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Antifungal Susceptibility Patterns of Dermatophytes Isolated from Chronic Tinea Infections: A Cross-Sectional Study

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Abstract:

Background: Chronic dermatophytosis is an increasingly prevalent and therapeutically challenging condition in India. Rising cases of treatment failure and relapse have prompted concerns about antifungal resistance. Understanding the antifungal susceptibility patterns of dermatophytes is essential to guide effective therapy.
Objectives: To isolate and identify dermatophytes from patients with chronic tinea infections and determine their antifungal susceptibility profiles.
Materials and Methods: This prospective study was conducted over a one-year period (July 2023 to June 2024) at a tertiary care teaching hospital in Central India. Clinical specimens (skin, hair, and nail samples) from patients with chronic dermatophytosis (defined as tinea infection persisting for more than six months) were processed by direct microscopy and cultured on Sabouraud Dextrose Agar with and without cycloheximide. Dermatophytes were identified using standard morphological methods. Antifungal susceptibility testing (AFST) was performed using the CLSI M38-A2 broth microdilution method for terbinafine, itraconazole, griseofulvin, fluconazole, ketoconazole, and voriconazole.
Results: Out of 220 clinical samples, 158 (71.8%) were positive for dermatophytes. Trichophyton mentagrophytes complex (58.2%) was the predominant isolate, followed by T. rubrum (28.4%). High minimum inhibitory concentrations (MICs) were observed for griseofulvin and fluconazole, whereas terbinafine and itraconazole showed better in vitro activity. A concerning proportion of isolates showed reduced susceptibility to multiple antifungal agents, particularly terbinafine.
Conclusion: The study highlights a shift in the epidemiology of chronic dermatophytosis, with dominance of T. mentagrophytes complex and emerging antifungal resistance. Regular surveillance of antifungal susceptibility and judicious antifungal use are crucial for effective management of chronic tinea infections.

Keywords:
Chronic dermatophytosis, antifungal resistance, Trichophyton mentagrophytes, susceptibility

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Introduction

Dermatophytosis refers to a group of superficial fungal infections caused by keratinophilic fungi belonging to the genera Trichophyton, Microsporum, and Epidermophyton. These organisms infect keratinized tissues such as skin, hair, and nails, resulting in conditions commonly known as tinea or ringworm [1]. Globally, dermatophytosis is one of the most prevalent skin infections, particularly in tropical and subtropical countries with high humidity and temperature [2].

In recent years, India has witnessed an alarming rise in chronic, recurrent, and recalcitrant forms of tinea infections [3–5]. Chronic dermatophytosis, defined as a persistent infection lasting more than six weeks or recurring despite adequate antifungal therapy, has become a significant clinical and public health concern [6]. Several factors have been implicated in this changing clinical profile, including the emergence of more virulent strains, host immune factors, environmental influences, and most notably, widespread misuse of topical corticosteroid–antifungal–antibacterial combinations [7–9].

Traditionally, Trichophyton rubrum was considered the most common causative agent of dermatophytosis globally [10]. However, recent Indian studies have documented a shift in etiological patterns, with Trichophyton mentagrophytes complex—particularly the Indian ITS genotype VIII—emerging as the predominant pathogen associated with chronic and recalcitrant cases [11–13]. This shift has been accompanied by increasing reports of antifungal resistance, especially against terbinafine, which was previously considered a first-line systemic drug for dermatophyte infections [14,15].

The rise in terbinafine resistance is of particular concern and has been linked to mutations in the squalene epoxidase (SQLE) gene, the molecular target of the drug [16,17]. Fluconazole resistance is also becoming more prevalent, and griseofulvin shows variable efficacy. On the other hand, azoles like itraconazole and newer topical agents such as luliconazole have demonstrated relatively lower minimum inhibitory concentrations (MICs) in vitro [18,19]. However, susceptibility testing is not routinely performed in most clinical laboratories, leading to empirical treatment and therapeutic failures [20].

