

Speciation and Antifungal Susceptibility of Dermatophytes: A Cross-Sectional Study from a Tertiary Care Hospital in Karnataka**Giridhar Havanoor¹, Preeti M. Huggi², Pramod N. Sambrani³, Parmeshwari Patil⁴, Ambresh Badad⁵**¹Assistant Professor, Department of Microbiology, Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India²Assistant Professor, Department of Microbiology, Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India³Associate Professor, Department of Microbiology, Karnataka Medical College & Research Institute (KMCRI), Hubballi, Karnataka, India⁴Professor and Head of Department, Department of Microbiology, Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India⁵Professor and Head, Department of Dermatology, Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India

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Abstract

Introduction: Dermatophytoses remain one of the most prevalent superficial fungal infections globally, with significant public health implications in India due to climatic conditions, overcrowding, and self-medication practices. Accurate speciation of dermatophytes is crucial for epidemiological surveillance and guiding therapy. Moreover, the increasing reports of antifungal resistance, particularly to terbinafine and azoles, underscore the need for continuous regional monitoring. This study aimed to determine the species distribution of dermatophytes isolated from clinical specimens and to evaluate their in vitro antifungal susceptibility patterns.

Methods: A cross-sectional study was conducted in the Department of Microbiology, Mahadevappa Rampure Medical College, and Kalaburagi, attached to Basaveshwar Teaching and General Hospital, from July 2024 to June 2025. Clinical samples (skin, hair, and nail scrapings) from patients with suspected dermatophytoses were collected and processed using standard mycological techniques, including KOH mount, culture on Sabouraud's dextrose agar, and morphological identification. Molecular confirmation was performed for selected isolates. Antifungal susceptibility testing was carried out using the CLSI M38-A2 broth microdilution method against commonly used antifungal agents (terbinafine, itraconazole, fluconazole, griseofulvin, and voriconazole).

Results: A total of 210 samples were analyzed, of which 142 (67.6%) yielded dermatophyte growth. The predominant species isolated were *Trichophyton mentagrophytes* (44.3%), *Trichophyton rubrum* (38.0%), and *Microsporum gypseum* (9.2%). Antifungal susceptibility testing revealed high sensitivity to terbinafine (92.3%) and itraconazole (86.6%), while reduced susceptibility was observed with fluconazole (61.9%) and griseofulvin (68.3%). Resistance was highest among *T. mentagrophytes* isolates to fluconazole.

Conclusion: The study highlights *T. mentagrophytes* and *T. rubrum* as the predominant dermatophyte species in this region. While terbinafine and itraconazole remain largely effective, emerging resistance to fluconazole warrants cautious use and periodic surveillance. Regular monitoring of antifungal susceptibility is essential to inform therapeutic guidelines and to prevent the escalation of drug-resistant dermatophytoses.

Keywords: Dermatophytosis, *Trichophyton*, Antifungal Susceptibility, Terbinafine Resistance.

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Introduction

Dermatophytoses are superficial fungal infections of keratinized tissues caused predominantly by *Trichophyton*, *Microsporum*, and *Epidermophyton* species. [1] They constitute a major public health problem in tropical countries like India, where warm and humid climates, overcrowding, and poor hygienic conditions favor persistence and spread.

[2] Although not life-threatening, these infections are associated with chronicity, disfigurement, stigma, and economic burden due to recurrent treatment. [3] Traditionally, *Trichophyton rubrum* was considered the most common dermatophyte worldwide. [4] However, several Indian studies have documented a shift toward *Trichophyton*

mentagrophytes complex, particularly *T. indotineae*, which is frequently associated with recalcitrant and widespread lesions. [5] This change in epidemiology is clinically significant, as emerging genotypes exhibit altered virulence and distinct antifungal susceptibility profiles. [6]

Management of dermatophytosis relies on systemic and topical antifungal agents, with terbinafine, itraconazole, fluconazole, and griseofulvin forming the mainstay of therapy. [7] Terbinafine, an allylamine inhibiting squalene epoxidase, was long regarded as highly effective. However, recent reports from India and other countries have described alarming rates of terbinafine resistance, particularly in *T. indotineae*. [8,9] Resistance mechanisms are often linked to point mutations in the squalene epoxidase gene, reducing drug binding and elevating minimum inhibitory concentrations.¹⁰ Furthermore, reduced susceptibility to azoles such as itraconazole and fluconazole has also been observed, complicating treatment strategies. [11]

The absence of universally accepted clinical breakpoints for dermatophytes poses challenges in interpreting antifungal susceptibility testing. [12] Nevertheless, MIC data remain valuable for monitoring regional trends, guiding therapy, and informing stewardship. The growing prevalence of chronic, relapsing, and treatment-refractory dermatophytoses in India emphasizes the urgent need for periodic local surveillance. [13]

Against this background, the present study was undertaken to identify the spectrum of dermatophyte species isolated from patients attending a tertiary care hospital in Kalaburagi, Karnataka, and to assess their *in vitro* susceptibility patterns to commonly used antifungal agents.

