

Dermatophyte infections in patients attending a tertiary care hospital in northern Italy

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SUMMARY

We studied dermatophyte infections in patients attending the Dermatology Outpatients Clinic of S. Matteo hospital Pavia, Italy, during the period 2004-2006. A total of 100 samples were collected from 95 patients; 97 dermatophytes and 3 keratinophilic fungi were isolated.

Trichophyton rubrum was the most frequent dermatophyte isolated (42.3%), followed by *Microsporum canis* (31%), *T. mentagrophytes* (14.5%) and *M. gypseum* (9.2%). Less frequently isolated were *Epidermophyton floccosum* and *T. violaceum*. The most common dermatophyte infections included tinea corporis, tinea pedis, tinea unguium, tinea capitis and tinea cruris.

Zoophilic dermatophytes were most commonly recovered from children and adolescents with tinea capitis and tinea corporis. Anthropophilic species were identified mostly in adults with tinea pedis, tinea cruris and onychomycosis.

KEY WORDS: Dermatophytes, Dermatophytosis, Epidemiology

Received April 30, 2008

Accepted June 04, 2008

INTRODUCTION

Superficial fungal infections are among the world's most common diseases and the prevalence of superficial mycotic infections has risen to such a level that skin mycoses now affect more than 20-25% of the world's population. The trend of living in communities, contact with animals, the use of antibiotics, corticosteroids, and antineoplastic drugs are some of the factors that contribute to the increase in the risk of infection by fungi especially by dermatophytes (Falahati *et al.*, 2003).

Dermatophytes are a unique group of fungi that infect keratinous tissue, with the skin, hair and nails being the most common sites. This group of

closely related fungi has the ability to invade keratinized tissue and produce an infection, dermatophytosis, commonly referred to as ringworm. Dermatophytes are among the most common parasites observed in human and animal skin's diseases. Infection is generally cutaneous and restricted to the nonliving cornified layers. Reaction to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors (Esquenazi *et al.*, 2004).

The distribution of the dermatophytosis and their etiological agents varies with geographical location and depends on several factors, such as lifestyle, type of the population, migration of people and climatic conditions, therefore some species are widely distributed whereas others are geographically restricted. Anthropophilic dermatophyte infections predominate in central and northern European countries, and infections

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caused by zoophilic species in some regions of south Europe and the Mediterranean. In the past decades, major changes in the epidemiological pattern of dermatophyte infections have occurred in Europe. In the postwar period, *Trichophyton rubrum* was the predominant pathogen causing dermatophytosis. In the 1980s, however, a sharp increase in the prevalence of *Microsporum canis* infections was recorded (Dolenc-Voljc, 2005).

The purpose of the present study was to establish the prevalence and distribution of dermatophytes recovered from skin, hair and nails of 95 patients examined in the Dermatology Outpatients Clinic of S. Matteo hospital Pavia during the period 2004-2006.

MATERIALS AND METHODS

Skin scales, nail and hair fragments were collected from patients with suspected dermatomycosis. A total of 100 cultures were obtained from 95 patients with dermatomycosis, 48 males (50.5%) and 47 (49.5%) females, during 2004-2006. All samples were obtained from patients attending the Dermatology Outpatients Clinic, S. Matteo hospital Pavia.

Fungal identification

Direct microscopic examination was performed using 20% (w/v) potassium hydroxide (KOH) with Blu Parker Super Quink 10%. A portion of specimen was placed on a microscopic slide and 50 µL of clarifying agent was added. After 20 min, the wet preparation from scales was examined for the presence of fungal elements and their diagnostic morphology such as arthroconidia hyphae, while the nail samples were microscopically observed one day later. Samples were also cultured on duplicate plates of Sabouraud Dextrose Agar (BioMérieux) and Dermatophyte Test Medium (DTM) made according to the manufacturer's instructions. The plates were inoculated with finely divided pieces of each sample and incubated at room temperature for recovery of dermatophytes or moulds.

The dermatophyte test medium (DTM) is an alternative culture method that suggests the presence of dermatophyte pathogens, even though it does not identify specific organisms. The culture medium was originally described by Taplin *et al.*

(Taplin *et al.*, 1969) as a test for the presence of dermatophytic molds. The DTM contains gentamicin and chlorotetracycline to inhibit bacterial growth and cycloheximide to inhibit growth of saprophytic fungi. Dermatophyte growth is indicated by a change in the colour of the DTM from yellow acid dye to red alkalized color in response to the alkaline catabolites produced by the growing dermatophytes.

