

Antifungal Susceptibility Patterns and Emerging Resistance in Dermatophytes: A Study from a Tertiary Care Centre in Central India

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ABSTRACT

Background: The rising incidence of recalcitrant dermatophytosis in India has necessitated the routine use of antifungal susceptibility testing (AFST). Understanding the susceptibility patterns of prevalent species is crucial for optimizing therapeutic regimens and addressing the challenge of antifungal resistance (AFR).

Objectives: This study aimed to determine the in vitro susceptibility of dermatophyte isolates to common antifungal agents using the microbroth dilution (MBD) method and to compare these results with Disk Diffusion (DDM) and E-test methods.

Methods: Eighty-three dermatophyte isolates (including *Trichophyton mentagrophytes/interdigitale* complex, *T. tonsurans*, and *T. rubrum*) obtained from clinical samples were tested against seven antifungal drugs: terbinafine, itraconazole, fluconazole, voriconazole, ketoconazole, clotrimazole, and griseofulvin. AFST was performed according to CLSI M38-A2 guidelines. Minimum Inhibitory Concentrations (MICs) were determined, and epidemiological cut-off values (ECVs) were analyzed.

Results: Voriconazole (GM MIC 0.16–0.29 mg/L) and itraconazole (GM MIC 0.31–1.99 mg/L) demonstrated the highest in vitro activity across all species. In contrast, fluconazole showed poor activity, with MICs consistently reaching 64 mg/L. Terbinafine exhibited a wide range of MICs (0.25–64 mg/L), with a significant proportion of *T. mentagrophytes* isolates (74.5%) showing reduced susceptibility based on established reference values. The MBD method showed substantial agreement with the E-test for most drugs, while DDM provided a useful screening alternative.

Conclusion: The study reveals a high prevalence of reduced susceptibility to terbinafine and fluconazole among dermatophytes in Central India. Itraconazole and voriconazole remain effective options. The findings emphasize the importance of standardized AFST in managing chronic dermatophytosis and the need for establishing clinical breakpoints to guide therapy.

INTRODUCTION

Dermatophytosis has traditionally been considered a easily treatable superficial fungal infection. However, the last decade has seen a dramatic shift in India, with clinicians encountering an increasing number of chronic, recurrent, and recalcitrant cases [2, 4]. This change is often attributed to the emergence of antifungal resistance (AFR), driven by the misuse of over-the-counter antifungal-steroid combinations and inadequate treatment durations [3, 9].

Antifungal susceptibility testing (AFST) is no longer just a research tool; it has become essential in clinical practice to guide the management of treatment-failure cases [5, 6]. The Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have provided standardized microbroth dilution (MBD) methods for testing filamentous fungi, including dermatophytes [5, 7].

Despite the availability of these standards, interpreting MIC values remains challenging due to the lack of established clinical breakpoints (CBPs) for dermatophytes. Instead, researchers often rely on epidemiological cut-off values (ECVs) or Upper Limit of Wild-Type (UL-WT) distributions to distinguish between wild-type isolates and those with potentially acquired resistance mechanisms [8, 9].

This study, conducted at Index Medical College Hospital & Research Centre, Indore, evaluates the susceptibility patterns of dermatophyte isolates from Central India against a panel of seven antifungal agents, providing critical data on the current state of antifungal resistance in the region.

MATERIALS AND METHODS

Fungal Isolates

A total of 83 dermatophyte isolates were included in the study. These were obtained from clinical samples (skin scrapings, nail clippings, and hair) of patients attending the dermatology outpatient department. The isolates were identified as *Trichophyton mentagrophytes/interdigitale* complex (n=55), *Trichophyton tonsurans* (n=20), and *Trichophyton rubrum* (n=8) using conventional and molecular methods [10].

Antifungal Agents

The following antifungal drugs (AFDs) were tested: 1. **Allylamines:** Terbinafine 2. **Azoles:** Itraconazole, Fluconazole, Voriconazole, Ketoconazole, Clotrimazole 3. **Others:** Griseofulvin

Microbroth Dilution (MBD) Method

AFST was performed following the CLSI M38-A2 guidelines. **Medium:** RPMI 1640 with L-glutamine, without bicarbonate, buffered to pH 7.0 with 0.165 M MOPS. **Drug Dilutions:** Stock solutions were prepared in DMSO. Serial two-fold dilutions were made to achieve a final concentration range (e.g., 0.03 to 64 mg/L). **Inoculum Preparation:** Conidial suspensions were prepared from 7- to 10-day-old cultures on Potato Dextrose Agar (PDA). The inoculum was standardized to 1×10^3 to 3×10^3 CFU/mL using a hemocytometer. **Incubation:** Microtiter plates were incubated at 28–30°C. **Endpoint Determination:** MICs were read after 4 to 7 days of incubation. For azoles and griseofulvin, the MIC was defined as the lowest concentration showing 80% growth inhibition. For terbinafine, a 100% inhibition endpoint was used [11, 12].

