T. s. 10M. c. 11M. g. 12M. s. 13T. s. 14F. s. 15P. s.</td>
</tr>
<tr>
<td>Nail (n= 1448, 68.1%)</td>
<td>Fingernail (n=216, 10.1%)</td>
<td>25</td>
<td>76</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Toenail (n=1232, 58.0%)</td>
<td>9</td>
<td>48</td>
<td>67</td>
<td>21</td>
</tr>
<tr>
<td>Skin scrappings  (n=625, 29.4%)</td>
<td>Hand, palm, interdigital (n=149, 7.0%)</td>
<td>3</td>
<td>15</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Arm (n=32, 1.4%)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Face, neck (n=28, 1.3%)</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Body (n=42, 2.0%)</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Foot sole, interdigital (n=286, 13.4%)</td>
<td>4</td>
<td>8</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Leg (n=52, 2.3%)</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Inguinal (n=28, 1.3%)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Gluteal (n=18, 0.8%)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Hair and scalp (n= 52, 2.4%)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total (n=2125), % in each group</td>
<td></td>
<td>46</td>
<td>160</td>
<td>135</td>
<td>46</td>
</tr>
</tbody>
</table>

1C.a: Candida albicans (22.3%); 1C.s: Candida spp (77.7%); 1T.r: T. rubrum (43.8%); 1T.m: T. mentagrophytes (14.9%); 1T.t: T. tonsurans (2.3%); 1T.v: T. verrucosum (0.6%); 1T.u: T. violaceum (0.6%); 1T.s: Trichophyton spp (33.4%); 1M.c: M. canis (1.9%); 1M.g: M. Gypseum (1.0%); 1M.s: Microsporum spp (1.3%); 1M.s: Malassezia spp (0.5%); 1T.s: Trichosporon spp (84.1%); 1F.s: Fusarium spp (14.3%); 1P.s: Phoma spp (1.6%)
(2.8%) Fusarium spp, 106 (16.4%) Trichosporon spp, 3 (0.5%) Malassezia spp and 2 (0.3%) Phoma spp. The distribution of fungi isolated to the samples and anatomic sites were enlisted in (Table 1).

Two significant fungi were cultured together from samples of 20 (3.1%) patients (Table 2). Of the samples cultured, non-albicans Candida species were the most prevalent (77.7%) yeasts. Among dermatophytes identified in species level, Trichophyton rubrum was founded to be the commonest etiological agent (43.8%) followed by T. mentagrophytes (14.9%).

Phoma spp was isolated from two patients in different years. The first patient [12] was a 37 years-old male teacher who dealt with gardening in summertimes. He presented with a history of greenish-yellow discoloration and subungual hyperkeratosis on all the toenails (Figure 2). There was no history of other diseases except for toenail dystrophy. The second patient was a 40 year old female nurse. Both of them were otherwise in good health and denied nail trauma or dystrophic nail abnormalities prior to the onset of the present lesions. In mycological examination septate hyphae were observed in 30% KOH preparation from the toenail samples. Rapid growing green-gray colonies were developed on SDA. Microscopical preparation revealed hyaline to brown septate hyphae, several picnidia with ostioles and unicellular conidia (Figure 3). The same fungus was isolated on a total of three consecutive cultures. Dermatophytes were absent. The isolated moulds were morphologically identified as Phoma spp.

Onycomycosis was the most common clinical form of dermatomycoses, and toenail onychomycosis (n=294, 47.2%) was the most prevalent type of infection. As agents of onychomycosis (n=442), dermatophytes were detected in (185, 41.9%), yeasts in (158, 35.7%) and non-dermatophyte fungi in (99, 22.4%) patients. Candida spp was isolated more frequently from fingernails than toenails, and females were affected more frequently with fingernail candidal infections than males.

Dermatophytes, non-dermatophytic fungi and Candida species are etiological agents of superficial infections. The etiology and frequency of dermatomycoses vary with changes in geographic and climatic conditions, different living habits and life style. Dermatophytes (47.9%) were the most common pathogens recovered from our patients with suspected dermatomycoses. In the present study, T. rubrum (21.0%) was the most common etiologic agent isolated from various cases of superficial mycoses and it was followed by T. mentagrophytes. The predominance of T. rubrum in our study represents global trend consistent with data from many other geographical regions [13, 14, 15, 16, 17, 18, 19, 20, 21, 22].

Discussion

Dermatophytes, non-dermatophytic fungi and Candida species are etiological agents of superficial infections. The etiology and frequency of dermatomycoses vary with changes in geographic and climatic conditions, different living habits and life style. Dermatophytes (47.9%) were the most common pathogens recovered from our patients with suspected dermatomycoses. In the present study, T. rubrum (21.0%) was the most common etiologic agent isolated from various cases of superficial mycoses and it was followed by T. mentagrophytes. The predominance of T. rubrum in our study represents global trend consistent with data from many other geographical regions [13, 14, 15, 16, 17, 18, 19, 20, 21, 22].

