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Clinico Mycological Study of Dermatophytosis in A Tertiary Care Hospital.

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Abstract

Background: Dermatophytosis is a common superficial fungal infection, particularly prevalent in tropical and subtropical regions. Changing epidemiological patterns, increasing predominance of Trichophyton mentagrophytes, recurrent infections and emerging antifungal resistance have increased the importance of accurate mycological diagnosis and antifungal susceptibility testing. The present study was undertaken to evaluate the clinico-mycological profile and antifungal susceptibility pattern of dermatophytes in patients attending a tertiary care hospital. Aim: To study the clinical and mycological aspects of dermatophytosis, including in-vitro antifungal susceptibility testing, in a tertiary care hospital. ***Materials and Methods***: A prospective clinico-mycological study was conducted among 100 clinically suspected cases of dermatophytosis. Clinical specimens including skin scrapings, hair and nail clippings were collected and subjected to direct microscopic examination using KOH wet mount. Samples were cultured on Sabouraud dextrose agar (SDA) for isolation and identification of dermatophytes, with potato dextrose agar used to facilitate sporulation where required. Species identification was performed using conventional mycological characteristics and appropriate biochemical tests. Antifungal susceptibility testing was performed by the microbroth dilution method, with minimum inhibitory concentrations (MICs) determined for terbinafine, clotrimazole, fluconazole and griseofulvin. ***Results***: Among the 100 patients, 60 (60%) were females and 40 (40%) were males, with a male-to-female ratio of 1:1.5. Homemakers constituted the largest occupational group (39%), followed by students (31%). A positive family history was present in 40% of patients, while diabetes mellitus was reported in 15%. Skin scrapings constituted 94% of specimens. KOH microscopy was positive in 58%, while fungal culture was positive in 60% of cases. Tinea corporis was the predominant clinical presentation (78%), followed by tinea cruris (16%), tinea capitis (4%) and tinea unguium (2%). Among culture-positive samples, Trichophyton mentagrophytes was the predominant isolate (46/60; 76.7%), followed by T. rubrum (16.7%). The MIC range for terbinafine was 0.03–0.5, compared with 0.06–2 for clotrimazole, 8–64 for fluconazole and 0.125–1 for griseofulvin among the major isolates. Terbinafine demonstrated the lowest MIC values, whereas fluconazole showed comparatively higher MIC values. ***Conclusion***: The study demonstrates a predominance of tinea corporis and T. mentagrophytes among dermatophytosis cases. Both KOH microscopy and fungal culture contribute to diagnosis, with culture providing species-level identification. The comparatively lower MIC values observed with terbinafine indicate greater in-vitro activity against the isolates studied, whereas fluconazole demonstrated higher MIC values. Periodic surveillance of dermatophyte species distribution and antifungal susceptibility patterns is important for guiding rational antifungal therapy and monitoring emerging resistance.

Keywords: Dermatophytosis; Trichophyton mentagrophytes; Tinea corporis; KOH mount; Fungal culture; Antifungal susceptibility; MIC; Terbinafine; Fluconazole.

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INTRODUCTION

Dermatophytosis are the most common superficial cutaneous fungal infections caused by filamentous fungal species of Arthodermataceae family. Based on ITS (internal transcribed spacer) of rRNA 7 CLADES of Arthodermataceae family have been identified which are Trichophyton, Microsporum, Epidermophyton, Lophophyton, Paraphyton ,Nanizzia and Gurramyces1.

Poor personal hygiene, overcrowding, tight clothing. hot humid climate and immunocompromised conditions are risk factors for dermatophytosis. In India, dermatophytosis caused by T. mentagrophyte species has shown an increased incidence as shown by recent epidemiological studies done by Kumaran G et al 20142, Agarwal et al 2014 3 Soniya Mahajan et al, 20174 Noronha TM et al 20165. Occurrence of dermatophytosis infection caused by T.rubrum show a reduced trend in India. In 1960 Ajello, said "species not only differ from region to region but may change with the passage of time"6

