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ORIGINAL ARTICLE

# Genotypic and Phenotypic Identification of Dermatophytes among Clinical Isolates

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## ARTICLE INFO

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## ABSTRACT

**Background:** The prevalence of superficial fungal infections varies based on environmental and hygienic conditions. Dermatophytes, particularly *Trichophyton rubrum* and *T. mentagrophytes*, are keratinophilic fungi responsible for the majority of these infections. Their ability to produce proteases and keratinases active at acidic pH facilitates colonization of human skin. This is a fundamental mechanism observed in *Trichophyton rubrum* and *Trichophyton mentagrophytes* in establishing superficial infection.

**Methods:** A total of 200 clinical samples from patients with suspected dermatophytosis were examined using potassium hydroxide (KOH) mount and culture techniques. Culture-positive isolates were further identified through conventional mycology and molecular methods, including PCR and sequencing using species-specific ITS2 primers for *T. rubrum* and *T. mentagrophytes*. Clinical profiles, treatment history, and recurrence patterns were also analyzed.

**Results:** Among 200 KOH-positive samples, 140 yielded culture-positive results, of which 60 isolates were identified as dermatophytes. Molecular identification confirmed 20 isolates as *T. rubrum* and 40 as *T. mentagrophytes*. Clinically, 43.34% of cases were naïve infections, 50% had ongoing antifungal treatment for 1–2 years (recalcitrant cases), and 6.66% were categorized as reinfections due to recurrence of lesions post-therapy. The mean patient age was 30.02 ± 10.95 years, with a male-to-female ratio of 2:1.

**Conclusion:** *Trichophyton mentagrophytes* was more prevalent than *T. rubrum* among dermatophyte-positive cases. The high rate of recalcitrant and recurrent infections highlights the need for accurate species-level diagnosis using molecular tools to guide effective antifungal management strategies.

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## Introduction

Dermatophytes are keratinophilic fungi that cause superficial fungal infection; grow on skin, mucous membranes, hair and nails. Dermatophytes are not a part of the normal human microbial flora but are well adapted to host keratin utilizing it as a source of nutrition for establishing infection [1].

The prevalence rate of superficial fungal infection differs from place to place according to environmental condition and also due to poor hygienic condition of population. In India most of the places are very hot and humid climate because of that our country has more prevalence rate of superficial fungal infections[2-4]. In spite of therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing [5]. Most of the people are living in very low socioeconomic status, crowded living environment, and poor medical care and wearing of dirty clothing which adds to the increased prevalence of cutaneous fungal infections [3]. This infection does not cause any death but it may lead to morbidity and plays a more important role in health related problems. Dermatophytes are mycelial group of closely related keratinophilic fungi, originally saprobial. Dermatophytoses is also termed as Tinea and the clinical conditions are named as "Tinea" followed by the anatomical site of infection in latin eg., tinea capitis, t inea corporis, and tinea pedis etc.

Dermatophytes comprise of 52 species of keratin degrading ascomycetes belonging to genera Trichophyton, Microsporum, Epidermophyton, Arthroderma, Lapophyton, Nanninzia, Ctenomyces, Guarromyces, Paraphyton. But human dermatophytosis is caused primarily by Trichophyton rubrum (*T. rubrum*), *Trichophyton mentagrophytes* (T. mentagrophytes), Trichophyton interdigitale, Epidermophyton floccosum and Microsporum canis and others. Globally Trichophyton rubrum commonly causes 50–80% of onychomycosis and tinea pedis but in India Trichophyton mentagrophyte is more prevalent in Tinea corporis and Tinea cruris [2].

During the initial stages of the infection, dermatophytes facilitate adhesions on host cell. It is initiated by a specific interaction of carbohydrate-binding proteins (lectin) with sugar (Sialic acid) as a terminal carbohydrate on the surface of the human cell. Proteases and keratinases are expressed by common dermatophytes: Trichophyton rubrum and *Trichophyton mentagrophytes* which have optimum activity at acidic pH values, and are able to establish contact with human acidic skin due to the environment. The secretion of these proteases and peptidase, have been identified as an important step in fungal pathogenicity and virulence. The metabolism of some amino acids shifts the extracellular pH from acidic to alkaline values at which most known keratinolytic proteases have optimal enzymatic activity. This is a fundamental mechanism observed in Trichophyton rubrum and *Trichophyton mentagrophytes* in establishing superficial infection [3].

Their ability to utilize keratin as major nutrient source and high affinity for keratin facilitates them to invade keratinized structures like nails, skin and hair causing superficial infections known as dermatophytose s in humans and animals. This is commonly known as ring worm infection. The infection is generally cutaneous and restricted to the nonliving cornified layers as the fungi is unable to penetrate the deeper tissues or organs of immunocompetent hosts.

