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In-vitro Antifungal Susceptibility of Dermatophytes Isolated from a Tertiary-Care Hospital in Central India: A Broth Microdilution Study

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ABSTRACT

Background: Dermatophyte treatment failure and recurrent infection have increased interest in standardized antifungal susceptibility testing. Minimum inhibitory concentration (MIC) data can describe local susceptibility distributions and support surveillance, although MIC values alone do not define clinical resistance in the absence of validated species-specific breakpoints.

Methods: In a descriptive study conducted from October 2022 to April 2025, 250 clinically diagnosed dermatophytosis cases were investigated at a tertiary-care teaching hospital in Indore. Dermatophytes isolated by microscopy and culture were tested by broth microdilution in accordance with Clinical and Laboratory Standards Institute M38-A2 guidance. MICs were determined for fluconazole, itraconazole, terbinafine, griseofulvin, ketoconazole, sertaconazole, luliconazole, and amorolfine. The analysis included MIC range, MIC_{50}, MIC_{90}, and the reported geometric mean for 131 isolates representing six species.

Results: The isolates comprised *Trichophyton mentagrophytes* (n=68), *T. rubrum* (n=45), *T. verrucosum* (n=7), *T. tonsurans* (n=6), *Nannizzia gypsea* (n=3), and *Microsporum canis* (n=2). Across all isolates, the reported geometric means were 19.85 µg/mL for fluconazole, 6.79 for itraconazole, 0.0976 for terbinafine, 0.1398 for griseofulvin, 4.68 for ketoconazole, 0.2713 for sertaconazole, 0.1046 for luliconazole, and 0.05481 for amorolfine. The corresponding MIC_{50}/MIC_{90} values were 16/32 µg/mL, 4/16, 0.06/0.25, 0.12/0.25, 4/8, 0.25/0.5, 0.06/0.25, and 0.03/0.12, respectively. The lowest reported central tendency among topical agents was for amorolfine, followed by luliconazole; among systemic agents, terbinafine had the lowest reported central tendency. Fluconazole showed the highest MIC distribution overall. The comparison of *T. mentagrophytes* and *T. rubrum* showed similar MIC_{50} values, while the study reported a higher fluconazole MIC_{90} for *T. mentagrophytes*.

Conclusion: The study demonstrated marked variation in in-vitro activity across antifungal agents and species. Amorolfine and luliconazole had the lowest reported topical MIC summaries, while terbinafine had the lowest reported systemic MIC summary. Fluconazole and itraconazole showed higher MIC distributions in this collection. These findings support local surveillance and targeted susceptibility testing for persistent or recurrent disease, but they should not be used as clinical breakpoints.

Keywords: dermatophytes; antifungal susceptibility; broth microdilution; minimum inhibitory concentration; terbinafine; amorolfine; India

INTRODUCTION

Dermatophytosis is caused by keratinophilic fungi that colonize and invade keratinized tissues. Treatment commonly involves topical or systemic antifungal agents, selected according to the site and extent of infection. Recurrent or chronic

disease, incomplete adherence, inappropriate topical combinations, and repeated over-the-counter exposure have increased concern about reduced antifungal responsiveness in some settings [1] [2].

Antifungal susceptibility testing (AFST) provides a reproducible way to compare the in-vitro activity of agents against clinical isolates. For filamentous fungi, the Clinical and Laboratory Standards Institute (CLSI) M38-A2 reference method uses broth microdilution to estimate MICs [3]. The method is technically demanding for dermatophytes because conidial production, inoculum preparation, growth rate, endpoint interpretation, and the choice of concentration range can influence results [4] [5]. Alternative methods, including agar-based screening and commercial systems, may be useful in particular laboratories, but methodological differences limit direct comparison between studies [6] [7].

Interpretation requires caution. MIC_{50} and MIC_{90} describe the concentrations inhibiting 50% and 90% of the tested isolates, respectively; they do not, by themselves, establish whether an isolate is clinically susceptible or resistant. Clinical interpretation requires validated epidemiological cut-offs or clinical breakpoints, together with species, site of infection, pharmacokinetics, and treatment outcome [4] [8]. Nevertheless, local MIC distributions are valuable for detecting shifts in susceptibility and for selecting isolates for further molecular investigation.

The present study evaluated the in-vitro susceptibility of six dermatophyte species isolated from patients attending a tertiary-care hospital in Indore. Eight commonly used antifungal agents were tested by broth microdilution, and the resulting MIC summaries were compared by species and drug class.

