



# Causative agents distribution of isolated from dermatomycoses in Eskisehir city hospital

✉ Rabiye Altinbas<sup>a,\*</sup>, ✉ Omer Ummetoglu<sup>b</sup>, ✉ Canan Sisman<sup>b</sup>

<sup>a</sup>Gaziantep Dr. Ersin Arslan Training and Research Hospital, Department of Medical Mycology, Gaziantep, Türkiye

<sup>b</sup>Eskisehir City Hospital, Department of Skin and Venereal Diseases, Eskisehir, Türkiye

## ARTICLE INFO

### Keywords:

Superficial fungal infections  
Dermatomycoses  
Dermatophytes  
Non-dermatophytes

Received: Jan 26, 2023

Accepted: Jun 05, 2023

Available Online: 23.06.2023

### DOI:

[10.5455/annalsmedres.2022.09.271](https://doi.org/10.5455/annalsmedres.2022.09.271)

## Abstract

**Aim:** Dermatomycoses are superficial fungal infections of the skin, hair and nails. The fungi responsible for dermatomycoses include dermatophytes, yeasts and non-dermatophytic molds (NDM). Onychomycosis is the most common nail disease caused by dermatophytes, NDMs, and yeasts with a worldwide. This study was under taken to investigate the current distribution of aetiological agents of dermatomycoses.

**Materials and Methods:** In this study, we evaluated the microscopic examination and culture results of the samples taken from the patients who applied to our clinic for two year. Species were identification on the basis of combined clinical pictures, culture and microscopic morphology characters of together.

**Results:** During the study period, 592 samples were examined. The distribution of isolates was found to be 47.2% (n=118) NDM, 26.4% (66) dermatophytes and 26.4% (66) yeasts, respectively. Tinea unguium was the most prevalent type of dermatophytoses. *Trichophyton rubrum* was most frequent aetiological agents of detected in patients. According to our study, 22% of non-dermatophyte hyaline molds are *Fusarium* spp. and 21% are *Aspergillus* spp. species.

**Conclusion:** Several studies have shown that both the prevalence and aetiology of dermatomycoses can change according to geographical location, climatic conditions, and personal factors. The objective of our study was to analyse 2 years of epidemiological data regarding dermatological mycology testing performed in a large tertiary care teaching hospital in Eskişehir. This report represents the most comprehensive analysis of this type from Eskişehir, and it is hoped that its findings will be of interest to, and inform, researchers and clinicians focused on mycoses and international epidemiology. To the best of our knowledge, we provide the first analysis from Eskisehir of fungal detections from all external body site (skin, hair and nail).



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## Introduction

Dermatomycoses are superficial fungal infections (SFI) of the skin, hair and nails [1]. The fungi responsible for dermatomycoses include dermatophytes, yeasts and non-dermatophytic molds (NDM). Approximately, 20–25% of the global human population is infected with a dermatophyte at least once per lifetime [2]. A number of epidemiological studies have demonstrated that most cases of SFIs are caused by dermatophytes [3–5]. Dermatophyte infections are responsible for at least half a billion dollars in health-care costs [1].

Dermatophytes are taxonomically grouped under the order Onygenales and the family Arthrodermataceae. Although the Arthrodermataceae family includes 7 genera,

only three genera; *Microsporum*, *Trichophyton* and *Epi-dermophyton* are commonly associated with dermatophytosis in humans and animals [4]. Depending on their host preferences and natural habitats, dermatophytes are also divided into three ecological groups: anthropophiles, zoophiles and geophiles. Dermatophytes grow on the keratinized tissues and leading to small to extended due to the characteristic red circular and concentric lesions which they are also known as tinea or ringworm infections [2,5,6].

Onychomycosis is the most common nail disease caused by dermatophytes, NDMs, and yeasts with a worldwide prevalence of 5.5%. More than half of these infections are caused by dermatophytes (tinea unguium) and the most common organism is *Trichophyton rubrum* [6]. NDMs are responsible for approximately 20% of fungal nail infections and the most common organisms are *Scopulariopsis brevicaulis*, *Aspergillus* spp., *Acremonium* spp., *Fusarium* spp.,

\*Corresponding author:

Email address: [rabiaguz@gmail.com](mailto:rabiaguz@gmail.com) (✉ Rabiye Altinbas)

*Alternaria alternata*, and *Neoscytalidium* spp. Yeasts, including *Candida* spp., are responsible for approximately 10% of fungal nail infections [7-9].

