Clinico-Mycological Profile of Dermatophytoses at a Tertiary Care Teaching Hospital of Central India

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Abstract

Background: Dermatophytosis is a disease of hair, nails, and stratum corneum of the skin caused by dermatophytes. The prevalence of dermatophytosis in a geographical area depends on a variety of factors such as climate, personal hygiene, and individual susceptibility. The clinical importance of isolating and identifying dermatophytes is to start appropriate treatment & to detect probable infection sources. Also, identification is important for prognostic consideration.
Objectives: Our study aims to know the clinico-mycological profile in suspected cases of dermatophytosis.
Patients and Methods: A total of 110 suspected cases of dermatophytoses that were diagnosed clinically by a dermatologist were included in this study. Specimen of skin scrapings, hairs & nail clippings wherever appropriate were collected from these patients. Specimens collected were subjected to standard mycological procedures.
Results: In our study, the most common age group affected was 21-30 years (31.82%). The majority of the cases were from the lower middle class (38%). The commonest clinical type was *Tinea corporis* (48%). In 72.73% of cases, we were able to detect fungi either by direct microscopy and/or culture. Out of 62 culture isolates, *T.rubrum* was found to be the commonest (59.7%), followed by *T.mentagrophytes* (24.2%), *E.floccosum* (6.5%), *T.tonsurans* (3.2%), *M. gypseum* (3.2%) and one isolate each of *M. audouinii* and *M. canis*.
Conclusion: With proper techniques, various species of dermatophytes can be identified. But conventional methods are time-consuming and a week to a month is required for identification to species level. So the development of rapid molecular techniques is the need of the hour.

Keywords: Dermatophytes, Dermatophytoses, Tinea, Trichophyton, KOH wet mount, Microscopy, Fungal culture.
DOI: 10.21608/svuijm.2022.129549.1297
***Correspondence**: abhishekmehta623@gmail.com
Received: 28 March,2022.
Revised: 19 April,2022.
Accepted: 21 April, 2022.
Cite this article as: Gaurav Saxena, Kalpana Sadawarte, Prafulla Songara, Abhishek Mehta. (2022). Clinico-Mycological Profile of Dermatophytoses at a Tertiary Care Teaching Hospital of Central India. *SVU-International Journal of Medical Sciences*. Vol.5, Issue 2, pp: 216-227 .

Introduction

Superficial fungal infections affect millions of people worldwide and dermatophytes are involved in the majority of them **(Rippon, 1988)**.

Dermatophytes are a broad group of closely related keratin-loving fungi. Dermatophytosis is a disease of hair, nails, and stratum corneum of the skin caused by dermatophytes commonly referred to as "tinea" or "ringworm". Tinea is a Latin word that stands for 'larva of small insect' **(Rippon, 1988; Hay, 2010; Chander, 2009)**.

Dermatophytes are classified as geophilic, zoophilic, and anthropophilic species depending on their usual habitat whether it is soil, animal, or human respectively. They are divided into three main anamorphic genera: *Trichophyton, Microsporum, and Epidermophyton* based on their morphological characters. The distribution of different species of dermatophytes varies markedly from one ecological niche to another depending on their primary natural habitat. Some dermatophytes are confined to & endemic only in particular areas. Some others are sporadic and worldwide in distribution **(Rippon, 1988)**.

The prevalence of dermatophytosis in a geographical area depends on a variety of factors such as climate, personal hygiene, and individual susceptibility. The clinical appearance of the infection varies with the site involved, the dermatophytic species involved and the immune reaction elicited by the host. The clinical features of dermatophytosis result from a combination of keratin destruction and inflammatory response generated in the host. The infections that are caused by animal species are more inflammatory and heal quickly while those caused by anthropophilic species have minimal inflammation and are likely to become chronic **(Hay, 2010; Chander, 2009)**.

The diagnosis of dermatophytosis relies upon clinical observations supported by laboratory investigations. Culture is considered the gold standard in diagnosing dermatophytoses but takes a long time and has low sensitivity. Recently molecular methods like PCR followed by restriction fragment length polymorphism (RFLP), Real-time PCR, and Multiplex PCR assay have been designed for the diagnosis of dermatophytosis. These methods are quick & seem promising but are yet to be standardized for use in routine diagnostic laboratories **(Chander, 2009; Yang et al., 2008; Arabatzis et al., 2007; Brillowska et al., 2007)**.