## Material and Method

The clinical isolates were collected for a period of four years, from August 2015 to july 2019. All the samples (n=280) received in the mycology laboratory from out patients department (OPD) of tertiary care Guru Teg Bahadur hospital, Delhi. Institutional ethical committee approval was obtained prior to commencement of the study. Written informed consent was taken from each participant. The patient information sheet for participation in the research project was provided to each participant.

### Clinical sample collection

Scrappings were collected from the affected nail and skin surface of patients referred from OPD with suspected dermatophytosis. The affected sites were decontaminated with 70% alcohol and the nail clippings from the discoloured, thickened nail bed, or nail plate was collected. The skin sample was taken from the advancing edge of lesions by scraping from the centre to edge using a sterile scalpel Samples were divided into two portions for direct microscopy and fungal culture.

### Microscopic identification

Microscopic identification of the samples were done using 40% Potassium Hydroxide (KOH) The specimen was briefly heated and then observed microscopically under 40 X magnification. The

presence of fungal hyphae, arthroconidia, was considered as a positive result for suspected dermatophyte infection.

**Phenotypic confirmation of the species**

A portion of the samples were cultured on Sabouraud's Dextrose agar (Hi-media, Mumbai) with antibiotics such as chloramphenicol (0.05 gm/l), Gentamicin (20mg/l) and Cyclohexamide (0.5 gm/l) (Hi-media, Mumbai). All inoculated tubes were incubated at 25°C for 3-4 weeks till optimal growth. After the growth, the etiological agent was confirmed by characteristic morphology of colony (pigmentation of the surface and reverse side, topography, texture and the rate of growth) and by studying the microscopic appearance of fungus on LPCB mounts. The species were confirmed by size and shape of macro conidia, microconidia, spirals, nodular organs and pectinate branches [1].

**Genotypic confirmation of the species**

These isolates were further confirmed by PCR and sequencing using species specific primers of *Trichophyton rubrum* and *Trichophyton mentagrophytes* [2].

**DNA extraction**

DNA was extracted from the cultures grown on SDA by using commercially available DNA extraction kit (Hi Yield genomic DNA kit, RBC, Taiwan).

**Polymerase Chain Reaction (PCR)**

On the basis of sequences of internal transcribed spacer to in NCBI nucleotide data base primer of *Trichophyton rubrum* and *Trichophyton mentagrophytes* were used shown in table-1 and the PCR product length of two primer sets were 203 base pair and 130 base pair respectively. For PCR each tube contained a total volume of 25 µl mix including 2.5 µl buffer (10X), 5 µl of Q-buffer, 0.5 µl dNTPs (200 µM), $MgCl_2$ 0.5 µl (1.5 mM), 0.15 µl Taq polymerase, 1 µl of each primer, forward and reverse (10 µM), 5 µl of the extracted DNA and nuclease free water to make up the volume. All PCR reagents were purchased from Qiagen, and amplification was performed on a thermo-cycler (Eppendorf) at the thermal profile shown in table 2 and 3 [3-4]. Standard ATCC control strains were used as positive controls (*T. mentagrophytes* ATCC. 28185) [5].

**Table 1** Primer sequences

| S. No. | Region | Primer name | Primer sequence |
|---|---|---|---|
| **1** | ITS 2 | *TR F | F 5′ TCTTTGAACGCACATTGCGCC 3′ |
| | | TR R | R 5′ CGGTCCTGAGGGCGCTGAA 3′ |
| **2** | ITS 2 | *TM F | F 5′-CAAACGTCCGTCAGGGTGAGC 3′ |
| | | TM R | R 5′-TAGCCACTAAAGAGAGGCTCGC 3′ |

***TR:** T. rubrum
***TM:** T. mentagrophyte

**Table 2** Thermal profile of *T. rubrum* -

| Temperature | Time | Cycles | Phase |
|---|---|---|---|
| **95°C** | 10 min | 1 | Initial Denaturation |
| **95°C** | 60 sec | 35 | Denaturation |
| **60°C** | **45 sec** | | **Annealing** |
| **72°C** | 60 sec | | Extension |
| **72°C** | 7 min | 1 | Final extension |
| **4°C** | ∞ | 1 | Hold |

**Table 3** Thermal profile of *T. mentagrophytes* –

| Temperature | Time | Cycles | Phase |
|---|---|---|---|
| **95°C** | 10 min | 1 | Initial Denaturation |
| **95°C** | 60 sec | 35 | Denaturation |
| **65°C** | **30 sec** | | **Annealing** |
| **72°C** | 60 sec | | Extension |
| **72°C** | 7 min | 1 | Final extension |
| **4°C** | ∞ | 1 | Hold |

The amplified PCR products were analyzed by electrophoresis on 1.2% agarose gel, at 125 V and 15 mA current in 10 slot apparatus for 40 minutes, stained with Ethidium Bromide.

