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ORIGINAL ARTICLE

MYCOLOGICAL PROFILE OF DERMATOPHYTES ISOLATED FROM CLINICAL SAMPLES IN KIMS HOSPITAL, BANGALORE

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ABSTRACT: The dermatophytoses constitute a group of superficial fungal infections of keratinised tissue-epidermis, hair and nails, caused by a closely related group of filamentous fungi, the dermatophytes. **OBJECTIVES:** Isolation and speciation of dermatophytes by culture and to determine its prevalence. **METHODOLOGY:** The study was conducted from January 2012-December 2013 at Kempegowda institute of medical sciences, Bangalore. All clinically suspected cases coming to the microbiology department where subjected to mycological work-up. Specimens like skin scraping, hair, and nail were collected and microscopically examined using 10%, 20% and 40% KOH respectively for fungal filaments. Culture was carried out using Sabouraud dextrose agar medium with and without cycloheximide. The growth in the culture tube was speciated based on the macroscopic and microscopic findings (with Lactophenol cotton blue staining). **RESULTS:** Total cases in the two year period were 609. The infection was found to be common in adults aged 20-30 years, the male to female ratio is 1.2:1. The KOH positives were 491(80%), and culture positives were 471(77%). Of the positive cultures Trichophyton rubrum (35%) was the common isolate followed by Trichophyton mentagrophytes (17%), and Epidermophyton floccosum (6%). **CONCLUSION**: In the present study the most common clinical presentation is Tinea corporis followed by Tinea unguium and Tinea cruris and the common isolate was T.rubrum. The present study was undertaken to determine the clinical pattern of dermatophytosis and the common species that was prevalent here.
KEYWORDS: Dermatophytes, Tinea infection, Sabouraud dextrose agar, Potassium hydroxide.

INTRODUCTION: The dermatophytoses constitute a group of superficial fungal infections of keratinised tissued, viz; the epidermis, hair and nails, caused by a closely related group of filamentous fungi, the dermatophytes. [1] Dermatophytes are closely related keratinophilic fungi. Infections caused by these fungi are also known by the names "Tinea" and "Ringworm." It is important to emphasize that "ringworm" is not caused by a worm, but rather by a type of fungus called, Dermatophyte[2] There are three genera of dermatophytes-Trichophyton, Microsporum and Epidermophyton[3] Depending on their ecological characteristics, dermatophytes are described as anthropophilic, zoophilic or geophilic. The fungal species exclusively affecting humans are known as anthropophilic while those inhabiting with domestic and wild animals as well as birds are called zoophilic dermatophytes. A third group isolated from soil is called as geophilic.[4] The infection caused by anthropophilic species tend to be chronic and intractable and the resultant inflammation will be minimal. Infection caused by zoophilic and geophilic species tend to be self-healing and the inflammation will be severe. Dermatophytes are the most common sources of Tinea infections.[2] The species of dermatophytes are differentiated by Microconidia & Macroconidia.[2]

Dermatophytosis has been reported from different parts of India.[1] The prevalence of Dermatophytosis is governed by environmental conditions, personal hygiene and individual's

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susceptibility from place to place. It is more common in tropical countries (63%) due to factors like heat and humidity.[1] The high humidity and temperature provides a fertile ground for the abundant growth of dermatophytes. Cutaneous dermatophyte infections are common in the general population with up to 20% of people being infected at any time.[2]

The organisms are transmitted by either direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months.[2] Most of these infections are not life threatening, but they can cause morbidity in immunocompromised, diabetic patients, people who use communal baths and people who are involved in contact sports such as wrestling. Outbreaks of infections can occur in schools, households and institutional settings. Such infections can spread usually through direct contact with an infected person or animal, clothing, bedding and towels can also become contaminated and spread the infection.

Dermatophyte infections can affect the skin on almost any area of the body, such as the scalp, legs, arms, feet, groin and nails. These lesions are usually itchy, scaly with redness and/ or fissuring of the skin, or a ring with irregular borders with a cleared central area. If the infection involves the scalp, an area of hair loss may result. More aggressive infections may lead to an abscess or cellulitis. Areas infected by dermatophytes may become secondarily infected by bacteria. Symptoms typically appear between 4 and 14 days following exposure.[2] Skin infection due to dermatophytes has become a significant health problem affecting children, adolescents and adults.[5]

The present study was undertaken to isolate and speciate the dermatophyte and to determine the clinical pattern of dermatophytosis that was prevalent here.

OBJECTIVES:

1. Isolation and speciation of dermatophytes based on culture.
2. To determine the prevalence of dermatophytes from the suspected skin lesions.
3. Compare clinical diagnosis with direct microscopy and culture positivity from clinically suspected cases.