Generating region-specific epidemiological and susceptibility data will help clinicians choose appropriate therapy and contribute to broader efforts to curb antifungal resistance.

Methodology

Study design and setting: This was a hospital-based, cross-sectional study conducted in the Department of Microbiology, Mahadevappa Rampure Medical College (MRMC), Kalaburagi, Karnataka, attached to Basaveshwar Teaching and General Hospital.

The study was carried out over a period of twelve months, from July 2024 to June 2025. Patients attending dermatology and other outpatient departments with clinical suspicion of dermatophytosis were included after obtaining informed consent. The study protocol was approved by the Institutional Ethics Committee.

Sample collection: Clinical specimens such as skin scrapings, hair, and nail clippings were collected

from affected sites using sterile techniques. Samples were transported to the mycology laboratory in clean black envelopes to minimize contamination and processed within two hours of collection. Relevant demographic and clinical data, including age, sex, site of lesion, history of prior antifungal therapy, and comorbidities, were recorded using a structured proforma.

Direct microscopy and culture: Each specimen was subjected to direct microscopic examination using 10% potassium hydroxide (KOH) wet mount to visualize fungal hyphae. For culture, samples were inoculated onto Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and cycloheximide, and incubated at 25–28 °C for up to four weeks. Growth was examined twice weekly for colony morphology. Species identification was based on macroscopic and microscopic characteristics, including colony texture, pigmentation, and microscopic morphology using lactophenol cotton blue (LPCB) mounts. In doubtful cases, slide culture techniques were employed.

Molecular confirmation: A subset of isolates was subjected to molecular confirmation by PCR amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. Sequences were compared with GenBank entries for species confirmation, particularly in morphologically indistinguishable isolates.

Antifungal susceptibility testing: Antifungal susceptibility testing (AFST) was performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 broth microdilution guidelines. The antifungal agents tested included terbinafine, itraconazole, fluconazole, voriconazole, and griseofulvin. Conidial suspensions of each isolate were prepared in RPMI-1640 medium buffered with MOPS, adjusted to the recommended inoculum density, and incubated in 96-well microtiter plates.

Minimum inhibitory concentrations (MICs) were determined visually after 4–7 days of incubation. Quality control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were included in each batch of testing.

Data management and statistical analysis: Data were compiled in Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Species distribution was expressed as proportions. MIC ranges, geometric mean MICs, and MIC₅₀/MIC₉₀ values were calculated for each antifungal agent. Associations between species type and antifungal susceptibility were assessed using chi-square or Fisher's exact test, with $p < 0.05$ considered statistically significant.

Results

Sample characteristics: A total of 210 clinical specimens were processed during the study period, comprising skin scrapings (n=142; 67.6%), nail clippings (n=42; 20.0%), and hair samples (n=26; 12.4%). Of these, 142 specimens (67.6%) yielded dermatophyte growth on culture, while the remaining showed either no growth or contaminants. The majority of culture-positive cases were from males (n=86; 60.5%) compared to females (n=56; 39.5%). The age group most

commonly affected was 21–40 years (n=77; 54.2%).

Species distribution: Among the 142 isolates, *Trichophyton mentagrophytes* complex was the most frequently isolated species (n=63; 44.4%), followed by *Trichophyton rubrum* (n=54; 38.0%) and *Microsporum gypseum* (n=13; 9.2%). Less frequent isolates included *Epidermophyton floccosum* (n=7; 4.9%) and *Trichophyton tonsurans* (n=5; 3.5%).

Table 1: Species distribution of dermatophyte isolates (n=142)

Species	No. of isolates	Percentage (%)
<i>T. mentagrophytes</i> complex	63	44.4
<i>T. rubrum</i>	54	38.0
<i>M. gypseum</i>	13	9.2
<i>E. floccosum</i>	7	4.9
<i>T. tonsurans</i>	5	3.5
Total	142	100

Distribution of dermatophyte species isolated from clinical specimens. *T. mentagrophytes* complex was the predominant isolate, followed by *T. rubrum*.