**Sequencing**

Purification of the PCR product was done using Gel purification kit protocol (Qiagen, Germany). Sequencing was done as per protocol used by big dye terminator method followed by Sanger sequencing method. The sequence analysis was performed by comparison of the nucleotide with dermatophyte reference nucleotide sequences obtained from the gene bank data base (http://www.ncbi.nih.gov/gene ), match sequence with data base and submit the sequence in gene bank data base for the accession number.

**Statistical analysis**

Bionumerics software (Applied Maths and Ghent) was used for consensus and concatenation of the sequences. The sequences and amino acid sequences depicted by using the ExPASy online tool (https://web.expasy.org/ translate/) were aligned.

## Results

**Identification of Culture**

A total of 280 samples were collected, which included 120 onychomycosis and 160 tinea infection. Out of 120 nail samples, 90 were found to be KOH positive, and 60 samples were culture positive for dermatophytes and non-dermatophytes. Among 60 isolates from nail sample (Figure-1), 19 isolates were identified as *T. rubrum* and 5 isolates as *T. mentagrophytes* complex. Similarly, of 160 skin samples, 110 cases were observed as KOH positive, of which 80 samples were culture positive. Of 80 culture isolates 35 isolates were characterized as *T. mentagrophytes* complex and 1 isolate as *T. rubrum*. A total of 60 culture isolates (nail and skin) as confirmed by LPCB, PCR and reconfirmed by sequencing, 40 isolates are of *T. mentagrophytes* complex and 20 isolates are of *T. rubrum* shown in figure 2.

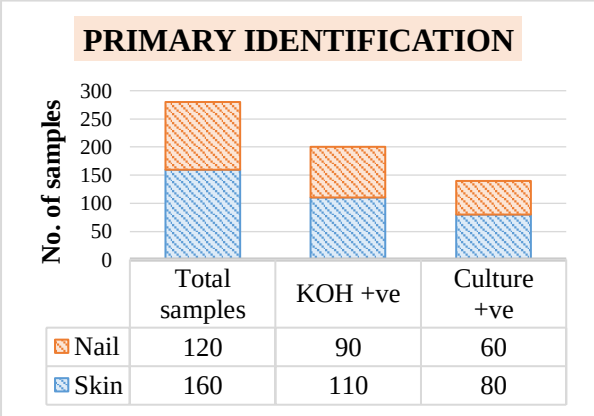



**Figure** 1 Screening of Dermatophytic samples

**Table 4** Distribution by KOH and culture among the patients-table and text *not matching*

| Cases | Total Cases | Skin (%) | Nail (%) |
|---|---|---|---|
| **Total Cases** | 280 | 160(57.14%) | 120(42.86%) |
| **KOH positive** | 200 | 110(55%) | 90(45%) |
| **Culture positive** | 140 | 80(57.14%) | 60(42.86%) |
| ***T. mentagrophytes*** | 40 | 35(87.5%) | 5(12.5%) |
| ***T. rubrum*** | 20 | 1(5%) | 19(95%) |

**Phenotypic Distribution of Isolates:**

In this study, a total of 280 samples suspected of dermatophytosis were collected, with 200 samples confirmed by KOH for fungal elements (110 cases of Tinea and 90 of onychomycosis).

From 110 Tinea cases 80 were found to be culture positive similarly out 90 onychomycosis cases 60 cases were culture positive.

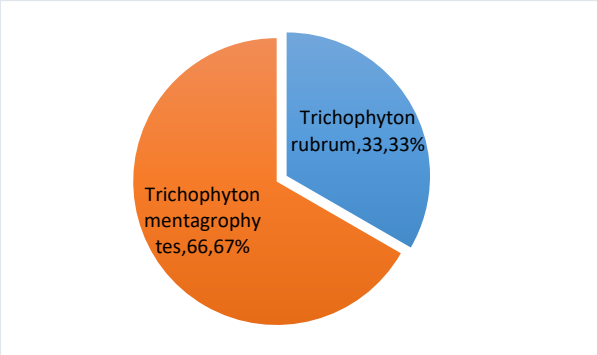



**Figure 2** Phenotypic distribution of the Culture isolates

Phenotypic Distribution of *T. mentagrophytes* 40/60 (66.67%) and *T. rubrum* 20/60 (33.33%) among the nail and skin samples.

- The patients mean age with standard deviation was 32.51±11.32 years. The duration of dermatophytic infection in the cases ranged from 3 months to 10 years (9.8 months ± 5.45 months).
- Out of 60 culture positive patients included skin and nail also, 43.34% cases were naïve, reported infection for the first time; 50% cases were on antifungal treatment for 1-2 years and 6.66% cases were with recurrence's which were presenting with lesions at the same or different site after completion of previous therapy. These were categorized as naïve, recalcitrant and reinfection respectively.