Exposure to a humid environment, obstructive shoes, and occupations such as frequent travel, hand washing, or shared bathing increase the risk of developing onychomycosis [10]. Predisposing factors for onychomycosis include trauma, diabetes, immunosuppression, and previous history of nail psoriasis [7].

Furthermore, onychomycosis has been reported to have a significant impact on patient quality of life due to a variety of physical changes (e.g., pain, discomfort, difficulty trimming thick nail plates, and difficulty walking). Onychomycosis is common in older people and prevalence increases with age [6,8].

Tinea capitis is a dermatophyte infection of the scalp and hair tends to affect children worldwide. *Microsporum canis* remains the dominant cause of tinea capitis [11].

Although dermatomycoses are not lifethreatening, they are important because therapeutic failures and repeated recurrences are frequently encountered in cases of dermatomycoses, and it is a public health problem that affects the quality in life of individuals [1,5].

History and clinical evaluation are not sufficient to make the diagnosis of dermatomycosis. A lot of conditions, including inflammatory disorders such as psoriasis and lichen planus which presenting nail changes that clinically mimic onychomycosis could be presenting similar nail changes therefore dermatomycose symptoms may sometimes be missed. Treatment duration is often long and is associated with potential adverse drug reactions, so it is necessary to confirm clinical diagnosis with laboratory examinations in order to identify the aetiological agent. The choice of treatment depends on the causative organism [12].

Although there has been a dramatic increase in the incidence of infections caused by dermatophytes in recent years, improvements in surveillance have resulted in a marked decrease in incidence in developed countries. Epidemiological information on dermatomycoses and determination of genera and species of causative agents are essential from the view point of infection control and public health. Determination of current aetiological agents of dermatomycoses according to region will provide epidemiological data as well as increase the success of treatment. A few studies have investigated the prevalence of causative agents of dermatomycoses in Türkiye [13-15].

This study was under taken to investigate the current distribution of aetiological agents of dermatomycoses in Eskisehir City Hospital, Türkiye. This is the first study conducted that monitors the spectrum of superficial fungal infections in our hospital, Eskisehir.

## Materials and Methods

In this study, we evaluated the microscopic examination and culture results of the all samples taken from the patients who applied to our clinic for two year (2019-2020) and were evaluated with a preliminary diagnosis of superficial fungal infection.

This was a retrospective study conducted in the Department of Mycology in collaboration with the Depart-

ment of Dermatology at Eskisehir City Hospital, Eskisehir, Türkiye. A total of 592 samples (560 nail, 32 scalp (skin scraping and hair) were received during this period. Species were identification on the basis of combined clinical, culture and microscopic morphology characters of together. Firstly, the lesional surface which including nail, skin or hair was cleaned with 70% alcohol. This simple precaution helps prevent the growth of bacteria and environmental molds that can be misinterpreted as pathogens rather than pollutants. After cleaning, sample clippings were collected from more viable hyphae in the proximal part of the nail. Skin scrapings were collected using scalpel blades, currettes, or the edge of a slide.

## Direct microscopic examination with KOH

Direct microscopy is used to confirm the presence of fungal pathogens on clinical samples. Although the sensitivity varies depending on the sampling quality and the experience and skill of the microbiologist, the easiest, fastest and most practical examination method in fungal infections of keratinized tissues is direct microscopic examination. The obtained cellular material should be placed on a microscopic slide and 10-40% KOH (10% for skin scrapings and 40% nail clippings) solution should be applied used to dissolve keratinocytes. After 15-30 minutes, the specimens can be examined under a light microscope to determine the presence of fungal elements such as arthroconidia, blastoconidia, true hyphae or pseudohyphae. KOH testing indicates whether fungus is present without species identification [5]. The procedure is simple, and efficient screening method but lacks sensitivity and will not determine fungal viability [8].

## Fungal culture

Fungal culture is a gold standard diagnostic method for laboratory testing [8]. The samples were inoculated in two Sabouroud dextrose agar (SDA-RTA, Türkiye) media containing chloramphenicol and two SDA media containing chloramphenicol-cycloheximide. The plates were wrapped in parafilm or protective bags. One SDA containing only chloramphenicol and one SDA containing chloramphenicol-cycloheximide were incubated at 25 °C, and the others (SDA containing only chloramphenicol and SDA containing chloramphenicol-cycloheximide) were incubated at 35 °C for 28 days. The plate observed for fungal growth daily for 1 week and twice weekly for the next 4 weeks.

