ORIGINAL ARTICLE

Clinicomycological Profile and *In Vitro* Antifungal Activity of Terbinafine and Griseofulvin against Clinical Isolates of Dermatophytes in a Tertiary Care Hospital

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Abstract

Background: Dermatophytes, primarily *Epidermophyton* spp., *Trichophyton* spp., and *Microsporum* spp., are responsible for superficial cutaneous mycoses, estimated to affect 20–25% of the people worldwide. The rise of antifungal resistance, especially to terbinafine, has made treating dermatophytosis increasingly difficult. This study aims to assess the clinical and mycological characteristics of dermatophytosis cases and evaluate the *in vitro* susceptibility of dermatophyte isolates to terbinafine and griseofulvin.

Materials and methods: A total of 118 samples were studied from patients with clinical suspicion of dermatophytosis. The samples were processed for KOH mount and fungal culture for further speciation. Susceptibility to terbinafine and griseofulvin was assessed using the microbroth dilution technique, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).

Results: Tinea corporis (57.6%) appeared as the leading symptomatology in our study, followed by tinea cruris (10.2%). KOH positivity was higher (70.3%) compared to positivity by culture (16.9%). *Trichophyton mentagrophytes* was the predominant species (85%) isolated, followed by *Trichophyton violaceum* (10%) and *Microsporum gypseum* (5%). Terbinafine resistance was observed in over 60% of *T. mentagrophytes* isolates, with moderate resistance detected in *T. violaceum*. Griseofulvin showed moderate resistance in *T. mentagrophytes* and higher resistance in *T. violaceum*.

Conclusion: This study highlights the increased resistance of *T. mentagrophytes* to terbinafine and *T. violaceum* to griseofulvin, stressing the critical role of routine susceptibility profiling. The findings highlight the growing challenge of antifungal resistance in dermatophytes and the importance of optimizing diagnostic and treatment strategies to improve patient outcomes.

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Introduction

Dermatophytes, filamentous fungi categorized under the genera *Epidermophyton* spp., *Trichophyton* spp., and *Microsporum* spp., are the primary causative agents of superficial cutaneous mycoses, impacting approximately 20–25% of the global population.[1] These fungi exhibit a unique ability to metabolize keratin, the predominant protein in skin, hair, and nails, which facilitates their role in causing dermatophytosis or ringworm infections. Dermatophytosis manifests as a host response to fungal enzymes released during keratin degradation, leading to inflammation and characteristic clinical presentations.

Globally, dermatophytosis remains a prevalent health concern, particularly in regions with high humidity, dense populations, and substandard hygiene.[2] Additional risk factors include close contact with animals harboring zoonotic dermatophyte infections. Despite the availability of effective antifungal therapies, treatment failures are increasingly reported due to poor adherence to prescribed regimens, improper use of topical steroids, and compromised host immunity. Among these challenges, drug resistance in dermatophytes poses a significant and growing public health threat.

The emergence of resistance to terbinafine, one of the most widely used antifungal agents, is particularly alarming.[3] Specific mutations in the squalene epoxidase (*SQLE* or *SE*) gene, which encodes the essential enzyme for ergosterol synthesis, are key to terbinafine resistance in dermatophytes. These genetic changes reduce the efficacy of terbinafine, thereby complicating treatment outcomes. Moreover, the lack of routine implementation of antifungal susceptibility testing (AFST) in clinical practice contributes to delays in detecting resistant strains and initiating suitable treatment strategies. The lack of antifungal policies in many hospitals worldwide has contributed to the increasing prevalence of antifungal-resistant strains of dermatophytes and inadequate infection control measures. For laboratory-based identification of antifungal resistance, the CLSI document provides guidelines for the broth microdilution method applicable to filamentous fungi. This method calculates the minimum inhibitory concentration (MIC), with higher MIC values indicating relative resistance to a particular drug.[4]

In light of these challenges, this study is designed to investigate both the clinical characteristics and mycological patterns of dermatophytosis in patients treated at a tertiary-care hospital and to evaluate the *in vitro* susceptibility of dermatophyte isolates to terbinafine and griseofulvin. This research addresses the significant gap in regional data on antifungal resistance and highlights the importance of adopting robust diagnostic and sensitivity-testing protocols to guide evidence-based management of dermatophytosis.