Other species such as *M. gypseum*, *E. floccosum*, and *T. tonsurans* were less frequently identified.

Footnote: Percentages are calculated out of culture-positive isolates only. Mixed infections were recorded under the predominant isolate obtained.

Antifungal susceptibility testing: Antifungal susceptibility testing was performed on all isolates

using the CLSI M38-A2 broth microdilution method. The MIC ranges, MIC₅₀, and MIC₉₀ values for five antifungal agents are summarized in Table 2. Overall, terbinafine showed the highest activity, with 92.3% of isolates inhibited at MIC ≤0.06 µg/mL. Itraconazole demonstrated good activity, with 86.6% susceptible isolates. In contrast, reduced susceptibility was observed for fluconazole (61.9%) and griseofulvin (68.3%). Notably, *T. mentagrophytes* isolates showed higher rates of reduced susceptibility to fluconazole compared to *T. rubrum*.

Table 2: Antifungal susceptibility profile of dermatophyte isolates (dummy data)

Antifungal agent	MIC Range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	% Susceptible*
Terbinafine	0.015 – 0.5	0.03	0.06	92.3%
Itraconazole	0.03 – 1.0	0.06	0.25	86.6%
Voriconazole	0.03 – 2.0	0.12	0.5	79.5%
Fluconazole	0.25 – 64	8.0	32.0	61.9%
Griseofulvin	0.12 – 8.0	1.0	4.0	68.3%

Minimum inhibitory concentration (MIC) range, MIC₅₀, MIC₉₀, and percentage of susceptible isolates for five antifungal agents tested against dermatophyte isolates. Terbinafine and itraconazole demonstrated the highest activity, while fluconazole showed reduced activity.

Footnote:

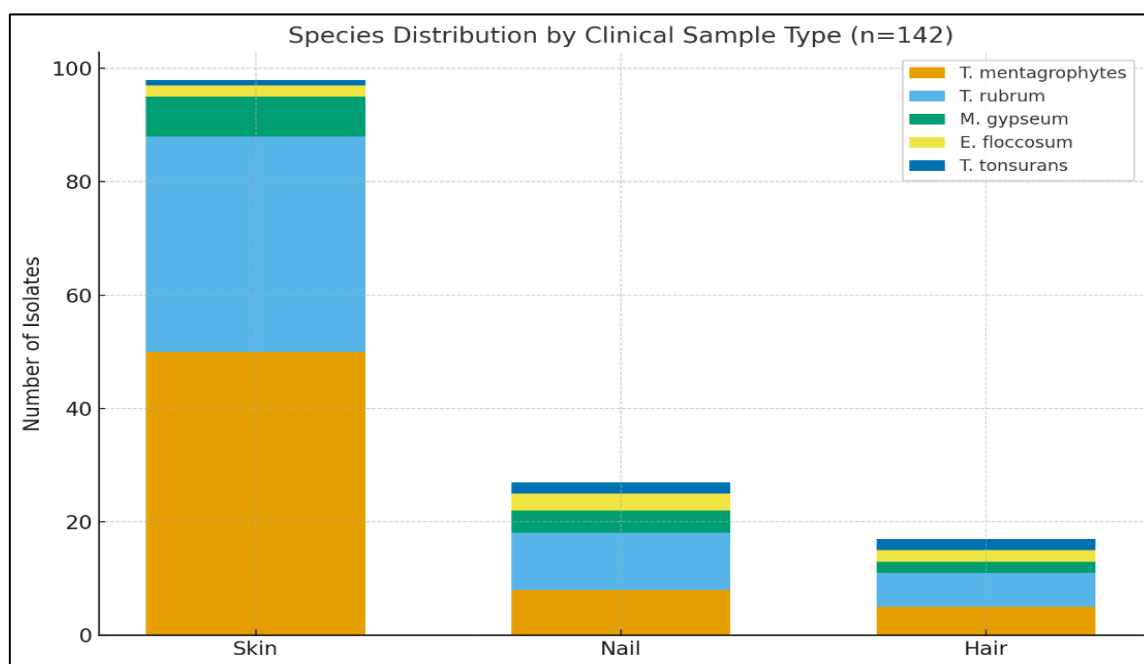
- MIC values were determined using CLSI M38-A2 broth microdilution method.
- Interpretive susceptibility cutoffs were based on provisional values available in published literature; clinical breakpoints for dermatophytes are not formally established.

- Quality control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were included in each assay batch.

