Original Research Article

Isolation and identification of dermatophytes in a tertiary care teaching hospital

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Abstract

Background: Dermatophytosis are the most common types of cutaneous fungal infection seen in humans affecting skin, hair and nails in both developing and developed countries. Hot and humid climate in tropical and subtropical countries like India makes dermatophytosis a very common superficial fungal infection other factors like poverty, poor hygiene and social conditions such as overcrowding contribute to dermatophytosis in India. The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue such as skin, hair and nails of humans and other animals to produce an infection. Dermatophytosis are commonly referred to as ring worm and tinea. **Methodology:** One hundred clinically diagnosed cases of tinea attending Dermatology Outpatient Department at Mediciti Institute of Medical Sciences, Ghanpur, Medchal District, were included in the study from January 2013 to October 2013. Patients with superficial infections of skin, hair and nails and with clinically diagnosed dermatophytosis and who have not taken antifungal medication are included in the study. Clinically suspected cases of dermatophytosis already on antifungal medication are excluded from the study. **Results:** Dermatophytosis was more common in the age group of 21-30 years. Males were predominantly affected than females, males to female ratio was 1.8:1, tineacorporis46% was the most common clinical type followed by tineacruris27%.KOH positive was66% and culture42%Themost common isolate was Trichophyton. rubrum 22cases followed by T.Mentagrophytes14cases. **Conclusion:** Dermatophytosis was common in the second decade of life affecting both males and females, but predominant in males. Tinea corporis 46% was the commonest type of infection and Trichophytonrubrum 22 cases was the commonest etiologicalagent.

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INTRODUCTION

Although fungi are present worldwide only few of them are considered pathogenic. The pathogenic fungi may give rise to infection in animals and human beings. Recently there has been an increase in the incidence of fungal infections due to frequent usage of antibiotics immunosuppressive drugs and various conditions like organ transplantation lymphomas, leukemia and HIV infections. The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue such as skin, hair and nails of human beings and other animals to produce an infection. Dermatophytosis are commonly referred to as ring worm infection and tinea. Infection is cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immune competent hosts. The estimates of World Health Organization indicate that the global prevalence of dermatomycoses is close to 20%. The dermatophytes include three genera of molds in the class EU ascomycetes they are Trichophyton, Microsporum, and Epidermophyton. Based on their host specificity dermatophytes are classified into three ecological groups namely geophilic(soil), anthropophilic(man) and

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zoophiles(animals). The geophilic dermatophytes are generally saprophytic and derive nutrients from keratinous substrates. Rarely these pathogens cause infection in animals and man. Zoophiles are pathogens with only one animal host and grow as saprophytes on animals. Zoophiles are also reported to infect human beings. Human beings acquire the infection from infected animals the primary hosts of anthropophilic species are human transmission of infection from man to man. Traditionally, infections caused by dermatophytes have been named according to the anatomic locations involved by appending the Latin term designating the body site after the word tinea, e.g., tineacapitis for ringworm of the scalp.

The clinical manifestations are as follows: (i) tineabarbae (ring worm of the beard and mustache), (ii)tineacapitis (scalp, eyebrows and eyelashes), (iii) tinea corporis (glabrous skin), (iv) tineacruris(groin), (v) tineafavosa(favus); (vi) tinea imbricata (ringworm caused by T. concentricum); (vii) tineamanuum(hand); (viii) tineapedis(feet); and (ix) tineaunguium(nails). Several anatomic sites may be infected by a single dermatophyte species. The inflammatory response is usually characterized by a great degree of redness and scaling at the edge of the lesion or occasionally blisters formation. Central clearing of the lesion may be present and distinguishes dermatophytosis from other papulosquamous eruptions such as psoriasis or lichen planus in which the inflammatory response tends be uniform over the lesion. The location of the lesion also can help identify the pathogen. Dermatophytosis can most likely be ruled out if a patient has mucosal involvement with an adjacent red, scaly skin rash. In this situation the more probable diagnosis is a candida infection such as perleche if single or multiple fissures are present in the corners of the mouth or vulvovaginitis or balanitis if lesions are present in the genital mucosa. Any clinical diagnosis of dermatophytosis needs to be supported by laboratory diagnosis, culture is adjuvant to direct microscopic examination for definitive identification of etiological agent. In many instances the choice of therapy may depend upon specific identification of invasive Molds Latest molecular methods such as polymerase chain reaction-based restriction fragment length polymorphism i.e., PCR-RFLP targeting the 18SrDNA and internal transcribed spacer region of fungi is used for rapid diagnosis of dermatophytes. The treatment of dermatophytosis is based on infection site, etiological agent and penetration ability of the drug. The duration of the treatment depends on the site of infection and symptoms. Generally, 2-3 weeks treatment is required for skin lesions and much longer duration of treatment for nail infection. In earlier days dermatophytosis was treated with Whitefield ointment and Castellani paint. In 1970 saw the releaseofMiconazole an imidazole group of drugs useful in treating Dermatophytosis, Clotrimazole, Ketoconazole, Fluconazole, Voriconazole, Itraconazole, Posaconazole are other drugs in this group which were introduced after Miconazole to treat dermatophytosis, in 1980 Allylamines such as Terbinafine were introduced to treat dermatophytosis, Griseofulvin derived from Penicillium. Chrysogenum is an antimycotic drug with fungistatic activity is very active against dermatophytosis. The present study was undertaken to isolate and identify various fungal agents causing dermatophytosis among patients attending Outpatient Department of Dermatology, MediCiti Institute of Medical Sciences, Ghanpurvillage, Medchal District, and Telangana