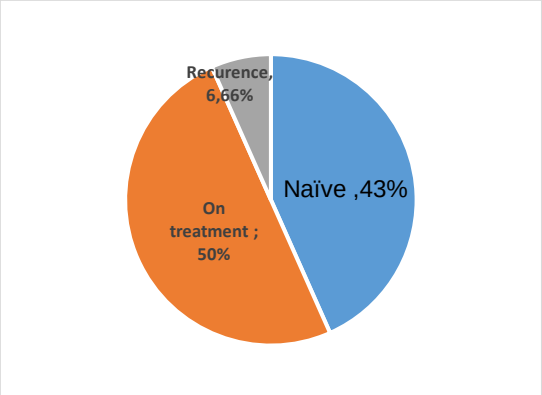



**Figure 3** Pie chart showing categorization of patients on the basis of their treatment

- **Phenotypic characteristics of *Trichophyton mentagrophytes***

  Microconidia of *Trichophyton mentagrophytes* complex were spherical, sessile, arranged in dense, grape-like clusters or along the hyphae. Macroconidia were 3-8 celled, smooth and thin walled, clavate to cigar-shaped (Figure 4a & 4b).

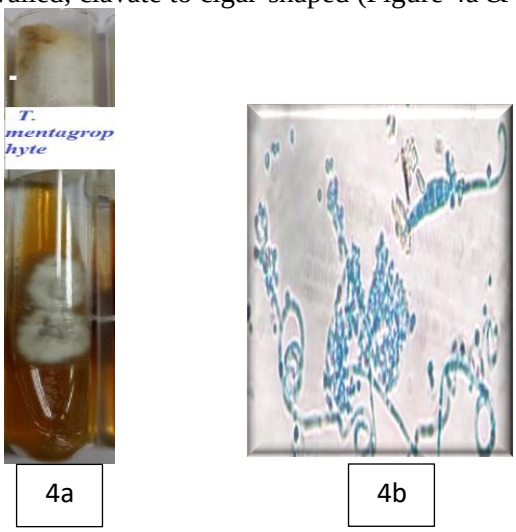



**Figure 4** (4a) culture of *T. mentagrophytes* and (4b) LPCB of *T. mentagrophytes*

- **Phenotypic Characteristics of *Trichophyton rubrum***

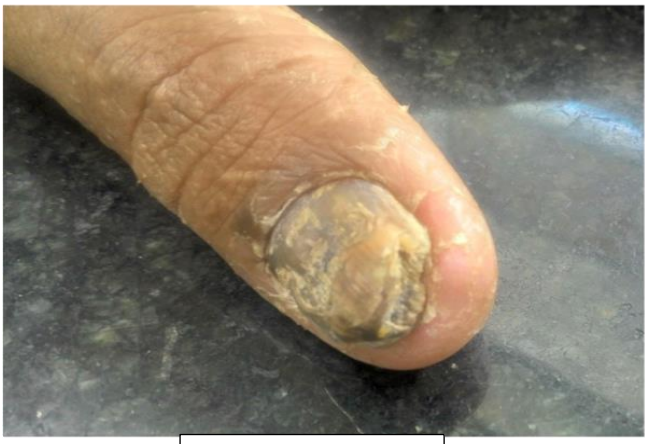
  Colonies were fluffy to cottony, white and most of the isolates turned rose when aging, reverse showed wine red pigmentation (Figure 5). Macroconidia seen occasionally, but when present are thin walled, poorly differentiated with variable size cylindrical to cigar-shaped. Microconidia were peg-shaped to pyriform. Sessile alongside undifferentiated hyphae (Figure 5a & 5b).

