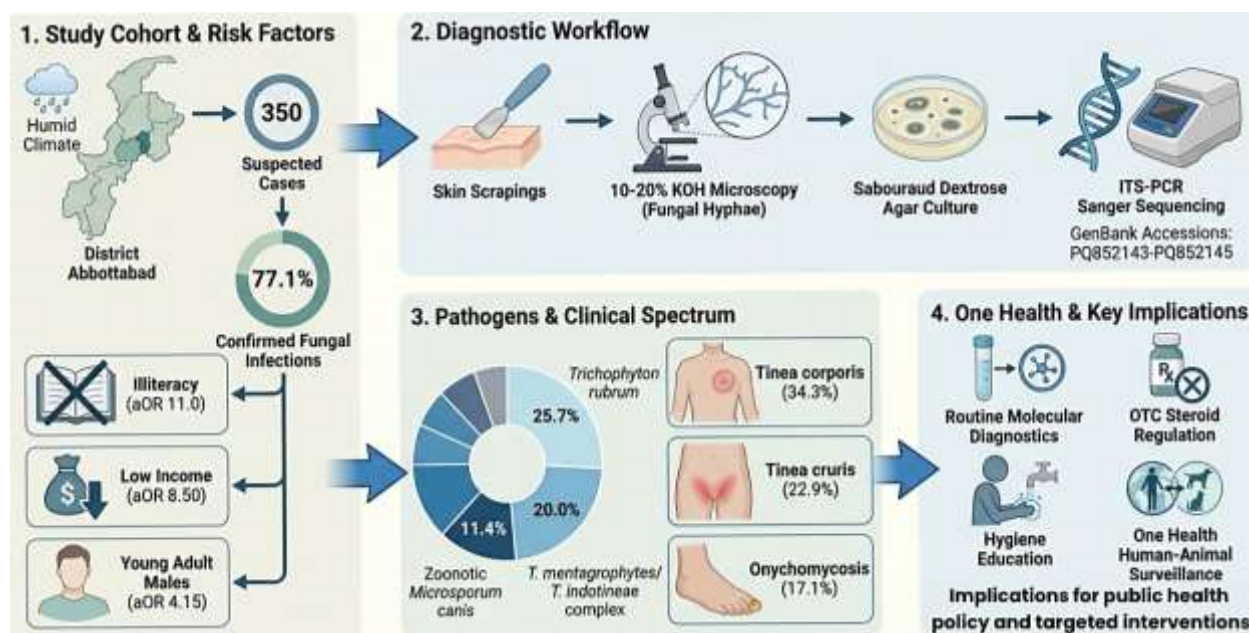


Molecular Epidemiology and Genomic Profiling of Dermatophytes in District Abbottabad: Resolving the *Trichophyton mentagrophytes* / *T. indotineae* Complex and Independent Socio-Demographic Predictors

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Graphical Abstract:



Abstract: Humid subtropical weather condition of District Abbottabad promotes fungal proliferation; hence, dermatomycoses are a growing public health problem in Pakistan. While there is regional molecular epidemiological data and knowledge of socio-demographic drivers is limited, there is a high clinical burden. The aim of this study was to find out the prevalence, clinical spectrum and molecular profile of fungal skin infections in Abbottabad through polyphasic diagnosis. A descriptive cross-sectional study was carried out in 350 cases clinically suspected. A triangulated approach was used for diagnostic confirmation: KOH microscopy, phenotypic culture on Sabouraud Dextrose Agar (SDA) and molecular identification using PCR-Sanger sequencing of the Internal Transcribed Spacer (ITS) region. Multivariate binary logistic regression was used to determine independent predictor(s) of infection. Laboratory confirmation of fungal etiology was made in 77.1% (n=270) cases. The highest prevalence was for *Trichophyton rubrum* (25.7%) followed by the *Trichophyton mentagrophytes*/*T. indotineae* complex (20.0%) and zoonotic *Microsporum canis* (11.4%). The most common clinical presentation was tinea corporis (34.3%). Multivariate analysis revealed that illiteracy (aOR 11.0; 95% CI 4.84–25.02), low income (aOR 8.50; 95% CI 3.92–18.43), and young adult age (aOR 4.15) were potent independent predictors of infection ($p < 0.05$). Representative sequences were lodged in GenBank (PQ852143 - PQ852145). The study highlights the high burden of dermatophytosis within the region, especially in terms of

socioeconomic differences, and the rise in potentially recalcitrant species. The study underscores the clinical need of conducting regular molecular diagnostics and the need for a "One Health" surveillance plan to curb the dissemination of multidrug-resistant fungal pathogens in Pakistan.