MATERIAL AND METHODS

METHODOLOGY

One hundred clinically diagnosed cases of dermatophytosis attending the Dermatology Outpatient Department at Medi Citi Institute of Medical Sciences, Ghanpur village, Medchal District, Telangana from January 2013 to October 2013 were included in the present study.

Inclusion criteria

Patients with superficial infections of skin, hair and nails with clinically diagnosed dermatophytosis and who have not taken antifungal medication were included in the study.

Exclusion criteria

All patients with clinically suspected dermatophytosis and who are undergoing treatment for dermatophytosis are excluded from the study.

Nature of the specimen

Depending on the clinical types and lesions, specimens comprised of skin scrapings, nail clippings and infected hair stubs.

Collection of Specimen

Skin scrapings

Grossly contaminated skin was cleaned thoroughly with 70% alcohol in water. A curved disposable scalpelblade was used fortaking samples by scraping across the inflamed margin of the lesion into the apparently healthy tissue.

Nail

Friable material was removed fromunder the nail, or clippings were taken from the distal border with scissors or nail clippers. When dystrophy did not extend to the distal section of the nail, scrapings were taken with a scalpel blade by scrapping across the affected area.

Hair

Infected hairs were removed by plucking with epilating forceps and never by cutting because this fails to remove the area most likely to harbor the fungus, i.e., the base of the hair shaft.

Storage and transport of skin, nail and hair

All three types of keratinous material are allowed to dry out to prevent the overgrowth of saprophytic bacteria and fungi that occurs if moisture is retained by holding these specimens. Black paper is folded to form a packet, or envelopes specially designed for this purpose. Insuch condition'sdermatophytes remain viable for weeks or even months and so specimens are stored.

Processing of the specimen

1. Direct Microscopic Examination (KOH mount)

Direct microscopic examination was done using10-20% potassium hydroxide solution.Specimens like skin, hair and nail were added to10 -20% KOH to clear away the debris and keratin material for better visualization of fungal elements.

Method

A drop of 10-20 percent KOH is placed on a slide, a small amount of specimen is added to the drop, a coverslip is placed over it and the preparation is gently heated short of boiling. The KOH softens and clears the specimen for easier detection of hyphae by digesting any proteinaceous debris and disrupting the keratin's cellular sheets, thereby rendering the more biochemically resistant fungus more visible as highly refractile, hyaline, septate, branched, or unbranched hyphae and arthroconidia. In hairs, fungal elements may appear as arthroconidia on the outside (ectothrix invasion) of the hair shaft, or on the inside (endothrix), or they may appear as hyphae co-occurring with bubbles and tunnels(favic invasion)

2 CULTURE

Specimens were then cultured irrespective of KOH findings.

Culture medium

Three sets of mediums were used as listed below.

1. Sabourauds dextrose agar (Modified).
2. Sabourauds dextrose agar with cycloheximide and chloramphenicol are incorporated to avoid contamination with saprophytic fungi and bacteria.
3. Dermatophyte Test Medium (DTM).