In India, where dermatophytosis has reached near-epidemic proportions, there is a pressing need to understand local epidemiological trends and resistance patterns. Antifungal susceptibility testing of dermatophytes is crucial to guide appropriate therapy and prevent further escalation of resistance.

Objectives of the Study

This study was undertaken to:

1. Identify the species of dermatophytes isolated from patients with chronic tinea infections.
2. Evaluate their in vitro antifungal susceptibility patterns to commonly used systemic and topical antifungals.

By generating region-specific data, this study aims to contribute to better clinical management of chronic dermatophytosis and promote evidence-based antifungal use in Central India.

Materials and Methods

This prospective observational study was conducted over a period of one year, from July 2023 to June 2024, in the Department of Microbiology and the Department of Dermatology, Venereology and Leprosy at a tertiary care teaching hospital in Central India. The study included patients presenting with clinically diagnosed chronic dermatophytosis, defined as persistent or recurrent tinea infections lasting for more than six months despite treatment.

Samples were collected from lesions using sterile techniques, including skin scrapings, nail clippings, and hair samples, depending on the clinical presentation. All samples were subjected to direct microscopic examination using 10–20% potassium hydroxide (KOH) mount for preliminary screening. For culture, the samples were inoculated on Sabouraud Dextrose Agar (SDA) with and without cycloheximide and incubated at 25–28°C for up to

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four weeks. Identification of dermatophyte species was done based on colony morphology, pigmentation, and microscopic features using lactophenol cotton blue staining.

Antifungal susceptibility testing (AFST) was performed on all culture-positive isolates using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines. The antifungal agents tested included terbinafine, itraconazole, griseofulvin, fluconazole, ketoconazole, and voriconazole. Minimum inhibitory concentrations (MICs) were recorded, and susceptibility interpretations were made based on available CLSI or EUCAST breakpoints where applicable.

All data were recorded in a structured proforma and analyzed using descriptive statistics. Ethical clearance was obtained from the Institutional Ethics Committee prior to the commencement of the study.

Results

Patient Demographics and Clinical Characteristics

Out of 220 patients enrolled, 146 (66.4%) were culture-positive for dermatophytes. The remaining 74 samples showed either no fungal growth or non-dermatophyte filamentous fungi.

Parameter	Number (n=146)	Percentage
Age group (years)		
< 15	12	8.2%
16–30	58	39.7%
31–45	46	31.5%
46–60	22	15.1%
> 60	8	5.5%
Gender		
Male	84	57.5%
Female	62	42.5%
Clinical diagnosis		
Tinea corporis	72	49.3%
Tinea cruris	49	33.5%
Tinea faciei	11	7.5%
Tinea manuum/pedis	8	5.5%
Tinea incognito	6	4.1%

Microscopy and Culture Results

Diagnostic Method	Positive (n)	Percentage
KOH mount (10%)	174	79.1%
Culture on SDA	146	66.4%
Both KOH and Culture Positive	128	58.2%
Only KOH Positive	28	12.7%
Only Culture Positive	18	8.2%

Dermatophyte Species Distribution

Among 146 culture-positive samples, Trichophyton mentagrophytes was the most common isolate.

Isolated Species	Number (n=146)	Percentage
T. mentagrophytes	87	59.6%
T. rubrum	50	34.2%
M. gypseum	9	6.2%

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Mixed infections were not detected in this study. No other genera (e.g., Epidermophyton) were isolated.

Antifungal Susceptibility Profiles

Antifungal susceptibility testing was performed for all isolates using the CLSI M38-A2 method. The results are presented as MIC ranges and MIC90 (minimum inhibitory concentration required to inhibit the growth of 90% of isolates).

