

# A Retrospective Study on Isolation and Identification of Various Species of Dermatophytes in Samples Received at Department of Microbiology at a Tertiary Care Hospital

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## Abstract

**Introduction:** Dermatophytes is the most common superficial mycoses affecting skin, hair, and nail; group of fungi that are capable of infecting keratinized tissues. Over the past decades, the prevalence of these infections has been rising. These infections are more common in the tropical countries due to humidity, sweating, and elevated temperature.

**Purpose:** The purpose of the study was to know the prevalence of dermatophytosis in different samples like skin, hair, and nail and to identify species of dermatophytes.

**Materials and Methods:** In this study, total 414 skin scrapings, nail clippings, and hair root samples by plucking were collected and processed by direct microscopy in potassium hydroxide and cultured on sabouraud dextrose agar (SDA) with antibiotics and cycloheximide. Identification of the species was done by colony morphology, lactophenol cotton blue mount, hair perforation test, and urease test.

**Results:** Out of 414 sample collection, 95 were either positive in direct microscopy or show growth in culture or both. Out of 95 samples, 45 were dermatophytes. Among this 55.6% of affected population were male and 44.4% were females. Out of 45 dermatophytes, 29 were positive in both direct microscopy and culture and 16 were only culture positive. Most common isolated dermatophytes is *Trichophyton* (89.5%) followed by microsporum (7.9%) and *Epidermophyton* (2.6%). Out of *Trichophyton* species, most common isolated species are *Trichophyton rubrum* (43.9%) followed by *T. mentagrophyte* (39.0%) and *T. violeceum* (12.2%).

**Conclusion:** *T. rubrum* is the commonly isolated dermatophytes in this area. Male has higher prevalence rate than females with 1.3:1 ratio. Tinea corporis is the most common type of infection. Identification of causative dermatophyte species would help with treatment approach and for the implementation of control measures.

**Key words:** Dermatophytes, Superficial mycoses, Tinea corporis, *Trichophyton rubrum*, *Trichophyton* species

## INTRODUCTION

Dermatophytosis is the most common type of superficial cutaneous fungal infection seen in men and animals.<sup>[1]</sup>

Over the past decades, the prevalence of these infections has been raised, accordingly, they have affected 20–25% of the world's population.<sup>[2]</sup> Dermatophytes have been recorded all over the world with variation in distribution, incidence, epidemiology, and target hosts from one location to another.<sup>[3]</sup> Geographic location, climate (temperature, humidity, wind, etc.), overcrowding, healthcare, immigration, environmental hygiene, culture, and socioeconomic condition have been incriminated as major factors for these variations.<sup>[3]</sup> The prevalence of the fungal infection of the skin is on a growing trend in

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India due to its climate conditions, such as its temperature and humidity setting the ground for the spread of this infection.<sup>[2]</sup>

Dermatophytes are aerobic fungi that produce proteases that digest keratin and allow colonization, invasion, and infection of the stratum corneum of the skin, hair shaft, and nail.<sup>[4]</sup> Infection is generally cutaneous and restricted to the nonliving cornified layers because fungi are not able to penetrate the deeper tissue or organ of a healthy immunocompetent host.<sup>[5]</sup> Dermatophytes are hyaline and septate molds with more than a hundred species. There are divided into three main anamorphic genera depending on their morphological characteristics, (1) *Trichophyton*-infect skin, hair, and nail, (2) *Microsporum*-infect skin and hair, and (3) *Epidermophyton*-infect skin and nail.<sup>[1,6]</sup>

The dermatophytes are referred to as zoophilic, anthropophilic, or geophilic depending on whether their primary source is an animal, human, or soil, respectively.<sup>[7]</sup> Of all the zoophilic dermatophytes, *Microsporum canis* is the most prevalent throughout the world, in both temperate regions and some tropical regions.<sup>[1]</sup> The distinction between geophilic and zoophilic species may not always be obvious and even may be controversial at times.<sup>[1]</sup> The infections caused by anthropophilic species tend to be chronic and inflammation is minimal. The infections caused by anthropophilic species tend to be chronic and inflammation is minimal and infections caused by geophilic and zoophilic species tend to be self-healing and resultant inflammation is more severe.<sup>[1]</sup> Dermatophytes grow in a centrifugal pattern in the stratum corneum, leading to the formation of well-demarcated annular-shaped pruritic scaly skin lesions with central clearing and raised edges.<sup>[8]</sup> Depending on the site of involvement, various clinical types of dermatophytes or tinea infections are produced.<sup>[1]</sup> (1) Tinea capitis – infection of the scalp, (2) Tinea corporis – infection of non-hairy skin of the body (trunk and limbs), (3) Tinea pedis – infection of web space between the toes, (4) Tinea cruris – infection of the groin area, (5) Tinea barbae – infection of the beard and mustache area, (6) Tinea faciei – infection of the non-bearded area of the face, (7) Tinea unguium – nail plate infection, and (8) Tinea Mannum – infection of the palmar aspect of hands.

