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

A Serda Kantarcıoğlu,[1*] PhD, Nilsen Güney,[1] MD, Nuri Kiraz,[1] MD, Arife S. Yaldız,[1] BSc, Zeynep Yazgan,[1] BSc, Deniz Turan,[1] MD, Ayşe B. Bayrı,[1] MD, Belkıs Yolburun,[1] MD, Zafer Habip,[2] MD, Burhan Engin,[2] MD, Fatma Coşkun,[1] MD, Fatma Özakkaş,[1] MD, Zekayi Kutlubay,[2] MD, Yalçın Tüzün,[2] MD

Address: Cerrahpaşa Medical Faculty, Department of [1]Medical Mycology, [2]Dermatology, Fatih, İstanbul, Turkey
E-mail: mycologist1@yahoo.com
** Corresponding Author:* Dr A. Serda Kantarcıoğlu, Cerrahpaşa Medical Faculty, Department of Medical Mycology, Istanbul, Turkey

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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 keratinophylic 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 usefull 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-

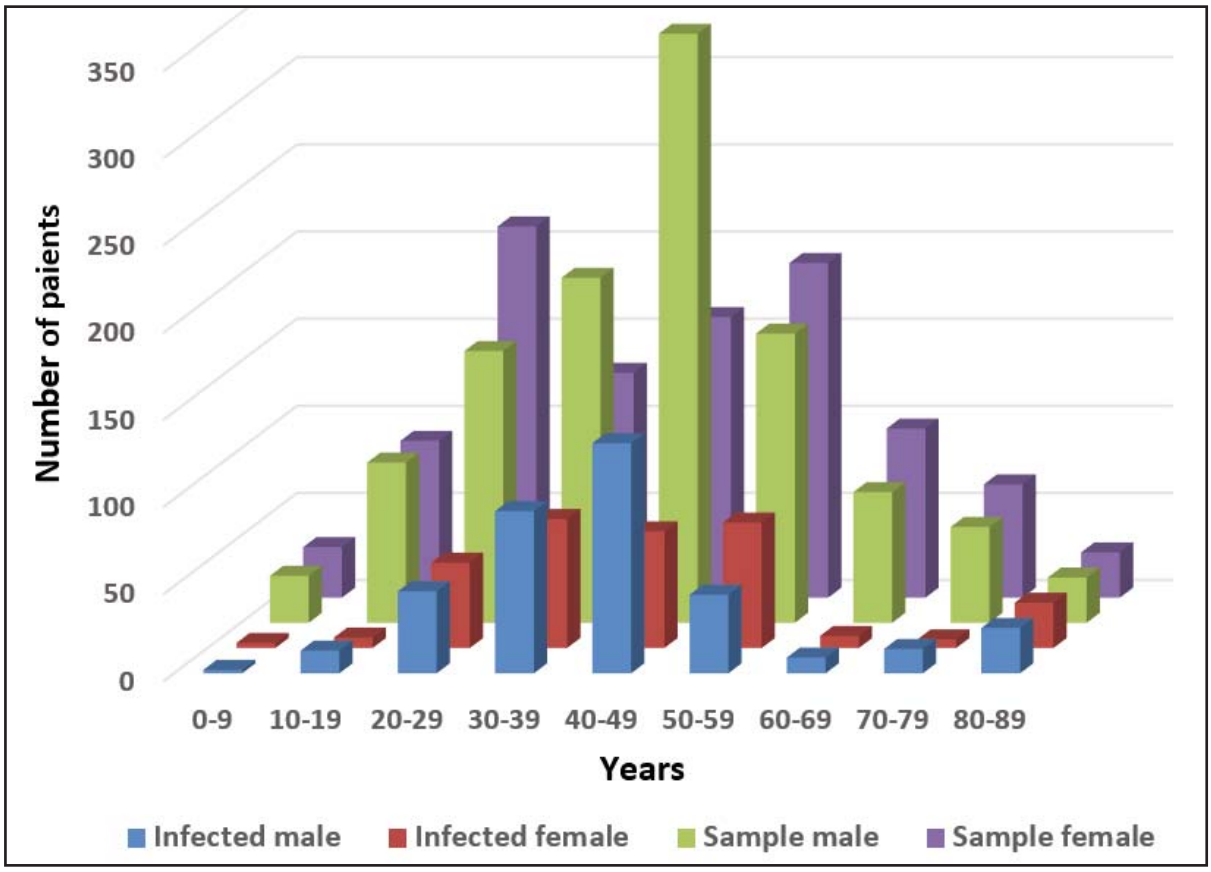

Figure 1. Age and gender distribution of patients with clinically suggestive lesions (no=2125) and of them those with microbiologically proven dermatomycosis (no=643)