Dermatophytosis is usually considered easy to treat, but the inadvertent use of antifungal drugs has led to emergence of resistant strains. In India there is increase in incidence of recalcitrant chronic & recurrent dermatophytosis in the recent years further worsens the scenario.4,7 Most dermatophytes which were usually susceptible to traditional azoles both in vivo and in vitro have developed resistance due to in adverent use of antifungals and poor patient's compliance. 7,4,2 Therefore, new antifungal agents are needed in order to improve safety and efficacy. Third generation

azoles have shown antifungal activities but clinical use has been sparsely reported. In vitro susceptibility testing would be useful for selecting the most suitable antifungal agents. Even though the wide range of antifungals are available, the treatment failure has been reported which results in poor treatment outcome. To avoid unnecessary usage of drugs for long time, routine investigation of antifungal susceptibility patterns have to be done4,7. So this prospective study has been undertaken to know about the local prevalence of fungal species causing dermatophyosis and it's susceptibility pattern.

Antifungal susceptibility testing therefore has an important role in understanding the susceptibility profile of locally prevalent dermatophytes. The concentration of an antifungal drug needed to stop fungal growth can be measured in vitro using the minimum inhibitory concentration (MIC) method. The inoculum size, culture medium, pH, incubation conditions, temperature, and length of incubation are some of the variables that affect the susceptibility testing process.8 For a meaningful comparison of susceptibility outcomes, standardized methodologies are especially crucial. The broth microdilution method is suggested by CLSI and EUCAST for standardized in-vitro antifungal susceptibility testing. The thesis explains broth dilution techniques, including macro- and microdilution, as well as agar dilution, disk diffusion, and E-test procedures.9

Because dermatophytosis can be mimicked by a number of dermatological disorders and clinical lesions may have unusual appearances, laboratory validation is crucial. Fungal elements may be easily and effectively demonstrated by direct microscopic inspection using potassium hydroxide (KOH). 10% KOH was employed for skin specimens and 20% KOH for hair and nail specimens in the thesis approach. Refractile, branched, septate hyphal elements with or without arthroconidia are seen in positive preparations.8,10,11

For isolation and species identification, culture is still crucial. In the current investigation, specimens were inoculated with antibiotics onto Emmons' variant of Sabouraud dextrose agar (SDA), and they were then identified using microscopic morphology and macroscopic colony features. Colony color, texture, growth rate, pigment production, and topography were assessed, and hyphae, microconidia, and macroconidia were studied under a microscope. For slide culture and the creation of conidial suspensions for antifungal susceptibility testing, further subculture on potato dextrose agar (PDA) was carried out.12,13

Species-level separation is aided by further identification techniques such urease testing, slide culture, and lactophenol cotton blue preparation. Slide culture can help with morphological identification since it maintains the unaltered arrangement of conidia, conidiophores, and hyphae. In the study methodology, urease testing is especially helpful in distinguishing T. mentagrophytes from T. rubrum.

Therefore, a more thorough evaluation of dermatophytosis can be obtained by combining a clinical, direct microscopy, culture-based, and antifungal susceptibility approach. Improved laboratory diagnosis and logical antifungal medication selection can be facilitated by identifying the local distribution of dermatophyte species and their antifungal susceptibility pattern. In order to assess the clinical and mycological profile of dermatophytosis and ascertain the in vitro antifungal susceptibility pattern of the isolated dermatophytes at a tertiary care hospital in Trichy, the current study was conducted.

AIMS AND OBJECTIVE

AIM:

To study the clinical and mycological aspects of dermatophytes including the in vitro antifungal susceptibility testing in a tertiary care hospital, Trichy.

OBJECTIVIES:

1. To do direct microscopic examination of samples with KOH wet mount

2. To perform fungal culture in SDA for identification of dermatophytes.
3. To do culture in Potato dextrose agar for sporulation
4. To do Antifungal susceptibility testing using micro broth dilution method for MIC determination (CLSI M38 -A)

MATERIALS AND METHODS

Study Setting

This study was carried out in the Dermatology Clinic of MGM Government Hospital (MGMGH), Trichy, in collaboration with the Department of Microbiology, K.A.P.V. Government Medical College, Trichy.