Materials and Methods

This hospital-based observational study was undertaken in the Department of Microbiology and Dermatology for a period of 6 months from February 2024 to July 2024, during which skin, hair, and nail samples were collected from clinically suspected cases of dermatophytosis of varying ages and both sexes. However, patients who were initiated on antifungal treatment were excluded from the study.

Institutional Ethics Committee Clearance

Ethical clearance for the study was obtained from the Institutional Ethics Committee under

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the reference number AV/IHEC/2024/006 dated January 3, 2024, prior to the initiation of research.

A total of 118 samples were studied, and patients were enrolled using a nonprobability sampling-convenient sampling technique. Each specimen was divided into two parts: one for a KOH mount and fungal culture. Varying concentrations of potassium hydroxide (KOH), 10%, 20%, and 40% were used for skin, hair, and nails, respectively and samples were subjected to microscopic evaluation to detect fungal elements (Fig. 1). Another part of the specimen was cultured on sabouraud dextrose agar (SDA) supplemented with cycloheximide and incubated at both 37 °C and 25 °C. The SDA slant was checked weekly for up to 4 weeks before being labelled negative for fungal culture (Fig. 2). Species identification was performed macroscopically by observing colony morphology and microscopically using a lactophenol cotton blue mount (LPCB) (Fig. 3).[5]

Antifungal drug susceptibility profile was determined for terbinafine and griseofulvin by microbroth dilution assay according to the CLSI guidelines-document M38-A of filamentous fungi.[6] The stock preparation of the antifungal agents was formulated for susceptibility testing. RPMI-1640 medium, developed by Rose Parker Memorial Institute, was employed as the culture medium for fungal growth. The dermatophyte isolate was subcultured on potato dextrose agar (PDA) to promote the development of conidia. For the inoculation, 100 µL of the conidial suspension in RPMI-1640 was added to each well of a sterile, flat-bottomed 96-well microtiter plate. The wells were then filled with 100 µL of the diluted antifungal drugs. There were control wells with a sterility control that included just RPMI-1640 to validate the lack of contamination and a growth control that contained only the conidial suspension to confirm normal fungal growth. To evaluate the antifungal susceptibility against the investigated drugs, fungal growth was monitored after 48 and 72 hours of incubation at 35 °C in the microtiter plates.

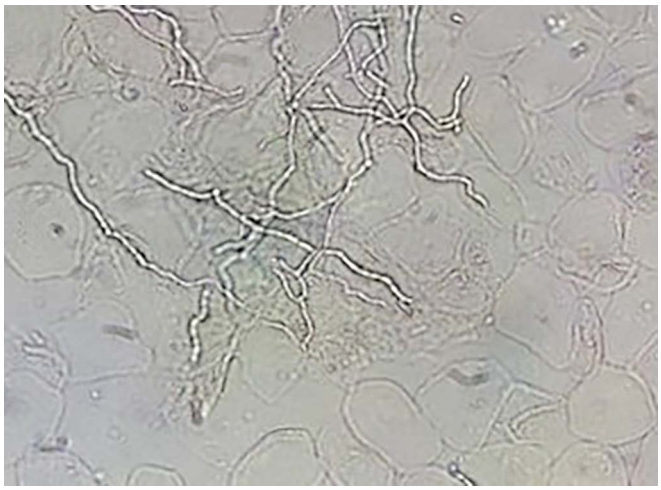
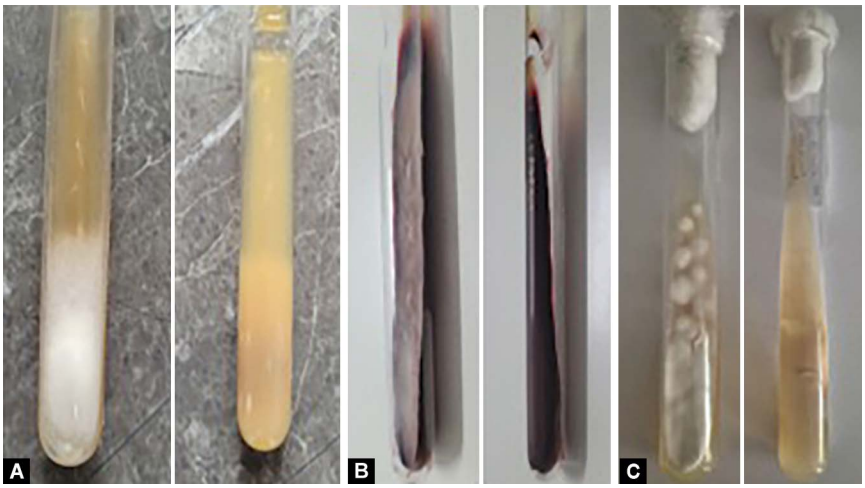