Keywords: Dermatomycosis, Molecular Epidemiology, ITS-PCR, Zoonotic Transmission, Abbottabad.

INTRODUCTION

Infections with fungi in humans have historically been an under-studied field of research, in spite of their devastating effects worldwide (Denning, 2024). Epidemiological estimates at present show that they cause over 1.5 million deaths annually (Brown et al., 2024). In 2022, the first Fungal Pathogen Priority List by the WHO was published, marking a significant recognition of this emerging threat (WHO, 2022). This classification of fungal pathogens according to clinical impact, resistance profile and grouped into critical risk, high risk and medium risk groups is based upon published data (Borman et al., 2025). The superficial fungal infections (dermatomycoses) are infections of the upper layer of the skin and currently infect more than 1.5 billion people worldwide (Urban et al., 2021). These infections involve keratinized tissues like the skin, hair, nails and result in high morbidity (Durdu et al., 2026). These were once thought of as insignificant, but now account for over 2 million deaths worldwide each year (Fisher et al., 2022). It is estimated that 20–25% of the world's total human population are affected by dermatophytosis (Klinger et al., 2021).

A high humidity and ambient temperature in South Asia offer a continuing ecological niche for fungal survival (Bhuiyan et al., 2024). Climate change is one of the main drivers and creating 'adaptive thermotolerance' amongst environmental fungi (Rokas, 2022). This thermotolerance means that species normally saprobic are able to overcome the thermal barrier of mammals (Fabri et al., 2023). The increase in temperature in urban areas and global warming is causing thermosensitive fungi to adapt to human body temperature (Casadevall, 2020). District Abbottabad has a humid subtropical climate and experiencing monsoons which make it a unique epidemiological setting in Pakistan (Mahmood et al., 2025). Although significant burden has been seen in local clinics, there is no real molecular surveillance in this part of the world (Mujeeba et al., 2025). The common phenotype attributes shared by fungal pathogens of mammals that facilitate growth within the host tissue support fungal pathogenicity in mammals (Dunker et al., 2021). Inducible morphogenesis, in the case of *Candida albicans*, from yeast to hyphae, often plays a role in virulence (Lachat et al., 2022).

It is a polymorphic switch that is critical for tissue invasion and evasion of host macrophages during infection (Naglik et al., 2023). Critical genes, such as those encoding the cytolytic peptide toxin candidalysin crucial to damage, are hypha-specific (Moyes et al., 2020). Secreted aspartic proteases (Sap4–6) are hydrolytic enzymes that are able to break down host proteins (Lionakis et al., 2023). Extracellular vesicles are also used by fungi to transport a range of biomolecules to the host and are known as "virulence-bags" (Brown Harding et al., 2024). In other species, such as *Cryptococcus*, cells differentiate into "titan cells" that are large, polyploid and resistant to immune clearance (Hommel et al., 2020). Up to 100 μm , these cells are able to block phagocytosis and to trigger non-protective immune responses in the lung (Reuwsaat et al., 2021). Unfortunately, a worrying trend towards chronic and resistant infections has compromised the clinical management of these infections to date (Chowdhary et al., 2022). The *Trichophyton mentagrophytes/T. indotineae* complex is a large concern for international issues (Tang et al., 2022). *Trichophyton indotineae* is a recently described pathogen with a high level of resistance to the 1st line drug, terbinafine (Kano et al., 2020).

MATERIALS AND METHODS

3.1. Study Design and Ethical Approval

The study was conducted in Abbottabad district, it was a descriptive cross-sectional study to find out the molecular profile of fungal skin infections (Uhrlab et al., 2022). The research protocol was carefully planned to meet the epidemiological requirements for the surveys in the South Asian region (Aho-Laukkanen et al., 2024). The Institutional Ethics Committee gave formal approval and gave ethical clearance according to the Declaration of Helsinki. Participants, and/or the legal guardians, gave informed consent in writing before they participated in the study.

3.2. Participant Recruitment and Clinical Assessment

A total of 350 patients with clinical suspicion of superficial mycoses were recruited from different health care facilities (Todd et al., 2026). Pathognomonic features, including scaly erythematous plaques and pruritus were used to make a clinical diagnosis (Khurana et al., 2021). A convenience sampling technique was used to select the participants for screening with a wide representation from the local population (Uhrlab et al., 2022). Data pertaining to detailed demographics, such as age, gender and socioeconomic background were collected through a structured questionnaire. To

minimize the chances of false negative results, patients taking systemic or topical anti-fungal in the previous two weeks were excluded (Todd et al., 2026).