Study Period

The study was conducted over a period of **one year,** from January 2019 to December 2019.

STUDY DESIGN

The study was designed as a prospective cross-sectional study.

STUDY GROUP

The study included 100 clinically suspected cases of dermatophytosis attending the Outpatient Department of Dermatology at MGM Government Hospital, Trichy.

Inclusion Criteria

Patients of all age groups attending the dermatology outpatient clinic with clinically diagnosed dermatophytic infections involving the skin, hair and/or nails were included in the study.

Exclusion Criteria

Patients who were already receiving topical or oral antifungal treatment were excluded from the study.

Specimen Collection

SKIN SCRAPPINGS

The affected area was thoroughly cleaned with 70% alcohol to remove surface contaminants and allowed to dry by evaporation. Using a sterilized blunt scalpel, scrapings were obtained from the active margin of the lesion. The scalpel was sterilized using spirit and flame and allowed to cool before use. Scrapings were collected from the margin of the lesion without injuring the skin, keeping the blade flat against the skin surface during scraping.

HAIR

The same procedure was followed for hair specimens. In addition, affected hairs were epilated using flame-sterilized forceps. Particular care was taken to obtain the basal portion of affected hairs, as the fungus is commonly present in this region.

NAIL

The affected nail was cleaned meticulously with 70% alcohol. Nail clippings or deep scrapings were obtained from recently invaded tissue. Specimens were collected from discoloured, dystrophic or brittle portions of the nail, including the full thickness when required. Scrapings were also obtained from white spots present on the nail.

TRANSPORT OF SAMPLES

The collected clinical specimens were transported to the microbiology laboratory in sterilized black paper sachets, allowing easy visualization of the collected scales.

Direct Microscopy

KOH Mount

Direct microscopic examination was performed using 10% potassium hydroxide (KOH) for skin specimens and 20% KOH for hair and nail specimens. The clinical specimen was applied to a clean glass slide along with a drop of KOH, which was then emulsified. To aid in cleansing, the preparation was gently heated over a flame and left for five to thirty minutes. The clearing time for specimens of hair and nails analyzed with 20% KOH may be as long as 24 to 48 hours. After carefully placing a coverslip, the preparation was inspected under 10× and 40× objectives. When a KOH preparation showed refractile, long, smooth, undulating, branching, and septate hyphal filaments—with or without arthroconidiospores—it was deemed positive.

Culture and Identification

Clinical specimens were inoculated onto Emmons' modification of Sabouraud dextrose agar (SDA) containing antibiotics, irrespective of the direct KOH findings. Cultures were incubated at 28°C and examined at the end of the first, second and third weeks. Fungal growth often happened in two weeks or less. The macroscopic and microscopic features of the fungal isolates were used to identify them. Colony color, texture, growth rate, pigment production on the obverse and reverse surfaces, surface texture, and colony topography were all evaluated macroscopically. The morphology of hyphae, microconidia, macroconidia, and their arrangement served as the basis for microscopic identification. In order to prepare conidial suspensions needed for antifungal susceptibility testing, isolated colonies were further subcultured on potato dextrose agar (PDA) for slide culture. The majority of isolates showed growth within a week of subcultures being incubated at 28°C. Following the appearance of growth, lactophenol cotton blue preparations were prepared.

IDENTIFICATION OF FUNGAL ISOLATES

a) Macroscopic Examination of Culture

Colony morphology was examined on SDA with assessment of:

- Colour of the aerial mycelium
- Colour of the obverse and reverse surfaces
- Production of diffusible pigment
- Surface texture, including glabrous, waxy, powdery, granular, suede-like, velvety, downy or fluffy appearance
- Colony topography, such as flat, cerebriform or crateriform appearance
- Growth rate.

b) Microscopic Examination of Culture

1) Tease Mount

A drop of lactophenol cotton blue (LPCB) stain was placed on a clean microscopic slide. A small portion of the fungal colony was removed from the agar and suspended in the stain. Using two teasing needles, the fungal hyphae were separated, a coverslip was applied and the preparation was examined under low- and high-power objectives.

The morphology of the hyphae, conidia and their attachment to conidiophores or hyphae was assessed for identification of the fungal species.