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 reffered 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 gentamycine (0.04 mg/ml) and one SDA with gentamycine and cycloheximide (0.05 mg/ml) and incubated 3 weeks at 25°C except one with gentamycine 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 morphologial 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 artroconidia. Germ tube test and chlamydospore formation test was performed for differentiating *Candida albicans* from non-albicans speciess. Der-

Table 1. Distribution of Isolated Significant Fungi (n= 643) to Samples Collected (n=2125) and Body Sites (01 April 2010 - 01 June 2014)

Specimen	Anatomical site	*Candida* spp (n= 206, 32.0 %)		Dermatophytes (n=308, 47.9%)										0ther keratinophylic fungi (n=126, 19.6%)			Total number of isolates per anatomical site
				Trichophyton spp (n=295, 95.8%)						*Microsporum* spp (n=13, 4.2%)							
		[1]*C. a*	[2]*C. s*	[3]*T. r.*	[4]*T. m.*	[5]*T. t.*	[6]*T. v.*	[7]*T. v.*	[8]*T. s.*	[9]*M. c.*	[10]*M. g.*	[11]*M. s.*	[12]*M.s.*	[13]*T.s.*	[14]*F.s.*	[15]*P.s.*	
Nail (n= 1448, 68.1%)	Fingernail (n=216, 10.1%)	25	76	12	3	1			10					16	5		148
	Toenail (n=1232, 58.0%)	9	48	67	21	4	2	1	64					64	12	2	294
Skin scrapings (n=625, 29.4%)	Hand, palm, interdigital (n=149, 7.0%)	3	15	7	1				5					20			51
	Arm (n=32, 1.4%)			3	3				1		1						8
	Face, neck (n=28, 1.3%)		3	3	2							1	2	1			12
	Body (n=42, 2.0%)	2	1	6					3	3							15
	Foot sole, interdigital (n=286, 13.4%)	4	8	32	13	1			13					4	1		76
	Leg (n=52, 2.3%)		5			1			2			2	1	1			12
	İnguinal (n=28, 1.3%)	2	1						2	3							8
	Gluteal (n=18, 0.8%)	1	2	4	1				1								9
Hair and scalp (n= 52, 2.4%)			1	1	2			1	2		2	1					10
Total (n=2125), % in each group		46 (7.2 %)	160 (24.9 %)	135 (21.0 %)	46 (7.2 %)	7 (1.0 %)	2 (0.3 %)	2 (0.3 %)	103 (16.0 %)	6 (1.0 %)	3 (0.5 %)	4 (0.6 %)	3 (0.5 %)	106 (16.4 %)	18 (2.8 %)	2 (0.3 %)	643 (100%)

[1]C.a.: *Candida albicans* (22.3%); [2]C.s.: *Candida* spp.(77.7%); [3]T.r: *T. rubrum* (43.8%); [4]T.m.: *T. mentagrophytes* (14.9%); [5]T.t.: *T. tonsurans* (2.3%); [6]T.v.: *T. verrucosum* (0.6%); [7]T.v.: *T. violaceum* (0.6%); [8]T.s.: *Trichophyton* spp (33.4%); [9]M.c.: *M. canis* (1.9%); [10]M.g.: *M. Gypseum* (1.0%); [11]M.s.: *Microsporum* spp (1.3%); [12]M.s.: *Malassezia* spp (0.5%); [13]T.s.: *Trichosporon* spp (84.1%); [14]F.s.: *Fusarium* spp (14.3%); [15]P.s.: *Phoma* spp (1.6%)

matophytes were subcultured on potato dextrose agar, ure agar slants and/or rice medium for further identification and nondermatophyte molds were identified by macroscopic and microscopic characteristics [**3, 4, 5, 6, 7**]. The patients from whose samples non dermatophite molds were cultured, were called two more times with two weeks intervals, to obtain fresh samples to confirm the pathogenic significance of the fungus by repeating cultures and to exclude contamination [**8, 9, 10, 11**].