The clinical importance of isolating and identifying dermatophytes is to start appropriate treatment & to detect probable infection sources. It is also important for prognostic consideration. In the light of these facts, this study was undertaken to isolate and identify the aetiological agents of dermatophytes using conventional diagnostic techniques.

Aim and objectives:

Aim: to know the clinico-dermatophytic profile in clinically suspected cases of dermatophytosis.

Objectives

- Isolation of dermatophytes from all the suspected cases of dermatophytosis.

- Identification of the isolates.
- To study age and sex distribution in the study group.
- To find out the relationship between the involved site and the causative agent associated.

Patients and methods

Study population: Clinically diagnosed cases of dermatophytoses visiting Skin OPD of People's Hospital, Bhopal.

Study period: - November 2013 to August 2015**.**

Place of study: Department of Microbiology, People's College of Medical Sciences & Research Centre, Karond Bypass Road, Bhanpur, Bhopal (M.P.). India-462037.

Inclusion criteria: Dermatophytes isolated from all the suspected cases of dermatophytosis. Patients of all age groups and both sex are included in the study.

Exclusion criteria: Bacterial isolates and fungi other than dermatophytes.

Study type: Hospital-based, cross-sectional study.

Sample size: Non-repetitive 110 suspected cases of dermatophytoses.

Specimen collection: The affected area or lesion was wiped with 70% ethanol. The specimen includes skin scales, hair, hair roots, nail clippings, and scraping beneath the nails. Samples were collected in clean black paper packets.

Specimen processing: Specimens collected were subjected to standard mycological procedures.

Direct Microscopic Examination

KOH wet mount –This was prepared by placing a portion of each sample collected (skin scales, hair, hair roots, nail clippings, and scraping beneath the nails) on a clean, grease-free, microscope glass slide. Then 1-2 drops of 10% KOH for the skin and hair sample while 20% or 40% KOH was applied for nail samples. The slide was then screened for 15-20 minutes for the presence of fungal hyphae.

Isolation of dermatophytes

After direct microscopy, the other portion of the collected sample was inoculated onto three test tubes slant in duplicate on;

- Sabouraud's dextrose agar (SDA) with chloramphenicol (0.005%) [HiMedia, Mumbai]
- Sabouraud's dextrose agar (SDA) with chloramphenicol (0.005%) and cycloheximide (0.05%) [HiMedia, Mumbai], and
- Dermatophyte test medium (DTM) [HiMedia, Mumbai].

SDA with chloramphenicol and SDA with chloramphenicol and cycloheximide were incubated at 25^{o}C and 37^{o}C for up to 4 weeks and observed regularly for growth. If there was no growth even after 4 weeks of incubation it was taken as negative. Dermatophyte test medium (DTM) was incubated at 25^{o}C and 37^{o}C for ten days and was observed for color change.

Identification of dermatophytes: Fungal isolates were identified based on distinctive colony characteristics, microscopy features (tease mount, slide culture), urease test, hair perforation test, and rice grain test **(Rippon, 1988; Chander, 2009)**.

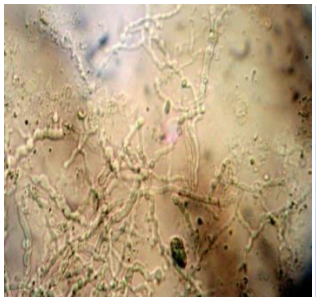

Fig.1:KOH mount

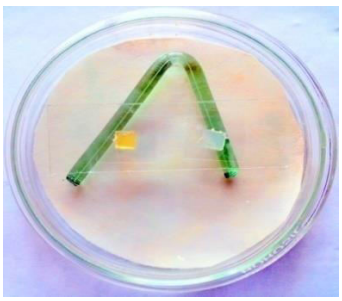

Fig.2: Slide culture

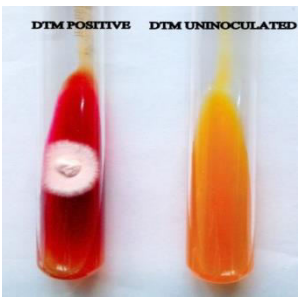

Fig.3: Growth on Dermatophyte Test Medium

Fig.4: Hair Perforation Test

Results

In this study of 110 cases, patients were distributed between the age ranges of 1.5-75 years. The mean age of the study group was 33.64 years. The age group of 21-30 years was most commonly affected (31.8%) followed by 31-40 years (23.6%) as depicted in (**Table.1**).

