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ORIGINAL ARTICLE

In vitro antifungal resistance and molecular analysis of the SQLE gene in dermatophytosis: a laboratory-based study

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ABSTRACT

Background and Objectives: Dermatophytosis is a common superficial fungal infection that affects the keratinised tissues. This study examines the antifungal resistance mechanisms and molecular detection of squalene epoxidase gene alterations in clinical isolates from patients.
Materials and Methods: A cross-sectional study conducted from January 2019 to December 2020 included 110 clinically suspected dermatophytosis specimens. Microscopy, culture, CLSI M38-A2 broth microdilution testing, and PCR-based ITS and SQLE (520 bp) amplification were performed. MIC50, MIC90, geometric mean, and ranges were analysed using SPSS version 19 with $P < 0.05$.
Results: A total of 110 suspected cases were evaluated, with 78 (70.9%) males. KOH positivity reached 100%, while culture positivity was 47.3% (52/110). *Trichophyton rubrum* accounted for 38.5% isolates. Fluconazole MICs were ≥64 µg/mL. 39/52 isolates showed terbinafine resistance, with 23 expressing the 520 bp SQLE gene.
Conclusion: Terbinafine and caspofungin showed consistent in vitro activity, while fluconazole showed limited activity. Routine susceptibility testing guides drug selection, improves treatment outcomes, and supports rational management of resistant dermatophytosis cases.

Keywords: Antifungal resistance; Gene expression; Minimum inhibitory concentration; Polymerase chain reaction; Squalene epoxidase

INTRODUCTION

Dermatophytosis, a superficial fungal infection affecting 20-25% of the global population, also called tinea or ringworm, is caused by keratinolytic dermatophytes inhabiting soil and human or animal keratinised tissues (1). It is classified into anthropophilic, zoophilic, and geophilic. Anthropophilic species are more often transmitted to humans through direct and indirect modes. Zoophilic species are transmitting infection mostly in animals and rarely in humans. Geophilic species rarely transmit infection in humans and animals; these are a saprophytic nature. There are three genera of dermatophytes: *Microspo-*

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rum, *Trichophyton*, and *Epidermophyton*. *T. rubrum*, *T. mentagrophytes/interdigitale*, *T. tonsurans*, *T. violaceum*, *M. gypseum* (*Nannizzia gypsea*), *M. canis*, *M. audouinni*, and *E. flocosum* are the species that are most often reported. In India, there has been an epidemic-like rise in dermatophytosis over the past few years. This increase is closely related to prolonged skin occlusion and moisture retention caused by non-breathable footwear and tight clothing, which favour fungal survival and keratin invasion, suboptimal personal hygiene that allows sustained fungal colonisation, and socioeconomic constraints that delay early diagnosis and treatment, leading to chronic and recurrent infections. Occupational exposure and climatic conditions with high temperature and humidity further create a favourable microenvironment for fungal growth and transmission. Predisposing host factors, including diabetes mellitus and immunosuppression, impair local cutaneous immunity and reduce fungal clearance, resulting in extensive or persistent disease. Improved access to healthcare facilities and wider availability of mycological diagnostic methods have increased the detection of cases rather than true incidence, therefore contributing to the increase in reported dermatophytosis cases (2-5).

The common clinical signs include annular erythematous scaly lesions that cause pigmentation changes, epidermal thickening, and desquamation. Atypical presentations may resemble other inflammatory or infectious dermatoses and thus mislead clinical diagnosis. These include impetigo, erythema multiforme, eczema, psoriasis, dermatitis herpetiformis, and polymorphous light eruption. Dermatophytosis affects multiple anatomical sites and is classified according to location as tinea corporis, tinea capitis, tinea unguium, tinea cruris, and tinea pedis (6-8).

The establishment of dermatophyte infection depends on fungal adaptation to the host surface. Adhesion occurs through fungal adhesins that bind host carbohydrates present on conidia and surface fibrils (9, 10). Conidia have minimal metabolic activity and occur in a dormant state until suitable germination conditions occur, thus facilitating survival on the keratinised tissues. After attachment, the fungus alters the local environment by increasing surface pH, which improves enzyme function. During invasion, dermatophytes release lipases, phosphatases, and keratinolytic proteases that degrade keratin and provide nutrients. Subsequent metabolic activation maintains tissue invasion and drives disease progression (Fig. 1) (11-14).