The first report of dermatomycosis in Turkey was by Unat in 1952 [23]. 60 years ago, the most widespread etiologic agent was reported to be Trichophyton schönleini [23], which was later succeeded by T. violaceum, M. canis and T. mentagrophytes. A seven year retrospective study in Istanbul by Koksal et al. [24] reported the most common isolate as T. rubrum, being Candida spp the most prevalent. In the

Figure 2. Nails infected with Phoma spp

Figure 3. Picnidium and oval shaped conidia (400x)
present study, T.schönleini was not isolated from specimens, and, T.violaceum was isolated very rare (0.6%), however T. rubrum was the most frequent dermatophyte isolated followed by T. mentagrophytes, suggesting the changes in epidemiology of dermatophytosis in Turkey in last 60 years. Similar findings were reported from Marmara [25], Easter Thrace [26], Middle Blacksea regions [27], and South Central Turkey [28], and Central Anatolia [29, 30]. This seems to be in accordance with the change in dermatophyte spectrum in dermatomycoses in Central and North Europe as underlined by Seebacher [17, 18]. The authors suggested this evolution to be connected with the increase in the incidence of tinea pedis, like in our study, tinea pedis was the most common clinical form, although tinea capitis superficialis and favus was in 1950s [23, 31].

In contrast, in Southern Europe, especially in Mediterranean and Arabic countries, zoophilic dermatophytes, such as Microsporum canis or T. verrucosum, are the most frequently isolated during the recent years and this dermatophyte is now the most prevalent in tinea capitis in children [17]. In our study, M. canis and T. verrucosum has very low frequency (1.0% and 0.3% respectively) and tinea capitis was rare (1.4%). This was in agree with the data reported from Aegean [32] and western Black Sea region of Turkey [33], but higher M. canis frequency was reported from Central Anatolia [29].

Candida spp (24.9%) was the second prevailing pathogen recovered from our patients with dermatomycoses, a rate correlating well with comparable studies [24, 28, 30, 34, 35]. Fingernails were affected than toenails and females were affected more than males, like findings reported by Kiraz [34] and this may probably attributed to frequent emersion of hands in water.

Non-dermatophytic fungi, Fusarium spp, Trichosporon spp and Phoma spp isolated from nail clippings (3.8%, 18.0% and 0.4% respectively) and the first two from skin scrapings (0.5% and 13.6 respectively) were previously regarded as contaminants, are now considered to be infectious agents. For Trichosporon spp, these rates were in agree with findings reported in Istanbul by Kiraz [34] and higher than reported by Koksal [24]. For Fusarium spp, our findings were correlating well with

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Fungi</th>
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<tbody>
<tr>
<td>Fingernail (n=7)</td>
<td></td>
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<tr>
<td>Candida sp</td>
<td>Trichosporon sp</td>
</tr>
<tr>
<td>Candida sp</td>
<td>Fusarium sp</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Fusarium sp</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Fusarium sp</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td>Toenail (n=10)</td>
<td></td>
</tr>
<tr>
<td>Trichophytum rubrum</td>
<td>Trichophytum sp</td>
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<td>Trichophytum sp</td>
<td>Trichophytum sp</td>
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<td>Trichophytum sp</td>
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<td>Candida sp</td>
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<td>Candida sp</td>
<td>Trichophytum sp</td>
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<tr>
<td>Candida glabrata</td>
<td>Fusarium sp</td>
</tr>
<tr>
<td>Foot interdigital (n=1)</td>
<td>Candida sp</td>
</tr>
<tr>
<td>Arm (n=1)</td>
<td>Microsporum sp</td>
</tr>
<tr>
<td>Hair (n=1)</td>
<td>Microsporum gypseum</td>
</tr>
</tbody>
</table>

Table 2. Specimens From Which Two Different Aetiological Agents Cultured (n=20)
In the current study, 643 of the total of 2125 clinically suspected cases were confirmed by mycological methods. From our overall data, dermatomycosis occurred mainly in adults (40-49 years), females were affected more than males (1207/898), which was similar to results of other studies [40, 41].

In conclusion, epidemiology of dermatomycoses was changed in Turkey in the last 60 years and the distribution of etiologic agents of superficial mycoses in this study was similar to the epidemiological pattern reported in North and Central Europe.

References

18. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia 2008; 166: 335-352. PMID 18478365


35. Aghamirian MR, Ghiasian SA. Dermatophytoses in outpatients attending the Dermatology Center of Avicenna Hospital in Qazvin, Iran. Mycoses 2007; 51: 155-160. PMID 18254753