Fungal isolates were examined macroscopically and microscopically in acid fucine and the dermatophyte species were identified by gross and microscopic morphology. Identification of moulds as pathogens was based on an examination of morphology of the thallus and of the microscopic structures following growth on cycloheximide-free Sabouraud Dextrose Agar medium.

RESULTS

Forty-eight (50.5%) out of 95 patients were males and 47 (49.5%) were females; the age range was from a few months to 73 years.

On direct microscopic examination 96.9% (94/97) of clinical samples showed the typical dermatophytic structures as cylindrical, sparsely branching filaments sometimes with regular chains of swollen cells, arthroconidia, or ectothrix and endothrix infection. In 3.1% of cases dermatophytes were isolated from skin scraping that were negative for fungal filaments on microscopic examination, probably because they were few and were missed in the observation.

The six-dermatophyte species isolated and their frequencies are shown in Table 1. *Trichophyton rubrum* was the most frequent isolate (42.3%) followed by *Microsporum canis* (31%), *T. mentagrophytes* (14.5%), *M. gypseum* (9.2%) *T. violaceum* (2%). *Epidermophyton floccosum* was isolated only once (1%).

On 1% peptone agar eleven isolates of *T. mentagrophytes* showed a suede-like to downy surface characteristic of the *interdigitale* variety whereas the other strains isolated had a granular appearance with, in microscopic observation, numerous large clusters of more spherical microconidia and more macroconidia typical of *T. mentagrophytes* var. *mentagrophytes*.

Figure 1 groups the results grouped according to

TABLE 1 - Prevalence of isolated dermatophyte species according to site of infection A adult, C children.

Species	Skin scales	Hair	Nails	Total	A	C
<i>Trichophyton rubrum</i>	23	-	18	41 (42.3%)	41	-
<i>Microsporium canis</i>	17	13	-	30 (31%)	11	19
<i>Trichophyton mentagrophytes</i>	11	-	3	14 (14.5%)	12	2
<i>Microsporium gypseum</i>	8	-	1	9 (9.2%)	7	2
<i>Trichophyton violaceum</i>	2	-	-	2 (2%)	2	-
<i>Epidermophyton floccosum</i>	1	-	-	1 (1%)	1	-
Total	62 (64%)	13 (13.4%)	22 (22.6%)	97 (100%)	74	23

the location of the dermatophytosis on the patients. Tinea corporis (26.8%) and tinea pedis (25.7%) were the most common type of cutaneous mycotic infections, followed by tinea unguium (22.7%), tinea capitis (14.5%) and tinea cruris (10.3%).

Table 2 shows that *M. canis* (65.4%) was the main etiological agent of tinea corporis and the most frequently isolated (92.8%) from tinea capitis. *Trichophyton rubrum* was the dominant agent of tinea pedis and tinea unguium.

The two strains of *T. violaceum* recorded in this study were isolated from tinea corporis and tinea capitis. *Epidermophyton floccosum* was isolated once from tinea cruris.

Nondermatophyte organisms were isolated in cultures of tinea unguium from three patients: *Chrysosporium keratinophilum*, *Scopulariopsis brevicaulis* and *Aspergillus niger* in one patient each. Direct mount of these nail samples showed

ambiguous filaments and on culture medium moulds grew from many separate nail pieces.

DISCUSSION

This survey on the prevalence and distribution of dermatophytes isolated from clinical materials in Dermatology Outpatients Clinic of S. Matteo hospital Pavia during the years 2004-2006 shows that the most common dermatomycoses are tinea corporis, tinea pedis and tinea unguium (Figure 1). The incidence of these forms of infection are closely related to the site of infection and consequently to the clinical materials collected. As a matter of fact in this study skin scales represent 64% of cases, while nails account for 22.6% and hair 13.4%.