Antifungal susceptibility testing guides therapy selection. Available agents include azoles, polyenes, allylamines, echinocandins, griseofulvin, and 5-flucytosine. Most act on ergosterol synthesis: azoles inhibit lanosterol 14α-demethylase, terbinafine targets squalene epoxidase, and griseofulvin interferes with microtubules (15, 16).

Terbinafine is extensively used to treat dermatophytosis, yet resistant infections now occur more frequently, causing treatment failure and recurrence. The drug inhibits squalene epoxidase, depleting ergosterol and accumulating toxic squalene. Resistance arises from point mutations at SQLE residues Leu393, Phe397, Phe415, and His440, reduced target affinity, and increased efflux activity. Enhanced degradation of antifungal agents through salicylate-1-monooxygenase has also been reported. Multidrug resistance is further mediated by ATP-binding cassette transporters (MDR2, MDR3, MDR5) and pleiotropic drug resistance proteins (PDR1). Biofilm formation contributes to antifungal tolerance, recurrent infections, and reduced drug efficacy, while nutrient availability influences antifungal response during biofilm growth (17-21). Therefore, this study aims to evaluate antifungal resistance patterns in dermatophytes with specific emphasis on molecular detection of the SQLE gene.

MATERIALS AND METHODS

The Institutional Ethics Committee approved this cross-sectional study conducted at Vinayaka Mission's Medical College and Hospital, Karaikal District, Puducherry, India, from January 2019 to December 2020. 110 specimens were obtained from clinically suspected patients of dermatophytosis from the outpatient Dermatology and Venerology.

The study included patients with a clinical diagnosis of superficial fungal infection and excluded individuals receiving antifungal therapy or declining consent. Written informed consent was obtained after explanation of procedures. Clinical information recorded comprised age, sex, lesion site and extent, lesion type, duration, prior antifungal exposure, and associated illnesses. Each patient underwent dermatological examination for lesion assessment and clinical classification. The specimens were collected un-