**LPCB of *T. rubrum.***

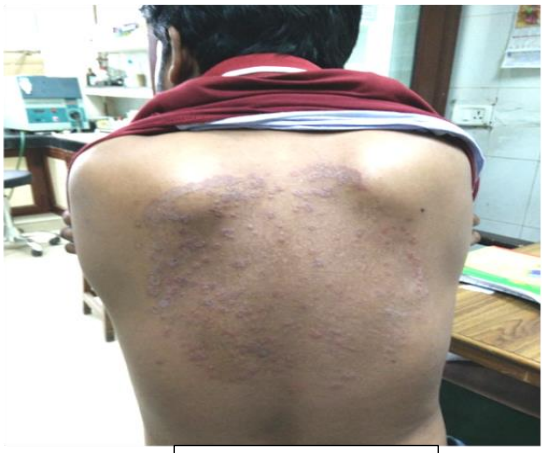

5a 5b

**Figure 5** (5a) culture of *T. rubrum* and (5b)

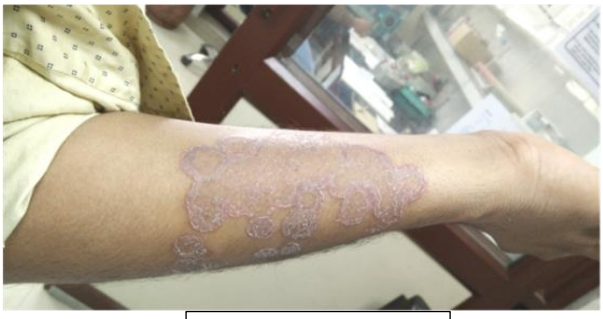
All isolates were subjected to PCR using species specific primer of the ITS region for further confirmation. The site of infection are shown in figure 6a- 6d before isolation of *T. rubrum* and *T. mentagrophytes* complex.



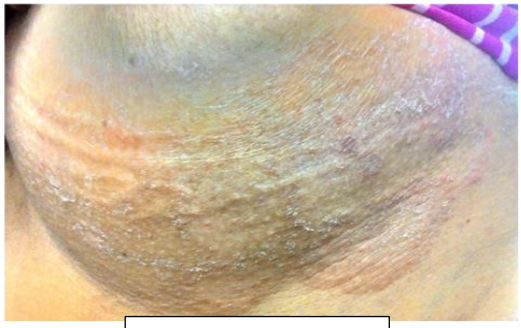
6a. Onycomycosis



6b. Tinea corporis

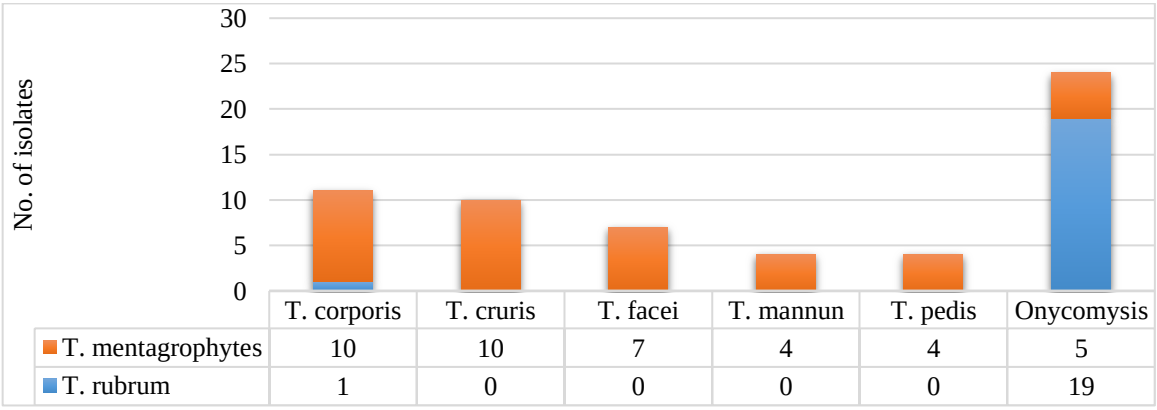


6c. Tinea mannun



6d. Tinea corporis

**Figure** 6 Lesion of Infection




| | T. corporis | T. cruris | T. facei | T. mannun | T. pedis | Onycomysis |
|---|---|---|---|---|---|---|
| T. mentagrophytes | 10 | 10 | 7 | 4 | 4 | 5 |
| T. rubrum | 1 | 0 | 0 | 0 | 0 | 19 |

**Figure 7** Distribution of *T. mentagrophyte* and *T. rubrum* with their site of infection.

**Clinical Manifestation:**

Amongst the 60 isolates, tinea corporis was predominant clinical presentation in 11 cases (18.33%) followed by T. cruris 10 (16.67%), T. facei 7 (11.67%) and T. pedis in 4 (6.67%) (Figure-7). *T. mentagrophytes* complex was the major causative agent except in one case of T. corporis, *T. rubrum* was isolated on culture. While in 24 (40%) cases of onychomycosis, *T. rubrum* 19/24 (79.17%) was isolated followed by *T. mentagrophytes* complex in 5 cases (20.83%).

**Table 5** Age and Sex distribution with species

| Age Group ( years) | *Trichophyton mentagrophytes* Complex | | | | *Trichophyton rubrum* | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | |
| | Skin | Nail | Skin | Nail | Skin | Nail | Skin | Nail |
| **10-20** | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **21-30** | 8 | 1 | 2 | 0 | 0 | 7 | 0 | 4 |
| **31-40** | 6 | 1 | 5 | 1 | 0 | 2 | 1 | 0 |
| **41-50** | 2 | 0 | 2 | 2 | 0 | 1 | 0 | 2 |
| **51-60** | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 0 |
| **Total** | **25** | **2** | **10** | **3** | **0** | **13** | **1** | **6** |

**Age Distribution:**

- The prevalence of Dermatophytic infection was more common in male patients 40/60 (66.67%) as compared to female patients 20/60 (33.33%) in both onychomycosis and Tinea infection.

