Original Article

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Microbiology Section

Profile of Dermatophytes and Antifungal Susceptibility Pattern: A Cross-sectional Descriptive Study in a Government Setup in Urban South Kerala, India

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ABSTRACT

Introduction: Recently, there has been a change in the spectrum of dermatophytosis, shifting from *Trichophyton rubrum*, which was previously the most common species, to the more drug-resistant and chronic *Trichophyton mentagrophytes*. Although there have been several recent studies on dermatophytosis, the majority have focused on clinical presentation and histopathological features. Therefore, a study that focuses on the current trends in species of dermatophytes causing infection and their antifungal susceptibility is the need of the hour. The treatment options can be tailored accordingly and newer options can be considered.

Aim: To determine the proportion of different species of dermatophytes affecting patients attending the Dermatology Outpatient Department (OPD) at a tertiary care centre in Kerala and to assess the antifungal susceptibility of the isolates to Fluconazole, Itraconazole and Terbinafine.

Materials and Methods: The present study was a cross-sectional descriptive study, conducted over a period of one year at the Department of Microbiology at Government Medical College, Thiruvananthapuram Kerala, India. Data collection for the study commenced in July 2018 and concluded in June 2019. The study included all patients attending the dermatology OPD with clinically suspected dermatophytosis during the study period. Specimens of skin, hair and nails were collected. Direct examination was performed using 10% Potassium Hydroxide (KOH) for skin and hair samples and 40% KOH for nail samples. Identification was achieved through culture on Sabouraud's Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). Antifungal susceptibility testing was conducted using Broth Microdilution testing (BMD).

Results: A total of 270 samples were collected. Of these, 53 (19.6%) were culture positive. Among the isolates, 40 (75.47%) were *T. mentagrophytes*, 7 (13.21%) were *T. rubrum* and only 6 (11.32%) were *Microsporum gypseum*. For *T. mentagrophytes*, antifungal susceptibility testing revealed the Minimum Inhibitory Concentration (MIC) ranges of 1-8 µg/mL, 0.031-0.25 µg/mL and 0.031-0.5 µg/mL for Fluconazole, Itraconazole and Terbinafine, respectively. Other species also exhibited a similar pattern of susceptibility.

Conclusions: In the present study, *T. mentagrophytes* was found to be the predominant species, followed by *T. rubrum*. Itraconazole was identified as the most effective drug according to antifungal susceptibility testing.

Keywords: Fluconazole, Itraconazole, *Trichophyton*

INTRODUCTION

In the last few decades, there has been an increase in the incidence of fungal skin infections in India. The reasons may include an increase in various predisposing conditions associated with fungal infections like immunosuppression (HIV), metabolic diseases like diabetes mellitus, solid organ transplant recipients and the use of steroids and anti-cancer drugs [1]. Among skin infections caused by fungi, the incidence of dermatophytosis has risen in recent years. Environmental factors, like humidity and the use of tight clothing, have also contributed to this increased incidence. Additionally, there has been a change in the clinical pattern of dermatophytosis [1].

Dermatophytes are hyaline septate moulds that are divided into three main genera: *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. *Trichophyton* species usually affect the skin, nails and hair. *Microsporum* species affect the skin and hair, while *Epidermophyton* species affect the skin and nails [2].

According to the site of the lesion, dermatophytoses can be classified into: Tinea capitis (scalp), Tinea corporis (trunk), Tinea pedis (foot), Tinea manuum (hands) and Tinea unguium (nails). Tinea corporis is the most common type in adults and is prevalent worldwide, particularly in tropical countries [3]. Previously, the most common species was *T. rubrum*; however, there has now been an increase in the incidence of *T. mentagrophytes*, which has emerged as a co-dominant pathogen [1].

Empirical treatment of dermatophytoses with Fixed Drug Combinations (FDCs) containing an antibacterial, antifungal and a steroid has resulted in partial resolution of the infection, followed by relapse. The commonly used antifungals for the treatment of dermatophytoses at present are terbinafine, fluconazole, itraconazole and griseofulvin. There has been an increase in the Minimum Inhibitory Concentrations (MIC) of isolates to terbinafine, fluconazole and griseofulvin. An increase in MIC values for commonly used antifungal drugs does not imply absolute resistance but rather warrants the use of higher dosages of these drugs for treatment over a longer duration [4].