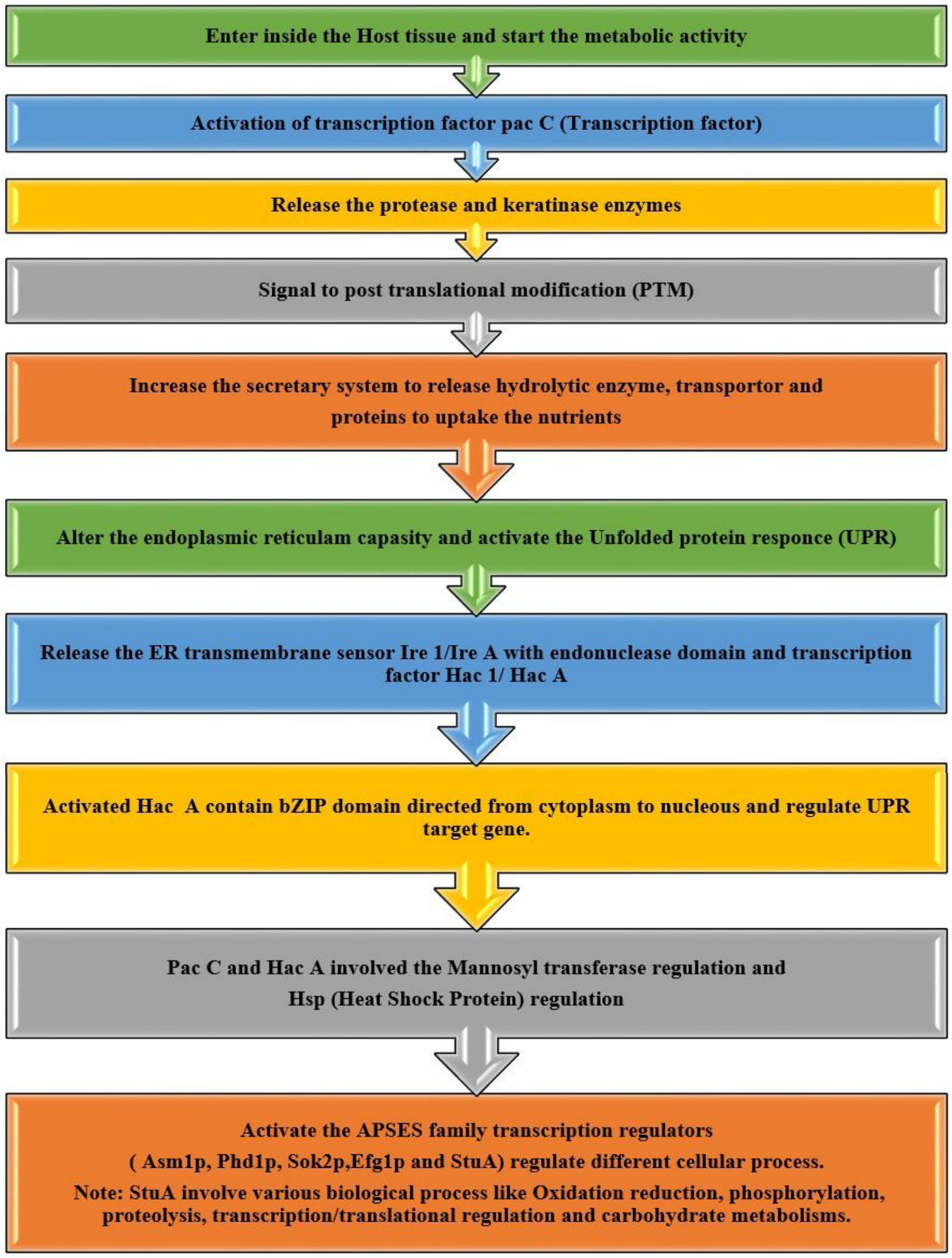

Fig. 1. Pathophysiology of dermatophyte infection.

der aseptic conditions by skin scraping with a sterile scalpel onto sterile thick black chart paper or butter paper, folded securely, and transported promptly to the Mycology section of the Department of Microbiology for further processing.

One portion of the scraping sample was processed with 10% KOH wet mount preparation and observed under 10X to 40X magnification for fungal elements. The remaining sample was inoculated on two sets of Saboraud's Dextrose Agar (SDA) with cycloheximide, and Dermatophyte Test Medium (DTM), incubated at 25°C and 37°C respectively, up to 4 weeks. In culture-positive cases, macroscopical observation, microscopical appearance were noted after performing a lactophenol cotton blue (LPCB) mount using tease mount preparation (22).

Antifungal susceptibility testing. Antifungal susceptibility tests were performed using the broth microdilution method, as suggested by the Clinical Laboratory Standards Institute (CLSI) approved standard M38-A2 guidelines.

Antifungal agents. Powdered fluconazole, terbinafine, griseofulvin, and caspofungin were used. Stock solutions (1000 mg/mL) were prepared in DMSO and stored at -80°C until use. Final drug concentration ranges tested were 0.03-64 for fluconazole, 0.004-8 for terbinafine, 0.125-64 for griseofulvin, and 0.03-16 µg/mL for caspofungin.

Medium. Broth microdilution experiments were conducted in RPMI 1640 medium with Morpholine-propane-sulfonic (MOPS) and without sodium bicarbonate (pH 7.0 ± 0.1 with NaOH). The medium was sterilised by using a sterile membrane filter (0.22 micron).

Inoculation preparation. The fungal isolates were subcultured on Potato Dextrose Agar and incubated at 30°C for 4-5 days to obtain adequate conidial growth. Sterile 0.85% saline (5 mL) was added, and colonies were gently dislodged using a transfer pipette to prepare a suspension. After 10 minutes of settling, conidia were counted with a haemocytometer, and the suspension was adjusted to 1-3 × 10^4 conidia/mL, following CLSI recommendations.

Inoculum quantitation of dermatophytes. A 0.01 mL dilution of the adjusted inoculum was plated on Sabouraud's Dextrose Agar to confirm viable colony-forming units per mL, followed by incubation at 30°C with daily growth monitoring.

Procedure. Dermatophyte inocula were tested in 96-well round-bottom microplates with growth and sterility controls at graded antifungal concentrations and incubated at 28°C for five days. MIC was defined as the lowest inhibitory concentration, with MIC-50 and MIC-90 representing 50% and 90% growth inhibition (23, 24).

Molecular identification. PCR was used to perform the genotypic technique on all clinical isolates that were identified using the phenotypic method.

