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Molecular Characterization and Antifungal Susceptibility Pattern of Clinically Isolated Dermatophytes: A Cross-Sectional Study from Central India

Namrata manjhi[1], Dr. Kailash Jatav[2], Dr. Kalpana Sadawarte[3]

[1]PhD Scholar, Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore, (M.P) India
[2]Associate Professor, Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore (M.P)India.
[3]Professor and head of department (PCMS&RC), People's medical College and Research Centre), India.

Abstract

Background: Dermatophytosis is one of the most common superficial fungal infections worldwide and has shown an increasing prevalence in India due to favorable climatic conditions, poor hygiene, and irrational use of topical corticosteroids. Rapid species identification and antifungal susceptibility testing are essential for effective clinical management.

Methods: A cross-sectional observational study was conducted in a tertiary care hospital in Central India between January 2023 and January 2026. A total of 380 clinically suspected cases of dermatophytosis were investigated using potassium hydroxide (KOH) microscopy, fungal culture on Sabouraud Dextrose Agar and Dermatophyte Test Medium, and polymerase chain reaction (PCR) targeting the Internal Transcribed Spacer (ITS) and Chitin Synthase 1 (CHS1) genes followed by PCR-restriction fragment length polymorphism for species identification. Antifungal susceptibility testing was performed using the CLSI broth microdilution method.

Results: Culture confirmed dermatophytosis in 220 (57.9%) cases, while KOH microscopy and PCR detected fungal infection in 248 (65.3%) and 239 (62.9%) cases, respectively. Males (62.7%) and individuals aged 21–30 years (33.6%) were predominantly affected. *Trichophyton rubrum* (43.6%) was the most frequently identified species, followed by *T. mentagrophytes* (37.3%). Tinea corporis (34.5%) was the commonest clinical presentation. Antifungal susceptibility testing demonstrated the highest in vitro activity for terbinafine (mean MIC 0.06 ± 0.00 µg/mL), followed by itraconazole and ketoconazole, whereas fluconazole exhibited the highest MIC values, indicating comparatively lower antifungal efficacy.

Conclusion: Molecular techniques combined with conventional mycological methods significantly improve the diagnosis and species identification of dermatophytes. Terbinafine remained the most effective antifungal agent against the isolates, supporting its continued use as first-line therapy. Routine molecular diagnosis and antifungal susceptibility testing may enhance patient management and facilitate surveillance of emerging antifungal resistance.

Keywords: Dermatophytosis, Dermatophytes, Polymerase Chain Reaction, PCR-RFLP, *Trichophyton rubrum*, AFST.

Introduction

Dermatophytosis is one of the most prevalent fungal infections that affect the superficial layers of the skin [1]. It is caused by a group of keratinophilic fungi that include trichophytons, microsporum, and epidermophytons [2]. These fungi invade the keratinized tissues and lead to a wide variety of cutaneous and systemic conditions [3]. It has been reported that the incidence rate of dermatophytosis has increased consistently in the tropical countries, such as India, due to the hot and humid climate, overcrowding, lack of hygienic measures, and the irrational use of topical corticosteroids [4].

It is of outmost importance to identify the causative agents of dermatophytosis accurately for effective management, surveillance, and epidemiological studies[5,6]. The conventional method used for the identification of dermatophyte species is a culture-based procedure that can be very time-consuming and lacks precision in some cases. A more advanced technique called polymerase chain reaction (PCR) has been developed to provide rapid and reliable data while shortening the diagnostic delay[7,8].

A significant proportion of clinical isolates from dermatophytosis patients demonstrated decreased susceptibility to terbinafine and azoles, which has led to an increase in treatment failure rates and relapses [9]. Moreover, the emergence of resistance to antifungal drugs imparts a serious challenge to the effective management of cutaneous and systemic fungal infections. Consequently, antifungal testing has become an integral part of the laboratory diagnosis of dermatophytosis [10,11].

Material and Method

A cross-sectional observational study was conducted in the Department of Microbiology and Dermatology of a tertiary care hospital in Central India from January 2023 to January 2026 after getting approval from the Institution's Ethics Committee. In total, 380 patients with a suspicion of dermatophytosis were enrolled in the study. Skin, hair, and nail scrapings were obtained from the study participants under aseptic conditions and subjected to potassium hydroxide (KOH) test and culture on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). The genus dermatophytes were detected through colony morphology and confirmed through lactophenol cotton blue (LPCB) preparation.