The clinical and mycological patterns of dermatophytoses are changing and the number of drug-resistant isolates is increasing. Studies focusing on the identification of dermatophytes and their antifungal susceptibility testing will assist clinicians in deciding the appropriate treatment. While there have been numerous studies in India regarding the clinical aspects of dermatophytosis, the findings in Kerala, particularly in urban areas, are markedly different from those in other states and rural areas. The antifungal susceptibility of *T. mentagrophytes*, a rapidly emerging species causing chronic dermatophytosis, needs to be studied in detail to tailor empirical

first-line treatment accordingly. The present study focuses on the urban demographic and the most common species at present, *T. mentagrophytes*.

Present study aimed to determine the profile of dermatophytes affecting patients visiting the Dermatology OPD of a tertiary care centre in South Kerala, India. The primary objective was to identify the proportion of different species of dermatophytes affecting patients attending the dermatology OPD of a tertiary care centre in South Kerala. and secondary objective was to assess the antifungal susceptibility of the isolates to terbinafine, fluconazole and itraconazole using the broth microdilution method.

MATERIALS AND METHODS

The present study was a cross-sectional descriptive study, conducted over a period of one year (July 2018 to June 2019) at the Department of Dermatology and Department of Microbiology at Government Medical College, Thiruvananthapuram, Kerala, India. The study was approved by Institutional Ethics Committee (IEC) (IEC No. 14/24/2017/MCT). Informed consent was included at the end of the submission.

Inclusion criteria: Patients attending the dermatology OPD with clinically suspected dermatophytosis (patients presenting with ring-like or annular lesions on their body or hair, or the presence of chalky yellowish-coloured nails, with or without a history of previous treatment) were included in the study.

Exclusion criteria: Patients who refused to give consent were excluded from the study.

Sample size: The sample size was calculated to be 270, using the formula $N=4pq/d^2$:

p=prevalence of *T. verrucosum*; 1.5%

q=100 - p; 98.5%

d=absolute precision; 1.5 (prevalence <10%)

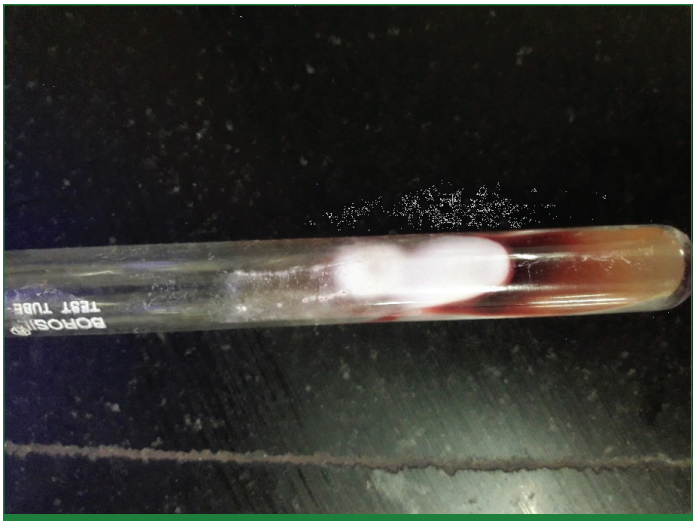
$d^2=2.25$

$N=(4\times1.5\times98.5) / 2.25=270$ [5].