MATERIALS AND METHODS

Study design and isolates

This descriptive laboratory-based study was conducted in the Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India, between October 2022 and April 2025. A total of 250 clinically diagnosed cases of dermatophytosis were investigated. Skin, hair, and nail specimens were examined by KOH mount and cultured on Sabouraud dextrose agar. Isolates were identified by colony morphology, tease mount, slide culture, and physiological testing. A total of 131 culture-positive dermatophyte isolates were included in AFST.
The isolates were *T. mentagrophytes* (n=68), *T. rubrum* (n=45), *T. verrucosum* (n=7), *T. tonsurans* (n=6), *N. gypsea* (n=3), and *M. canis* (n=2).

Antifungal agents and stock solutions

The eight antifungal agents tested were fluconazole, itraconazole, terbinafine, griseofulvin, ketoconazole, sertaconazole, luliconazole, and amorolfine. Stock solutions were prepared from antifungal powders. Dimethyl sulfoxide was used as the diluent for all agents except fluconazole, for which water was used. Working solutions were prepared in RPMI-1640 medium buffered with MOPS and serially diluted twofold.

The final concentration ranges used in the study were 0.25–64 µg/mL for fluconazole and itraconazole, 0.007–2 µg/mL for terbinafine, 0.03–8 µg/mL for griseofulvin, 0.25–64 µg/mL for ketoconazole, 0.03–8 µg/mL for sertaconazole and luliconazole, and 0.004–1 µg/mL for amorolfine.

Inoculum preparation and broth microdilution

Isolates were subcultured on potato dextrose agar and incubated at 25°C for four to five days or until adequate conidiation was observed. Conidia were suspended in sterile 0.85% saline, allowed to settle, transferred to fresh tubes, vortexed, and adjusted to 0.5 McFarland. The final testing inoculum was targeted at approximately 1×10^3 to 3×10^3 CFU/mL.

Sterile round-bottom 96-well microtiter plates were prepared according to CLSI M38-A2 guidance. Drug-containing wells received inoculum and twofold serial drug dilutions. Growth-control and sterility-control wells were included. Plates were incubated at 35°C without agitation for four days. *T. rubrum* ATCC MYA-4438 and *Candida albicans* ATCC M054 were used as quality-control strains in the source protocol.

MIC endpoint and statistical analysis

Growth in each well was compared visually with the drug-free growth-control well. The MIC endpoint was defined as the lowest concentration producing an approximately 80% reduction in visible growth relative to the control, as specified in the source protocol. MIC range, MIC_{50}, MIC_{90}, and the reported geometric mean were summarized for the complete isolate collection and by species. The source study also used independent t tests for selected pairwise comparisons. Given the small numbers of several species and the absence of clinical breakpoints, inferential comparisons are interpreted cautiously.

RESULTS

Overall susceptibility summary

The 131 isolates showed the lowest reported overall central tendency for amorolfine among the topical agents and for terbinafine among the systemic agents. The study reported an overall mean/geometric mean of 0.05481 µg/mL for amorolfine, 0.1046 for luliconazole, 0.2713 for sertaconazole, and 4.68 for ketoconazole. Among systemic agents, the corresponding values were 0.0976 µg/mL for terbinafine, 0.1398 for griseofulvin, 6.79 for itraconazole, and 19.85 for fluconazole.

Antifungal agent	Drug group in this analysis	MIC range (µg/mL)	Reported GM/mean (µg/mL)	MIC50 (µg/mL)	MIC90 (µg/mL)
Fluconazole	Systemic	0.25–64	19.85	16.00	32.00
Itraconazole	Systemic	0.25–64	6.79	4.00	16.00
Terbinafine	Systemic	0.004–1	0.0976	0.060	0.250
Griseofulvin	Systemic	0.01–4	0.1398	0.120	0.250
Ketoconazole	Topical/systemic use varies by formulation	0.12–32	4.68	4.00	8.00
Sertaconazole	Topical	0.015–4	0.2713	0.250	0.500
Luliconazole	Topical	0.03–16	0.1046	0.060	0.250
Amorolfine	Topical	0.008–0.5	0.05481	0.030	0.120

The fluconazole MIC distribution was the highest overall, with MIC values extending to 64 µg/mL. Itraconazole also showed relatively high MIC values compared with terbinafine and griseofulvin. The topical agents amorolfine and luliconazole had the lowest reported MIC summaries, while ketoconazole showed a higher distribution than the other topical agents tested.