3.3. Specimen Collection and Processing

The focus for specimen collection was the affected areas such as skin scrapings, nail clippings and hair roots (Harel et al., 2024). The saprophytic contamination was removed from the affected area with thorough cleaning with 70% isopropyl alcohol to maximize the amount of information that will be available for diagnosis (Pospischil et al., 2022). Skin scrapings were collected from the edges of the lesions using a sterile scalpel blade (Uhrlab et al., 2022) from the front of the advancing edges of the lesions. Distal nail clippings and subungual debris were obtained from the patient with onychomycosis and placed in sterile containers. (Khurana et al., 2021). Sterile forceps were used to pluck the samples which included the root bulb which is essential for the diagnosis of endothrix and ectothrix infections (Lin et al., 2021). Both specimens of each were split into two halves, with one half being used for parallel phenotypic and molecular analysis (Tang et al., 2021).

3.4. Phenotypic Diagnostics: Standardization

Initial laboratory analysis began with a 10–20% Potassium Hydroxide (KOH) microscopy which quickly allows for the visualization of fungal elements (Khurana et al., 2021). This method is based on the standard dermatological procedure to make immediate clinical decisions. The microscopic examination of slides under 10X and 40X magnification was done to find septate hyphae or arthroconidia (Jeon et al., 2024). Fungal culture was carried out in the standard way with inoculating the specimens into Sabouraud Dextrose Agar (SDA). Bacterial overgrowth was avoided by adding the medium with chloramphenicol (100 µg/mL) (Pham et al., 2024). So, the growth of saprophytic moulds was also inhibited by the addition of cycloheximide (500 µg/mL) (Aho-Laukkanen et al., 2024). Culture plates were kept at a constant temperature of 28°C–30°C until the plates were cultured for up to four weeks (Uhrlab et al., 2022). Growth rate, surface texture and reverse pigmentation were the site of these macromorphological assessments of the colonies (Tang et al., 2021). The micromorphological features were confirmed by preparations of the tease mount using the Lactophenol Cotton Blue (LPCB).

3.5. Molecular Identification (ITS-PCR)

The genomic DNA was purified from the isolates or the clinical specimens directly by using column-based isolation kits (Pospischil et al., 2022). A bead-beating homogenizer was added to the steps for the recovery of DNA from tough keratinized tissues, to improve DNA recovery (AhoLaukkanen et al., 2024). The Internal Transcribed Spacer (ITS) regions of the ribosomal DNA (rDNA) were the PCR targets used. The universal fungal primers, ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') were used for amplification (Burmester et al., 2022). A high fidelity Taq polymerase master mix along with 2 µL of template DNA was used in the total reaction volume of 25 µL. Thermal cycling conditions were: denaturation phase of 5 minutes at 94°C. The denaturation, annealing and extension were repeated 30 times at 94°C, 55°C to 60°C and 72°C, respectively (Burmester et al., 2022). A final extension step was carried out at 72°C for 10 minutes, to make sure that the product was obtained (Uhrlab et al., 2022).

3.6. Gel Electrophoresis and Visualization

The PCR products were separated on 1.5% (w/v) agarose gel in 1X TBE buffer. To confirm the size of the fragments, a molecular size marker, namely 100 bp DNA ladder was used. Ethidium bromide was used to stain the gel and the gels were viewed on a UV transilluminator with a gel documentation system. The distinct bands of 360 bp to 700 bp was an indication of successful amplification of the ITS region (Uhrlab et al., 2022).

3.7. Refinement: Sanger Sequencing and Bioinformatics

To be precise in the species and genotype, representative amplicons were sequenced using Sanger sequencing. When dealing with species in the *Trichophyton complex*, this step is crucial in research to differentiate between the species (Khurana et al., 2021). The sequence data was used to search against curated sequences of the NCBI GenBank database (BLASTn tool) (Aho-Laukkanen et al., 2024). Definitive species identification was required to be $\geq 99\%$ sequence identity (Pospischil et al., 2022). To determine the appearance of the terbinafine resistant species *Trichophyton indotineae* (Aho-Laukkanen et al., 2024) a phylogenetic analysis was done. For this, the sequenced isolates of *T. rubrum* and *T. mentagrophytes* were placed in the phylogenetic tree assembled in MEGA software (neighbor-joining method, 1000 bootstrap replicates) in comparison to other

global *T. rubrum* and *T. mentagrophytes* reference strains retrieved from GenBank, to visually confirm their clustering with the expected clade. Matches returned for the top BLASTn hits were unambiguously species-level matches; there were no matches with an E-value of 0 or coverage of 100%. As part of this study, representative sequences have been deposited in the NCBI GenBank database (PQ852143 for *T. rubrum*, PQ852144 for *T. mentagrophytes* and PQ852145 for *M. canis*), allowing independent confirmation and comparison of genomes in future studies.