The plate that grew mold was identified on the basis of morphological features like growth rate, texture, and color of the colony on obverse and reverse of SDA.

The growing colonies were examined macroscopically and microscopically to identify the possible causative agent. Microscopic examination by lactophenol cotton blue mount and slide cultures, and identified by standard mycological methods [16]. The identification of dermatophyte and non-dermatophyte molds was made through conventional methods and according to their biochemical characteristics. For this purpose, inoculation was carried out in secondary isolation medium such as urea agar for urease activity.

Yeasts isolates were identified by germ tube test, microscopic morphology on corn-meal agar with tween 80, growth on chromogenic medium Candida CHROMagar (RTA, Türkiye), commercial system which carbon and nitrogen assimilation test by VITEK-2 (ID-YEST card; BioMérieux, France).

Plates without growth, even after 4 weeks of incubation were considered negative.

In order for NDM to be accepted as the causative agent of onychomycosis, it has been suggested to show the growth of NDM in the culture for the second time [17]. In our study, the cases in which NDM growth was shown for the second time were accepted as the causative agent.

This study was approved by Ethics Committee Chairmanship on Noninterventional Clinical Research of Eskişehir Osmangazi University (approval number: 04; approval date: 06.04.2021).

**Results**

During the study period, 592 skin, hair, and nail samples were examined. related to suspected Superficial Fungal Mycosis (SFM) cases. Of the clinical samples requested for fungal culture, 94.6% (n=560) were nails and 5.4% (n=32) were scalp. 96% of them were sent from the Dermatology Department.42,2% of the samples yielded in culture. The distribution of isolates was found to be 47.2% (n=118) NDM, 26.4% (n=66) dermatophytes and 26.4% (n=66) yeasts.

Of the patients diagnosed with onychomycosis, 138 female (average age 48), 88 were male (average age 51).

Tinea unguium was the most prevalent type of dermatophytoses at 74.24% (n=49), followed by tinea capitis 25.76% (n=17). *T. rubrum* 42.4% (n=28) was most frequent aetiological agents of detected in patients. When cases were examined according to age groups, tinea unguium was occurred mostly in the age groups ranging between 7 and 95 years (average age 49.1). Tinea capitis occurred mostly in the age groups ranging between 3 and 11 years (average age 6.4).

Evaluations of the samples the agreement between the direct microscopic examination with KOH, and the growth in culture results was found to be 73% (n=432) (Table 1). The rate of samples direct microscopic examination with KOH is positive without growth in culture is 16% (n=95) and the rate of samples direct microscopic examination with KOH is negative with growing in culture is 11% (n=65).

**Table 1.** The compatible of the samples direct examination evaluations with KOH and Growth in Culture results.

Direct Examination with KOH	Growth in Culture	Sample (n)	Compatible (%)
Negative	Negative	241	+ (40.7)
Positive	Positive	191	+ (32.3)
Positive	Negative	95	- (16)
Negative	Positive	65	- (11)

**Table 2.** Superficial Fungal Culture Requests and results.

	n	%
Dermatophytes	66	11.1
Non-Dermatophyte Molds	118	20
Hyalen Molds	86	73
Dematiaceous Molds	32	27
Yeasts	66	11.1
Candida Species	53	80
Non-Candida Species	13	20
Contamination	6	1
No growth	336	56.8
All	592	

As a result of direct microscopic examination, the incidence of fungal element was found to be the highest in tinea capitis 100%, and it was found 83.7% in tinea unguium.

The distribution of culture requests is shown in Table 2. Accordingly, no growth was detected in 56.8% of the samples. 1% was considered as contamination. Growth was detected in 42.2% of the samples. Hyaline molds 34.4% (n=86), dermatophytes 26.4% (n=66), *Candida* species 21.2% (n=53), dematiaceous molds 12.8% (n=32), and yeast like fungus 5.2% (n=13).

Dermatophytes grown in clinical samples (n=66/250); they were identified as 74% (n=49) *Trichophyton* species and 26% (n=17) *Microsporum* species. 65.3% of *Trichophyton* species are at species level; 57.1% were identified as *T. rubrum* and 8.2% as *T. mentagraphytes*. At the species level, 76.5% of *Microsporum* species were identified as *M. canis*.