Males (53%) were slightly more affected than females. The ratio of affected males to females was 1.12:1.

Table 1. Age-wise distribution of dermatophytes

Age in years	Number of cases	Percentage
≤ 10	09	8.2%
11-20	09	8.2%
21-30	35	31.8%
31-40	26	23.6%
41-50	19	17.3%
51-60	09	8.2%
61-70	02	1.8%
71-80	01	0.9%
Total	**110**	**100%**

Out of 110 clinically diagnosed cases of dermatophytoses, the most common clinical type was tinea corporis(48%), followed by tinea unguium (18%), tinea cruris (14%), tinea capitis (7%), tinea pedis (5%), tinea corporis with tinea cruris (4%), tinea manuum (3%) and tinea barbae (1%) as depicted in (**Table.2**).

The majority of the cases were from the lower middle class (38% cases), followed by the lower class (27% cases) and middle class (25% cases). The upper class and the upper-middle class were the least affected with 4% and 8% cases respectively, (**Table.3**)

Overall dermatophytoses were most common in manual workers (35.5% cases) followed by students (24.6% cases), housewives (18.2% cases), professionals (13.6% cases), and others (8.2% cases). Tinea corporis was more common in manual workers. Tinea unguium was more common in manual workers and students. Tinea cruris and tinea pedis were most commonly seen in students. Tinea capitis was most common in preschool children, (**Table.4**).

Table 2. Gender-wise distribution in clinical types

S. No.	Clinical type	Gender		Total	%
		Male	Female		
1	Tinea corporis	33 (62.3%)	20 (37.7%)	53	48.2
2	Tinea unguium	10 (50%)	10 (50%)	20	18.2
3	Tinea cruris	05 (31.3%)	11 (68.8%)	16	14.6
4	Tinea capitis	05 (62.5%)	03 (37.5%)	08	7.3
5	Tinea pedis	01 (20%)	04 (80%)	05	4.6
6	Tinea manuum	01 (33.3%)	02 (66.7%)	03	2.7
7	Tinea barbae	01 (100%)	00 (0%)	01	0.9
8	Tinea corporis + Tinea cruris	02 (50%)	02 (50%)	04	3.6
	Total	**58**	**52**	**110**	**100**

Table 3 : Socio-economic status pattern in Dermatophytoses cases

Socio-economic status[a]	Number of cases	Percentage
Lower Class (LC)	30	27.3%
Lower Middle Class (LMC)	42	38.2%
Middle class (MC)	25	22.7%
Upper Middle Class (UMC)	09	8.2%
Upper Class (UC)	04	3.6%
Total	**110**	**100**

[a] Socio-economic status is based on Modified BG Prasad classification (1961) [154] [revised for the year 2014 as per all India consumer price index (AICPI)] [AICPI (IW) All India (base 2001) = 237]

Table 4. Clinical types and their relation to occupation

Clinical type	Occupation					Total
	Manual worker	Housewife	Students	Professio-nal	Others	
Tinea corporis	24 (45.3%)	11 (20.8%)	05 (9.4%)	10 (18.9%)	3 (5.7%)	**53 (48.2%)**
Tinea unguium	08 (40%)	03 (15%)	08 (40%)	01 (5%)	00 (0.0%)	**20 (18.2%)**
Tinea cruris	02 (12.5%)	03 (18.8%)	08 (50%)	02 (12.5%)	01 (6.3%)	**16 (14.6%)**
Tinea capitis	00 (0.0%)	00 (0.0%)	03 (37.5%)	01 (12.5%)	04 (50%)	**08 (7.3%)**

Tinea pedis	00 (0.0%)	01 (20%)	02 (40%)	01 (20%)	01 (20%)	**05 (4.6%)**
Tinea manuum	01 (33.3%)	01 (33.3%)	01 (33.3%)	00 (0.0%)	00 (0.0%)	**03 (2.7%)**
Tinea barbae	01 (100%)	00 (0.0%)	00 (0.0%)	00 (0.0%)	00 (0.0%)	**01 (0.9%)**
Tinea Corporis + Tinea Cruris	03 (75%)	01 (25%)	00 (0.0%)	00 (0.0%)	00 (0.0%)	**04 (3.64%)**
Total	**39 (35.5%)**	**20 (18.2%)**	**27 (24.6%)**	**15 (13.6%)**	**09 (8.2%)**	**110 (100%)**