METHODOLOGY: This study was undertaken for a period of 2 years from January 2012 to December 2013 in Kempegowda Institute of Medical Sciences and research centre, Bangalore. All the clinically suspected cases were subjected to mycological work-up.

SPECIMEN COLLECTION: After selection of an appropriate site**,** the affected area was cleaned aseptically with 70% ethyl alcohol and allowed to dry. Skin scales, crusts and pieces of nail were collected in clean black paper and hair is collected on clean white paper packets.

Skin specimen was collected by scraping across the inflamed margin of the lesion. Nail specimen was collected by taking clippings of the infected part and scrapings beneath the nail surface. Hair specimen was collected by plucking with epilating forceps along with the base of the hair shaft around the follicle and scalp scraping was also collected

MICROSCOPIC EXAMINATION: Direct microscopic examination was undertaken with potassium hydroxide (KOH). A wet mount preparation of the specimens was done. For skin scale 10% KOH was

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employed, for hair sample 20% and for nail sample 40% was used. It was placed in the incubator at 37^0 C for 15 minutes, 1 hour and 5 hour depending upon the specimen.

CULTURE STUDY: After selection of an appropriate site, the area is cleaned aseptically with 70% ethanol and the scales were collected on a sterile slide with the help of sterile scalpel. The culture was performed in two different sets of Sabouraud dextrose agar (SDA) media, one tube with chloramphenicol 50 mg/L and cycloheximide 500 mg/L and the second tube with chloramphenicol without cycloheximide. The culture tubes were incubated at 37^0C and the culture growth was observed and the tubes were discarded only after six weeks in the absence of growth.

The mycological identification was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and pigment production on the reverse.

The microscopic examination of fungal growth was observed with lactophenol cotton blue stain. Nature of mycelium and conidia formation (macro and micro conidia) helped to differentiate various genera and species.

Budding yeast cells of Candida spp. were identified microscopically. Candida species were classified as albicans and non-albicans group by the production of the chlamydospores on corn meal agar and germ tube formation. Olive oil (2%) was over laid on the media for the isolation of Malassezia spp. in clinically diagnosed cases of Pityriasis versicolor. Plain SDA medium was used in cases of Pityriasias versicolor. In case of difficulty in the identification, slide culture was also performed.

RESULTS: Total cases of clinically suspected dermatophyte infection in the two year period was 609
Male to female ratio – 1.2: 1.