In tinea corporis and tinea capitis *Microsporium canis* was the most commonly isolated etiologic

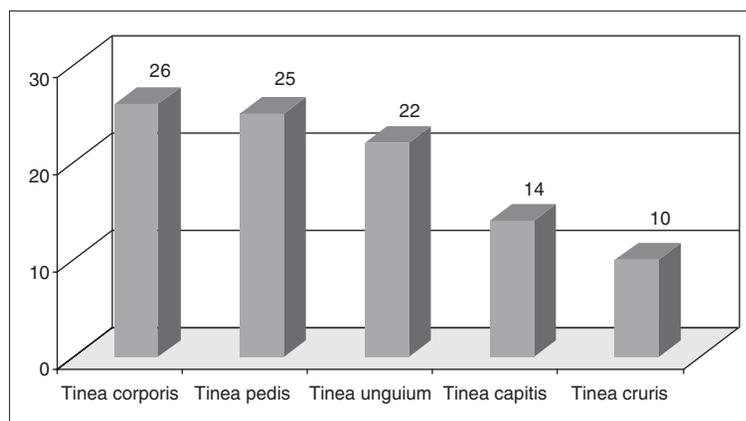


FIGURE 1 - Distribution of tinea types in 97 positive cases.

TABLE 2 - Frequency of dermatophytes according to type of tinea. Number of isolates, () Distribution percentage of species, A adult, C children.

Species of dermatophytes	Type of lesion										Total	
	Tinea corporis		Tinea pedis		Tinea unguium		Tinea capitis		Tinea cruris			
<i>Trichophyton rubrum</i>	-	-	16 (64)	-	18 (81.8)	-	-	-	7 (7)	-	41	-
<i>Microsporum canis</i>	17 (65.4)	-	-	-	-	-	13 (92.8)	-	-	-	30	-
<i>T. mentagrophytes</i>	3 (11.5)	-	8 (32)	-	3 (13.6)	-	-	-	-	-	14	-
<i>M. gypseum</i>	5 (19.3)	-	1 (4)	-	1 (4.6)	-	-	-	2 (2)	-	9	-
<i>T. violaceum</i>	1 (3.8)	-	-	-	-	-	1 (7.2)	-	-	-	2	-
<i>Epidermophyton floccosum</i>	-	-	-	-	-	-	-	-	1 (1)	-	1	-
Total	26	-	25	-	22	-	14	-	10	-	97	-
Patient age	A	C	A	C	A	C	A	C	A	C	A	C
	16	10	25	-	22	-	1	13	10	-	74	23

agent. This zoophilic dermatophyte is usually acquired from infected domestic pets, such as dogs and cats, typically associated with urban life-style, and was found to be the most frequent isolated species in several countries (Frangoulis *et al.*, 2004).

In Europe *M. canis* is still the most common re-

ported causative agent of tinea capitis and percentages of isolation from this clinical form in Italy range from 82.7% to 90.5%, followed by *T. mentagrophytes*.

Cats, especially kittens, are the main source of human infections. The knowledge of ringworm epidemiology in pets is very important to reduce

TABLE 3 - Number of dermatophyte isolates according to type of tinea and patient sex.

Species of dermatophytes	Type of lesion												
	Tinea corporis		Tinea pedis		Tinea unguium		Tinea capitis		Tinea cruris		Total		
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
<i>Trichophyton rubrum</i>	-	-	14	2	4	14	-	-	0	7	18	23	41
<i>Microsporum canis</i>	10	7	-	-	-	-	7	6	-	-	17	13	30
<i>T. mentagrophytes</i>	1	2	3	5	2	1	-	-	-	-	6	8	14
<i>M. gypseum</i>	2	3	1	0	0	1	-	-	0	2	3	6	9
<i>T. violaceum</i>	1	0	-	-	-	-	0	1	-	-	1	1	2
<i>Epidermophyton floccosum</i>	-	-	-	-	-	-	-	-	0	1	0	1	1
	14	12	18	7	6	16	7	7	0	10	45	52	97
Total	26	-	25	-	22	-	14	-	10	-	97	-	

the spread of zoophilic fungal infections to humans (Ginter-Handelmayer *et al.*, 2007; Bassiri Jahromi *et al.*, 2006; Mancianti *et al.*, 2002; Romano, 1999; Aste *et al.*, 1997).

The present study has confirmed this dermatophyte as being the most common species (92.8%) among all dermatophytes isolated in tinea capitis and only patients in the prepubertal stage were affected by this clinical form with an equal distribution among sexes (Table 3).

The findings on this type of dermatophytosis are in line with the data collected in Siena and reported in the retrospective study of Romano (Romano, 1999).

Considering tinea unguium, tinea pedis and tinea cruris the main species isolated from this dermatophytosis was *Trichophyton rubrum* (Table 2). All 41 *T. rubrum* infections in this study were found in adults and this agreed with the observations of other authors (Ng *et al.*, 2001) that adults have a higher susceptibility to *T. rubrum* infections than children.