### Objectives

The objectives of the study are as follows:

1. To determine the prevalence of dermatophytosis in different samples like skin, hair, and nail
2. To identify the species of dermatophytes isolated in culture.

## MATERIALS AND METHODS

This study was conducted in the department of microbiology at the tertiary care center. In this study, a total of 414 samples (Skin scraping, nail, and hair root samples) were collected from patients attending various outpatient department with the suspicion of fungal infection.

### Direct Microscopic Examination (Potassium Hydroxide [KOH] Mount)

#### Scraping

In all cases, cleaning the local site with 70% alcohol, which is allowed to evaporate before taking the specimen, may be helpful and should always be done if greasy ointments or powders have already been applied as medication.<sup>[1]</sup> The material should be collected by scraping outward from the edges of active lesions with a scalpel blade held at an angle of 90° of the skin surface.<sup>[1]</sup> The specimens from the scalp are obtained by scraping with a blunt scalpel so that they include hair stubs, contents of plugged follicles, and scales.<sup>[1]</sup> In the case of onychomycosis, after disinfecting with 70% alcohol, the nail is clipped from the free edge, taken as far back as possible, and should include its full thickness.<sup>[1]</sup>

#### KOH Mount

The samples were subjected to direct microscopic examination for the presence of fungal elements using KOH (10% for skin scrapings or hair and 20–40% for nail clippings<sup>[1]</sup>). These specimens were mounted under cover slip in KOH on slide.<sup>[1]</sup> This clears material within 5–20 min, depending on its thickness.<sup>[1]</sup>

#### Culture

All specimens were cultured irrespective of positive or negative direct microscopic examination SDA containing chloramphenicol with cycloheximide to prevent bacterial contamination then inoculated plates were incubated for 10 days to 3 weeks at 25°C and 37°C. Culture plates were examined on an alternate day for any fungal growth. Cultures of dermatophytes were identified on the basis of colony characteristics and pigmentation.<sup>[1]</sup>

#### Lactophenol cotton blue (LCB) Stain

Take a transparent tape and touch its sticky surface to the fungal colony grown in the culture plate and place it on the slide where a drop of LCB is already added and examine it under a microscope.<sup>[1]</sup>

#### Urease Test

Christensen's urea agar was used for the differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum*.<sup>[1]</sup> The identification of dermatophytes was accomplished using fungal morphology, hyphal organization (septate/aseptate),

and the presence and arrangement of microconidia and macroconidia.

## RESULTS

Out of 95 samples, 45 were dermatophytes. Among this 55.6% of affected population were male and 44.4% were females. Male to female ratio was 1.3:1. The most common age group affected with dermatophytosis was 21–30 years with 11 cases [Tables 1 and 2].

Out of 95 sample collected, 73 were skin scraping, 13 were nail scraping and clippings, and nine were hair stubs [Table 3].

Out of 45 dermatophytes, 29 were positive in both direct microscopy and culture and 16 were only culture positive [Table 4].

Most common isolated dermatophytes is *Trichophyton* (89.5%) followed by microsporum (7.9%) and *Epidermophyton* (2.6%). Out of *Trichophyton* species, the most common isolated species are *T. rubrum* (43.9%) followed by *T. mentagrophytes* (39.0%), *T. violaceum* (12.2%) [Table 5].

Most common infection was tinea corporis 37.8% followed by tinea cruris 24.5% [Table 6].

**Table 1: Frequency of dermatophytes in relation to sex**

Sex	Total	Dermatophytes (%)
Male	55	25 (55.6)
Female	40	20 (44.4)
Total	95	45 (47.4)

**Table 2: Age-wise distribution of clinically diagnosed dermatophytosis**

Age (years)	Number of cases
<10	6
11–20	6
21–30	11
31–40	6
41–50	7
>50	9
Total	45

**Table 3: Distribution of clinical samples and results of microbiological investigation**

Sample	Total number	Dermatophytes (%)
Skin scraping	73	38 (84.4)
Nail	13	2 (4.5)
Hair follicle	9	5 (11.1)
Total	95	45 (47.4)

## DISCUSSION

In the present study of 45 cases of dermatophytic infections, the following clinical forms were observed: Tinea corporis, tinea cruris, tinea pedis, tinea mannum, tinea unguium, tinea barbae, and tinea faciei.