3.8. Statistical Analysis

The data obtained from all demographic and experimental variables were processed using Statistical Package for the Social Sciences (SPSS) version 25.0. The prevalence of different fungal pathogens was summarized using descriptive statistics, while the Pearson Chi-square test was used to assess associations between risk factors and infection rates. The p value <0.05 was considered statistically significant for all tests.

RESULTS

4.1. Cohort Characteristics and Laboratory Confirmation: In this study, a total of 350 people with clinically suspected superficial fungal infections were enrolled. 270 cases (77.1%) were confirmed as fungal, using conventional microscopy and molecular ITS-PCR in the laboratory. Of the 80 cases (22.9 %), the rest were found negative across all the modalities of diagnosis, which may be due to other dermatological conditions or from self-medication in the past.

4.2. Demographic and Socioeconomic Risk Factors

The statistical evaluation found that gender, age and socioeconomic status were significant factors in Abbottabad for determining infection prevalence, as summarized in Table 1.

Gender Predominance: A significantly higher prevalence of infection was found in males (81.0%, 170/210) than in females (71.4%, 100/140) with a statistically significant difference between the two with a p-value of 0.012.

Age-wise Distribution: Young adults were the most impacted age group. High level of prevalence was observed in 16--30 years age group (85.7%) and 31-45 years age group (83.3%) implying that high risk is present in the active working age group with $p = 0.001$.

Socio-educational Impact: Very strong inverse correlations were found between educational attainment and rates of infection. The most severely infected were the illiterate (91.7%), compared to the least infected group, which had a higher education (College/University) (50.0%; $p < 0.001$). In addition, low socioeconomic group participants were the most vulnerable (89.5%) as compared to high socioeconomic group participants (50.0%) ($p = 0.007$). These associations are illustrated in Figure 1.

In order to get past univariate percentages, a multivariate binary logistic regression was conducted to find out which socio-demographic factors were still independently predictive of infection (Table 2), after accounting for the other factors. This approach helps determine for instance, if low income is still an important risk factor after controlling for illiteracy, and provides the magnitude of each independent risk as an adjusted odds ratio (AOR) $\pm$ 95 % confidence interval.

4.3. Clinical Presentation and Clinical Diagnosis

Tinea corporis (34.3%), *Tinea cruris* (22.9%) and *Onychomycosis* (17.1%) were the most frequent diseases in the clinical spectrum. Other positive cases were less common, such as *pityriasis versicolor* (14.3%) and *cutaneous candidiasis* (11.4%). The distribution of clinical presentations is shown in Figure 2.

4.4. Molecular Profile of Etiological Agents

The species-level identification by ITS-PCR was used to identify 270 fungal isolates. The main causative agents were the dermatophytes, which were found in 51.4% of the total tested samples. Representative microscopic findings are shown in Figure 3, while the molecular identification profiles are presented in Figure 4 and summarized in Table 3.

- The most common pathogen was *Trichophyton rubrum* which gave a characteristic 400 bp amplicon and was found in 25.7% of samples ($p = 0.001$).
- *Trichophyton mentagrophytes* was the next most common species found with 20.0% and the amplicon size was 360 bp ($p = 0.005$).
- In 11.4% of the cases (400 bp; $p = 0.030$), the zoonotic *Microsporum canis* was detected.
- Non-dermatophyte fungi, such as *Candida albicans* (8.6%, 520 bp) and *Malassezia furfur* (5.7%, 700 bp) were also detected.