**Genotypic confirmation of *Trichophyton mentagrophytes* by PCR**

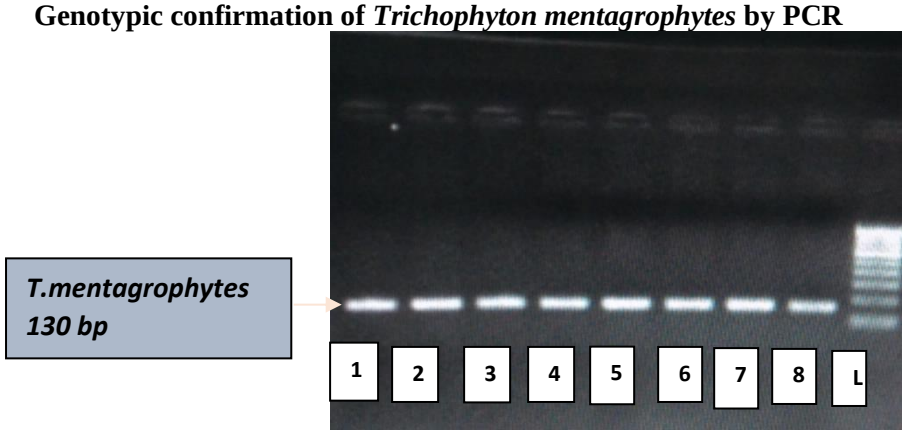



**Figure 8a**: Gel picture below showing bands at 130 bp for 8 isolates of *T. mentagrophytes* (figure:- 8a and figure:-8b).

Figure showing Lane 1-8 and L (ladder). Where lane 1-8 showing bands of samples of *T. mentagrophytes*, and L is 100 base pair molecular weight marker (Fermentas).

- **Genotypic Confirmation of *Trichophyton rubrum* by PCR**

**Figure:-8b**

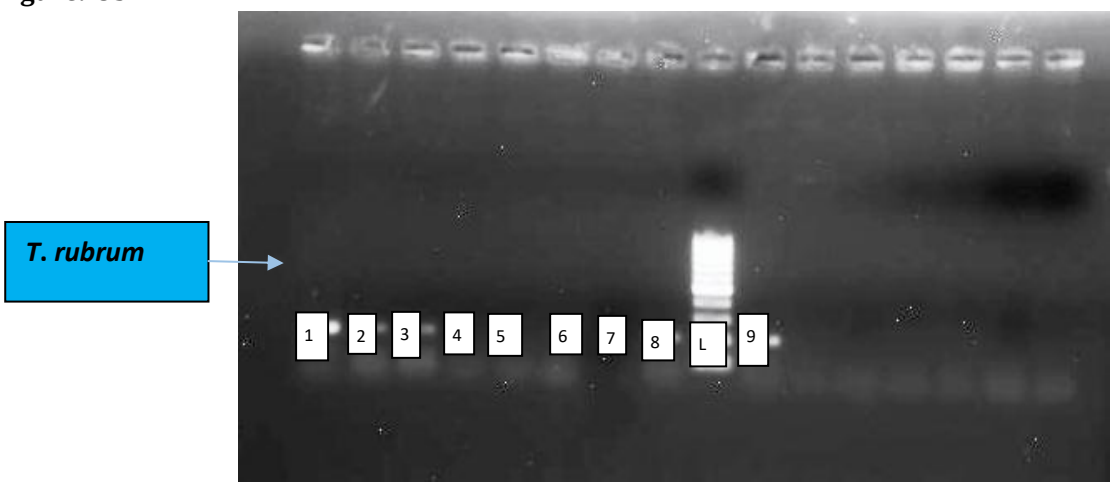



**Figure 8b:** Gel picture showing bands at 203 bp for 5 PCR products of *T. rubrum* strains

Figure showing Lane 1-7, L (ladder) and 8. Where lane 1-3, 7 & 8 are samples of *T. rubrum*, Lane 4-6 are negative control (no template) and L is 100 base pair molecular weight marker (Fermentas).