Results

A total of 2125 samples were collected from patients with symptoms compatible with superficial mycosis. Of those 1227 (57.7%) were female, 898 (42.3%) male. Age range was from 1 to 80 years and mean age was 49. The distribution of patients with clinically dermatomycosis suspected lesions and with mycologically confirmed dermatomycosis according to age and gender shown in (**Figure 1**). Of them, 72 (11.6%) were diagnosed and treated with topical or systemic antifungals in the past and relapsed in 38 (6%) patients and the remainings did not responded to the therapy.

Pathogen fungi (n=643) were isolated from 623 patients' samples by direct microscopy and culture. Of the totally 643 pathogen isolates, 308 (47.9%) were dermatophytes, 206 (32.0%) *Candida* spp, and 129 (19.8%) non dermatophyte fungi, as 18

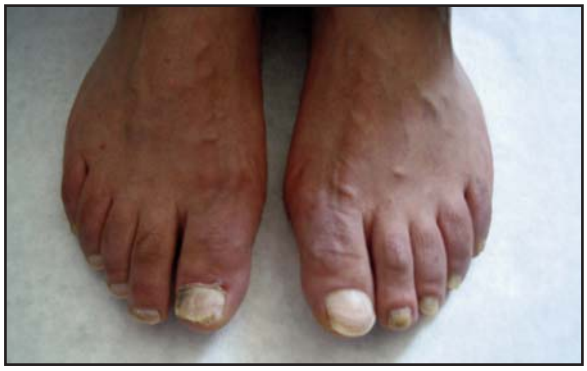

Figure 2. Nails infected with *Phoma* spp

(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 dealed 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.

Figure 3. Picnidium and oval shaped conidia (400x)

Dermatophytosis was present in family members of 166 (26.6%) patients, contacts with animals occured in 89 (14.3%), with soil in 24 (3.9%). Diabetes mellitus was found in 50 (8.0%), psoriasis in 12 (1.9%) of 623 patients.

Tinea capitis due to *T. mentagrophytes* was detected in two males and due to *T. rubrum, T. Violaceum, Microsporum* sp each in one female pediatric patients. *Trichophyton* rubrum was isolated from a generalized tinea corporis and tinea pedis case.

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

Table 2. Specimens From Which Two Different Aetiological Agents Cultured (n=20)

Specimen	Fungi	
Fingernail (n=7)	*Candida* sp	*Trichosporon* sp
	Candida sp	*Trichophyton rubrum*
	Candida glabrata	Fusarium sp
	Candida glabrata	*Candida tropicalis*
	Candida glabrata	*Fusarium* sp
	Candida glabrata	*Fusarium* sp
	Candida glabrata	Candida tropicalis
Toenail (n=10)	*Trichophyton rubrum*	*Trichophyton* sp
	Trichophyton rubrum	*Trichophyton* sp
	Trichophyton sp	*Trichophyton* sp
	Trichophyton sp	*Trichophyton* sp
	Candida sp	*Trichophyton* sp
	Candida sp	*Trichophyton* sp
	Candida sp	*Trichophyton* sp
	Candida sp	*Trichophyton* sp
	Candida sp	*Trichophyton* sp
	Candida glabrata	*Fusarium* sp
Foot interdigital (n=1)	*Candida* sp	*Trichophyton rubrum*
Arm (n=1)	*Microsporum* sp	*Trichosporon* sp
Hair (n=1)	*Microsporum gypseum*	*Trichophyton rubrum*

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

comparable studies [**36, 37, 38**]. In the present study, two different significant fungi were isolated together in 20 (3.1%) cases, probably representing mixed infections.

Onychomycosis is caused mainly by dermatophytes but occasionally by nondermatophytic fungi. Traditionally moulds other than dermatophytes have been considered as contaminating fungi of the skin and nails. *Phoma* is a typicall genus of Coelomycetes with over 200 known species which were occasionally recovered in cases of human subcutaneous disease, endophthalmitis and deep tissue infection [**5**] and very rarely reported from onychomycosis [**39**]. In our laboratory, *Phoma* spp was isolated from toenails of two different patients in two different years, and clinically mimiced the signs and symptoms of dermatophyte infections. Careful diagnostic attention is required when identifying non dermatophytes as an etiologic agent of onychomycosis.

In the current study, 643 of the total of 2125 clinically suspected cases were confirmed by mycological methods. From our overall data, dermatomycosis occured 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.

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