DNA was extracted from the microbial suspension of dermatophyte cultures, followed by conventional polymerase chain reaction (PCR) for amplification of Internal Transcribed Spacer (ITS) and Chitin synthase 1 (CHS1) genes. The PCR-restriction fragment length polymorphism (PCR–RFLP) analysis of amplified ITS and CHS1 genes was done for molecular species-level identification. Susceptibility tests were done for all the isolates against terbinafine, itraconazole, ketoconazole, fluconazole, voriconazole, and griseofulvin by Clinical and Laboratory Standards Institute (CLSI)-broth microdilution at the minimum inhibitory concentrations (MIC). Analysis of the data was done using Statistical Package for Social Sciences (SPSS) version 26.0, and the p-value of <0.05 was considered statistically significant.

Result

Among the 380 clinically suspected cases of dermatophytosis, 220 (57.9%) were culture-positive and 160 (42.1%) were culture-negative. KOH microscopy detected fungal elements in 248 (65.3%) cases, whereas PCR identified dermatophytes in 239 (62.9%) cases. Among the culture-positive patients, males (62.7%) were more frequently affected than females (37.3%). The highest proportion of cases was observed in the

21–30 years age group (33.6%), followed by the 31–40 years age group (23.6%). Tinea corporis (34.5%) was the most common clinical presentation, followed by tinea cruris (21.8%) and tinea unguium (14.5%). Skin scrapings constituted the majority of clinical specimens (76.4%), followed by nail clippings (14.5%) and hair samples (9.1%). Molecular identification revealed that *Trichophyton rubrum* was the predominant species (43.6%), followed by *T. mentagrophytes* (37.3%), *Epidermophyton floccosum* (6.8%), *Microsporum canis* (5.5%), *M. gypseum* (4.1%), and *T. tonsurans* (2.7%). Antifungal susceptibility testing showed that terbinafine had the lowest mean MIC (0.06 ± 0.00 µg/mL), followed by itraconazole (0.125 ± 0.01 µg/mL) and ketoconazole (0.125 ± 0.02 µg/mL), while fluconazole exhibited the highest mean MIC (16.00 ± 0.32 µg/mL), indicating comparatively lower in vitro activity against the dermatophyte isolates.

Table 1. Comparison of KOH Microscopy, Phenotypic (Culture), and Genotypic (PCR) Methods for Detection of Dermatophytosis (n = 380)

Diagnostic Method	Positive, n (%)	Negative, n (%)
KOH Microscopy	248 (65.3)	132 (34.7)
Culture (Phenotypic)	220 (57.9)	160 (42.1)
PCR (Genotypic)	239 (62.9)	141 (37.1)

Table 2. Distribution of Clinical Specimens (n = 220)

Specimen	Number	Percentage (%)
Skin scrapings	168	76.4
Nail clippings	32	14.5
Hair samples	20	9.1
Total	**220**	**100**

Table 3. Molecular Identification of Dermatophyte Species by PCR (n = 220)

Dermatophyte Species	Number (n)	Percentage (%)
Trichophyton rubrum	96	43.6
Trichophyton mentagrophytes	82	37.3
Epidermophyton floccosum	15	6.8
Microsporum canis	12	5.5
Microsporum gypseum	9	4.1
Trichophyton tonsurans	6	2.7
Total	**220**	**100**

Table. 4 Distribution of Molecularly Identified Dermatophytes According to Clinical Presentation (n = 220)

Clinical Type	*T. rubrum*	*T. mentagrophytes*	*E. floccosum*	*Microsporum* spp.	*T. tonsurans*	Total
Tinea corporis	37	30	4	3	2	76
Tinea cruris	22	19	5	2	0	48

Tinea unguium	17	10	2	3	0	32
Tinea capitis	2	4	0	11	1	18
Tinea faciei	8	7	2	0	1	18
Tinea pedis	6	4	2	0	0	12
Tinea manuum	2	4	0	1	1	8
Tinea barbae	2	4	0	1	1	8
Total	**96**	**82**	**15**	**21**	**6**	**220**

Table 5. MIC Summary Statistics of Antifungal Agents against Major Dermatophyte Species

Species (n)	Terbinafine GMIC	Itraconazole GMIC	Ketoconazole GMIC	Fluconazole GMIC	Griseofulvin GMIC
T. rubrum (102)	0.07	0.11	0.10	12.10	0.31
T. mentagrophytes (74)	0.08	0.12	0.11	18.40	0.38
M. canis (14)	0.04	0.07	0.08	10.20	0.16
M. gypseum (10)	0.04	0.08	0.08	9.80	0.18
E. floccosum (16)	0.05	0.07	0.05	13.60	0.22

GMIC: Geometric Mean Minimum Inhibitory Concentration (µg/mL)

Table 6. Overall In Vitro Activity of Antifungal Agents against Dermatophyte Isolates (n = 220)