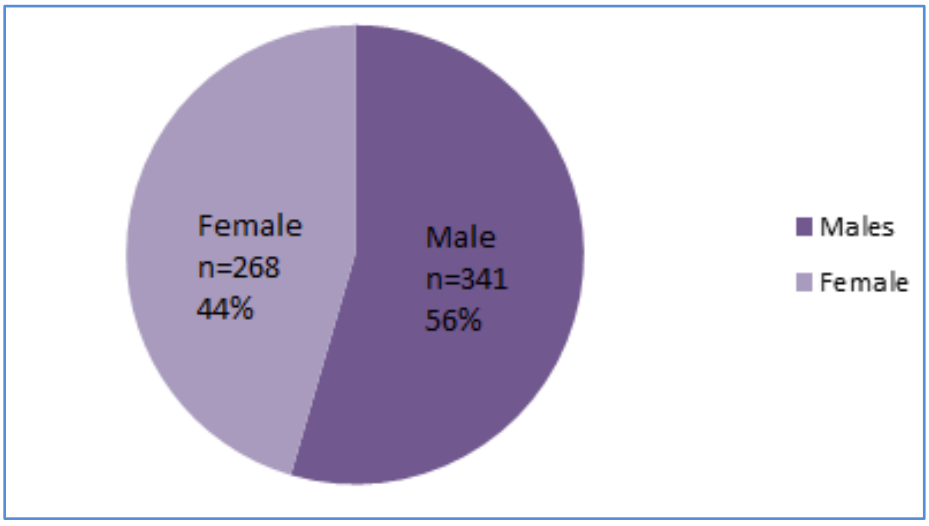

Figure 1: SEX DISTRIBUTION OF CLINICALLY SUSPECTED CASES

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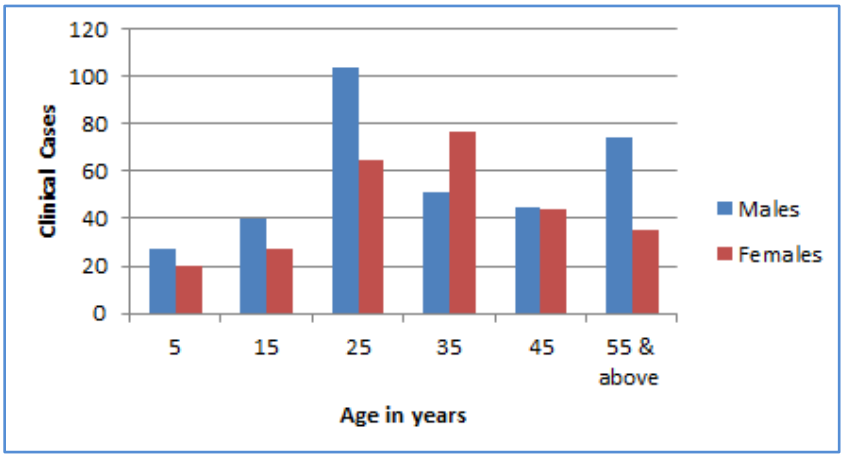

Figure 2: AGE AND SEX DISTRIBUTION OF CLINICALLY SUSPECTED CASES

- Of the 609 clinically suspected cases.
- KOH positives – 491.
- Culture positives – 471.

KOH	CULTURE	CASES
Positive	Negative	76
Positive	Positive	415
Negative	Positive	56
Negative	Negative	62

Table 3

CLINICAL CONDITION	NO.OF CASES
Tinea corporis	162
Tinea unguium	147
Tinea cruris	106
Tinea capitis	53
Tinea pedis	49
Tinea corporis + Tinea cruris	31
Tinea mannum	16
Tinea faciea	13
Tinea barbae	11
Tinea incognito	11
Tinea faciea+Tinea cruris	10
Total	**609**

Table 4: The Various Clinical Condition Presented To The Department Of Microbiology

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Cases	T. rubrum	T. mentagrophyte	E. floccossum	M. Gypseum	T. tonsurans	M. audouinii.	T. schoenleinii	T. verrucosum	T. violaceum
Tinea corporis	70	24	-	14	9	-	-	-	-
Tinea cruris	62	20	7	3	2	1	-	-	-
Tinea capitis	4	5	-	8	5	7	-	-	1
Tinea pedis	4	2	2	-	3	-	1	-	-
Tinea unguium	4	25	20	-	4	-	-	-	-
Tinea mannum	-	2	-	2	1	-	-	-	-
Tinea corporis+ Tinea cruris	9	2	3	-	1	-	-	-	-
Tinea faciea	6	-	-	-	-	-	-	-	-
Tinea barbae	2	1	-	-	-	-	-	1	-
Tinea faciea + tine cruris	-	-	4	-	-	-	-	-	-
Tinea incognito	6	-	1	-	-	-	1	-	-
Total	**167**	**81**	**37**	**27**	**25**	**8**	**2**	**1**	**1**

Table 5: Dermatophytes Isolated From Different Clinical Types

Out of the 471 culture positives few of them could not be speciated because it was overgrown with contamination and some even after 3 to 4 weeks of incubation, specific morphology could not be appreciated.

Trichophyton (Not speciated) -100

Non-dermatophytes - 22

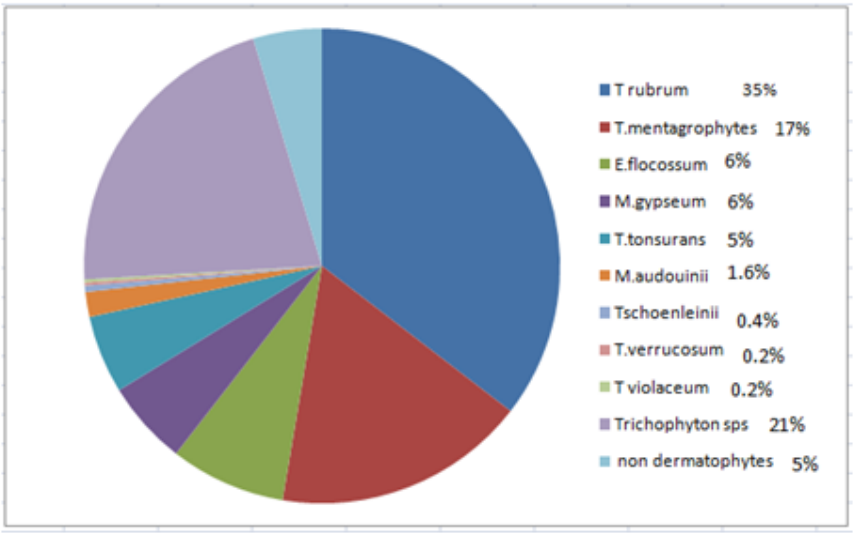

Figure 6: DISTRIBUTION OF DERMATOPHYTES FROM CULTURE POSITIVE

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DERMATOPHYTE	DURATION OF GROWTH
T. rubrum	21 days
T. mentagrophyte	15 days
E. floccossum	15days
M. gypseum	20days
T. tonsurans	28 days
M. audouinii	21days
T. schoenleinii	28days
T. verrucosum	28days
T. violaceum	30days

Table 7: Comparison Of Dermatophytes Based On Their Duration Of Growth

DISCUSSION: The total cases that came to the department of microbiology during the 2year period were 609.