Out of 110 clinically suspected cases of dermatophytoses, in 80 cases (72.73%) we were able to detect fungi either by direct microscopy and/or culture. In 59 cases (53.64%) both microscopy and culture were positive. 18 cases (16.36%) were positive only by microscopy but culture turned out to be negative. In 3 cases (2.73%) culture was positive but microscopy was negative. In 30 cases (27.27%) both microscopy and culture were negative. **(Table.5**).

Table 5: Correlation of direct microscopy (KOH) findings with culture

Variables	Culture positive	Culture negative	Total	p-value, Chi-square ($\chi 2$) Value
KOH Positive	59	18	77	**p- value is < 0.001 $\chi 2 = 42.83$**
KOH Negative	3	30	33	
Total	62	48	110	
Sensitivity = 95.16%, Specificity = 62.5%, PPV= 76.62%, NPV= 90.91%				

Considering fungal culture as the gold standard, the diagnostic utility of direct microscopy (KOH) findings was evaluated.The sensitivity and specificity of Direct Microscopy (KOH) were found to be 95.16% and 62.5% respectively. The predictive value of a positive KOH test (PPV) was 76.62%. The predictive value of the negative KOH test was 90.91%. The diagnostic utility of KOH mount for laboratory diagnosis of dermatophytoses was found to be significant with a p-value of < 0.001. (**Table.5**)

Overall out of 62 culture isolates, *T. rubrum* was found to be the commonest (59.7%), followed by *T. mentagrophytes* (24.2%), *E. floccosum* (6.5%), *T. tonsurans* (3.2%), *M. gypseum* (3.2%) and one isolate (1.6%) each of *M. audouinii* and *M. canis***.**

In tinea corporis, tinea unguium, tinea cruris, and tinea corporis with tinea cruris mixed infection, *T. rubrum* was the commonest isolate followed by *T.*

Mentagrophytes. In tinea capitis, *T.tonsurans* was the most common isolate. In both cases of tinea pedis, *T. rubrum* was isolated. From the 2 cases of tinea manuum, one case yielded *T. rubrum* isolate and the other yielded *T. mentagrophytes*. In tinea barbae, the only culture isolate was *T. rubrum*, (**Table.6**).

Table 6. Percentage distribution of Dermatophytes in various clinical types

Clinical type	No. of cases	T. rubrum No.(%)	T. mentagrophyte No.(%)	T. tonsurans No.(%)	M. audouinii No.(%)	M. canis No.(%)	M. gypseum No.(%)	E. floccosum No.(%)	Total isolated No.(%)
Tinea corporis	53	21 (65.6)	7 (21.9)	0 (0)	0 (0)	1 (3.1)	2 (6.3)	1 (3.1)	**32 (51.6)**
Tinea unguium	20	5 (50)	4 (40)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	**10 (16.1)**
Tinea cruris	16	5 (55.7)	2 (22.2)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (22.2)	**9 (14.5)**
Tinea capitis	08	0 (0)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0 (0)	**3 (4.8)**
Tinea pedis	05	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**2 (3.2)**
Tinea manuum	03	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**2 (3.2)**
Tinea barbae	01	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**1 (1.6)**
Tinea corporis + cruris	04	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**3 (4.8)**
Total	**110**	**37 (59.7)**	**15 (24.2)**	**2 (3.2)**	**1 (1.6)**	**1 (1.6)**	**2 (3.2)**	**4 (6.4)**	**62 (100)**