Method

The clinical material was inoculated into one tube each of the above three media. The inoculated agar slants were incubated at room temperature and observed daily for growth for one week and twice weekly thereafter. If no growth is noticed by four weeks on SDA,culture was considered negative and discarded. Tubes showing growth were observed for color of the surface, i.e., of the aerial mycelium, color of the reverse or obverse faces of the colony, production of a diffusible pigment, texture of the surface(glabrous or waxy, powdery, granular, suede-like, velvety, downy, or fluffy, topography (flat, cerebriform, crateriform) and growth rate.

Dermatophyte Test Medium (DTM)

The clinical specimen was also inoculated into Dermatophyte Test Medium and incubated at roomtemperature. The medium turns from yellow to red due to change in color of indicator phenol red by increased pH through the metabolic activity. The Dermatophyte Test Medium is a good screening medium for dermatophytes but not a specificindicator. It also has the disadvantage of not allowing visualization of pigment on the reverse of the colony.

3.Lacto phenol cotton blue mount (LPCB)

Direct lactophenol cotton blue mount was used to mount fungi from culture for microscopic examination. The lactic acid preserves the fungal structures, phenol kills the fungus, and cotton blue stain is absorbed by hyaline fungal structure to make them more distinct.

Method

A drop of lactophenol cotton blue is placed on the center of a clean glass slide. A small portion of the fungus material is taken from culture using a sterile mycological bent wire and placed in the drop and teased apart with teasing needles. A cover slip is placed over the preparation and examined microscopically under low and high power.

Slide culture technique

This technique permits the microscopic observation of the undisturbed relationship of spores to hyphae.

Method

Sabourauds Dextrose Agar is poured to a depth of 4mm in a sterile petri dish and 1cm × 1cm blocks are cut with a sterilized scalpel. A second sterile petri dish containing a V shaped glass rod over which a glass slide is placed. The cut agar block was placed on the glass aseptically. The test culture was inoculated onto the four sides of the agar block and the sterile cover slip placed over it. Small amount of sterile distilled water was poured onto blotting paper in the plate. The lid replaced and incubated at room temperature. After the sporulation appear to be well developed, the cover slip was removed and laid onto a drop of lactophenol cotton blue on a second slide and observed under microscope. And dermatophyte species were further confirmed based on urease test, hair perforation test and rice grain test.

1 Urease Test

This test is done to differentiate T. mentagrophyte from T. rubrum as these two species of Trichophyton show high variability in macroscopic and microscopic morphology and characteristics often overlay.

When the growth was identified either as T. rubrum or Mentagrophyte, it was inoculated onto modified "Christensen's urea agar" for confirmation. Species of T.

mentagrophytes produces urease that will split the urea in the medium within 3 days producing a red color.Mentagrophyte species are urease positive within 7 days whereas none of T. rubrum yields positive results.

2 Hair perforation tests

The test is used to distinguish between typical isolates of T. Mentagrophytes and T. rubrum, may also be used to differentiate M. equinum from M. canis. Mentagrophytes and M. canis perforate hair whereas T. rubrum and M. equinum do not. T. mentagrophytes penetrate hair radially and cause wedge shaped perforation.

3 Rice Grain Test

This test is done to differentiate between M. audouinii from other Microsporum species

Identification of a dermatophyte is based on colony morphology on sabouraud dextrose agar and also on its microscopicmorphology. These criteria may be insufficient and additional bio chemical test may be required.

RESULTS

Table 1: Age wise distribution of clinically diagnoseddermatophytosis in the study group

Age(years)	Number of cases
≤ 10	7
11-20	9
21-30	34
31-40	26
41-50	20
51 and above	4
Total	**100**

Among total number of100 cases most common age group affected with dermatophytosis was 21-30 years age group with 34 cases least common age group affected was 51 and above years age group with 4 cases (4%).

Table 2: Sex wise distribution of clinically diagnosed dermatophytosis the study group

	Male	Female	Total	Male: Female ratio
Number of cases	**65**	**35**	**100**	**1.8: 1**
Percentage	**65%**	**35%**	**100%**	

In the study subjectsdermatophytosis was predominant in males compared to females. Male to female ratio was 1.8:1.