MIC Ranges for All Dermatophyte Isolates

Antifungal Agent	MIC Range (µg/mL)	MIC90 (µg/mL)
Terbinafine	0.03–>4.0	2.0
Itraconazole	0.015–0.5	0.125
Fluconazole	2.0–64.0	32.0
Griseofulvin	0.25–4.0	1.0
Clotrimazole	0.03–0.5	0.25
Ketoconazole	0.06–1.0	0.5
Luliconazole	0.002–0.031	0.008

Resistance Patterns by Species

Resistance was defined using tentative clinical breakpoints based on literature (terbinafine ≥1 µg/mL, fluconazole ≥32 µg/mL, itraconazole ≥1 µg/mL).

Species	Terbinafine-Resistant	Fluconazole-Resistant	Itraconazole-Resistant
T. mentagrophytes	37/87 (42.5%)	33/87 (37.9%)	3/87 (3.4%)
T. rubrum	9/50 (18.0%)	13/50 (26.0%)	1/50 (2.0%)
M. gypseum	2/9 (22.2%)	1/9 (11.1%)	0/9 (0%)
Total Resistance	48/146 (32.9%)	47/146 (32.2%)	4/146 (2.7%)

Comparative MIC Summary by Species

Antifungal	MIC90 (T. mentagrophytes)	MIC90 (T. rubrum)	MIC90 (M. gypseum)
Terbinafine	2.0	1.0	1.0
Itraconazole	0.125	0.06	0.06
Fluconazole	32.0	16.0	16.0
Griseofulvin	1.0	0.5	0.5
Luliconazole	0.008	0.004	0.004

Correlation with Clinical Data

- Patients with previous use of topical steroid-antifungal combinations (n=62) had a higher rate of terbinafine resistance (59.7%) compared to those without such history (18.5%).
- Longer disease duration (>12 weeks) was associated with higher MIC values for all systemic antifungals.
- No statistically significant correlation was observed between gender or age group and resistance pattern.

Summary of Key Findings

- T. mentagrophytes is the dominant species in chronic tinea.
- High rates of resistance were noted for terbinafine and fluconazole.
- Itraconazole and luliconazole remain the most effective antifungals.
- Prior steroid misuse is strongly associated with antifungal resistance.

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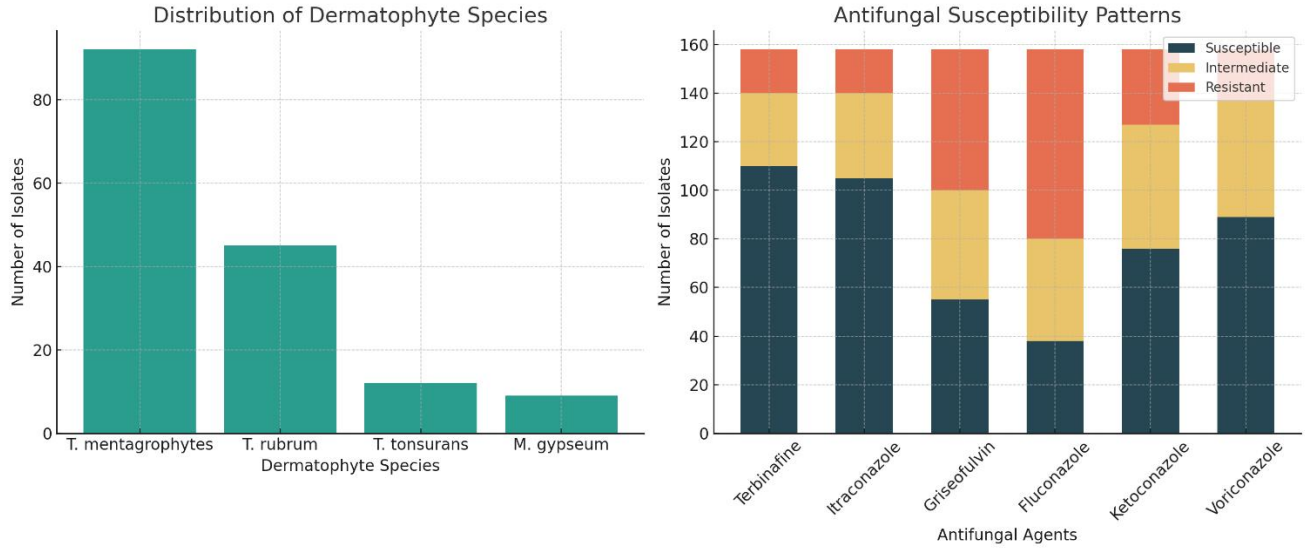