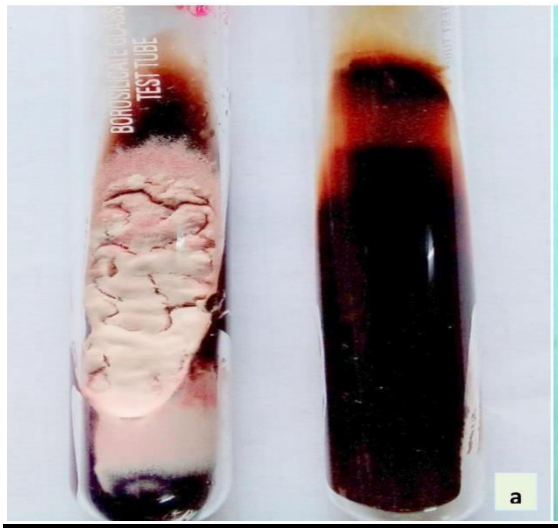

Fig.5a: Growth of Trichophyton rubrum on SDA slant

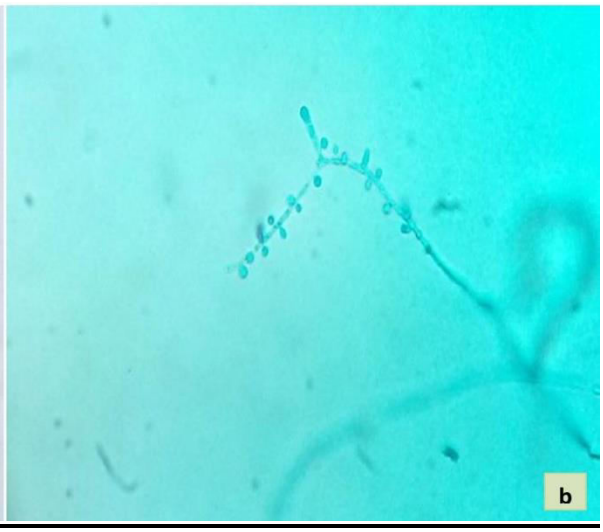

Fig.5b: LCB mount showing typical arranement of microconidia

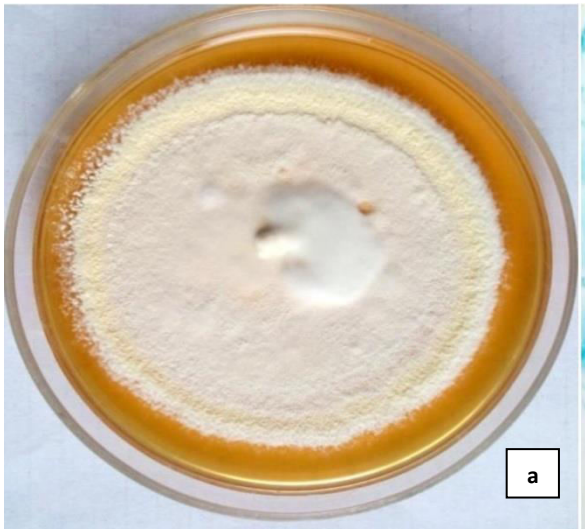

Fig.6a: Growth of *Trichophyton mentagrophytes*

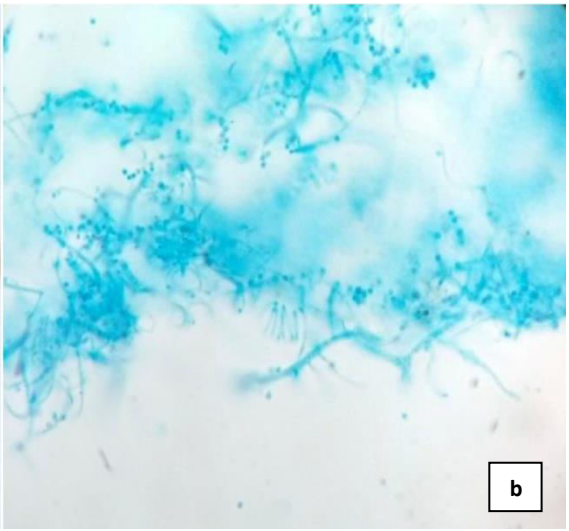

Fig.6b: LCB mount showing numerous microconidia and spiral hypha

Discussion

In our study, 110 suspected cases of dermatophytoses were studied. The age group of 21-30 years was most commonly affected with 35 cases (31.8%) followed by 31-40 years with 26 cases (23.6%) followed by 41-50 years with 19 cases (17.3%). In the majority of cases 80(72.7%) were in the 21-50 years age group. Extremes of ages are least commonly affected. This is in concordance with many studies reported across different parts of India **(Patwardhan and Dave, 1999; Peerapur et al., 2004; Sen and Rasul, 2006; Kumar et al., 2014; Najotra et al., 2015).** The reason for the high incidence of dermatophytosis in this age group (21-50 years) might be due to hormonal factors & active lifestyle.