DNA isolation and molecular analysis. Genomic DNA was extracted from all clinical dermatophyte isolates using a modified phenol–chloroform method. Fungal mycelium was suspended in lysis buffer containing sodium dodecyl sulfate, EDTA, Tris buffer, and sodium chloride, then homogenised and transferred to a microcentrifuge tube. A phenol–chloroform–isoamyl alcohol mixture was added, followed by mixing and centrifugation to separate phases. The aqueous phase was transferred to a fresh tube, and DNA was precipitated using chilled isopropanol and sodium acetate. After centrifugation, the supernatant was discarded, and the DNA pellet was washed with cold ethanol to remove residual impurities. The pellet was air-dried and resuspended in sterile nuclease-free water. Extracted DNA was stored at -20°C until further molecular analysis.

PCR-based identification used amplification of the ITS1 and ITS4 regions with universal fungal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). Each reaction volume was 10 µL and contained PCR master mix, primers, template DNA, and Milli-Q water. Thermal cycling comprised an initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C, annealing at 55°C, and extension at 72°C, with a final extension at 72°C. PCR products were separated on 1.5% agarose gel electrophoresis at 50 V for 45-60 minutes. Bands were examined under ultraviolet illumination to confirm fungal DNA amplification and species-level identification.

SQLE gene detection was performed using primers Drsq1 (TTGCCAACGGAGGTGTAAAG) and Drsq2 (GGGGCCATCTATAAGTCCAAGTT), obtained commercially (Barcode Bioscience, Bangalore, India). PCR reactions were set up in a final volume of 10 µL containing PCR master mix, primers, template DNA, and Milli-Q water. Amplification involved an initial denaturation at 95°C, followed by 30 cycles of denaturation, annealing at 58°C, and extension, with a final extension step at 72°C. The expected 520 bp amplicons were separated on 1.5% agarose gel electrophoresis at 50 V for 45-60 minutes. Bands were visualised under ultraviolet light to identify SQLE-positive isolates (Fig. 2) (25).

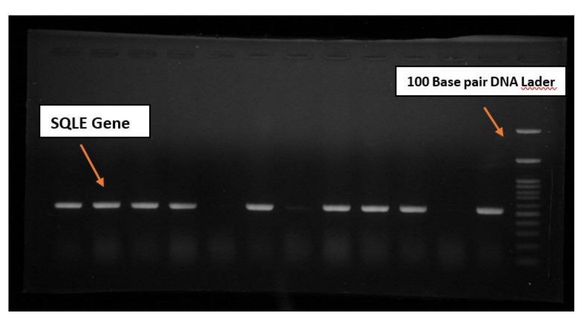

Fig. 2. SQLE gene expression (520bp)

Statistical analysis. Statistical analysis was performed using multiple comparison tests in SPSS software version 19, and statistical significance was set at a P value less than 0.05.

Ethics statement. The Institutional Ethics Committee granted ethical permission for the study (IEC permission No: VMMC/2018/36).

RESULTS

Demographic characteristic. A total of 110 suspected cases were analysed, comprising 78 males (70.9%) and 32 females (29.1%). Male predominance was observed across all age groups. Statistical analysis showed no significant association between age group and gender distribution ($\chi^2 = 5.377$; $p = 0.251$) (Fig. 3). All 110 clinical specimens were positive by direct microscopy using 10% KOH mount. Fungal culture yielded growth in 52 samples (47.3%), while 58 samples (52.7%) were culture negative. The difference between KOH positivity and culture positivity was statistically significant (Fisher's exact test = 78.765; p = 0.0001), indicating higher detection by direct microscopy (Fig. 4).

Species identification of dermatophytes. Dermatophyte identification was performed for all culture-positive isolates using macroscopic colony morphology and microscopic features on lactophenol cotton blue mounts. *T. rubrum* was the most common species, with 20 isolates (38.5%), *T. mentagrophytes* with 14 isolates (26.9%). *Microsporum gypseum* had 8 isolates (15.4%). Less frequently isolated species included *T. tonsurans* (5 isolates, 9.6%), *T. violaceum* (3 isolates, 5.8%), and *M. canis* (2 isolates, 3.8%). *Trichophyton* species constituted the majority of isolates recovered (Table 1).