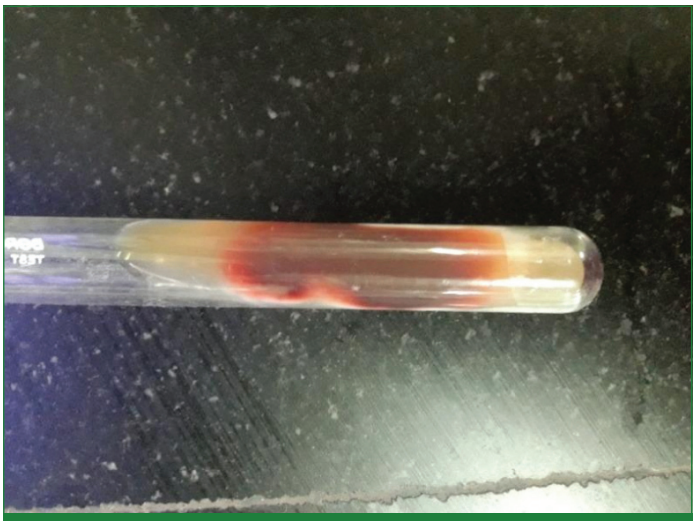
Study Procedure

A proforma was used to collect the relevant patient details, including age, sex, education, occupation, duration of the present illness, history of prior treatment and any other relevant history such as household contacts with the same disease or history of contact with pets. Specimens were collected by the dermatologist from the patients after obtaining informed consent. Skin scrapings were taken from the active margin of a lesion using a sterile scalpel. Nail clippings were obtained using a clipper and debris was collected from the underside of the nails. Hair samples were taken using epilatory forceps. The specimens were packed, folded into black paper, placed in a sterile envelope and transported to the microbiology laboratory [6].

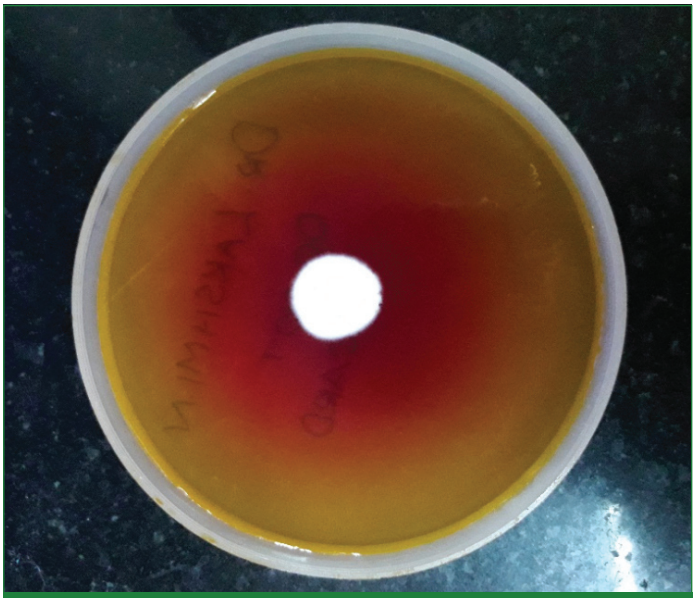
Direct microscopic examination was performed using a KOH wet mount. A 10% KOH solution was used for skin samples [2], while a 40% KOH solution was used for nail samples. The media inoculated included SDA with cycloheximide at a concentration of 0.5 g/litre and gentamicin at 50 mg/litre, plain SDA and DTM. The samples were incubated at both 37° Celsius and at room temperature, with better growth observed at room temperature. Growth typically occurred within a period of 7-14 days. Samples were incubated for a total of 30 days before a negative report was issued. Genus identification was based on macroscopic appearance on SDA and microscopic appearance on Lactophenol Cotton Blue (LPCB) mount. The macroscopic appearance of *T. rubrum* is shown in [Table/Fig-1,2], while the macroscopic appearance of *T. mentagrophytes* on DTM is illustrated in [Table/Fig-3]. The appearance of *T. mentagrophytes* on the LPCB mount, showing spiral hyphae, is displayed in [Table/Fig-4].



[Table/Fig-1]: Macroscopic appearance of *Trichophyton rubrum* on SDA obverse.