2) Slide Culture

Slide culture was performed to study the undisturbed morphological details of the fungi, particularly the relationship between conidial arrangement, conidiophores and hyphae.

Procedure

A sterile microscopic slide was placed on a bent glass rod inside a sterile Petri dish. A approximately 1 cm^2 block of potato dextrose agar was placed on the slide. The four sides of the agar block were injected with fungal growth derived from SDA and covered with a sterile coverslip. To keep the Petri dish damp, a tiny amount of sterile water was poured to the bottom. The preparation was evaluated on a regular basis while being incubated at 28°C. The coverslip was gently removed and put over a drop of lactophenol cotton blue for microscopic inspection after growth had occurred, typically after 5–7 days.

Urease Test Procedure

The urease test was performed for differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. A small amount of fungal culture was inoculated onto urea agar and incubated at 28°C for seven days, along with an appropriate control. Urea hydrolysis by *T. mentagrophytes* produced a colour change from yellow to magenta red, whereas *T. rubrum* remained negative.

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR DERMATOPHYTES

MICROBROTH DILUTION METHOD

Antifungal susceptibility testing was performed by the microbroth dilution method for determination of the minimum inhibitory concentration (MIC). The thesis specifies the use of the CLSI M38-A methodology. The antifungal agents tested were clotrimazole, fluconazole, griseofulvin and terbinafine.

INOCULUM PREPARATION

A fungal culture grown on PDA for approximately 48 hours to seven days was used for preparation of the inoculum. To suspend the conidia, the fungal colony was coated with sterile saline and gently scraped. Heavy particles were allowed to settle after the suspension was vortexed. After being moved to a sterile container, the top homogenous suspension was vortexed once again. As per the study protocol, the conidial concentration was adjusted to around $1–3 \times 10^3$ CFU/mL.

NUMBER OF CONCENTRATIONS TESTED

The antifungal concentration ranges used in the study were:

Antifungal agent	Concentration range
Clotrimazole	0.0313–16 µg/mL
Griseofulvin	0.0313–16 µg/mL
Fluconazole	0.0125–64 µg/mL
Terbinafine	0.0313–16 µg/mL

RPMI-1640 medium containing glutamine without bicarbonate was used as the test medium, with **MOPS (3-N-morpholino propanesulfonic acid)** used as the buffer.

ANTIFUNGAL STOCK SOLUTION

For water-soluble antifungal agents such as fluconazole, two-fold dilutions were prepared volumetrically in broth. Water-insoluble antifungal agents were prepared using dimethyl sulphoxide (DMSO) as the diluent according to the study protocol.

INOCULUM PREPARATION FOR SENSITIVITY TESTING

The fungal colony grown on PDA for approximately seven days was used for in-vitro susceptibility testing. A little amount of Tween 80 was added to sterile normal saline to suspend the colony. The homogenous top suspension was vortexed after the heavier particles were gently scraped and settled. Spectrophotometric measurements of turbidity were made at 530 nm and corrected to an optical density of 0.09–0.11, or 0.5 McFarland. After then, the stock inoculum was diluted 1:50 in RPMI-1640 medium.

INOCULATING RPMI-1640 MEDIUM

Each well was inoculated with 0.1 mL of the 2× inoculum suspension. This produced the required final drug and inoculum concentrations. Growth-control wells contained the corresponding diluted inoculum without antifungal drug.

TEST PROCEDURE

The susceptibility test was performed using sterile microtitre plates. Aliquots of 100 µL of antifungal drug dilutions were added to the designated wells, followed by 100 µL of inoculum. Fluconazole was tested over a concentration range of 0.125–64 µg/mL, while the other antifungal agents were tested over the specified concentration range of approximately 0.039–16 µg/mL. A growth-control well containing inoculum without antifungal drug was included.

INCUBATION

The microdilution trays were incubated at 28°C without agitation.

INTERPRETATION

The MIC for azole antifungal agents was determined as the lowest concentration producing approximately 80% inhibition of fungal growth, whereas for terbinafine, the MIC was determined by 100% inhibition of growth.