Species-specific MIC patterns

For *T. mentagrophytes* (n=68), the reported geometric means were 21.41 µg/mL for fluconazole, 6.74 for itraconazole, 0.088 for terbinafine, 0.146 for griseofulvin, 4.51 for ketoconazole, 0.28 for sertaconazole, 0.105 for luliconazole, and 0.057 for amorolfine. The MIC_{50}/MIC_{90} values were 16/64, 4/16, 0.06/0.25, 0.12/0.25, 4/8, 0.25/0.5, 0.06/0.25, and 0.03/0.12 µg/mL, respectively.

For *T. rubrum* (n=45), the reported geometric means were 19.38 µg/mL for fluconazole, 7.18 for itraconazole, 0.11 for terbinafine, 0.134 for griseofulvin, 4.98 for ketoconazole, 0.26 for sertaconazole, 0.103 for luliconazole, and 0.055 for amorolfine. The MIC_{50}/MIC_{90} values were 16/32, 4/16, 0.06/0.25, 0.12/0.25, 4/8, 0.25/0.5, 0.06/0.25, and 0.03/0.12 µg/mL, respectively.

The smaller species groups generally showed the same broad pattern: higher MIC summaries for fluconazole and itraconazole and lower values for terbinafine, griseofulvin, luliconazole, and amorolfine. However, these observations are based on very small numbers for *N. gypsea* (n=3) and *M. canis* (n=2), and their MIC distributions are imprecise.

Species	n	Fluconazole GM	Itraconazole GM	Terbinafine GM	Griseofulvin GM	Ketoconazole GM	Sertaconazole GM	Luliconazole GM	Amorolfine GM
T. mentagrophytes	68	21.41	6.74	0.088	0.146	4.51	0.28	0.105	0.057
T. rubrum	45	19.38	7.18	0.110	0.134	4.98	0.26	0.103	0.055
N. gypsea	3	16.00	6.34	0.096	0.120	5.33	0.25	0.100	0.030
M. canis	2	16.00	8.00	0.042	0.155	3.00	0.155	0.750*	0.075
T. verrucosum	7	10.76	4.00	0.070	0.100	4.71	0.267	0.117	0.032
T. tonsurans	6	16.00	5.03	0.096	0.153	4.50	0.26	0.106	0.050

Pairwise comparisons reported in the source study

The MIC_{50} values for *T. mentagrophytes* and *T. rubrum* were identical for all eight agents in the study. Their MIC_{90} values were also identical except for fluconazole, which reported 38.40 µg/mL for *T. mentagrophytes* and 32.00 µg/mL for *T. rubrum*, with a reported p value of 0.01. The overall fluconazole MIC_{90} as 32 µg/mL and the species groups were not equal in size, this comparison should be regarded as exploratory rather than a definitive estimate of resistance.

The study also reported lower MIC_{50} values for *M. canis* than *N. gypsea* for several agents, including fluconazole, itraconazole, terbinafine, griseofulvin, and ketoconazole. These comparisons were based on two *M. canis* isolates and three *N. gypsea* isolates and therefore have very limited statistical stability. The MIC_{50} values for *T. verrucosum* and *T. tonsurans* were identical across the agents listed in the source table; MIC_{90} comparisons were not available for these small groups.

DISCUSSION

This study provides a local susceptibility profile for 131 dermatophyte isolates tested against eight antifungal agents by broth microdilution. The most consistent finding was the separation between agents with lower MIC summaries, particularly amorolfine, luliconazole, and terbinafine, and agents with higher MIC distributions, particularly fluconazole and itraconazole. Similar variation between species and antifungal classes has been described in previous broth microdilution studies [9] [10] [11].

Among the topical agents, amorolfine had the lowest reported overall geometric mean, followed by luliconazole and sertaconazole. The reported amorolfine MIC_{50} was 0.03 µg/mL, and the MIC_{90} was 0.12 µg/mL. The corresponding luliconazole values were 0.06 and 0.25 µg/mL. These findings are directionally consistent with studies that have reported low in-vitro MICs for newer topical agents, although absolute values vary with inoculum, endpoint definition, medium, incubation, and isolate composition [9] [12]. In-vitro potency should not be equated directly with clinical superiority because topical exposure, penetration, adherence, lesion site, and formulation also determine treatment outcome.