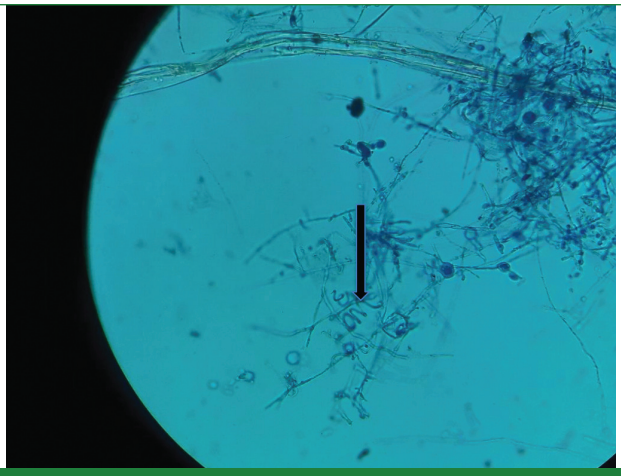
[Table/Fig-2]: Macroscopic appearance of *Trichophyton rubrum* on SDA reverse.



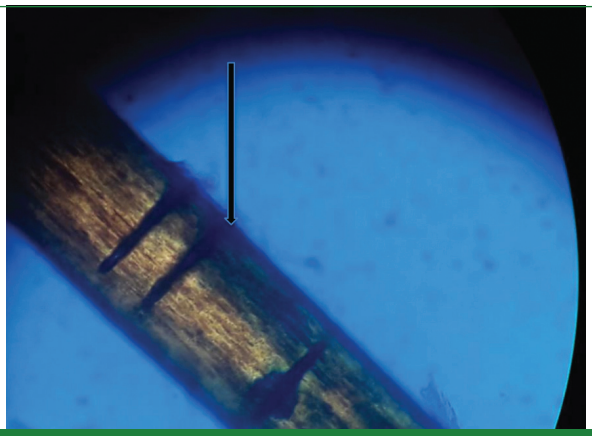
[Table/Fig-3]: Macroscopic appearance of *Trichophyton mentagrophytes* on DTM.

Additional tests used for the identification of the species included the urease test, which was used to identify *T. mentagrophytes*, producing a positive result in 2-3 days. The hair perforation test was also employed for the identification of *T. mentagrophytes*, which produced wedge-shaped perforations observed under a light microscope (high power X40) [7]. A positive hair perforation test is displayed in [Table/Fig-5].

Isolates were also sent to the National Culture Collection of Pathogenic Fungi under the Mycology Division of the Postgraduate



[Table/Fig-4]: LPCB mount showing spiral hyphae of *Trichophyton mentagrophytes* (10×) (arrow represents spiral hyphae).



[Table/Fig-5]: Positive hair perforation test in *Trichophyton mentagrophytes* (40×) (arrow represents wedge shaped perforations).

Institute of Medical Education and Research in Chandigarh for species-level identification.

Antifungal susceptibility testing of isolates was performed using the broth microdilution technique, in accordance with Clinical and Laboratory Standards Institute (CLSI) M38-A2 [8].

The procedure for conducting antifungal susceptibility testing by BMD can be explained under the following headings:

I. Preparation of Antifungal Agents

- **Source:** The pure substances were obtained commercially, directly from the manufacturer.
- **Weighing of antifungal powders:** The formulas used for weighing the antifungal drugs are as follows:

$$\text{Weight in mg} = \frac{\text{Volume (mL)} \times \text{Concentration (µg/mL)}}{\text{Potency (µg/milligm)}}$$

Or

$$\text{Volume (mL)} = \frac{\text{Weight (mg)} \times \text{Potency (µg/milligm)}}{\text{Concentration (µg/mL)}}$$

For example, to prepare 100 mL of a stock solution containing 1280 µg of the antifungal agent per mL, using antifungal powder with a potency of 750 µg/mg, the following formula was used:

$$\text{Weight} = \frac{100 \times 1280}{750} = 170.7 \text{ mg}$$

Drug dilution for the stock solution was prepared at 100 times higher than the highest concentration tested. For fluconazole, the diluent used was distilled water, while for itraconazole and terbinafine, the diluent used was DMSO.

- **Filtration:** To ensure additional sterility, the prepared stock solution was filtered using a membrane filter.
- **Storage:** The stock solution was stored in sterile polypropylene vials at -80° Celsius.