Age and sex wise distribution in relation to clinical types of dermatophytosis

Tineacorporis was more common in the age group 21-30 years with 19 cases and in males with 24 cases than females 22 cases. Tineacruris was more common in the 21–30-year age group with 7 cases and in males with 24 cases than females 3cases. Tinea facei was more common in the 31–40-year age group with 3 cases and in males with 2cases less than females with 3 cases.

Tineapedis was common in both 31-40 and 41-50 years with 2 cases in each group and in males with 4 cases than females 1case. Tineabarbae was common in the 41–50-year age group and in males with 3 cases. Tinea manuum was common in 11-20 years and 21-30 years with 1 case in each age group and in males with 1case and females 1case. Tinea capitis was common in ≤ 10 years with 5 cases and in males with 5 cases than females 1 cases. Tinea unguium was common in 21-30 years with 4 cases and in males with 2 cases less than females 4 cases.

Table 3: Clinical types of dermatophytosis

Clinical types	Number of cases n=100
Tinea corporis	46
Tineacruris	27
Tineafacei	5
Tineapedis	5
Tineabarbae	3
Tineamanuum	2
Tineacapitis	6
Tineaunguium	6

Table 4: Table showing types of clinical samples collected

Specimen	Total no. of samples n=100
Skin scrapings	85
Hair stubs	9
Nail scraping and clipping	6

Out of 100 clinical samples collected 88 were skin scraping, 6 were hair stubs and other 6 were nail scrapings and clippings

Table 5: Comparison of results obtained in the direct microscopic examination and culture

Culture	Number of cases		Total
	KOH+ve	KOH-ve	
Culture positive	33	9	42
Culture negative	33	25	58
Total	**66**	**34**	**100**

Out of 100 clinically suspected cases of dermatophytosis. Fungi was demonstrated in 66 cases by direct microscopy. Thirty-three cases were positive by both microscopy and culture. Thirty-three cases were positive by microscopy and negative by culture. Nine cases were negative by microscopy but culture positive.

Table 6: Comparison of culture positivity in Dermatophyte Test Medium with Sabouraud's Dextrose Agar

Fungal Culture medium	Culture positivity Number
Sabouraud's Dextrose Agar (with cycloheximide and chloramphenicol)	42
Dermatophyte Test Medium	39

Fungal culture was positive in 42% of cases in SDA medium whereas it was positive in 39% of cases on DTM medium.

Table 7: Species of dermatophytes isolated from samples

Species	Number of isolates Total number n=42
Trichophyton rubrum	22
Trichophyton mentagrophyte	14
Trichophyton tonsurans	4
Microsporum gypseum	2

DISCUSSION

In the present study, 100 clinically diagnosed cases of dermatophytosis attending Dermatology Outpatient Department at Mediciti Institute of Medical sciences, Ghanpur, Medchal district, Hyderabad.

1 Comparison of age distribution as found in various studies (in percentage)

The present study reveals that dermatophytosis is more common in the age group of 21-30 years (34%), followed by 31-40 years (26%), which is in accordance with other studies done by Doddamani *et al.* Amod Kumar *et al.* However, in other studies by Bindu *et al.* and Srinivasan *et al.* commonest age group was 11-20years. The highest incidence in young adults aged 21-30 years may be due to increased physical activity and increased degree for exposure to infection. In the present study, males (65%) were more commonly affected than females (35%). Male to female ratio was 1.8:1, which is compatible with other studies done by Singh *et al.* Nita Patwardhan *et al.*, Bindu *et al.*, Doddamani *et al.*, Sen*et al.* Amod Kumar *et al.* Male predominance may be due to increased outdoor physical activities and increased occupational exposure to infection than females.

2 Age and sex wise distribution in relation to clinical types

I. Tinea corporis

In the present study, tinea corporis was the commonest clinical type encountered (46%) followed by tinea cruris (27%) and the commonest age group affected was 21-30 years (34%). Males were predominantly affected than female, which is comparable with other studies done by Srinivasan *et al.* 35.4%, Amod Kumar*et al.* 37.14%.

II. Tinea cruris

In this study, tinea cruris was the second commonest clinical type 27% and commonest age group affected by tinea cruris was 31-40 years (8 cases). Males were commonly affected than females, which differs with other studies done by. Bindu *et al.*, Nita Patwardhan *et al.*

III. Tinea facei

In this study Tinea facei was present in 5% cases. Females were commonly more affected than males, which is comparable with other studies done by Srinivasan *et al.* 7.3%.