Alternative Methods

- **E-test:** Performed on non-supplemented Mueller-Hinton Agar (MHA). The MIC was read where the elliptical zone of inhibition intersected the strip.
- **Disk Diffusion Method (DDM):** Performed on MHA using standard antifungal disks. Inhibition zone diameters (IZD) were measured in millimeters [13, 14].

Data Analysis

Geometric Means (GM), MIC ranges, and MIC_{50} and MIC_{90} values were calculated. The Upper Limit of Wild-Type (UL-WT) was determined at the 97.5% percentile. Statistical analysis, including Kappa statistics for agreement between methods, was performed using SPSS software.

RESULTS

Susceptibility Profiles by Species

1. *Trichophyton rubrum* (n=8)

Voriconazole showed the highest potency (GM MIC 0.29 mg/L). High MICs were observed for terbinafine (GM 13.18 mg/L), griseofulvin (GM 41.50 mg/L), and fluconazole (all isolates at 64 mg/L). The UL-WT for terbinafine was 32 mg/L.

2. *Trichophyton mentagrophytes/interdigitale* complex (n=55)

This complex showed significant resistance. Voriconazole (GM 0.16 mg/L) and itraconazole (GM 0.31 mg/L) were the most active. Terbinafine showed a very high GM MIC of 12.13 mg/L, with a UL-WT of 64 mg/L. Fluconazole was largely ineffective (GM 59.33 mg/L).

3. *Trichophyton tonsurans* (n=20)

Similar to the other species, voriconazole (GM 0.25 mg/L) and clotrimazole (GM 0.25 mg/L) were potent. Terbinafine and griseofulvin showed high MICs, with GM values of 17.75 mg/L and 27.84 mg/L, respectively.

Comparison with Reference Values

Using the UL-WT values from a landmark Indian study (Shaw et al., 2020) as a reference, the resistance rates for *T. mentagrophytes* in our study were: Terbinafine: 74.5% Fluconazole: 94.5% Griseofulvin: 43.6% Itraconazole: 32.7% Voriconazole: 30.9%

Method Correlation

The E-test showed substantial agreement with the MBD method for itraconazole and voriconazole. The Disk Diffusion Method also demonstrated a good correlation, particularly for identifying highly resistant isolates, making it a viable screening tool for busy clinical laboratories.

DISCUSSION

The most striking finding of this study is the high level of in vitro resistance to terbinafine among dermatophyte isolates in Central India. Terbinafine has long been the first-line systemic agent for dermatophytosis due to its fungicidal action. However, our data shows that nearly three-quarters of *T. mentagrophytes* isolates have MICs well above the historical wild-type range. This resistance is often linked to point mutations in the squalene epoxidase (SE) gene, such as F397L and L393F, which have been increasingly reported across India [15, 16, 17].

Fluconazole, despite being widely used, showed almost no activity in our study. [21-24] This suggests that its role in the management of dermatophytosis should be re-evaluated, especially in regions where the *T. mentagrophytes* complex is dominant. [30-33]

Itraconazole and voriconazole remain the most effective azoles. Itraconazole's low MICs support its current use as a preferred systemic agent for recalcitrant cases [18, 19]. Voriconazole, while highly effective in vitro, is generally reserved for deep-seated or systemic fungal infections due to its cost and side-effect profile. [26-30]

The comparison of UL-WT values with previous studies (e.g., Shaw et al., 2020) indicates a clear upward shift in MICs over a short period. This rapid evolution of resistance is a major concern and highlights the "tip of the iceberg" phenomenon of antifungal resistance in the Indian subcontinent [20].

The role of efflux pumps in contributing to this resistance cannot be ignored. Studies have shown that the upregulation of ABC transporters can lead to multi-drug resistance, affecting both azoles and allylamines [21, 22].

CONCLUSION

This study documents a significant prevalence of antifungal resistance among dermatophytes in Central India, particularly against terbinafine and fluconazole. The *Trichophyton mentagrophytes/interdigitale* complex is the primary driver of this resistance. Itraconazole and voriconazole continue to show good in vitro activity and remain reliable therapeutic options. Standardized AFST is crucial for monitoring these trends and for the personalized management of patients with chronic and recurrent infections. There is an urgent need for multicenter studies to establish clinical breakpoints and to develop strategies to mitigate the further spread of resistance.

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