DISCUSSION

The epidemiological surveillance of superficial fungal infections in District Abbottabad showed a high laboratory-confirmed prevalence of 77.1%, underlining the substantial burden of dermatomycoses in the region. The humid subtropical climate, with increased temperature and humidity, may favour the survival and transmission of dermatophytes. Males showed a higher prevalence (81.0%) than females, while young adults aged 16–30 years were the most affected group, possibly because of greater physical activity and perspiration. Similar epidemiological concerns have been reported in recent literature, with *Trichophyton indotineae* increasingly recognized as an emerging cause of chronic and recurrent superficial dermatophytosis across different geographical regions (Jabet et al., 2023; Gupta et al., 2026). Global burden analyses have also demonstrated substantial sex- and age-related variation in fungal skin diseases, emphasizing the importance of demographic and environmental factors in disease distribution (Qin et al., 2024; Verma et al., 2021).

Low literacy and socioeconomic status were important factors associated with infection. A large proportion of affected individuals were illiterate (91.7%), while poverty may contribute to unhygienic practices, including sharing towels and bedding, which can facilitate person-to-person transmission. These findings indicate that clinical treatment alone may be insufficient and should be supported by community-based hygiene education to reduce transmission (Verma et al., 2021).

The cohort was dominated by the *Trichophyton complex*, which is concerning because of the increasing emergence of antifungal resistance. South Asia has experienced the emergence of recalcitrant dermatophytosis associated with *Trichophyton indotineae*. Although the present study identified particularly strong associations with low literacy and low income (aOR 11.0 and 8.50, respectively), recent global analyses similarly indicate that fungal skin diseases impose a substantial burden in low- and middle-socioeconomic settings (Qin et al., 2024). These findings suggest that clinical treatment alone may be insufficient and should be complemented by community-based health education, improved access to appropriate diagnosis, and preventive hygiene measures (Dos Santos et al., 2025).

The cohort was dominated by the *Trichophyton complex*, which is concerning because of the increasing emergence of antifungal-resistant dermatophytes. The widespread use of over-the-counter corticosteroid creams may contribute to difficult-to-treat dermatophytosis and tinea

incognito by suppressing inflammation while allowing fungal growth to continue. In addition, 22.9% of cases were negative by KOH microscopy, culture and ITS-PCR. These cases may represent steroid-modified infections or clinical mimics such as eczema and psoriasis. Molecular testing therefore remains valuable for distinguishing active fungal infection from non-fungal or steroid-modified lesions and for supporting rational antifungal treatment (Rouhi et al., 2025; Gupta et al., 2026).

ITS-PCR provided a more precise approach for fungal identification than conventional KOH microscopy and culture, particularly for species-level discrimination. The detection of *Microsporum canis* in 11.4% of cases is epidemiologically important because of its zoophilic nature and potential transmission from domestic or stray animals. Similarly, Blanchard et al. (2023) demonstrated that molecular detection of resistance-associated markers can facilitate rapid identification of terbinafine-resistant dermatophytes. These findings support a One Health approach integrating human diagnostic services with veterinary surveillance, particularly in the humid conditions of Abbottabad that may support fungal persistence and transmission (Dos Santos et al., 2025).

Overall, District Abbottabad represents an important public health setting for dermatomycoses, with a high prevalence of 77.1% and *tinea corporis* as the predominant clinical presentation. Low literacy (aOR 11.0), low income (aOR 8.50), young adult age, and the presence of potentially resistant *Trichophyton* species highlight the need for integrated control measures. The increasing international recognition of *T. indotineae*, its frequent association with terbinafine resistance, and the difficulty of distinguishing it from closely related *Trichophyton* species further emphasize the importance of molecular surveillance (Jabet et al., 2023; Gupta et al., 2024). Future strategies should therefore include affordable molecular diagnostics, appropriate antifungal stewardship, regulation and public education concerning inappropriate corticosteroid-containing preparations, community hygiene education, and One Health surveillance of *M. canis* and other emerging dermatophytes. Recent genomic and diagnostic studies support the feasibility of strengthening laboratory surveillance to detect emerging resistant dermatophytes before they become more widely established (Abdolrasouli et al., 2025; Gupta et al., 2026).

In conclusion, the present study demonstrated a high laboratory-confirmed prevalence of dermatomycoses (77.1%) in District Abbottabad, with *tinea corporis* as the predominant clinical

presentation. Young adult males, low literacy (aOR 11.0), and low income (aOR 8.50) were important associated factors. The identification of *T. mentagrophytes* (20.0%) and *M. canis* (11.4%) highlights the need for accurate molecular identification and surveillance of emerging resistant and zoonotic dermatophytes. Overall, affordable molecular diagnostics, rational antifungal use, community hygiene education, and One Health surveillance are recommended to reduce the disease burden.

