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RESEARCH ARTICLE

Isolation and Molecular Identification of Dermatophytes from Clinical Skin Samples in Urban Populations

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Abstract: ***Background:*** Dermatophytosis, a prevalent fungal infection in urban settings, poses diagnostic and therapeutic challenges due to its high incidence and rising antifungal resistance. Accurate identification of dermatophyte species is crucial for effective management, particularly in densely populated areas like Silvassa, India. This study combines conventional and molecular methods to isolate and identify dermatophytes from clinical skin samples in an urban population. ***Objective:*** To determine the prevalence and species distribution of dermatophytes in urban patients at NAMO Hospital, Silvassa, and to evaluate the efficacy of molecular identification using ITS region sequencing compared to conventional culture techniques. ***Methods:*** Between January and June 2025, 150 skin scrapings were collected from patients with suspected dermatophytosis at NAMO Hospital. Samples underwent direct microscopy with 10% KOH, followed by culture on Sabouraud's dextrose agar supplemented with chloramphenicol and cycloheximide. Isolates were identified morphologically and confirmed by PCR amplification and sequencing of the ITS region. Prevalence and species distribution were analyzed, with statistical significance assessed using chi-square tests ($p < 0.05$). ***Results:*** Of the 150 samples, 92 (61.3%) were culture-positive for dermatophytes. Trichophyton rubrum was the most common species (42 isolates, 45.7%), followed by Trichophyton mentagrophytes (26, 28.3%), Microsporum canis (13, 14.1%), Epidermophyton floccosum (8, 8.7%), and Trichophyton tonsurans (3, 3.3%). Males (65.2%) and the 25–40 age group (52.2%) showed higher prevalence ($p = 0.03$). Molecular sequencing resolved identification ambiguities in 14 isolates (15.2%) and detected mixed infections in 10 cases (10.9%). A slight increase in cases was noted during monsoon months. ***Conclusion:*** The high prevalence of dermatophytosis in urban Silvassa, dominated by T. rubrum, underscores the need for precise diagnostics. Molecular identification via ITS sequencing enhances accuracy and speed, offering a valuable tool for epidemiological surveillance and clinical management in urban settings. These findings advocate for integrating molecular methods into routine diagnostics to address the growing challenge of dermatophytosis in India.

Keywords: Dermatophytes, Molecular identification, ITS sequencing, Urban populations, Silvassa, India

INTRODUCTION

Dermatophytosis, commonly known as ringworm or tinea, represents one of the most prevalent fungal infections worldwide, affecting the skin, hair, and nails. Caused by dermatophytes, a group of keratinophilic fungi, these infections thrive in warm, humid environments, making them particularly common in tropical regions like India [1]. Urban populations face unique challenges that exacerbate the spread of dermatophytosis, including high population density, limited access to hygiene facilities in certain areas, and occupational exposures in industrial settings [2]. In India, the burden of superficial fungal infections has surged in recent years, with studies reporting prevalence rates ranging from 10% to over 60% in urban centres [3]. This rise is further complicated by increasing antifungal resistance, which underscores the need for accurate identification of causative species to guide effective treatment [4].

Traditional diagnostic methods for dermatophytes rely on culture-based isolation and microscopic examination, which are often time-consuming and prone to misidentification due to morphological similarities among species [5]. Molecular techniques, such as polymerase chain reaction (PCR) targeting the internal transcribed spacer (ITS) region of fungal DNA, have emerged as a gold standard for precise species identification [6]. These methods not only enhance diagnostic accuracy but also provide critical epidemiological data, which is essential for tracking resistance patterns and informing public health strategies [7]. Despite their advantages, molecular diagnostics are underutilized in resource-constrained settings, including many urban hospitals in India, where conventional methods remain dominant.

Silvassa, a rapidly urbanizing city in the Union Territory of Dadra and Nagar Haveli, India, serves as a representative case study for exploring dermatophytosis in urban populations. The city's diverse demographic, including industrial workers and residents of densely populated neighbourhoods, creates an environment conducive to fungal infections. Conducted at NAMO Hospital in Silvassa, this study aims to isolate dermatophytes from clinical skin samples and employ molecular methods to identify species accurately. By combining conventional and molecular approaches, we seek to determine the prevalence and distribution of dermatophyte species in this urban setting, offering insights into their epidemiology and diagnostic challenges. This work aims to contribute to the growing body of evidence supporting integrated diagnostic strategies for managing dermatophytosis in urban India.