In our study, males were slightly more affected than females. Most studies

across different parts of India also reported male preponderance **(Patwardhan and Dave, 1999; Sen and Rasul, 2006; Bindu, 2002; Singh and Beena, 2003)**. This might be due to more involvement of males in outdoor physical work. But some investigators have reported female preponderance **(Sarada and Kumari, 2015)**.

In our study, dermatophytoses were more prevalent in the lower middle class (38 % cases), followed by the lower class (27 % cases) & middle class (25% cases). The upper class and the upper-middle class were the least affected with 4 % and 8 % cases respectively. Together, the lower class, the lower middle class, and the middle class account for 88.18 % of cases. This is probably due to unhygienic conditions, overpopulated houses, sharing of the same towels, bed sheets, etc., nutritional deficiencies, and poor access to healthcare facilities among lower economic classes of society.

In this study, dermatophytoses were most prevalent in manual workers (35.5% cases) followed by students (24.6% cases), housewives (18.2% cases), professionals (13.6% cases), and others (8.2% cases). This was also reported in the study done by **Veer et al. (2015)**. The reason for more prevalence in manual workers might be due to more physical activity leading to excessive sweating & also there is increased chance of exposure.

In this study, out of 110 suspected cases of dermatophytoses, the fungus was demonstrated in 80 cases (72.73%) either by direct microscopy & / or culture, 59 cases (53.64%) were positive by both microscopy and culture, 18 cases (16.36%) were positive by microscopy only, 3 cases (2.73%) cases were only culture positive, 30 cases (27.27%) were negative both by microscopy and culture. Similar findings were also reported in studies done by **Singh and Beena (2003)** and **Yadav et al. (2013)**. The sensitivity of KOH was found to be 95.16% and the specificity was 62.5%. The positive predictive value (PPV) was 76.62% while the negative predictive value (NPV) was 90.91%. Similar sensitivity of direct microscopy was also reported by **Gupta et al. (2014)** in their study at Jaipur. As per our study, direct microscopy is highly sensitive & is a good screening test for the diagnosis of dermatophytoses. This is useful for the early initiation of treatment in suspected cases of dermatophytoses as culture is often time-consuming. The culture which is considered a confirmatory test in the diagnosis of dermatophytoses has a drawback of high false negativity. This high false negativity can be attributed to nonviable fungal hyphae in the specimen or inadequate and improper specimen collection.

In the present study, out of 62 culture isolates *Trichophyton rubrum* (59.7%) was most common which was followed by *Trichophyton mentagrophytes* (24.2%). Comparable findings have also been reported in studies done by **Najotra et al. (2015)** and **Ranganathan et al. (1995)**. In contrast, **Karmakar et al. (1995)** reported *T. violaceum* (55.76%) as the most common isolate followed by *T. rubrum* (42.3%). **Bhatia and Sharma (2014)** at Himachal Pradesh reported *T. mentagrophytes* (63.5%)

as the most common isolate followed by *T. rubrum* (34.6%). This shows that the prevalence of dermatophyte species varies with geographical location.

Conclusion:

Dermatophytoses are very common in our country & there is a lot of variation in the prevalence of different species of dermatophytes in different geographical locations.

Trichophyton sp. was found to be the most common aetiological agent of dermatophytoses in this geographical area.

Trichophyton rubrum was the most common isolate and also the most common agent of tinea corporis, tinea unguium, tinea pedis, tinea cruris, and tinea barbae in our study. *Trichophyton tonsurans* is the most common agent of tinea capitis in our study. The second most common isolate was *Trichophyton mentagrophytes* in our study.

The clinical presentation of dermatophytoses, though typical, is often confused with other skin infections. This is attributed to self-medication, application of steroid and antifungal ointments inadvertently & irregularly. This often results in an incorrect diagnosis due to the lack of typical signs & symptoms of the disease at the time of presentation.

Hence there is a dire need for correct and quick laboratory diagnosis. With proper diagnostic techniques, various species of dermatophytes can be identified. But conventional methods are time-consuming and a week to a month is required for identification to species level. So the development of rapid molecular techniques is the need of the hour.

Conflict of Interest:- The authors declare that there is no conflict of interest.

Funding:- None.

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