Fig. 1: KOH mount of skin scraping showing fungal filaments (40× magnification)



Figs 2A to C: SDA showing white powdery-cottony growth of *Trichophyton mentagrophytes* (A), wrinkled, purple pigmented colonies of *Trichophyton violaceum* (B), and white powdery colonies of *Microsporum gypseum* (C)

The isolates were further observed visually for 50% (MIC_{50}) and 90% (MIC_{90}) inhibition in growth, and the MIC calculated was established as the concentration at which the growth of dermatophytes was reduced by 80% for antifungal agents when compared to the control strains, *Trichophyton rubrum* PTCC 5143 and *T. mentagrophytes* PTCC 5054. Each test was conducted in triplicate.

Statistical Analysis

Frequency and percentage were used to summarize categorical variables. The mean ± standard deviation was used to describe continuous variables. Data analysis was conducted using SPSS version 28.

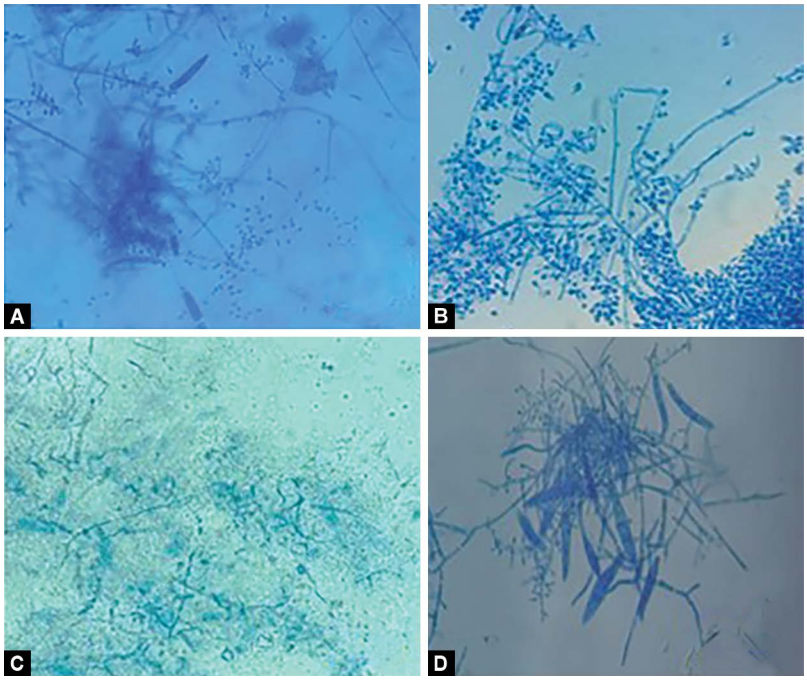
Results

Table 1 illustrates the distribution of individuals by gender across different age groups. The largest group, aged 21–30 years, comprised 25 people, with a nearly equal split between males and females. The 41–50 years group followed with 23 individuals. Age groups 11–20 and 31–40 years had 22 and 20 people, respectively, also showing balanced gender proportions. The 61–70 years group and the 51–60 years had 11 individuals each. The youngest (0–10 years) included only 5, and the oldest (71+ years) recorded just one male. Males generally outnumbered females, except in the 11–20, 51–60, and 61–70 years groups. This shows a very slight male (50.9%) predominance over females (49.1%).

The most commonly encountered clinical presentation was tinea corporis, accounting for 57.6% of cases. This was followed by a combined presentation of tinea corporis and tinea cruris in 13.6% and isolated tinea cruris in 10.2% of cases. Other presentations, such as tinea capitis (7.6%), tinea pedis (4.2%), tinea faciei (3.4%), tinea incognita (1.7%), and tinea barbae (1.7%), were less frequent. These results highlight tinea corporis as the predominant clinical manifestation among the study population (Table 2).

KOH positivity was significantly higher, with 83 samples (70.3%) testing positive, while culture positivity was much lower, with only 20 samples (16.9%) yielding positive results. All those 20 samples which was culture positive were also KOH positive. Out of 83 KOH-positive samples, 63 were culture negative. From the total 118 samples, both KOH and culture-negative were 35 samples (Table 3).