**Sequencing**

Purification of PCR products was done by Sodium Acetate Manual method and DNA sequence analysis was performed and by comparison of the nucleotides with dermatophytes reference nucleotide sequences obtained from the gene bank database (http://www.ncbi.nih.gov/gene), *T. mentagrophytes* and *Trichophyton rubrum* were showing 99% similarity with the reference strain. The representative sequence obtained were submitted to the gene bank database; and Accession numbers were obtained, total sequence submitted 12 numbers that are *T. mentagrophytes* accession no. are (9 sequences ) **MW497552 to MW497560,** (3 sequences ) **MH644185, MH745112 & MH778308** and total sequence submitted 19 numbers that are *Trichophyton rubrum* accession no.- (16 sequences) **MW506332 to MW506347 and** (3 sequences ) **MH497367 to MH497369.**

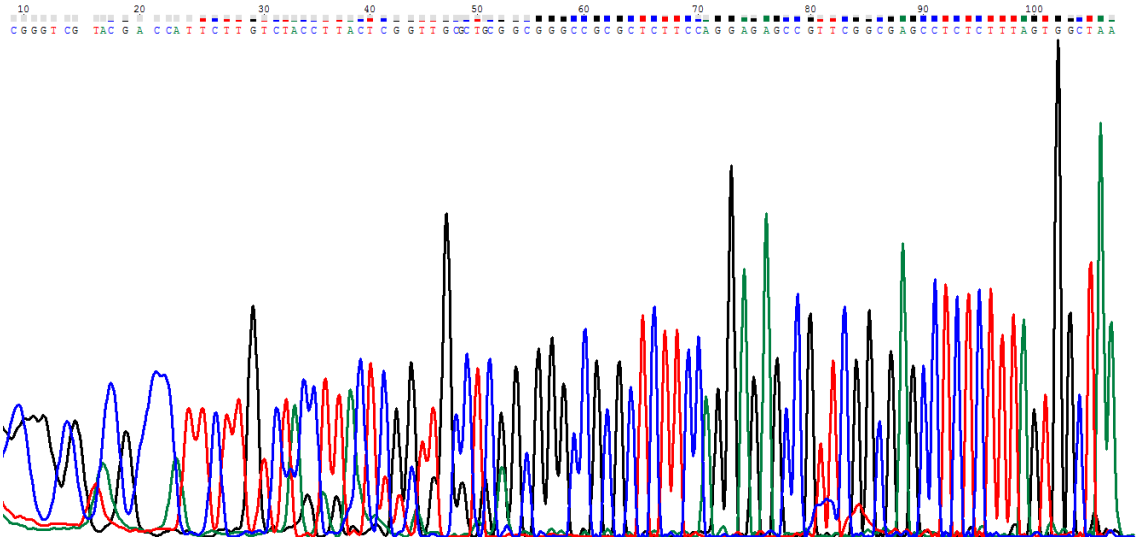


**Figure 9** Showing Electropherogram of *T. mentagrophtes*

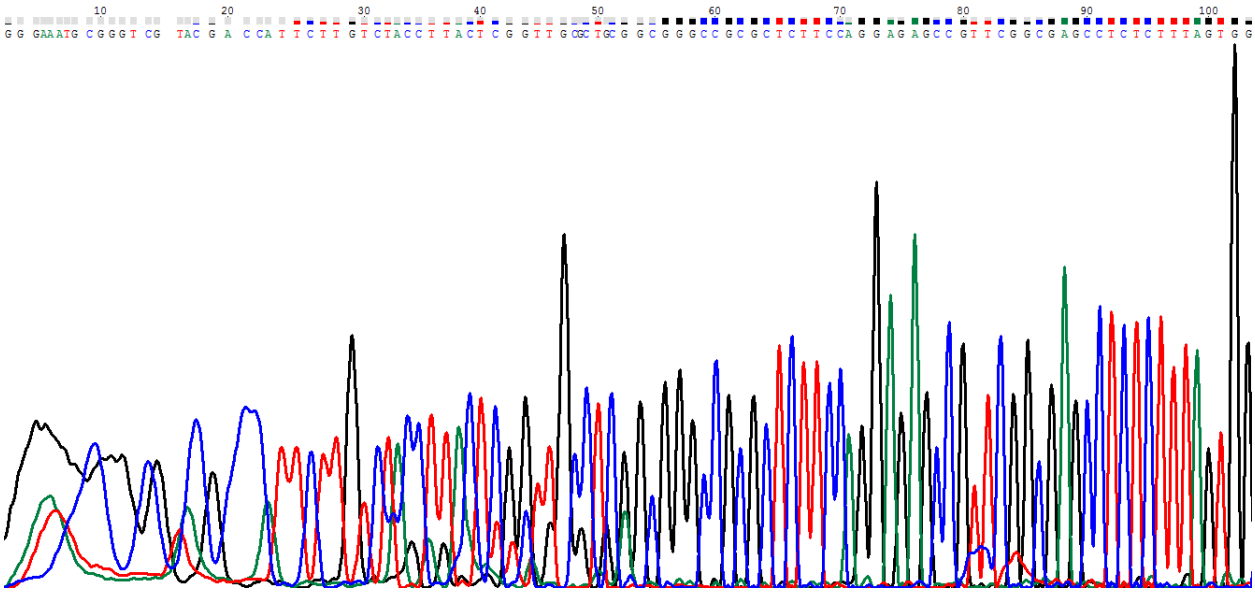


**Figure 10** Showing Electropherogram of *T. rubrum*

## Discussion

The demographic and microbiological profile observed in this study reflects current epidemiological patterns of dermatophytosis. The mean age of 30.02 ± 10.95 years indicates a higher prevalence among young adults, potentially due to greater exposure to shared public spaces and increased perspiration, which creates an ideal environment for fungal proliferation. The male predominance (2:1 ratio) is consistent with recent findings, suggesting that occupational exposure, outdoor activities, and behavioral factors may contribute to higher infection rates in males [6]. Although 200 samples were positive by KOH examination, only 140 yielded positive cultures, highlighting the limitations of microscopy alone and the need for complementary culture and molecular diagnostics. Interestingly, *T. mentagrophytes* was more frequently isolated than *T. rubrum*, indicating a potential shift in dominant species distribution, which has also been reported in recent studies across Asia and Europe [7-8]. Molecular identification using ITS2-based PCR and sequencing enhances diagnostic accuracy, particularly in distinguishing closely related species within the *T. mentagrophytes/T. interdigitale* complex, and is critical in the context of increasing antifungal resistance and clinical recalcitrance [9]. The discrepancy between KOH and culture positivity is consistent with previous findings and may be attributed to nonviable fungal elements, sample quality, or contamination during transport or processing [10].

The predominance of *Trichophyton mentagrophytes* (66.7%) over *T. rubrum* (33.3%) is notable and suggests a shift in the species distribution of dermatophytes, a trend increasingly reported in India and other parts of Asia. Traditionally, *T. rubrum* was the most prevalent dermatophyte worldwide. However, emerging data now show a rising incidence of *T. mentagrophytes* complex, particularly strains associated with chronic and treatment-resistant cases [11-12]. Molecular methods such as ITS2 sequencing provide superior resolution in distinguishing species within the *T. mentagrophytes/T. interdigitale* complex, which is vital for epidemiological monitoring