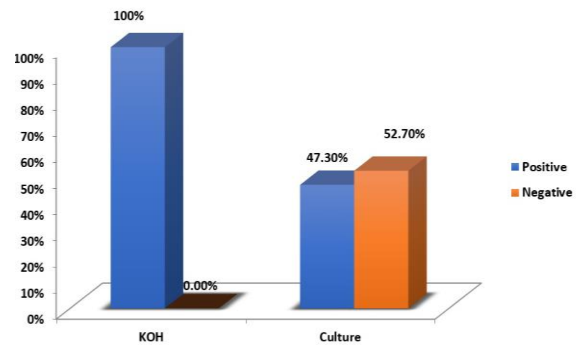

Fig. 4. Overall KOH and culture positive percentage.
Fisher's exact test value-78.765; p-value-0.0001 Sig

Table 1. Dermatophyte isolate distribution

Name of the isolate	Number of isolates, n (%)
T. rubrum	20 (38.5)
T. mentagrophytes	14 (26.9)
T. tonsurans	5 (9.6)
T. violaceum	3 (5.8)
M. gypseum	8 (15.4)
M. canis	2 (3.8)
Total	52 (100)

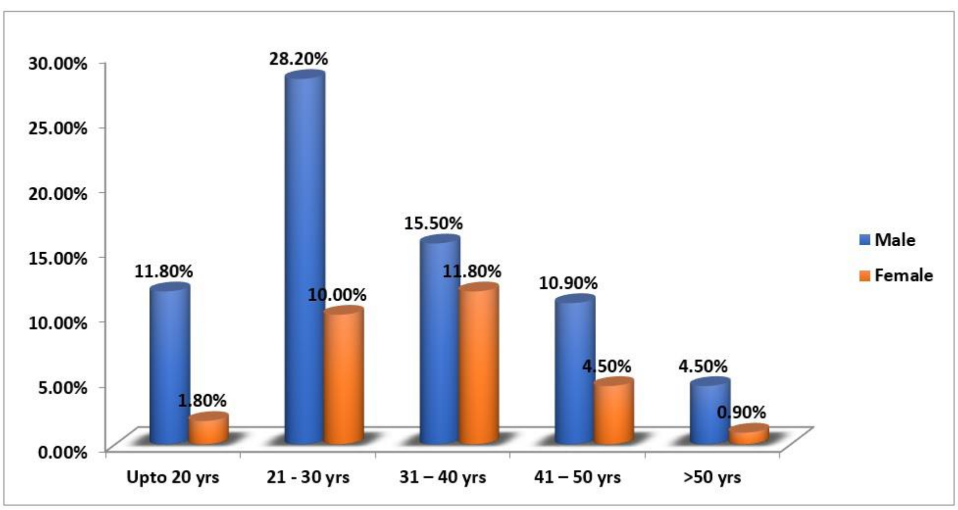

Fig. 3. Age distribution with gender of the patient comparison
Chi Square test value - 5.377; p-value - 0.251 (Not Sig)

Antifungal susceptibility testing. Testing followed the CLSI M38-A2 broth microdilution method, with results interpreted using CLSI criteria. Resistance was defined by high inhibitory values, and fluconazole demonstrated poor activity against most isolates. All isolates showed MIC values ≥64. Terbinafine MICs ranged from 0.125–16 for *T. rubrum* and 0.25-16 for *T. mentagrophytes* (µg/mL). Griseofulvin and caspofungin showed lower MIC distributions across species. Detailed MIC distribution data for each antifungal agent and dermatophyte species are presented in Table 2.

Table 2. Minimum inhibitory concentration (MIC) distribution of antifungal drugs against dermatophyte isolates, n (%)