Ethical Considerations

The original thesis included institutional ethical clearance from the Institutional Ethics Committee of K.A.P. Viswanatham Government Medical College, Tiruchirappalli, and the study used informed consent for participation and collection of clinical specimens. The thesis consent form specifically states that patients consented to use of their clinical data and specimens for analysis and further investigation.

RESULTS

Table 1: Demographic and Epidemiological Characteristics of the Study Population

Characteristic	Category	Number	Percentage (%)
Sex (n=100)	Male	40	40%
	Female	60	60%
Occupation (n=100)	Homemaker	39	39%
	Student	31	31%
	Office employee	7	7%
	Outdoor labourer	23	23%
Diabetes mellitus (n=100)	Present	15	15%
	Absent	85	85%
Family history (n=100)	Present	40	40%
	Absent	60	60%

Among the 100 patients studied, 60 (60%) were females and 40 (40%) were males, giving a male-to-female ratio of 1:1.5. Homemakers constituted the largest occupational group (39%), followed by students (31%), outdoor labourers (23%) and office employees (7%). Diabetes mellitus was reported in 15% of patients, while a positive family history of dermatophytosis was present in 40%.

Table 2: Clinical and Mycological Characteristics of the Study Population

Parameter	Category	Number	Percentage (%)
Clinical specimen (n=100)	Skin scraping	94	94%
	Hair	4	4%
	Nail clipping	2	2%
KOH mount (n=100)	Positive	58	58%
	Negative	42	42%
Culture (n=100)	Positive	60	60%
	Negative	40	40%
Clinical type (n=100)	Tinea corporis	78	78%
	Tinea cruris	16	16%
	Tinea capitis	4	4%
	Tinea unguium	2	2%

Skin scrapings constituted the majority of clinical specimens (94%), followed by hair (4%) and nail specimens (2%). Direct KOH microscopy was positive in **58%** of cases, whereas fungal culture was positive in **60%**. Tinea corporis was the predominant clinical presentation (78%), followed by tinea cruris (16%), tinea capitis (4%) and tinea unguium (2%).

Table 3: Distribution of Dermatophyte Species Isolated from Culture-Positive Samples

Dermatophyte species	Number (n=60)	Percentage (%)
Trichophyton mentagrophytes	46	76.7%
Trichophyton rubrum	10	16.7%
Trichophyton tonsurans	2	3.3%
Trichophyton violaceum	1	1.7%
Microsporum canis	1	1.7%
Total	**60**	**100**

Among the 60 culture-positive samples, *T. mentagrophytes* was the predominant isolate, accounting for 46 (76.7%) isolates, followed by *T. rubrum* in 10 (16.7%). *T. tonsurans*, *T. violaceum* and *M. canis* accounted for 3.3%, 1.7% and 1.7%, respectively.

DISCUSSION

The present clinico-mycological study evaluated 100 patients with clinically suspected dermatophytosis in a tertiary care hospital. The distribution of dermatophyte species, clinical presentation, direct KOH microscopy, fungal culture, and demographic and epidemiological features were all evaluated. The results show that among culture-positive cases, there is a significant preponderance of Trichophyton mentagrophytes, tinea corporis, and females. The male-to-female ratio in the current study was 1:1.5, with 60% of the participants being female and 40% being male (Table 1). In contrast to a number of Indian research where males were more commonly impacted, this majority is female.14,15 This variability may be attributed to differences in geographic location, occupational exposure, healthcare-seeking behavior, and the clinical spectrum of patients attending different facilities. Homemakers made up the biggest group (39%), followed by students (31%), outdoor laborers (23%), and office workers (7%), according to the occupational distribution (Table 1). Although work exposure was not independently assessed in the current study, frequent exposure to water, perspiration, occlusive clothing, and home environmental conditions may contribute to dermatophyte transmission among homemakers. 40% of patients had a favorable family history (Table 1). The possible significance of home transmission in dermatophytosis is supported by this data. Transmission between family members may be facilitated by sharing towels, clothing, soaps, and other personal items. Shared home items and bathing habits were noted by Bhatia et al. as potential contributing variables to the family incidence of dermatophytosis.16 Similarly, research from India on recurring and chronic dermatophytosis has shown that a significant percentage of patients have family relatives who are afflicted.17 Fifteen percent of the participants in this research had diabetes mellitus (Table 1). The current study was not intended to identify diabetes as a separate risk factor, even though it may coexist with dermatophytosis.