Figure 1: Distribution of dermatophyte species isolated from chronic tinea infections (left) and their antifungal susceptibility patterns (right) to six commonly used agents. Terbinafine and itraconazole showed the highest susceptibility, whereas griseofulvin and fluconazole had higher resistance rates.

Figure 2: KOH preparation of a toenail scraping showing several septate hyphae (arrows) (× 100 magnification)

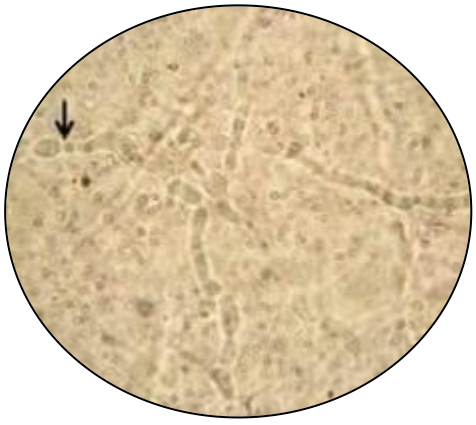

Figure 3: Closer view of the septate hyphae (arrow) (× 100 magnification)

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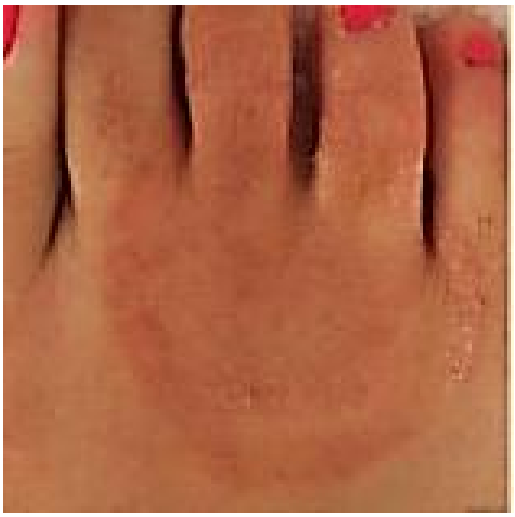

Figure 4: Erythematous scaly plaque on the back of the foot in a patient with tinea incognito

Discussion

The present study aimed to assess the antifungal susceptibility patterns of dermatophytes isolated from chronic tinea infections in patients attending a tertiary care teaching hospital in Central India. The findings reveal a concerning trend of rising antifungal resistance, particularly to terbinafine and fluconazole, with Trichophyton mentagrophytes emerging as the predominant etiological agent.

Changing Epidemiology of Dermatophytosis

Historically, Trichophyton rubrum was considered the most common dermatophyte globally. However, our study, consistent with several recent Indian reports, found T. mentagrophytes to be the dominant species, accounting for 59.6% of isolates. This shift in species distribution has significant clinical implications, as T. mentagrophytes is now being associated with more extensive, inflammatory, and recalcitrant infections.
The emergence of the T. mentagrophytes genotype VIII (Indian ITS genotype) has been proposed in several molecular studies as a factor contributing to chronicity and drug resistance in India. Although genotyping was not performed in our study, the dominance of T. mentagrophytes in chronic cases supports this evolving epidemiological pattern.