II. Preparation of Medium

The medium used was RPMI 1640 (with glutamine, without bicarbonate and phenol red as a pH indicator). The powdered medium was dissolved in 900 mL of distilled H_2O and MOPS buffer was added to obtain a final concentration of 0.165 mol/L. The pH was adjusted to 7.0 at 25° Celsius using 1 mol/L NaOH. The medium was then filtered and stored at 4° Celsius.

III. Preparation of Inoculum

The fungal isolate was grown on potato dextrose agar at a temperature of 30° Celsius for four to five days or until good conidial growth was obtained. The colonies were then covered with 1 mL of sterile 0.85% saline and a suspension was prepared by gently probing the colonies with the tip of a transfer pipette. After allowing the resulting suspension to settle for five to ten minutes, it was adjusted to be two times more concentrated than the density needed for testing (1×10^3 to 3×10^3).

IV. Preparing Drug Dilutions and Inoculating the Medium

- **Preparation of Drug Dilutions:** Sterile plastic test tubes were used to make the drug dilutions and sterile disposable multiwell microdilution plates (96 U-shaped wells) were used to perform the tests.

The dilutions were prepared in the following manner [Table/Fig-6].

Antimicrobial solution						
Step	**Concentration (µg/mL)**	**Source**	**Volume (mL)**	**Solvent (mL) e.g., DMSO**	**Intermediate concentration (µg/mL)**	**Final concentration at 1:50 (µg/mL)**
1	6400	Stock	0.5	0.5 1.5 3.5	6400	128
2 3 4	6400 6400 6400	Stock Stock Stock	0.5 0.5 0.5	0.5 1.5 3.5	3200 1600 800	64 32 16
5 6 7	800 800 800	Step 4 Step 4 Step 4	0.5 0.5 0.5	0.5 1.5 3.5	400 200 100	8 4 2
8 9 10	100 100 100	Step 7 Step 7 Step 7	0.5 0.5 0.5	0.5 1.5 3.5	50 25 12.5	1 0.5 0.25
11 12 13	12.5 12.5 12.5	Step 10 Step 10 Step 10	0.5 0.5 0.5	0.5 1.5 3.5	6.25 3.125 1.56	0.125 0.0625 0.0313
14 15 16	1.56 1.56 1.56	Step 13 Step 13 Step 13	0.5	0.5 1.5 3.5	0.78 0.39 0.195	0.0156 0.0078 0.0039
17	0.195	Step 16		0.5	0.0975	0.0019

[Table/Fig-6]: Schematic diagram for preparing antifungal dilutions.

- **Inoculation of the medium:** On the day of the test, each well was inoculated using 0.1 mL of the 2× conidial inoculum. This step diluted the drug concentrations, inoculum densities and solvent to the final desired test concentrations. The growth control wells contained 0.1 mL of the corresponding diluted inoculum suspension and 0.1 mL of drug diluent without any antifungal agent.

V. Incubation and Interpretation of Results

The microdilution trays were incubated at 35° Celsius for a period of four days. Following incubation, the results were interpreted by assessing the MIC for each antifungal agent, which is defined as the lowest concentration of the antifungal agent that inhibits fungal growth [8].

The range of drug dilutions tested for the drugs Fluconazole, Itraconazole and Terbinafine according to CLSI M38-A2 was as follows:

- Fluconazole: 0.125-64 µg/mL
- Terbinafine: 0.001-0.5 µg/mL
- Itraconazole: 0.001-0.5 µg/mL [8]

The MIC values of *T. mentagrophytes* ATCC 4439 are as follows:

- Terbinafine: 0.002-0.008 µg/mL
- Itraconazole: 0.03-0.25 µg/mL
- Fluconazole:2-64 µg/mL