Among 20 culture positive samples *T. mentagrophytes* was the most prevalent species, accounting for 17 isolates (85%). *T. violaceum* was identified in two isolates (10%), and *M. gypseum* was the least common, with only one isolate (5%) (Table 4).



Figs 3A to D: LPCB mount showing grape-like clusters of microconidia, cigar-shaped macroconidia of *Trichophyton mentagrophyte* (A and B), highly distorted hyphae of *Trichophyton violaceum* (C), and numerous spindle-shaped macroconidia of *Microsporum gypseum* (D)

Table 2: Clinical presentation of dermatophytes infection (*n* = 118)

Clinical presentation	*Number*	*Percentage (%)*
Tinea corporis	68	57.6
Tinea cruris	12	10.2
Tinea corporis and cruris	16	13.6
Tinea pedis	5	4.2
Tinea incognita	2	1.7
Tinea capitis	9	7.6
Tinea faciei	4	3.4
Tinea barbae	2	1.7

Table 3: Comparison of KOH and culture results of dermatophytosis

KOH	*Culture*	
	Positive	*Negative*
Positive (83)	20	63
Negative (35)	–	35
Total (118)	20	98

Table 1: Age and sex-wise distribution of cases (*n* = 118)

Age group	*Male*	*Female*	*Total*
0–10 years	3	2	5 (4.2%)
11–20 years	10	12	22 (18.6%)
21–30 years	13	12	25 (21.1%)
31–40 years	10	10	20 (16.9%)
41–50 years	13	10	23 (19.4%)
51–60 years	5	6	11 (9.3%)
61–70 years	5	6	11 (9.3%)
>71 years	1	0	1 (0.8%)
Total	60 (50.9%)	58 (49.1%)	118

Table 4: Isolation rate of dermatophyte species (*n* = 20)

Species	*Number*	*Percentage (%)*
Trichophyton mentagrophytes	17	85
Trichophyton violaceum	2	10
Microsporum gypseum	1	5
Total	20	100

The minimum inhibitory concentration testing for terbinafine showed that *T. mentagrophytes* had MIC_{50} and MIC_{90} values of 4 µg/mL and 8 µg/mL, respectively. *T. violaceum* demonstrated lower MIC_{50} (0.6 µg/mL) and significantly higher MIC_{90} (32 µg/mL) values, indicating variable susceptibility. However, in *M. gypseum*, both MIC_{50} and MIC_{90} were recorded at 8 µg/mL. The findings revealed varied susceptibility patterns to terbinafine among different dermatophyte species. More than 60% of *T. mentagrophytes* (*n* = 17) isolates showed resistance to terbinafine. *T. violaceum* (*n* = 2) and *M. gypseum* (*n* = 1) shows 50% and 100% resistance, respectively (Table 3).

Griseofulvin susceptibility testing showed that *T. mentagrophytes* had MIC_{50} and MIC_{90} values of 4 µg/mL and 16 µg/mL, respectively, indicating moderate resistance. *T. violaceum* demonstrated a higher MIC90 (64 µg/mL) compared to its MIC_{50} (2 µg/mL), suggesting greater resistance at higher concentrations. For *M. gypseum*, both MIC_{50} and MIC_{90} values were 0.5 µg/mL, reflecting good susceptibility to griseofulvin. These findings highlighted significant variations in griseofulvin susceptibility across dermatophyte species (Table 5).

Discussion

Fungal infections of the skin, hair, and nails that result in cutaneous mycoses are caused by dermatophytes. These infections are more prevalent in developing countries and are commonly reported in various regions of India. The warm, humid climate of tropical and subtropical areas is believed to favor their growth and spread. A wide range of antifungal agents has been used for its treatment, but susceptibility varies among different dermatophyte strains. Failure of treatment and emergence of resistance are frequently associated with decreased drug absorption, changes at the phenotypic or genetic level, and increased activity of drug

Table 5: *In vitro* susceptibility testing of terbinafine and griseofulvin antifungal agents against dermatophytes species by microbroth dilution assay ($n = 20$)

Species	*Antifungal drug concentration (µg/mL)*		
		Terbinafine	*Griseofulvin*
T. mentagrophytes ($n = 17$)	Range	0.03–64	0.03–64
	MIC_{50}	4	4
	MIC_{90}	8	16
T. violaceum ($n = 2$)	Range	0.03–64	0.03–64
	MIC_{50}	0.6	2
	MIC_{90}	32	64
M. gypseum ($n = 1$)	Range	0.03–64	0.03–64
	MIC_{50}	8	0.5
	MIC_{90}	8	0.5

efflux systems.[4,7,8] The present study draws attention to the rising concern of antifungal resistance in dermatophytes, with particular interest taken in susceptibility to terbinafine and griseofulvin.