The bulk of specimens (94%) were skin scrapings, with hair (4%) and nail clippings (2%) coming in second and third, respectively (Table 2). The high prevalence of cutaneous dermatophytosis is consistent with the preponderance of skin specimens. Fungal culture was positive in 60% of instances, whereas direct KOH microscopy was positive in 58% of cases (Table 2). Poluri et al. showed similar KOH and culture positivity, with KOH positivity of around 58% and culture positive of roughly 56%.15 The supportive significance of culture in individuals with clinically suspected dermatophytosis is

highlighted by the slightly greater culture positive in this research. Culture enables recovery and species identification, whilst KOH testing offers quick detection of fungal elements.15,18

With 78% of patients, tinea corporis was the most common clinical manifestation in this investigation. It was followed by tinea cruris (16%), tinea capitis (4%), and tinea unguium (2%) (Table 2). The prevalence of tinea corporis aligns with other research conducted in India.15,19 Climate, referral patterns, length of illness, and prior antifungal therapy may all have an impact on variations in the prevalence of certain clinical categories between studies.

T. mentagrophytes was the most common isolate among the 60 culture-positive samples, accounting for 46 cases (76.7%), followed by T. rubrum in 10 cases (16.7%) (Table 3). Microsporum canis, T. tonsurans, and T. violaceum were rather rare. The prevalence of T. mentagrophytes is consistent with recent Indian research showing this species' growing role in dermatophytosis.14,20 Nenoff et al. reported a significant epidemiological change in favor of T. mentagrophytes in India, and additional research has shown that this species is predominant in some regions.20,21 Nonetheless, T. rubrum is still a significant dermatophyte, and multicentric research has shown that the relative distribution of T. rubrum and T. mentagrophytes varies geographically.22 Overall, the current study shows that tinea corporis was the clinical manifestation of dermatophytosis, which primarily affected females. In 60% of clinically suspected cases, dermatophyte infection was confirmed by culture, with T. mentagrophytes appearing as the most common isolate. These results highlight how crucial it is to combine clinical evaluation with KOH microscopy and culture for precise dermatophytosis diagnosis and epidemiological surveillance.

CONCLUSION

The present clinico-mycological study demonstrates that dermatophytosis remains a common superficial fungal infection in the study population. With a male-to-female ratio of 1:1.5, women made up the majority of the 100 patients assessed. Forty percent of patients reported having a favorable family background, while homemakers made up the biggest occupational category. The most common clinical manifestation was tinea corporis, which was followed by tinea cruris, tinea capitis, and tinea unguium. The complimentary significance of direct microscopy and fungal culture is highlighted by the fact that direct KOH microscopy revealed fungal elements in 58% of instances whereas culture was positive in 60%. With 76.7% of culture-positive cases, Trichophyton mentagrophytes was the most common dermatophyte identified, followed by T. rubrum (16.7%). According to the results of the antifungal susceptibility tests, fluconazole had the highest MIC values and terbinafine had the lowest MIC range. The significance of species identification and routine antifungal susceptibility surveillance is highlighted by the observed susceptibility pattern. Treatment failure and the development of resistance may be decreased by using antifungal medications sensibly and avoiding inappropriate or extended therapy.

LIMITATIONS OF THE STUDY

The study was conducted in a single tertiary care hospital with a relatively small sample size of 100 patients, which may limit the generalizability of the findings. Larger multicentric studies with molecular identification, extended susceptibility testing, and long-term clinical follow-up are advised because the study population was hospital-based and might not accurately reflect the prevalence of dermatophytosis in the community; antifungal susceptibility testing was limited to the four medications examined in the study; the study evaluated in-vitro MIC values and did not correlate them with clinical treatment response; and long-term follow-up was not carried out to assess recurrence, treatment failure, or development of resistance.

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