Dermatophyte species (n)	Antifungal drug (µg/ml)	64	32	16	8	4	2	1	0.5	0.25	0.125	0.062	0.031
T. rubrum (18)	Fluconazole	18 (100)	–	–	–	–	–	–	–	–	–	–	–
	Terbinafine	–	–	8 (44.4)	4 (22.2)	–	–	3 (16.6)	2 (11.1)	–	1 (5.5)	–	–
	Griseofulvin	–	–	–	–	6 (33.3)	2 (11.1)	3 (16.6)	1 (5.5)	4 (22.2)	2 (11.1)	–	–
	Caspofungin	–	–	–	–	–	2 (11.1)	8 (44.4)	7 (38.8)	1 (5.5)	–	–	–
T. mentagrophytes (16)	Fluconazole	16 (100)	–	–	–	–	–	–	–	–	–	–	–
	Terbinafine	–	–	11 (68.75)	1 (6.25)	–	–	1 (6.25)	2 (12.5)	1 (6.25)	–	–	–
	Griseofulvin	–	–	2 (12.5)	–	5 (31.25)	4 (25)	2 (12.5)	3 (18.75)	–	–	–	–
	Caspofungin	–	–	–	–	1 (6.25)	5 (31.25)	3 (18.75)	4 (25)	3 (18.75)	–	–	–
T. tonsurans (5)	Fluconazole	4 (80)	–	1 (20)	–	–	–	–	–	–	–	–	–
	Terbinafine	–	–	–	3 (60)	2 (40)	–	–	–	–	–	–	–
	Griseofulvin	–	–	–	–	–	1 (20)	–	2 (40)	1 (20)	–	1 (20)	–
	Caspofungin	–	–	–	–	–	–	1 (20)	1 (20)	2 (40)	1 (20)	–	–
T. violaceum (3)	Fluconazole	1 (33.3)	1 (33.3)	–	1 (33.3)	–	–	–	–	–	–	–	–
	Terbinafine	–	–	2 (66.6)	–	1 (33.3)	–	–	–	–	–	–	–
	Griseofulvin	–	–	–	–	–	–	2 (66.6)	1 (33.3)	–	–	–	–
	Caspofungin	–	–	–	–	–	1 (33.3)	–	2 (66.6)	–	–	–	–
M. gypseum (8)	Fluconazole	6 (75)	1 (12.5)	1 (12.5)	–	–	–	–	–	–	–	–	–
	Terbinafine	–	–	4 (50)	2 (25)	1 (12.5)	–	–	–	–	1 (12.5)	–	–
	Griseofulvin	–	–	–	–	1 (12.5)	–	3 (37.5)	1 (12.5)	1 (12.5)	2 (25)	–	–
	Caspofungin	–	–	–	–	4 (50)	2 (25)	1 (12.5)	1 (12.5)	–	–	–	–
M. canis (2)	Fluconazole	–	1 (50)	1 (50)	–	–	–	–	–	–	–	–	–
	Terbinafine	–	–	–	–	–	–	1 (50)	–	1 (50)	–	–	–
	Griseofulvin	–	–	–	–	–	–	–	–	1 (50)	1 (50)	–	–
	Caspofungin	–	–	–	–	–	–	–	1 (50)	1 (50)	–	–	–

MIC summary statistics. Fluconazole exhibited uniformly high MIC_{50} and MIC_{90} values (≥64 µg/mL) for *T. rubrum* and *T. mentagrophytes.* Terbinafine showed MIC_{90} values of 16 µg/mL for both species, with a geometric mean of 2.83 µg/mL. Griseofulvin demonstrated lower MIC_{50} values across species, ranging from 0.031 to 0.5 µg/mL. Caspofungin showed the lowest values, with MIC_{50} values between 0.062 and 0.25 µg/mL and MIC_{90} values not exceeding 2 µg/mL in most species. Species-dependent variation in antifungal susceptibility was observed, with higher MIC values consistently noted for fluconazole and increasing MIC_{90} values for terbinafine among *Trichophyton* species (Table 3).

SQLE gene detection and interpretation. Among the 52 dermatophyte isolates tested, 39 isolates demonstrated elevated terbinafine MIC values consistent with phenotypic resistance. PCR amplification of the squalene epoxidase (SQLE) gene revealed that 23 of these 39 terbinafine-resistant isolates showed amplification of the SQLE gene fragment at 520 bp. The remaining resistant isolates lacked SQLE amplification. Gel electrophoresis confirmed the specific target band, with fragment size verified using a 100 bp molecular size marker (Fig. 2). The detection of the SQLE gene in a subset of phenotypically resistant isolates suggests a molecular basis for terbinafine resistance in these strains, while the absence of amplification in other resistant isolates indicates the possible involvement of alternative resistance mechanisms.

DISCUSSION

Dermatophytosis in the present study showed a clear male predominance, with young adults forming the most affected group. A similar age and sex distribution has been reported earlier, where males constituted nearly 81% of cases, and most patients belonged to the 21-40-year age range (26). Greater occupational exposure, increased sweating, frequent physical activity, and prolonged use of tight or occlusive clothing likely contribute to sustained fungal exposure and cutaneous colonisation in this population.

Of the 52 culture-positive cases, 38.46% were *T. rubrum*, followed by *T. mentagrophytes* at 26.92%, *M. gypseum* at 15.38%, and *T. tonsurans* at 9.61%. A similar study shows that *T. rubrum* was a highly isolated human pathogen, followed by *T. mentagrophytes* (27-29). These findings confirm the continued dominance of anthropophilic *Trichophyton* species in dermatophytosis, consistent with global epidemiological trends.