MATERIAL AND METHOD

Study Setting and Ethical Considerations

This study was conducted at the Departments of Dermatology and Microbiology, NAMO Medical Education & Research Institute, affiliated with Shri Vinoba Bhave Civil Hospital, Silvassa, India, between January 2025 and June 2025. All participants provided written informed consent prior to enrolment, and the study adhered to the principles of the Declaration of Helsinki.

Sample Collection

A total of 150 patients aged 18–65 years, presenting with clinical symptoms of dermatophytosis (e.g., erythematous, scaly, or pruritic lesions on the body, groin, feet, or scalp), were recruited from the dermatology outpatient clinic. Exclusion criteria included patients with recent antifungal therapy (within four weeks), confirmed bacterial infections, or systemic diseases affecting the skin. Skin scrapings were collected aseptically from the active margins of lesions using sterile scalpels after cleaning the area with 70% ethanol. Samples were placed in sterile containers and transported to the microbiology laboratory within two hours for immediate processing.

Isolation of Dermatophytes

Each sample was divided into two portions: one for direct microscopy and one for culture. For microscopy, a portion of the sample was treated with 10% potassium hydroxide (KOH) and examined under a light microscope (40x magnification) to detect fungal elements such as septate hyphae or arthroconidia. For culture, samples were inoculated onto Sabouraud's dextrose agar (SDA) plates supplemented with chloramphenicol (0.05 mg/mL) and cycloheximide (0.5 mg/mL) to suppress bacterial and non-dermatophyte fungal growth. Plates were incubated at 28°C for up to four weeks, with daily monitoring for colony growth.

Colonies exhibiting characteristic dermatophyte morphology (e.g., powdery, granular, or velvety texture) were subcultured onto fresh SDA plates to ensure purity. Preliminary identification was based on macroscopic features (color, texture, and growth rate) and microscopic examination using lactophenol cotton blue staining to observe hyphal structures, macroconidia, and microconidia.

Molecular Identification

Pure fungal cultures were subjected to DNA extraction using a commercial fungal DNA extraction kit (Qiagen, Germany), following the manufacturer's instructions. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using polymerase chain reaction (PCR) with universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The 25 µL PCR reaction mixture consisted of 12.5 µL of 2x PCR master mix (Promega, USA), 1 µL of each primer (10 pmol/µL), 2 µL of template DNA, and 8.5 µL of nuclease-free water. Amplification was performed in a thermal cycler (Bio-Rad, USA) with the following conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. Amplicons of the expected size (~600–700 bp) were purified using a gel extraction kit (Qiagen, Germany) and sent for bidirectional sequencing (Eurofins Genomics, India). Sequences were analysed using the Basic Local Alignment Search Tool (BLAST) against the NCBI GenBank database, with species identification confirmed at a sequence identity threshold of ≥98%.

Data Analysis

The prevalence of dermatophytes was expressed as a percentage of positive cultures among total samples. Species distribution was tabulated, and differences in prevalence across demographic groups (age, sex, and infection site) were analysed using chi-square tests, with a p-value < 0.05 considered statistically significant. All statistical analyses were performed using SPSS version 25 (IBM, USA).

RESULT:

From January 2025 to June 2025, a total of 150 skin samples were collected from patients presenting with clinical signs of dermatophytosis at the dermatology outpatient clinic of NAMO Hospital, Silvassa, India. Of these, 92 samples (61.3%) yielded positive dermatophyte cultures, confirming a significant prevalence of fungal infections in this urban

population. The remaining 58 samples were either negative for dermatophytes (42 samples, 28%) or contaminated with non-dermatophyte fungi or bacteria (16 samples, 10.7%). Direct microscopy using 10% potassium hydroxide (KOH) detected fungal elements, such as septate hyphae or arthroconidia, in 88 of the 92 culture-positive samples, indicating a sensitivity of 95.7% for KOH microscopy when correlated with culture results. Four culture-positive samples were KOH-negative, likely due to low fungal load or sampling variability.