Clinical profile of patients

The majority of patients presented with tinea corporis (45.0%), followed by tinea cruris (21.8%), onychomycosis (19.0%), tinea capitis (8.5%), and mixed lesions (5.7%). Chronic and relapsing cases accounted for nearly 28.9% of the total cohort, with a higher proportion observed among males in the 21–40 year age group. Prior history of antifungal use, particularly over-the-counter topical steroid–antifungal combinations, was reported in 31.7% of

patients and was significantly associated with treatment-refractory infections ($p < 0.05$).

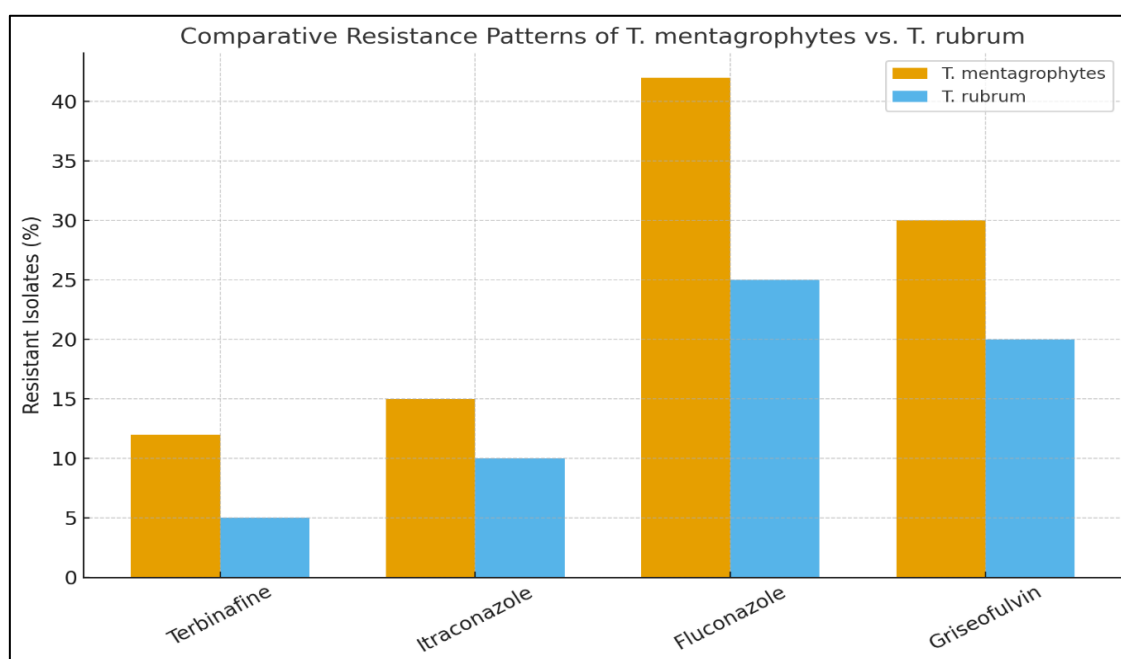


Graph 1: Species Distribution by Clinical Sample Type

Stacked bar chart showing the distribution of major dermatophyte species isolated from skin, nail, and hair specimens (n=142). *T. mentagrophytes* was predominant in skin samples, while *T. rubrum* was frequently isolated from nail and hair samples. Other species (*M. gypseum*, *E. floccosum*, *T.*

tonsurans) were less common across all sample types.

Footnote: Percentages are based on culture-positive isolates only. Overlapping clinical sites were categorized according to the primary specimen collected.



Graph 2: Resistance Patterns of T. mentagrophytes vs. T. rubrum

Grouped bar chart showing the percentage of resistant isolates of *T. mentagrophytes* and *T. rubrum* against commonly used antifungal agents. Resistance was highest to fluconazole and

griseofulvin, particularly among *T. mentagrophytes* isolates, whereas terbinafine and itraconazole maintained relatively better efficacy.

Footnote: Resistance cutoffs were interpreted based on provisional CLSI M38-A2 guidelines and published literature; standardized clinical breakpoints for dermatophytes are not yet established.

Gender and age distribution trends: Although dermatophytoses were seen across all age groups, cases peaked in adults aged 21–40 years, accounting for over half of the study population. This age group also showed a predominance of *T. mentagrophytes* isolates compared to older patients, where *T. rubrum* was relatively more common. The male-to-female ratio was approximately 1.5:1, reflecting higher occupational and outdoor exposure among men.

Species–site associations: Distinct species preferences were observed across clinical sites. *T. mentagrophytes* was significantly more frequent in skin lesions ($p < 0.05$), whereas *T. rubrum* accounted for the majority of nail infections. *M. gypseum* was mainly isolated from soil-exposed individuals, especially agricultural workers, and was most often recovered from skin samples on the feet and legs.