In this study, dermatophytosis is more commonly seen with age group of 21–30 years which is same as other studies done by Doddamani *et al.* While in other study by Bindu and Pavithran, the most common age group was 11–20 years.<sup>[9]</sup> The highest incidence in age group of 21–30 years may be due to increased physical activity and increased degree of exposure to infection.

In the present study, males were commonly affected than females. Male to female ratio was 1.3:1, which is compatible with other studies done by Singh *et al.*, Bindu and Pavithran, and Doddamani *et al.* The higher incidence in males could be due to greater physical activity and increased sweating.<sup>[5]</sup>

**Table 4: Potassium hydroxide and culture analysis of clinical specimen of dermatophytes**

Samples	Number of culture positive cases	Number of cases positive by KOH	Number of cases negative by KOH
Skin	73	25	48
Nail	13	1	12
Hair	9	3	6
Total (%)	45	29 (64.44)	16 (35.55)

KOH: Potassium hydroxide

**Table 5: Prevalence pattern of dermatophytes**

Species	Positive (%)
<i>Trichophyton</i>	41 (89.5)
<i>T. rubrum</i>	18 (43.9)
<i>T. mentagrophytes</i>	16 (39.0)
<i>T. violaceum</i>	5 (12.2)
<i>T. scholenii</i>	2 (4.9)
<i>Microsporum</i>	3 (7.9)
<i>Epidermophyton</i>	1 (2.6)
Total	45

*T. rubrum*: *Trichophyton rubrum*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *T. violaceum*: *Trichophyton violaceum*, *T. scholenii*: *Trichophyton scholenii*

**Table 6: Clinical types of dermatophytosis**

Clinical types	Number of cases (n=45), n (%)
Tinea corporis	17 (37.8)
Tinea cruris	11 (24.5)
Tinea capitis	5 (11.0)
Tinea faciei	4 (8.9)
Tinea barbae	1 (2.2)
Tinea mannum	3 (6.7)
Tinea pedis	2 (4.5)
Tinea unguium	2 (4.5)

**Table 7: Dermatophytes isolated in various studies**

Studies	<i>T. rubrum</i> (%)	<i>T. mentagrophytes</i> (%)	<i>Microsporum</i> (%)	<i>Epidermophyton</i> (%)
Upadhyay <i>et al.</i> <sup>[2]</sup>	73.27	17.24	0	7.75
Surendran <i>et al.</i> <sup>[5]</sup>	67.5	20	5	5
Doddamani <i>et al.</i> <sup>[10]</sup>	46.87	36.46	6.24	8.33
Singh and Beena <sup>[11]</sup>	73.27	17.24	0	7.75
Patel <i>et al.</i> <sup>[12]</sup>	60.71	23.80	1.19	1.19
Hanumanthappa <i>et al.</i> <sup>[13]</sup>	58.9	24.6	8.2	0.7
Kalyanappa and Surekha <sup>[14]</sup>	52.38	33.33	4.76	0
Belurkar <i>et al.</i> <sup>[15]</sup>	53.52	0	8.92	0
Present study	43.9	39	7.9	2.6

*T. rubrum*: *Trichophyton rubrum*, *T. mentagrophytes*: *Trichophyton mentagrophytes*

Among clinical types, tinea corporis was highest (17 cases) followed by 11 cases of tinea cruris. This is in accordance with the Bindu and Pavithran.

In the present study, it was possible to demonstrate fungi on direct microscopy with KOH in 29 cases but overall positivity by culture was 45 which is same as other studies done by Belurkar *et al.* and Huda *et al.*<sup>[16]</sup>

Species identification findings of the present study are compared with other studies as shown in [Table 7].

## CONCLUSION

The prevalence of dermatophytes infections depends on environmental factors, personal hygiene, and individual susceptibility. In this study, dermatophytes had an isolation rate of 10.9%, which is quite lower than the rates reported in other studies. This can be due to the fact that the majority of the investigated patients who were from urban regions and hygienic condition predispose the inhabitants to low risk of cutaneous fungal infections.

Among all cases, Tinea corporis was the predominant type of dermatophytosis with 37.8% followed by tinea cruris. KOH mount were positive in 64.4% of cases and all cases were culture positive. In 35.6% of KOH negative cases culture were positive. Hence, it is advisable always go for fungal culture irrespective of KOH results. *T. rubrum* is the commonly isolated dermatophytes in this area. Male have higher prevalence rate than females with 1.3:1 ratio.

Identification of causative dermatophytes species would help with treatment approach and for implementation of control measures.

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