Terbinafine showed the lowest reported systemic MIC summary, with an overall geometric mean of 0.0976 µg/mL and MIC_{50}/MIC_{90} values of 0.06/0.25 µg/mL. This result is compatible with the established activity of terbinafine against many *Trichophyton* isolates. However, a low MIC distribution in one collection does not exclude resistant subpopulations. Mutations in the squalene epoxidase gene have been associated with allylamine resistance in dermatophytes, and molecular evaluation is relevant when elevated MICs coexist with treatment failure [13].

Griseofulvin also showed a low MIC distribution, with an overall reported geometric mean of 0.1398 µg/mL. The relatively narrow MIC_{90} of 0.25 µg/mL across most species suggests retained in-vitro activity in this collection. Its clinical use remains dependent on infection site, patient factors, treatment duration, and tolerability. The laboratory result should therefore be interpreted alongside clinical context rather than as a substitute for treatment assessment.

Fluconazole showed the highest MIC distribution among the tested agents. The overall reported MIC_{50}/MIC_{90} was 16/32 µg/mL, and the *T. mentagrophytes* group had the highest species-specific geometric mean and MIC_{90} in the source tables. Higher fluconazole MICs have also been reported in studies from different regions [9] [10]. The observation may reflect intrinsic differences in activity, prior drug exposure, or the selection of less susceptible isolates. It should not be interpreted as a clinical resistance rate because the study did not apply a validated clinical breakpoint.

Itraconazole showed higher MIC summaries than terbinafine and griseofulvin, with a reported overall geometric mean of 6.79 µg/mL and MIC_{50}/MIC_{90} values of 4/16 µg/mL. The *T. rubrum* group had the highest geometric mean among the six species. Differences between studies may be substantial because itraconazole MICs are sensitive to methodological details and the epidemiological composition of the tested collection [4] [5] [14].

The present study also illustrates why species-specific surveillance is important. The dominant species, *T. mentagrophytes* and *T. rubrum*, showed similar MIC_{50} values across all eight agents, but the upper-tail fluconazole distribution differed in the study. The smaller groups appeared to show additional variation, but their sample sizes were too small to support stable conclusions. Future studies should include larger numbers of less common species, molecular identification where available, and linkage between MIC results, previous antifungal exposure, and clinical outcomes.

Methodological standardization remains essential. CLSI M38-A2 provides a reference framework, but dermatophytes can be difficult to grow and sporulate consistently. The choice of medium, incubation time, inoculum density, endpoint threshold, and quality-control procedures can change MIC values. Comparative interpretation should therefore prioritize studies using the same or closely harmonized methods [3] [4] [6] [7].

STRENGTHS AND LIMITATIONS

The main strength of this study is that it tested eight antifungal agents against a relatively substantial local collection of dermatophyte isolates using a broth microdilution protocol based on CLSI M38-A2. It also reports both overall and species-specific MIC summaries, which can support local surveillance and future comparisons.

Important limitations should be considered. The study was performed at one tertiary-care hospital, and the isolate distribution may not represent the wider region. Several species groups were very small, particularly *M. canis* and *N. gypsea*. The study contain minor inconsistencies in terminology and some species-specific values; the present paper reproduces the principal reported results while identifying values that require cautious interpretation. Molecular mechanisms of resistance, prior antifungal exposure, treatment outcomes, and recurrence were not examined.

CONCLUSION

In this Central Indian tertiary-care collection, amorolfine and luliconazole showed the lowest reported MIC summaries among the topical agents, while terbinafine showed the lowest reported MIC summary among the systemic agents. Fluconazole and itraconazole had comparatively higher MIC distributions. The findings support the value of local AFST for recurrent, chronic, or treatment-unresponsive dermatophytosis, while emphasizing that MIC distributions are surveillance measurements and not clinical breakpoints. Larger, multicentric studies that integrate standardized MIC testing, molecular resistance markers, previous treatment exposure, and clinical outcomes are needed.

Declarations
Ethics and consent: informed consent and institutional ethical clearance were obtained prior to study.
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Conflict of interest: No conflict of interest was reported in the source study.
Data availability: Aggregate MIC results are presented in this manuscript.

Author contributions: Ms Pooja Chouhan contributed to the study work and manuscript preparation. Dr Ramanath K contributed to supervision, interpretation, and critical review. Both authors approved the manuscript.

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