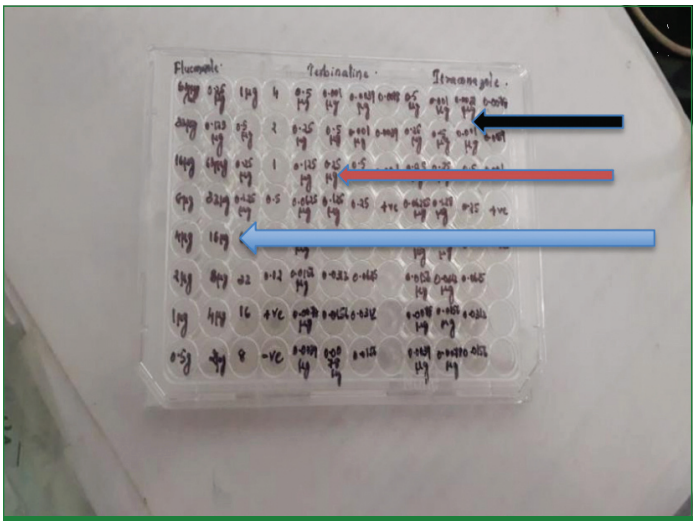
The MIC values of *T. rubrum* ATCC 4438 are as follows:

Fluconazole: 0.5-4 µg/mL [8]

Terbinafine: ≤0.015µg/mL

Itraconazole: 0.015-0.25 µg/mL

After four days of incubation, the results were interpreted by examining the MIC for each antifungal agent, which indicates the lowest concentration of the antifungal agent that inhibits fungal growth. The microtitre plate used for performing BMD is shown in [Table/Fig-7].



[Table/Fig-7]: Microtitre plate for antifungal susceptibility testing using BMD: The dilutions are shown increasing down the columns. Blue arrow: Fluconazole, red arrow: Terbinafine, black arrow: Itraconazole

RESULTS

Samples were taken from 270 clinically suspected dermatophytosis patients. This included 238 (88.1%) skin samples, 31 (11.5%) nail samples and 1 (0.4%) hair sample. The total number of culture-positive samples was 53 (19.6%). Most species of dermatophytes grew in DTM within 2-3 days, while growth on SDA was slower, occurring within 7-14 days.

The sex-wise distribution of dermatophyte isolates showed that, out of the total isolates, 25 (47.17%) were males and 28 (52.83%) were females. The highest number of isolates belonged to patients in the age group of <20 years, with 18 (33.96%). The greatest number of isolates was found among students, totalling 23 (43.40%). [Table/Fig-8] shows the distribution of isolates according to clinical specimens.

S. No.	Clinical specimen	Number of isolates	Percentage (%)
1.	Skin	52/238	21.84
2.	Hair	1/1	100
3.	Nail	0	0
Total		53/270	

[Table/Fig-8]: Isolates from different clinical specimens.

The distribution of isolates according to clinical diagnosis indicated that 36 (67.9%) patients had lesions on only a single site, whereas 17 (32%) patients had lesions on multiple sites. The highest number of isolates was from patients presenting with Tinea corporis, with 37 (56.92%). The distribution of isolates according to clinical diagnosis is detailed in [Table/Fig-9].

S. No.	Clinical presentation	n (%)
1.	Tinea corporis	37 (56.92)
2.	Tinea cruris	19 (29.23)
3.	Tinea fascei/barbae	4 (6.15)
4.	Tinea manuum	1 (1.53)
5.	Tinea capitis	2 (3.07)
6.	Tinea incognito	2 (3.07)

[Table/Fig-9]: Distribution of isolates according to clinical diagnosis.

Three species of dermatophytes were identified in the study. [Table/Fig-10] shows the distribution according to species.

Species	n (%)
Trichophyton mentagrophytes	40 (75.47)
Trichophyton rubrum	7 (13.21)
Microsporum gypseum	6 (11.32)

[Table/Fig-10]: Species-wise distribution of isolates.

Out of the 53 isolates, 47 (88.68%) belonged to *Trichophyton* species, while 6 (11.32%) were *Microsporum gypseum*.

Antifungal susceptibility testing revealed the range of MIC obtained in the isolates tested in the present study for various drugs, as shown in [Table/Fig-11]. The MIC of standard strains was used to interpret the susceptibility results of the isolates obtained in this study.