The significant interspecies variation in susceptibility profiles was observed. Generally, fluconazole and terbinafine are considered a common therapeutic strategy for treating dermatophytes. Fluconazole demonstrated limited efficacy across most isolates, with all species showing high MIC values (≥64 µg/ml). *T. rubrum* and *T. mentagrophytes* exhibited complete resistance (100% isolates with MIC at 64 µg/ml), indicating poor activity of fluconazole against these species. Even *T. tonsurans*, *M. gypseum*, and *M. canis* showed reduced susceptibility, therefore suggesting that fluconazole may not be a reliable option for dermatophytosis treatment, particularly in recalcitrant cases. These findings show that fluconazole has inadequate utility in dermatophytosis management due to widespread resistance.

Terbinafine has also been found to be the most resistant overall. For *T. mentagrophytes*, 68.75% of isolates were inhibited at 16 µg/ml, with MIC values distributed across the range, including lower MICs (≤2 µg/ml) in some isolates. *T. rubrum* also showed moderate susceptibility, with 38.8% of isolates exhibiting MICs ≤2 µg/ml. *T. tonsurans* and *M. canis* demonstrated susceptibility at lower MICs, reflecting terbinafine clinical effectiveness. This heterogeneity in MIC distribution indicates species- and strain-specific differences in terbinafine response rather than uniform resistance. Badali H et al. (16), Sharma R et al.(30), Nweze EI et al.(31), Fernandez-Torres B et al. (32), and Adimi P et al. (33) reported high fluconazole values of 32, 64, and 256 µg/mL. Maurya S et al. (34) and Adimi P et al. (33) reported terbinafine values up to 16 µg/mL, with 21.33% and 29.33% of isolates at 2 and 4 µg/mL. These reports were in accordance with

Table 3. Minimum inhibitory concentration (MIC) summary of antifungal drugs against dermatophyte isolates
GM: Geometric Mean

Dermatophyte species (n)	Fluconazole Range (µg/ml)	MIC-50	MIC-90	GM	Terbinafine Range (µg/ml)	MIC-50	MIC-90	GM	Griseofulvin Range (µg/ml)	MIC-50	MIC-90	GM	Caspofungin Range (µg/ml)	MIC-50	MIC-90	GM
T. rubrum (18)	>64	>64	>64	0	0.125–16	0.5	16	2.83	0.125–4.0	0.25	4	1.00	0.25–2.0	0.25	1	0.50
T. mentagrophytes (16)	>64	>64	>64	0	0.25–16	0.5	16	2.83	0.25–8.0	0.5	8	2.00	0.125–2.0	0.125	2	0.50
T. tonsurans (5)	16–64	2	16	5.7	1.0–8.0	0.5	8	2.00	0.031–1.0	0.031	1	0.18	0.062–0.5	0.062	0.5	0.18
T. violaceum (3)	8.0–64	2	16	5.7	2.0–8.0	2	8	4.00	0.250–0.500	0.25	0.5	0.35	0.250–1.0	0.25	1	0.50
M. gypseum (8)	16–64	1	16	4.0	0.125–16	0.125	16	1.41	0.125–4.0	0.125	4	0.71	0.25–2.0	0.25	2	0.71
M. canis (2)	16–32	1	16	4.0	0.125–0.500	0.125	0.5	0.25	0.062–0.125	0.062	0.125	0.09	0.125–0.250	0.125	0.25	0.18

the present findings with reduced susceptibility rather than sudden resistance. Elevated values in selected isolates point to emerging reduced response linked to antifungal exposure and selective pressure.

Griseofulvin showed moderate activity, with better response in *T. rubrum*, *T. mentagrophytes*, and *M. gypseum*, showing values from 0.25 to 2 µg/mL in vitro isolates. However, resistance was observed in some of the species, like *T. tonsurans* and *T. violaceum* thus indicating variable efficacy. This variability could be affected by species-specific differences in drug uptake and metabolic adaptation. Indira G et al. (35) reported the lower range of griseofulvin MIC 0.32 µg/ml and the high range of MIC was 32 µg/ml in other studies (33, 36).

Caspofungin showed activity against most dermatophyte species tested. *T. mentagrophytes*, *T. rubrum*, and *M. gypseum* showed MIC values mainly between 1 and 4 µg/ml. *T. tonsurans*, *T. violaceum*, and *M. canis* showed lower MIC values, reaching 0.5 µg/ml. These findings indicate a measurable in vitro susceptibility of dermatophytes to caspofungin. Echinocandins are not routinely used for dermatophytosis since their data is limited. The low MIC values indicate possible application in infections thus exhibiting poor response to standard antifungal drugs.