Of all hyaline molds, 61% (n=52) were identified at the species level. Molds were found that the most grew among them *Fusarium* spp. 22% (n=19) and *Aspergillus* spp. 21% (n=18). Others are *Acremonium* spp. (n=7), *Scopulariopsis* spp. (n=2). *Trichoderma* spp. (n=2), *Saprochaete* spp. (n=1), *Scedosporium* spp. (n=1), *Scytalidium* spp. (n=1), *Sporobolomyces* spp. (n=1).

Of all dematiaceous molds, 90.6% (n=29) were identified at the species level. *Alternaria* spp. 38% (n=11) is the most grown dematiaceous mold among them. Others are *Chaetomium* spp. (n=7), *Cladosporium* spp. (n=5), *Phaeoacremonium* spp. (n=5), *Phoma* spp. (n=1).

All yeast has been described at the species level. Non-albicans *Candida* was the most prevalent species 86.8% (n=46). *C. parapsilosis* complex 43.5% (n=20) was found most frequently among non-albicans. Other species were also detected *C. glabrata* 24% (n=11), *C. tropicalis* (n=4), *C. melibiosica* (n=4), *C. kefyr* (n=3), *C. pelliculosa* (n=2), *C. kruseii*, (n=1) and *C. lipolytica* (n=1). Non-albicans *Candida* 86.8% (n=46) overproduced *C. albicans* 13.2% (n=7).

The most frequently detected yeast-like microorganisms other than *Candida* species growing in superficial clinical specimens were *Trichosporon* species 38.5% (n=5/13) and 38.5% *Rhodotorula* species (n=5/13). Others are

*Magnusiomyces capitatus* (n=2/13). and *Malassezia* spp. (n=1/13). From 2 of the *Trichosporons*; it has been identified at species level, one of them *T. asahii* and the other *T. inkin* has been identified.

Dermatophyte reproduction rate in scalp samples was 53% and all of them were found to be *Microsporum* species (100%). The growth rate of dermatophyte in nail samples was 8,8%, and all of them were *Trichophyton* species. 71% (n=13) of dermatophytes growing in scalp samples could be identified at the species level. All *Microsporum* species defined at the species level were found as *M. canis*. 65% of dermatophytes growing in nail samples could be identified at the species level. They were found as *T. rubrum* (57% n=28) and *T. mentagraphytes* (8% n=4). According to our study, 22% of non-dermatophyte hyaline molds are *Fusarium* spp. and 21% are *Aspergillus* spp.

Household transmission was seen in two patients. A sample of these was the scalp. No household transmission related to nail infection was observed.

## Discussion

Several studies have shown that both the prevalence and aetiology of dermatomycoses can change according to geographical location, rural or urban area, climatic conditions, life style, personal factors such as age, gender, heredity, hygiene, migration, socioeconomic status [8,12,18].

It is critical not to share personal items such as slippers, towels, combs for the prevention of superficial dermatomycotic infections; there are greatest risk of when breaks in skin barrier or nail trauma. Dermatophytes attract attention due to their high contamination and shared wet surfaces, such as swimming pools, showers, and bathtubs may provide sources for superficial dermatomycotic infections [10].

The pathogenesis of dermatophyte infection involves complex interaction between the host, agent and the environment [5]. However, antifungal systemic therapy is expensive and may be accompanied by unwanted side effects such as drug interaction. Therefore, reliable diagnostic methods are important to verify fungal infection prior treatment [4].

Although the sensitivity varies depending on the sampling quality, the experience and skill of the microbiologist, the easiest, fastest and most practical examination method in fungal infections of keratinized tissues is direct microscopic examination. The sensitivity of direct microscopic examination is lower than culture but combination with culture (53%) and direct microscopy (48%), higher sensitivity (74%) was found in the diagnosis of onychomycosis [19].

In studies conducted in our country, positivity detection rates in direct microscopic examination are reported to be between 16-63% [15,20-22]. In our study, similar to the previous results, a positivity of 48.3% was found between these values.

In studies conducted in our country, the rates of detection of growth in culture are reported to be 11-22% [11,14,15,20,23]. In our study, growth was detected in 42.2% of the cultured samples. This rate is higher than the studies conducted in our country.

In our study, the false negative rate was found to be 11%. The most common reason for false negative microscopic examination is the examination of inappropriate specimens containing no fungal hyphae. In addition, antifungal treatment before sample collection, insufficient amount of samples, and insufficient time to examine samples can be counted among the reasons for false negative microscopic examination.