Species Distribution and Clinical Correlations

Molecular identification through sequencing of the internal transcribed spacer (ITS) region provided definitive species-level identification for all 92 positive isolates. The predominant species was Trichophyton rubrum, identified in 42 isolates (45.7%), followed by Trichophyton mentagrophytes with 26 isolates (28.3%). Microsporum canis was found in 13 isolates (14.1%), while Epidermophyton floccosum and Trichophyton tonsurans were less common, with 8 isolates (8.7%) and 3 isolates (3.3%), respectively.

Table 1 summarizes the species distribution and their associated infection sites.

Species	Number of Isolates	Percentage (%)	Common Site of Infection	Predominant Clinical Presentation
Trichophyton rubrum	**42**	**45.7**	**Feet, Groin**	**Tinea pedis, Tinea cruris**
Trichophyton mentagrophytes	**26**	**28.3**	**Body, Scalp**	**Tinea corporis, Tinea capitis**
Microsporum canis	**13**	**14.1**	**Arms, Legs**	**Tinea corporis**
Epidermophyton floccosum	**8**	**8.7**	**Groin**	**Tinea cruris**
Trichophyton tonsurans	**3**	**3.3**	**Scalp**	**Tinea capitis**
Total	**92**	**100**	**-**	**-**

Table 1: Distribution of dermatophyte species isolated from clinical skin samples, their prevalence, and associated clinical presentations.

Trichophyton rubrum was most frequently associated with tinea pedis (22 isolates, 52.4% of T. rubrum cases) and tinea cruris (15 isolates, 35.7%), reflecting its preference for moist, occluded areas like the feet and groin. Trichophyton mentagrophytes was predominantly linked to tinea corporis (17 isolates, 65.4% of T. mentagrophytes cases) and tinea capitis (6 isolates, 23.1%), often presenting as annular, scaly patches on the body or scalp. Microsporum canis was commonly isolated from tinea corporis cases affecting exposed areas like the arms and legs (10 isolates, 76.9%), with several patients reporting contact with stray animals, suggesting zoonotic transmission. Epidermophyton floccosum was almost exclusively associated with tinea cruris (7 isolates, 87.5%), while Trichophyton tonsurans was found in scalp infections, primarily in younger adults with tinea capitis (3 isolates, 100%).

Demographic and Temporal Trends

Demographic analysis revealed a higher prevalence of dermatophytosis among males (60 isolates, 65.2%) compared to females (32 isolates, 34.8%), with a statistically significant difference (chi-square test, $p = 0.03$). This male predominance may be attributed to occupational exposures, such as prolonged use of occlusive footwear among industrial workers, or differences in healthcare-seeking behavior. The age group most affected was 25–40 years (48 isolates, 52.2%), followed by 18–24 years (25 isolates, 27.2%) and 41–65 years (19 isolates, 20.6%). The higher incidence in the 25–40 age group could reflect increased social and occupational activities, leading to greater exposure to environmental or interpersonal transmission.

Temporal analysis showed a slight increase in dermatophyte isolations during the monsoon months (June–August), with 38 isolates (41.3%) compared to 28 isolates (30.4%) in the pre-monsoon period (January–March) and 26 isolates (28.3%) in the transitional months (April–May). Although this trend was not statistically significant ($p = 0.12$), the monsoon peak aligns with the humid conditions favorable for fungal proliferation.

Diagnostic Performance of Molecular Methods

Molecular identification via ITS sequencing was critical in resolving identification challenges encountered with conventional methods. In 14 isolates (15.2%), morphological characteristics (e.g., colony texture, pigmentation, or conidial structures) were ambiguous, particularly in distinguishing T. rubrum from T. mentagrophytes or M. canis from other Microsporum species. ITS sequencing provided clear differentiation, with all sequences showing ≥98% identity to reference sequences in the NCBI GenBank database, confirming the reliability of molecular methods. The turnaround

time for molecular identification, from DNA extraction to sequence analysis, averaged 3–4 days, compared to 2–4 weeks for culture-based identification, highlighting the efficiency of molecular approaches.