Chronic infections caused by *T. rubrum* in the glabrous skin, crural region, feet and nails are possibly due to occlusive footwear and tight underwear (Valdigem *et al.*, 2006). Risk factors are on the basis of the higher frequency of *T. rubrum* among middle-aged people, and in most of the examined clinical forms showed a preference for male patients, especially in the case of tinea unguium and tinea cruris, while in the case of tinea pedis a contrary situation was recorded (Table 3). The second etiologic agent of tinea pedis and tinea unguium isolated from the patients surveyed was *Trichophyton mentagrophytes*. It was mainly isolated from tinea pedis and corresponded to 32% of all cases of this clinical form. Its distribution over male and female patients was relatively equivalent, five males and three females in tinea pedis and eight males and six females of all isolates belonging to this species (Table 3).

If compared with the study of Aste *et al.* focused on tinea pedis in Cagliari (Aste *et al.*, 2000), the prevalence of dermatophyte species in this clinical form was the exact opposite: *T. rubrum* was the predominant pathogen followed by *T. mentagrophytes*. In the examined cases of tinea unguium the frequency of species agreed with that found by Romano *et al.* in northern and central Italy along with the isolation of moulds in this superficial fungal infection.

In tinea corporis *Trichophyton mentagrophytes* represented the third species isolated, while *Microsporum gypseum* was the second one. This ubiquitous geophilic dermatophyte is an established human pathogen found in the soil and from 2004 to 2006 the organism was isolated from 9 patients with cutaneous infections. It was mainly found in cases of tinea corporis and only one isolation came from tinea pedis and tinea unguium (Table 2) and it was recorded from two prepuberty patients and seven adults (Table 1).

In one case of tinea corporis and tinea capitis *Trichophyton violaceum* was isolated which "with *Epidermophyton floccosum*" represents the species with the lowest predominance in the study period considered. This result could be interpreted as indicative of species that nowadays have a low clinical relevance and may tend to disappear from our community.

In Europe, reports of *T. violaceum* are only sporadic in Poland and Spain, but in Holland infections are increasing in parallel with immigration, and increases are reported in some areas of the UK. *Trichophyton violaceum*, which was one of the most frequent anthropophilic mycetes until the 1950s, subsequently decreased, disappearing completely in some regions and becoming only sporadic in others. As reported by Romano *et al.* (Romano *et al.*, 2000) the epidemiological data of the last 20 years for tinea capitis indicate very few cases, all in residents, with a prevalence in adults of Apulia and Sardinia, but the cases of dermatophytosis due to *T. violaceum* in Tuscany from 1985 to 1997 described by the authors indicated a constant increase in the number of infections due to this etiological agent. This trend was not observed during the years considered in the present study.

Considering the survey conducted two decades ago in Pavia by Di Silverio *et al.* (Di Silverio *et al.*, 1989) tinea corporis remains the prevalent dermatophytosis, while the percentage of tinea pedis and unguium are exactly opposed. Noteworthy is the increase of cases of tinea capitis observed in our study.

Some dissimilarities are noticeable from the study conducted in Rome by Panasiti and coworkers (Panasiti *et al.*, 2007) in which tinea corporis, the prevalent dermatophytosis, was followed in frequency by tinea cruris and tinea capitis, *M. canis* was the species with high predominance during

the study period and *T. mentagrophytes* represented the most common etiologic agent isolated from tinea pedis and tinea unguium.

In conclusion, the results of the present study indicated that tinea corporis, tinea pedis and tinea unguium are the clinical forms mainly present in the considered period and that the consequent distribution of dermatophytes in this study area was dominated by the anthropophilic and zoophilic dermatophytes, while geophilic ones made up 9.2%. Moreover, following the changing patterns of dermatophyte infections globally, *T. rubrum* was the most common dermatophyte isolated, followed by *Microsporum canis*. This finding concurred with that of Romano (Romano *et al.*, 2000) who found that these two species were the organisms responsible for most cases of dermatophytosis.

The data also showed that while anthropophilic dermatophytes infected mainly adults, geophilic and zoophilic species preferentially affected prepubertal individuals. In addition, the high predominance of tinea corporis may be partly explained by the dispersal of fungi from other lesions of the trunk as a result of sharing facilities.

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