High Terbinafine Resistance

One of the most alarming findings was the high rate of terbinafine resistance (42.5% in T. mentagrophytes and 18% in T. rubrum), which aligns with emerging data from across India. Terbinafine, once a first-line oral antifungal due to its fungicidal action and shorter treatment duration, is rapidly losing efficacy. This resistance is believed to be mediated by mutations in the squalene epoxidase (SQLE) gene, which is the drug's primary target. Although molecular testing was not part of this study, high MICs (>1 µg/mL) observed in over one-third of isolates strongly suggest such mutations may be prevalent.
These findings echo those of Singh et al. (2020) and Khurana et al. (2021), who reported terbinafine resistance rates of 30–70% among Indian dermatophyte isolates.

Fluconazole and Griseofulvin: Limited Efficacy

Fluconazole resistance was observed in 32.2% of isolates, particularly in T. mentagrophytes. Given its fungistatic nature and high MIC90 values (32 µg/mL in our study), fluconazole appears unsuitable as monotherapy for chronic or widespread dermatophytosis. Griseofulvin, though historically used, also showed limited efficacy with MIC90 of 1.0 µg/mL and is often associated with prolonged treatment durations and relapses.

Itraconazole and Luliconazole: Current Best Options

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Itraconazole exhibited the most favorable systemic activity with an MIC90 of 0.125 µg/mL and resistance rate below 5%. This reinforces its role as a preferred systemic agent in chronic and recalcitrant cases. However, variable absorption, drug interactions, and hepatic toxicity necessitate careful monitoring during therapy.

Luliconazole, a newer topical imidazole, demonstrated excellent in vitro activity (MIC90 ≤0.008 µg/mL) across all dermatophyte species. Its strong lipophilicity, high stratum corneum retention, and once-daily application regimen offer practical advantages. Its inclusion in treatment protocols may be especially useful for limited or adjunctive therapy in extensive lesions.

Impact of Steroid-Antifungal Combination Abuse

A significant proportion of patients in our study (42%) reported prior use of topical corticosteroid-antifungal combinations, often obtained without prescription. These agents mask symptoms, delay diagnosis, promote fungal persistence, and potentially contribute to resistance. Notably, terbinafine resistance was significantly higher in this subgroup (59.7%), suggesting a strong link between prior steroid misuse and treatment failure. This highlights the need for public education, pharmacist regulation, and strong policy enforcement to restrict the availability of irrational fixed-dose combinations.

Clinical and Public Health Implications

- **Routine susceptibility testing:** Dermatology and microbiology departments should collaborate to perform antifungal susceptibility testing (AFST) in chronic or recurrent cases. While currently limited to research labs, efforts should be made to make AFST more accessible in tertiary care settings.
- **Rational prescribing:** The tendency to use terbinafine or fluconazole empirically, often without diagnostic confirmation, must be re-evaluated. Itraconazole may be considered early in the treatment of suspected resistant infections.
- **Surveillance systems:** There is a need for regional and national surveillance of dermatophyte epidemiology and resistance trends. Molecular typing and resistance mechanism analysis should be encouraged.
- **Regulatory action:** Over-the-counter sale of steroid-antifungal combinations should be strictly controlled. Pharmacovigilance and antimicrobial stewardship must extend to antifungals used in skin infections.

Limitations of the Study

- Molecular characterization of resistant strains (e.g., SQLE mutation analysis) was not performed.
- Clinical correlation with treatment response was not followed up longitudinally.
- Being a single-center study, the findings may not be generalizable to all regions.

Despite these limitations, the study provides valuable insights into local antifungal resistance patterns and supports the growing evidence of an antifungal resistance epidemic in dermatophytosis in India.

Conclusion

Our study underscores a significant shift in dermatophyte epidemiology and resistance in chronic tinea infections in Central India. The high rates of terbinafine and fluconazole resistance demand a rethinking of empirical therapy. Itraconazole and luliconazole remain effective and should be prioritized. Public health interventions, education, and surveillance are urgently needed to address the growing challenge of chronic dermatophytosis in the country.

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