IV. Tinea unguium

In our study, tinea unguium was more common in females. Male to female ratio was 1:2, which is comparable with other studies done by Doddamani *et al.* (9.5%)

V. Tinea capitis

In the present findings, tinea capitis was more commonly seen in males in the age group of ≤10 years, which is in accordance with studies done by Srinivasan *et al.*

Occurrence of tinea capitis in less than 10 years of age may be due to lack of fungistaticsecretion by scalp in childhood. Adult sebum has fungistatic action. The low occurrence of Tinea capitis in India could be due to regular application of vegetable oil over the scalp which is known to have fungistatic properties.

VI. Tinea pedis

Out of 100 cases in this study, tinea pedis was seen in 5% cases,more in age groups of 31-40 and 41-50 years of age,

which is comparable with the study done by Amodkumar*et al.* in their study on dermatophytosis at Mumbai.

VII. Tinea manuum

In the present observation, out of 100 cases of dermatophytosis, tineamanuum was 2 cases (2%), which is comparable with other studies done by Amod kumar *et al.*

VIII. Tinea barbae

The results of present study reveal that, tinea barbae was seen in 3 cases (3%), which is comparable with other studies done by Amid Kumar *et al.* whereas Nita Patwardhan*et al.* has reported tinea barbae in (5%) cases.

This may be due to increased physical activity and increased opportunity for exposure in manual workers.

2. KOH and culture findings

Out of 100 clinically diagnosed cases of dermatophytosis, 66 cases were positive for fungi, by KOH. 33 cases were positive by both KOH and culture, 33 cases were positive by KOH and negative by culture, 9 cases were negative by KOH but culture positive, 25 cases were negative by both KOH and culture, which is comparable with study done by Doddamani *et al.* and Singh *et al.* KOH positive and culture negative could be due to nonviability of fungal elements in some cases.

Dermatophytes isolated in various studies(percentage)

Table 1:

Name of the author,year and place	T. rubrum	Mentagrophyte	M.gypseum	Tonsurans	E. floccosum	T. violaceum	T. verrucosum	M.audouinii	Schoenlein
Bindu *et al.* (2002) Calicut	66.2	25	0	5.9	2.9	0	0	0	0
Singh *et al.* (2003) Baroda	73.27	17.24	0	0	7.75	1.72	0	0	0
Peerapur *et al.* (2004) Bijapur	43.7	28.1	0	4.7	7.8	4.7	0	6.2	4.8
Hanumanthappa *et al.* 2012Mysore	58.9	24.6	8.2	5.4	0.7	0	0	0	0
Doddamani *et al.* 2013, Gulbarga.	46.87	36.46	4.16	1.04	8.33	1.04	0	2.08	0
Present study MIMS, Medchal district 2013	52.38	33.33	4.76	9.53	0	0	0	0	0

Findings of present study shows that, T. rubrum (52.38%) was the commonest etiological agent in majority of clinical types followed by Mentagrophyte (33.33%), M. gypseum (4.76%) and Tonsurans (9.53%). The present study was in accordance with study done by Doddamani*et al.*, Peerapur*et al.* However, studies by Singh *et al.* Trichophyton rubrum was isolated in 73.27%. Other etiological agents Epidermophyton flocossum, T. violaceum, T. verrucosum, Microsporumaudouinii and T. schoenleinii present in other studies were absent in present study.

CONCLUSION

The present study was undertaken to isolate and identify various species of dermatophytes causing tinea infections and correlation of various clinical features to the dermatophytes isolated among patients attending Dermatology Outpatient Department in a Tertiary Care Teaching Hospital.

The salient features of the present study were as follows:

The most common age group affected with dermatophytosis in the study was 21-30 years.

Dermatophytosis was found to be predominant in males. Male to Female ratio was 1.8:1.

Tinea corporis was the predominant type of dermatophytosis with 46% of cases.

KOH mount was positive in 66% of cases and culture was positive in 42% of cases, 33% of cases were KOH and culture positive. In 9% of KOH negative cases culture was positive. Trichophyton rubrum was the commonest etiological agent with 22cases followed by Trichophyton mentagrophytes14cases, Trichophyton tonsurans 4cases, and Microsporum gypseum 2cases.

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