341 males and 268 females were affected. The male to female ratio is 1.2:1.All other studies also show the male predominance. It may be due to increased outdoor physical activities and increased opportunity for exposure to infection than females. Also in rural India, males may visit the hospital to a greater extent than females who may not be very open for hospital visit for dermatological infections.[2,3] Males in the age group of 20-30 years and females 30-40 years are commonly affected in our study. This data is comparable with other studies such as Sen SS. etal,[1] Ranganathan etal,[2] Mishra M etal,[6] Singh S etal,[7] Jain Neetu etal,[8] Bhavna singla eta,[9] H hanumanthappa etal.[10]

491 cases were KOH positive (80%) and 471 cases were culture positive (77%). Of the positive culture 56 were KOH negative and 62 suspected cases were both KOH and culture negative. 68% were both KOH and culture positive. This result is comparable with other studies of Mishra M etal[6] were direct microscopy positives were 85%, and culture positives were 64%.,Bindu V etal[11]direct microscopy positives were 64% and culture positives were 45%, In Singh S etal[7] direct microscopy was 60% positive and culture was 44% positive. Jain Neetu[8] et al study direct microscopy was 72% positive and culture was 62% positive, Bhavna singla[9] etal shows direct microscopy 60% and culture 49% positivity. These studies shows that both direct microscopy and culture play an important role in the diagnosis of fungal infection.

In our study the most common clinical presentation is Tinea corporis (162, 26%), followed by Tinea unguium(147, 24%),Tinea cruris(106,17%), Tinea capitis (53,8%), Tinea pedis (34,8%), mixed site infection 5% and Tinea incognito 2%. In most of other study Tinea corporis is the most common clinical presentation except in Peerapur B.V etal[12] were Tinea corporis with Tinea cruris is the common presentation and in a study of Bhavna singla[9] etal Tinea cruris is the common clinical presentation.

Trichophyton rubrum (35%) is the common dermatophyte that is isolated in our study. This is isolated from Tinea corporis followed by Tinea cruris. This result is comparable with other studies. Predominantly chronic nature of the infection and the adaptation of the dermatophyte to the human skin can explain the higher predominance of T.rubrum in India. Trichophyton mentagrophytes is the second most common isolate (17%) in our study and is seen in Tinea unguium followed by Tinea

corporis. In other study of Sen SS.etal,[1] Ranganathan etal[2] Madhavi S,[3] Kannan P,[5] Mishra M et. al,[6] Jain Neetu etal[8] Singh S et. al,[13] Trichophyton mentagrophytes is isolated from Tinea corporis. Epidermophyton floccosum (6%) is isolated from Tinea unguium followed by Tinea cruris. Other studies like Kannan P,[5] Bhavna singla[9] et.al, Bindu V[11] ,Peerapur B.V,[12] Singh S etal,[13] were able to isolate this species but mostly from Tinea Corporis and cruris. M. gypseum (6%) from Tinea corporis. T.tonsurans (5%) from Tinea corporis .M audouinii (2%) isolated from Tinea capitis was comparable with other studies. 2 cases of T.schoenleinii was isolated from Tinea pedis and Tinea incognito in our study which is comparable to another study of Jain Neetu with similar finding. One case T verrucosum was isolated from Tinea barbae and 1 case of T.violaceum was isolated from Tinea capitis.

22 non-dermatophytes were isolated during our study but some of them could not be speciated due to the following limitations- 1) the cultures in the SDA tube was overgrown with contamination,hence could not be speciated. 2) Even after 3 to 4 weeks of incubation, specific morphology could not be appreciated.

CONCLUSION: Cutaneous fungal infections have been reported worldwide as being one of the most common human infectious diseases in clinical practice. In spite of therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing. In the present study, of the 609 cases, Tinea corporis was the most common clinical presentation followed by Tinea unguium, Tinea cruris, Tinea capitis, Tinea pedis, Tinea corporis and cruris followed by other presentations. The common dermatophyte that was isolated is Trichophyton rubrum 35%, T. mentagrophytes 17% and E.floccosum6%. Some of the growth could not be speciated due to over growth of contaminant, so proper collection of specimen is advised and for the better appreciation of the morphology, special media need to be used.

The present study has given us a clear insight into the mycological aspect of dermatophytosis as compared to other parts of India

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