S. No.	Dermatophyte species	Fluconazole (µg/mL)	Itraconazole (µg/mL)	Terbinafine (µg/mL)
1.	*Trichophyton mentagrophytes*	1-8	0.031-0.25	0.031-0.5
2.	*Trichophyton rubrum*	2-8	0.031-0.125	0.031-0.25
3.	*Microsporum gypseum*	0.25-4	0.031-0.125	0.031-0.125

[Table/Fig-11]: Range of MIC in the present study for various antifungal agents.

A comparison was made between the MIC of standard strains [8] and the isolates obtained in the study, highlighting the percentage of isolates with higher MIC.

The number and percentage of isolates of each species showing MIC greater than that of the standard strain is presented in [Table/Fig-12].

However, it should be noted that, as of now, the CLSI has not defined breakpoints for dermatophytes due to a lack of data on clinical correlation, pharmacokinetics, pharmacodynamics, or epidemiological cut-off MIC values [9]. Hence, the term “resistant” cannot be used in the case of dermatophytes and the observation of higher MIC for a certain drug may imply only the need for a higher

Species	Fluconazole (MIC >4 µg/mL)	Itraconazole (MIC >0.25 µg/mL)	Terbinafine (MIC >0.008 µg/mL)
Trichophytoton mentagrophytes	16 (30.18%)	10 (18.86%)	40 (100%)
Trichophyton rubrum	1 (1.8%)	0	7 (100%)
Microsporum gypseum	0	0	6 (100%)
Total	17 (32.07%)	10 (18.86%)	53 (100%)

[Table/Fig-12]: The number and percentage of isolates of each species which show higher MIC for Fluconazole, Itraconazole and Terbinafine.

concentration or prolonged use, rather than indicating absolute resistance to the drug.

DISCUSSION

In the present study, it was found that 238 (88.1%) out of 270 samples were skin samples, 31 (11.5%) were nail samples and only 1 (0.4%) was a hair sample. This was similar to the study conducted by Poluri LV et al., which reported that 101 (91.81%) were skin samples and only 9 (8.18%) were hair samples [10]. Among the 270 clinically suspected cases of dermatophytosis in the present study, 53 (19.6%) showed culture positivity. In a study conducted by Narayanan MP et al., in Calicut, a culture positivity rate of 61 (47%) was noted [5]. Additionally, a study by Surekha A et al., in Tamil Nadu in 2015 reported a culture positivity rate of 30%, which was comparable to the findings of the present study [11]. Notably, the culture positivity rates in recent studies are lower compared to older studies. Since this was a tertiary care centre, the application of topical steroids and over-the-counter antifungal agents for shorter durations than recommended may have led to many patients presenting with partially healed lesions. This factor may also have contributed to the lower culture positivity rates observed.

Of the 53 specimens that were culture positive, 25 (47.17%) were from males, while 28 (52.83%) were from females. In a study by Bhangra S et al., conducted in and around the Shimla Hills, this variation was more pronounced, with a male-to-female ratio of 4:1 [12]. In another study conducted by Sirisha NL et al., it was found that 4,469 (52.3%) of the patients were female, while 4,076 (47.7%) were male, which was similar to the present study's findings [13].

In the present study, it was noted that 17 (32%) of the patients had a history of lesions in multiple sites, while a total of 36 (67.9%) patients had lesions in only a single site. Among the 53 isolates, 37 (56.92%) were from patients with tinea corporis, 19 (29.23%) were from patients with tinea cruris, 4 (6.15%) were from patients with tinea barbae, 2 each were from patients with tinea capitis and tinea incognito and 1 was from a patient with tinea manuum. A study conducted by Kumar S et al., found that tinea corporis was the most common clinical type, accounting for 119 (47.6%), followed by tinea cruris with 60 (24%), tinea pedis with 15 (6%), tinea capitis with 11 (4.4%), tinea manuum with 10 (4%), tinea faciei with 8 (3.2%) and tinea barbae with 3 (1.2%) [14].