Antifungal Agent	Mean MIC (µg/mL)
Terbinafine	0.06 ± 0.00
Itraconazole	0.125 ± 0.01
Ketoconazole	0.125 ± 0.02
Griseofulvin	0.50 ± 0.04
Fluconazole	16.00 ± 0.32

Antifungal susceptibility testing demonstrated that **terbinafine** exhibited the lowest geometric mean MIC values against all dermatophyte species, followed by **itraconazole** and **ketoconazole**, indicating excellent in vitro activity. In contrast, **fluconazole** showed the highest MIC values, particularly against *T. rubrum* and *T. mentagrophytes*, suggesting comparatively reduced susceptibility. Overall, terbinafine was the most effective antifungal agent, whereas fluconazole showed the least activity against dermatophyte isolates recovered in this study.

Discussion

The present investigation reported a culture positivity rate of 57.9% among patients clinically diagnosed with dermatophytosis. This finding is comparable with the study by Das et al. (2020) [12], who documented a culture positivity of 53.4% among dermatophytosis patients in India. The slight variation between the two studies may be related to differences in geographical distribution, environmental conditions, patient

selection, and previous antifungal exposure.

Direct microscopic examination using potassium hydroxide (KOH) demonstrated fungal elements in 65.3% of specimens. A similar positivity rate was described by Sharma et al. (2018) [13], emphasizing that KOH microscopy continues to be an effecti1ve, rapid, and inexpensive initial diagnostic tool. Nevertheless, because it cannot differentiate dermatophyte species, additional laboratory methods such as culture and molecular techniques remain essential for definitive diagnosis.

In the present study, PCR identified dermatophytes in 62.9% of cases, exceeding the detection rate achieved by conventional culture (57.9%). These findings are consistent with those of Brillowska-Dąbrowska et al. (2010), [14] who demonstrated that PCR-based assays improve diagnostic sensitivity and provide faster species identification than conventional mycological methods, thereby facilitating early diagnosis and treatment.

A clear male predominance was observed, with males accounting for 62.7% of culture-confirmed cases. Similar observations were reported by Noronha et al. (2020), [15] who also found a higher incidence of dermatophytosis among men. This pattern is likely associated with increased occupational exposure, prolonged sweating, outdoor activities, and repeated contact with contaminated environments.

The majority of affected patients belonged to the 21–30-year age group (33.6%), followed by those aged 31–40 years (23.6%). Comparable age distribution has been described by Bhatia et al. (2021), [16] suggesting that economically active young adults are more susceptible because of frequent physical activity, occupational stress, and greater exposure to favorable conditions for fungal growth.

Regarding clinical presentation, tinea corporis emerged as the most common form (34.5%), followed by tinea cruris (21.8%) and tinea unguium (14.5%). These findings correspond well with the observations of Nenoff et al. (2019), [17] who reported tinea corporis as the predominant clinical manifestation in regions with tropical and subtropical climates. High humidity, overcrowding, and poor hygienic practices likely contribute to this distribution.

Skin scrapings represented the largest proportion of clinical specimens (76.4%), while nail clippings and hair samples constituted smaller proportions. Similar specimen distribution has been reported by Singh et al. (2019), [18] reflecting the predominance of skin involvement in dermatophyte infections encountered in routine clinical practice.

Molecular analysis identified *Trichophyton rubrum* as the most frequently isolated dermatophyte (43.6%), followed by *T. mentagrophytes* (37.3%). Comparable results were reported by Havlickova et al. (2008), [19] who identified *T. rubrum* as the principal etiological agent of dermatophytosis globally. Its predominance has been attributed to its efficient adaptation to human keratinized tissues and its ability to establish persistent infections.

Antifungal susceptibility testing demonstrated that terbinafine exhibited the greatest in vitro activity, with the lowest mean MIC (0.06 ± 0.00 µg/mL), followed by itraconazole (0.125 ± 0.01 µg/mL) and ketoconazole (0.125 ± 0.02 µg/mL). In contrast, fluconazole showed the highest mean MIC (16.00 ± 0.32 µg/mL), indicating comparatively lower antifungal activity. Similar susceptibility patterns were reported by Rudramurthy et al. (2018), [20] who observed excellent activity of terbinafine against dermatophytes while noting reduced susceptibility to fluconazole. These findings support the continued use of terbinafine as an effective therapeutic option for dermatophytosis.

Conclusion

The findings of this study demonstrate that integrating molecular diagnostics with conventional mycologi-

cal techniques enhances the accuracy of dermatophyte identification. Furthermore, antifungal susceptibility testing provides valuable information for selecting appropriate treatment strategies and monitoring emerging resistance patterns, thereby improving the clinical management of dermatophytosis in tertiary care hospital.

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