and therapeutic decision-making. Clinically, the high proportion of recalcitrant cases (50%) highlights growing concerns regarding antifungal resistance and inappropriate or prolonged treatment regimens. This is in line with several studies that have identified reduced susceptibility to commonly used antifungal agents—especially terbinafine—among *T. mentagrophytes* isolates [13]. Inadequate dosing, poor adherence, and empirical treatments without species confirmation may contribute to chronicity and relapse. These findings reinforce the need for targeted antifungal therapy based on species identification and antifungal susceptibility testing in persistent cases.

The recurrence rate of 6.66% and the presence of reinfections also underscore the challenges in managing dermatophytosis, especially in tropical climates where reinfection risk is high due to humidity, crowded living conditions, and poor hygiene. Reinfection may also reflect environmental reservoirs, fomite transmission, or untreated household contacts. Public health measures focusing on hygiene education, avoidance of self-medication, and treatment of close contacts are essential to control reinfection cycles [14-15].

The demographic profile of patients in this study, with a mean age of 30.02 ± 10.95 years and a male-to-female ratio of 2:1, is consistent with existing literature. Young adult males tend to have higher occupational exposure, greater physical activity, and more frequent use of occlusive footwear or clothing, increasing their vulnerability to dermatophyte infections [16]. This trend is frequently attributed to increased occupational exposure, physical activity, and the use of occlusive footwear or tight clothing, which create warm and moist environments conducive to fungal growth [14-15]. Men are also more likely to work in outdoor or industrial settings, where hygiene conditions may be suboptimal, increasing the risk of acquiring superficial fungal infections. Moreover, gender-based differences in health-seeking behavior can influence diagnosis rates; women may be more likely to seek early medical attention, while men often delay treatment until the infection becomes severe or widespread [16]. Hygiene practices and cultural norms may also play a role in differential exposure and susceptibility between genders. Additionally, dermatophytosis is frequently reported among individuals in their second and third decades of life due to increased social interaction, use of shared facilities (e.g., gyms, dormitories), and lifestyle habits that promote transmission [10]. Understanding these demographic patterns is critical for designing effective prevention and education

strategies that are tailored to the most affected populations, particularly in resource-limited settings where recurrence and chronicity are common [17-18]. Gender-based differences in healthcare-seeking behavior and hygiene practices may also contribute. Understanding the demographic and clinical characteristics of affected populations is essential for designing preventive strategies and tailoring health education efforts.

## Conflict of Interest

No conflict of interest in this study

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