According to the present study, 47 (88.67%) of the isolates were from *Trichophyton* species. Of these, 40 (75.47%) were identified as *Trichophyton mentagrophytes* and 7 (13.21%) were *Trichophyton rubrum.* Six (11.32%) were *Microsporum gypseum*. These findings are in agreement with those of Venkatesh VN and Kotian S, who reported that the predominant dermatophyte was *T. mentagrophytes*, with 214 (13.46%), followed by *T. rubrum* with 55 (3.46%), *T. mentagrophytes* var. *interdigitale* with 56 (3.52%), *T. violaceum* with 28 (1.76%), *T. soudanense* with 26 (1.64%), *T. schoenleinii* with 8 (0.50%), *T. tonsurans* with 14 (0.88%), *T. verrucosum* with 13 (0.82%), *M. audouinii* with 7 (0.44%), *M. gypseum* with 8 (0.50%), *M. canis* with 3 (0.19%) and *E. floccosum* with 13 (0.82%) [15].

The findings in the present study were also comparable to those of the study conducted by Pavani A et al., where the predominant species isolated was *T. mentagrophytes*, accounting for 41 (69.5%) of the total isolates, followed by *T. rubrum* with 9 (15.25%) [16]. This might be due to the increasing incidence of *Trichophyton mentagrophytes* infection in our country, attributed to its ability to survive for a long time on fomites and its relative resistance to the common antifungal drugs used.

In earlier studies conducted in India, *Trichophyton rubrum* was found to be the predominant species isolated. Doddamani PV et al. reported that *T. rubrum* (45, 46.87%) was the most common agent, followed by *T. mentagrophytes* (35, 36.46%), *E. floccosum* (8, 8.33%) and *M. gypseum* (4, 4.16%) [17].

In the present study, antifungal susceptibility testing was performed by BMD for fluconazole, itraconazole and terbinafine. The results of present study were similar to those from the study conducted by Singh SK et al., [18] two studies are compared in [Table/Fig-13,14]. The results for *Trichophyton mentagrophytes* have also been compared with those from Mahajan S et al., in [Table/Fig-13] [19]. Here, the percentage of isolates with higher MIC for *Trichophyton mentagrophytes* was calculated by taking the total number of *T. mentagrophytes* isolates as 40, noting the number of isolates with an MIC greater than that of standard strains and then calculating the percentage. Similarly, for *T. rubrum*, the total number of isolates was taken as seven and the percentage of isolates with an MIC greater than that of standard strains was calculated. In other studies, the criteria used to classify an isolate as resistant or showing a higher MIC can vary, as there are no uniform guidelines.

Antifungals	Singh SK et al., [18]	Mahajan S et al., [19]	Present study
Fluconazole	26.8	22	40
Terbinafine	65.9	18	100
Itraconazole	17.1	6	25

[Table/Fig-13]: Comparison for *T. mentagrophytes* (percentage of isolates with higher MIC) [18,19].

Antifungal	Singh SK et al., [18]	Present study
Fluconazole	33.3	14.3
Terbinafine	100	100
Itraconazole	0	0

[Table/Fig-14]: Comparison for *T. rubrum* (percentage of isolates with higher MIC) [18].

In this study, as well as in other similar studies, it was found that fluconazole and terbinafine showed a higher rate of resistance compared to Itraconazole [18,19]. However, since breakpoints have not been defined by the CLSI, it is difficult to compare individual studies.

Limitation(s)

Patients with a prior treatment history were not excluded from the study. As this was a tertiary care centre, most of the patients had taken some form of topical or systemic antifungal drugs before presenting for treatment. Newer antifungals, such as Isavuconazole and Sertaconazole, were not tested in the study. Additionally, since most of the patients were outpatients, proper follow-up could not be conducted. These factors represent the limitations of the study.

CONCLUSION(S)

In the present study, it was found that *Trichophyton mentagrophytes* was the predominant species causing dermatophytosis in South Kerala, India. Through antifungal susceptibility testing, Itraconazole was identified as having more susceptible isolates and is likely to be more effective than the other agents tested. This study could be expanded further to assess the antifungal susceptibility of rarer species like *Trichophyton tonsurans*, *Epidermophyton floccosum* and *Trichophyton verrucosum*, which were not isolated in the present study. Additionally, newer antifungal agents can also be tested and compared against established treatments. This approach will help to prevent the overuse of agents like Fluconazole.

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