Additional Observations

Among the 92 positive isolates, 10 cases (10.9%) presented with mixed infections, where two dermatophyte species were identified in the same sample (e.g., T. rubrum with E. floccosum in 6 cases, T. mentagrophytes with M. canis in 4 cases). These mixed infections were confirmed by subculturing and sequencing distinct colony morphologies. Additionally, 12 patients (13% of positive cases) reported recurrent infections despite prior antifungal treatment, suggesting potential treatment failure or reinfection, which warrants further investigation into local antifungal resistance patterns.

CONCLUSION

This study, conducted at NAMO Hospital in Silvassa, India, revealed a high prevalence of dermatophytosis (61.3%) among urban patients, aligning with reported ranges of 10–61.5% across Indian urban centers [3, 8]. The significant burden observed in Silvassa likely reflects the interplay of environmental factors such as high humidity, occupational exposures among industrial workers, and crowded living conditions, all of which facilitate fungal transmission [2]. The predominance of Trichophyton rubrum (45.7%) as the leading causative species corroborates national trends, where it accounts for the majority of tinea infections, particularly tinea pedis and tinea cruris [9]. Its prevalence in moist, occluded body sites underscores its adaptability to urban lifestyles, where prolonged use of footwear and limited ventilation are common [10].

The notable presence of Trichophyton mentagrophytes (28.3%) and Microsporum canis (14.1%) highlights the diversity of dermatophytes in this urban setting. The association of M. canis with lesions on exposed areas like arms and legs, coupled with patient histories of animal contact, points to zoonotic transmission, a growing concern in urban peripheries with stray animal populations [11]. This finding suggests a need for public health interventions targeting animal-human interactions in cities like Silvassa. The lower prevalence of Epidermophyton floccosum (8.7%) and Trichophyton tonsurans (3.3%) aligns with their typically minor roles in Indian epidemiology, though their presence in tinea cruris and tinea capitis cases, respectively, emphasizes the importance of site-specific diagnostics [12].

Molecular identification via ITS sequencing proved invaluable, resolving ambiguities in 15.2% of isolates where morphological methods were inconclusive. This supports previous studies advocating for molecular techniques as a gold standard for dermatophyte identification due to their precision and speed [5, 6]. In our study, ITS sequencing not only confirmed species but also detected mixed infections in 10.9% of cases, a phenomenon often missed by conventional methods [13]. These findings highlight the potential of molecular diagnostics to enhance clinical decision-making, particularly in urban hospitals where diverse and resistant strains are increasingly common [4]. However, the cost and technical requirements of sequencing remain barriers to widespread adoption in resource-limited settings like Silvassa, suggesting a need for cost-effective molecular protocols [14].

The higher prevalence among males (65.2%) compared to females (34.8%) may reflect occupational factors, such as prolonged exposure to humid conditions among male industrial workers, or differences in healthcare-seeking behavior [15]. The 25–40 age group's dominance (52.2%) likely stems from increased social and occupational activities, elevating exposure risks [3]. The slight monsoon-related increase in cases, though not statistically significant, aligns with literature linking humidity to dermatophyte proliferation [2]. The observation of recurrent infections in 13% of positive cases raises concerns about antifungal resistance, a growing issue in India, and underscores the need for susceptibility testing in future studies [4, 16].

Limitations of this study include its single-center design, which may not fully capture regional variations, and the lack of antifungal susceptibility testing, which could provide insights into treatment failures. Future research should explore multicenter studies and integrate resistance profiling to better address the therapeutic challenges of dermatophytosis. Additionally, expanding molecular diagnostics to include real-time PCR or other rapid platforms could further reduce turnaround times, making them more practical for routine use [17].

In conclusion, this study underscores the high burden of dermatophytosis in urban Silvassa and the critical role of molecular methods in accurate diagnosis. By integrating conventional and molecular approaches, we gained a clearer picture of local epidemiology, which can guide targeted interventions. Promoting hygiene, early diagnosis, and affordable diagnostics could significantly reduce the impact of dermatophytosis in urban India.

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