Similar to other studies, *T. rubrum* was found to be the most frequently isolated superficial mycosis agent in our study. The worldwide distribution of *T. rubrum* may be associated with its ability to cause of chronic infections, and by its resistance to treatment [13]. *M. canis* remains the predominant cause of tinea capitis. The fungistatic fatty acids in the scalp sebum of children may also be associated with higher carriage rates in children [24]. Considering the samples coming to our laboratory, it can be interpreted that the most common nail dermatophytosis is encountered in patients admitted to our hospital. However, it is noteworthy that the culture positivity in nail samples is lower than in scalp samples.

In our study, *C. parapsilosis* complex and *Fusarium* species were found to be the second and third most frequently detected agents. *M. canis* which were reported to be isolated at a rate of 0.9-5% in various studies were found at a similar rate of 5.2% in our study [15,20,25]. All *Microsporum* species were isolated from tinea capitis lesions.

In our study, the prevalence of superficial fungal infection was 42.2%, and 11.6% of them were dermatophytosis. Şaşmaz et al. [26] investigated the prevalence of superficial fungal infection was found in 20% of the soldiers, 81.6% of them were reported as dermatophytosis.

A similar study was conducted among workers in a textile factory [13]. Superficial mycoses were detected in 16.9% of the workers; 76.7% of these were dermatophytosis. It is noteworthy that the rates of dermatophytosis were reported much higher than our study in both of these studies. However, *T. rubrum* and *T. mentagraphytes* were two of the most frequently isolated dermatophytes in all three studies.

Although both positive direct examination and growth in culture are present, it can not confirm the diagnosis of mycosis with non-dermatophyte mold, because the surface of nails or skin fragments may be colonized with contaminated molds. However, dermatomycoses caused by true non-dermatophyte molds can occur [27].

One of the most important problems in dermatomycoses caused by non-dermatophyte molds such as *Aspergillus*, *Fusarium*, *Scopulariopsis*, or *Acremonium* species is to determine the pathogenicity of the isolated fungus. Therefore, isolation of the same species from consecutive specimens from infected material is critical to confirm the diagnosis. There is very little chance of grown in culture the same "contaminant" when sampling is repeated [11].

The most frequent organisms isolated from dermatomycoses were NDM (20%), followed by dermatophytes (11%) and yeasts (11%) in our study. Contrary to our findings, many studies have reported that superficial fungal infections of dermatophytes are the most common etiological

agents [3–5]. Yeast has also been reported to play an important role in superficial mycoses, especially in onychomycosis. A high rate (56.8%) of yeasts isolated from onychomycosis was reported by Ataides et al. [28] in a study conducted in Brazil. Also, similar to our study, both Ataides et al. and Segal et al. [29] reported *C. parapsilosis* as the most common yeast strain in onychomycosis.

The dermatophytes isolated in our study, 74% of the genus *Trichophyton*, 26% of the genus *Microsporium*. The dermatophytes isolated in the studies of Eryılmaz et al. [3] 94.7% are *Trichophyton* genus and 5.3% are *Microsporium* genus. In our study, *Trichophyton* genus ratio was found to be lower than these study, and *Microsporium* genus ratio was found to be higher.

Dermatophytic infections are commonly spread in family members, especially in the case of tinea capitis. In our study, intrafamilial transmission was detected in two patients isolated from *M. canis*. Familial dermatophytosis might be an important contributor to treatment failure [10]. Although it is stated that intrafamilial transmission is common in onychomycosis, we did not encounter any case of onychomycosis transmitted within the family in our study [6].

The objective of our study was to analyse 2 years of epidemiological data regarding dermatological mycology testing performed in a large tertiary treating hospital in Eskişehir. This report represents the most comprehensive analysis of this type from Eskişehir, and it is hoped that its findings will be of interest to, and inform, researchers and clinicians focused on mycoses and international epidemiology.

To the best of our knowledge, we provide the first analysis from Eskişehir of fungal detections from all external body sites (skin, hair and nail). In addition, the cooperation of dermatologists and clinical microbiologists are extremely important.

#### Limitations

First, this is a single-center study that may not accurately reflect the general of Türkiye. Furthermore, second limit of the present study was the small sample size that may not accurately reflect the general of superficial mycotic infections. Last, since conventional methods were used in our study, it was difficult to identify some atypical and unusual isolates according to their in vitro properties. The use of molecular methods will also contribute to the elimination of this problem. Conventional methods also require a high degree of specialist skill.

#### Ethical approval

This study was approved by Ethics Committee Chairmanship on Noninterventional Clinical Research of Eskişehir Osmangazi University (approval number: 04; approval date: 06.04.2021).

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