In vitro susceptibility testing of six dermatophyte species against fluconazole, terbinafine, griseofulvin, and caspofungin showed clear species-level differences in antifungal response, supporting the need for accurate species identification before treatment selection. Fluconazole demonstrated uniformly poor activity against all tested dermatophytes, indicating widespread reduced responsiveness. Both *Trichophyton* and *Microsporum* species showed limited inhibition, consistent with previous reports of poor fluconazole performance in dermatophytosis (33). These findings confirm that fluconazole has restricted usefulness for dermatophyte infections and should not be relied upon as single-agent therapy, particularly in recurrent or persistent disease.

Terbinafine demonstrated variable in vitro activity across the dermatophyte species tested. Several isolates retained good susceptibility, particularly among *Microsporum* species, while *Trichophyton rubrum* and *T. mentagrophytes* showed heterogeneous responses, with a subset displaying reduced sensitivity. This non-uniform pattern indicates that terbinafine effectiveness differs by species and even among isolates of the same species. Similar variability in terbinafine response has been documented earlier (32-36), suggesting that reduced susceptibility is not uniform but occurs in selected strains. These observations underline the importance of species-level identification and laboratory guidance when selecting terbinafine for therapy.

Griseofulvin demonstrated moderate activity against the dermatophytes examined. Reduced effectiveness was noted in a proportion of *T. mentagrophytes*, *T. rubrum*, and *M. gypseum* isolates, while better responses were observed in *T. tonsurans* and *M. canis*, indicating that drug performance varied by species. Earlier reports also described both good response and resistance in different settings (33, 36), suggesting that griseofulvin activity differs by species and geographic exposure patterns rather than showing uniform effectiveness.

Caspofungin showed good laboratory activity against most dermatophyte species. Lower inhibitory concentrations were observed, particularly in *T. tonsurans* and *M. canis*, while higher values in other species remained lower than those seen with fluconazole or terbinafine. Similar laboratory findings have been reported earlier (16). These results show measurable antifungal activity of caspofungin; however, it is not routinely recommended for dermatophytosis because clinical evidence is limited.

In this study, 39 of 52 dermatophyte isolates (75%) showed phenotypic resistance to terbinafine. PCR analysis identified SQLE gene amplification in 23 of these 39 resistant isolates (58.97%), indicating a major role of SQLE-related mechanisms in terbinafine resistance. A comparable observation was reported by Zahra Salehi et al., where SQLE expression was detected in 2 of 5 terbinafine-resistant isolates (25).

Terbinafine acts by inhibiting squalene epoxidase, which disrupts ergosterol synthesis and causes intracellular accumulation of squalene, leading to fungal growth inhibition. Alterations in the SQLE gene, including point mutations or increased gene expression, have been reported as major resistance mechanisms, particularly in *Trichophyton interdigitale* and *T. rubrum* (37). Increasing resistance has been reported in settings with frequent terbinafine exposure and incomplete treatment courses. The detection of resistant isolates lacking SQLE amplification in this study indicates that terbinafine resistance does not depend on a single mechanism. Other factors, including efflux pump activity and metabolic adaptation, may contribute to resistance and require further evaluation (38, 39).

CONCLUSION

Prolonged incubation for dermatophyte culture limits routine isolation and susceptibility testing, promoting empirical therapy; evaluation of local antifungal susceptibility patterns is therefore essential to guide effective, rational treatment decisions clinically. In vitro testing identified terbinafine and caspofungin as the most consistently active agents, with greater activity against non-*Trichophyton* species. Griseofulvin showed intermediate activity with species-dependent variability. Fluconazole demonstrated poor activity, and reduced terbinafine susceptibility was observed in *T. rubrum* and *T. mentagrophytes*, reflected by elevated MIC-90 values. The findings indicate resistance in frequently isolated species; integrating clinical findings, species identification, and susceptibility results supports accurate antifungal drug selection. Detection of SQLE-related mechanisms provides useful information when phenotypic testing is unavailable. Therapy guided by laboratory and molecular data may improve response, reduce recurrence, and limit unnecessary antifungal exposure. Evaluation of alternative agents remains necessary for resistant dermatophytosis.

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