In the present investigation, dermatophytosis was predominantly observed in individuals aged 21–30 years, with a higher incidence among males. These findings are consistent with those documented by Das et al.[8] The observed distribution pattern may be attributed to factors such as increased outdoor exposure and physical activity commonly associated with this age group.[9] Furthermore, tinea corporis emerged as the most frequently encountered clinical manifestation, aligning with the observations reported by Poluri et al.[10]

In the current study, we noted a discrepancy between KOH positivity (70.3%) and culture positivity (16.9%). Such trends have also been reported in another study,[11] where they have substantiated that the elements may fail to grow because of previous antifungal exposure or due to nonviability. The discrepancy between KOH and culture positivity shows the importance of optimizing diagnostic methodologies for accurate identification. In the present study, *T. mentagrophytes* emerged as the most prevalent dermatophyte species, aligning with observations from a study in Western India, where it comprised 47.2% of the total isolates.[12] One of the key findings of this study is the moderate resistance of *T. mentagrophytes* to terbinafine, with MIC_{50} and MIC_{90} values of 4 µg/mL and 8 µg/mL, respectively. This similar pattern of terbinafine resistance in dermatophytes, particularly in *T. mentagrophytes*, has been reported in several other studies.[13–15] The rising occurrence of terbinafine resistance is often associated with mutations in the squalene epoxidase gene, leading to reduced therapeutic effectiveness. The research conducted by Turner and McLellan[16] highlighted that the growing resistance to terbinafine is a major concern in managing dermatophyte infections.

Sensitivity pattern of griseofulvin demonstrated a higher resistance among *T. violaceum* with MIC_{90} of 64 µg/mL. The outcome of the current study matches the study by Van et al.,[17] who reported similar resistance profiles for griseofulvin across different *Trichophyton* species. On the contrary, the study conducted by Sowmya et al.[7] showed 100% susceptibility of the isolates to terbinafine, griseofulvin, and other antifungal agents, and another study by Amin et at.[18] documented lower MIC values for dermatophytes isolated from chronic and recurrent dermatophytosis.

The emergence of antifungal resistance calls for the routine testing of antifungal susceptibility, especially in recurrent dermatophytosis.[19] The performance of broth microdilution testing has been shown to yield reliable and reproducible results, as observed in previous studies,[20] when compared to other methods for AFST.

Conclusion

The findings from this study enhance current knowledge on dermatophyte characterization and resistance trends, which are critical for guiding effective antifungal therapy in dermatophytosis. *T. mentagrophytes* is the most prevalent dermatophyte observed with significant resistance to terbinafine, underscoring a growing concern regarding antifungal resistance in dermatophyte infections. Although griseofulvin remains effective against certain species, such as *M. gypseum*, its efficacy is compromised in others, notably *T. mentagrophytes*, where moderate resistance was detected.

The findings highlight the critical role of performing routine AFST in clinical practice, both to guide evidence-based therapeutic decisions and to ensure the selection of the most effective antifungal agents, thereby improving treatment outcomes. Given the increasing prevalence of dermatophytosis and the rise in antifungal resistance, AFST can aid in tailoring treatment strategies, minimizing treatment failures, and improving patient outcomes. The study also highlights the need for region-specific strategies to combat dermatophytosis, as the prevalence and resistance patterns may vary across different geographical locations. Enhanced diagnostic protocols, regular surveillance of antifungal resistance, and the adoption of more stringent antifungal stewardship practices in clinical settings are crucial to combat the threat of emerging antifungal resistance.

Author's Contributions

- Jaishma Rajni J: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing—Original draft preparation.
- Pramodhini S: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing—Original draft preparation, Supervision, Visualization, Reviewing, and Editing.
- Sheela Kuruvila: Conceptualization, Methodology, Investigation, Resources, Supervision, Visualization, Reviewing, and Editing.
- Latha R: Conceptualization, Methodology, Formal analysis, Supervision, Visualization, Reviewing, and Editing.
- Kavitha K: Conceptualization, Methodology, Investigation, Resources, Supervision, Visualization, Reviewing, and Editing.
- Sherief Shebeena: Investigation, Resources, Supervision, Visualization, Reviewing, and Editing.

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