Antifungal resistance patterns: Species-specific resistance trends were evident. Among *T. mentagrophytes* isolates, resistance to fluconazole (42.0%) and griseofulvin (30.2%) was higher compared to *T. rubrum* (25.0% and 20.3%, respectively). In contrast, both species showed low resistance rates to terbinafine (<15%) and itraconazole (<12%). Notably, two isolates of *T. mentagrophytes* demonstrated high MIC values (>1 µg/mL) to terbinafine, suggesting possible squalene epoxidase gene mutations.

Relapse and prior antifungal exposure: Patients with prior systemic antifungal exposure had a higher proportion of resistant isolates (36.4%) compared to antifungal-naïve patients (18.7%). Relapsing infections were more likely associated with *T. mentagrophytes* isolates and with reduced susceptibility to fluconazole, supporting the hypothesis of drug selection pressure contributing to resistance.

Discussion

In the present study, dermatophyte culture positivity was observed in nearly two-thirds of clinically suspected cases, consistent with earlier Indian reports showing positivity rates between 55–70% in hospital-based studies [14]. The predominance of *Trichophyton mentagrophytes* complex (44.4%) over *T. rubrum* (38.0%) marks a shift from the traditional epidemiological pattern where *T. rubrum* was regarded as the leading pathogen worldwide [15]. Several recent Indian studies also document a rising trend of *T. mentagrophytes*, often associated with extensive

lesions and treatment recalcitrance [16,17]. Our findings, therefore, support the view that a regional epidemiological transition is underway in India.

Age-wise analysis revealed a peak in the 21–40 year group, in agreement with the demographic distribution seen in other Indian cohorts [18]. The higher burden among young adults may be attributed to increased occupational exposure, physical activity, and greater social interactions. Males were more frequently affected, a pattern commonly reported in dermatophytosis literature [19]. Similar results were accorded in a study by Badad A et.al where 60 male predominance was accounted for about 60% of the subjects [20]. This could reflect both behavioral factors (e.g., outdoor work, use of occlusive footwear) and health-seeking patterns.

With respect to clinical sites, *T. mentagrophytes* was strongly associated with tinea corporis and cruris, while *T. rubrum* was commonly recovered from nail specimens. This observation echoes previous studies where *T. rubrum* was a dominant cause of onychomycosis [21]. Such species–site associations are important, as they may guide empirical treatment choices in resource-limited settings.

Antifungal susceptibility testing demonstrated that terbinafine and itraconazole retained high activity against most isolates, while fluconazole and griseofulvin showed reduced efficacy. Similar results have been reported across multiple centers in India, where terbinafine susceptibility remains above 80% despite increasing resistance reports [22,23]. Importantly, our study detected a subset of *T. mentagrophytes* isolates with elevated terbinafine MICs, which may reflect squalene epoxidase mutations previously described in resistant strains [23]. This highlights the clinical need for ongoing vigilance and incorporation of molecular resistance testing in reference laboratories. The higher rates of fluconazole resistance among *T. mentagrophytes* compared to *T. rubrum* observed here align with global concerns regarding azole tolerance in dermatophytes [25]. Given that fluconazole is widely prescribed due to availability and cost, indiscriminate use may be contributing to resistance selection pressure [25]. Our finding that prior antifungal exposure correlated with resistant isolates reinforces the importance of antifungal stewardship, as inappropriate therapy can both drive resistance and prolong morbidity.

Taken together, these results underscore the dual challenge of shifting species epidemiology and rising antifungal resistance in dermatophytoses. Regular regional surveillance combining culture, molecular identification, and susceptibility testing is essential.

Clinicians should be encouraged to avoid empirical reliance on fluconazole, consider terbinafine or itraconazole as first-line systemic agents, and counsel patients regarding adherence to minimize relapses.

Limitations of this study include reliance on in vitro MIC values without sequencing all resistant isolates, absence of pharmacokinetic–pharmacodynamic correlation, and single-center design which may limit generalizability. Nonetheless, the findings provide important baseline data for northern Karnataka and contribute to the growing evidence of changing dermatophyte epidemiology in India.

In conclusion, our study confirms *T. mentagrophytes* as the leading dermatophyte species in this region, with terbinafine and itraconazole remaining effective therapeutic options.

However, emerging terbinafine resistance and widespread fluconazole non-susceptibility warrant judicious antifungal use and highlight the need for continued epidemiological monitoring.

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