CONSENT TO PUBLISH: The authors have no conflict and agree to publish.

AUTHORS CONTRIBUTIONS: Mujeeba and Uqba Mahmood carried out the investigation; Iqra Muzaffar and Jaweria Akram wrote the main manuscript and data analysis; Uma Laila and Fatima Bashir formatted the research paper and prepared all the figures; All authors reviewed the manuscript.

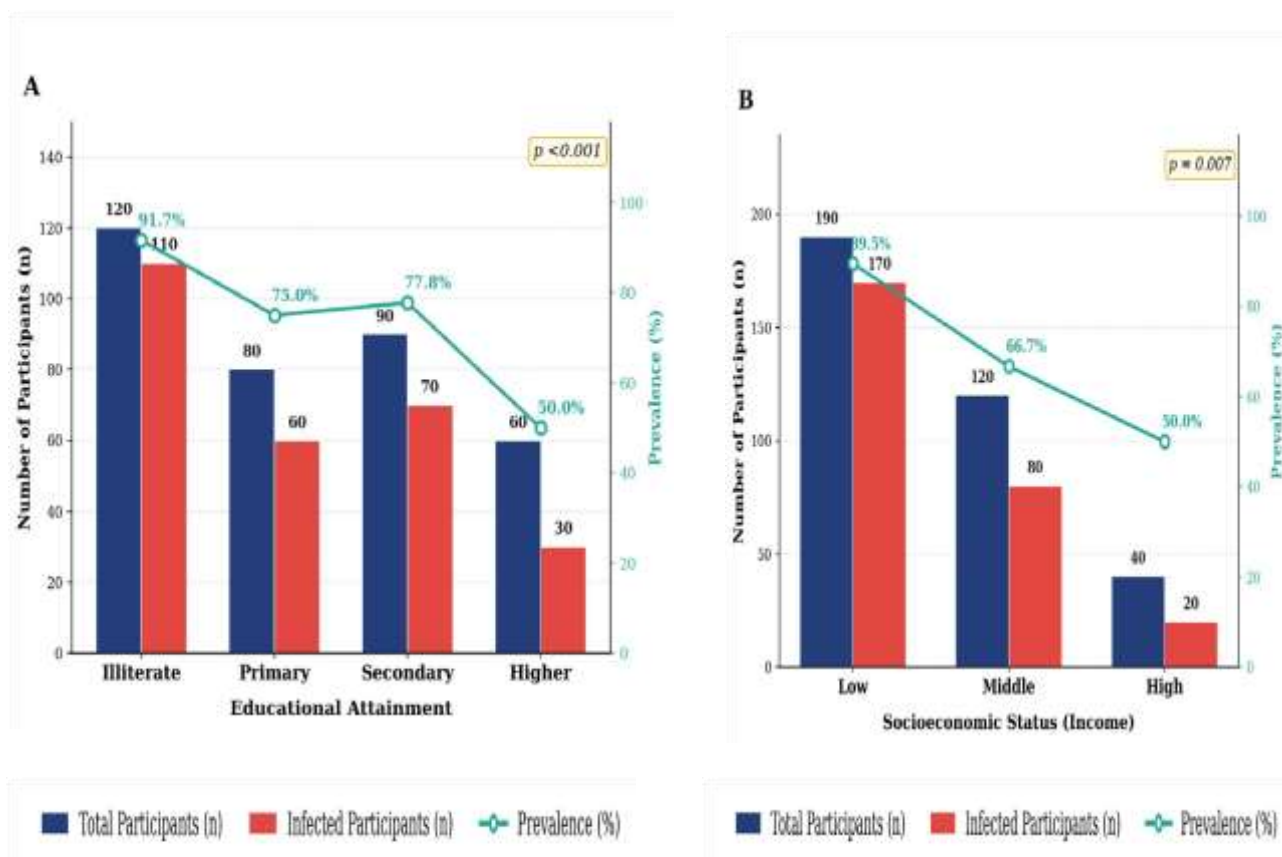


Figure 1. Rate of superficial fungal infection by educational status and socioeconomic status (SES). The educational level (illiterate, primary, secondary, higher education) was compared with the prevalence (n = 350). Prevalence by socio-economic status of the household as measured by income level (Low, Middle, High; n = 350) (B). Educational attainment (χ^2 , $p < 0.001$) and

socioeconomic status (χ^2 , $p = 0.007$) both had an inverse association with the prevalence of infection that was considered to be statistically significant.

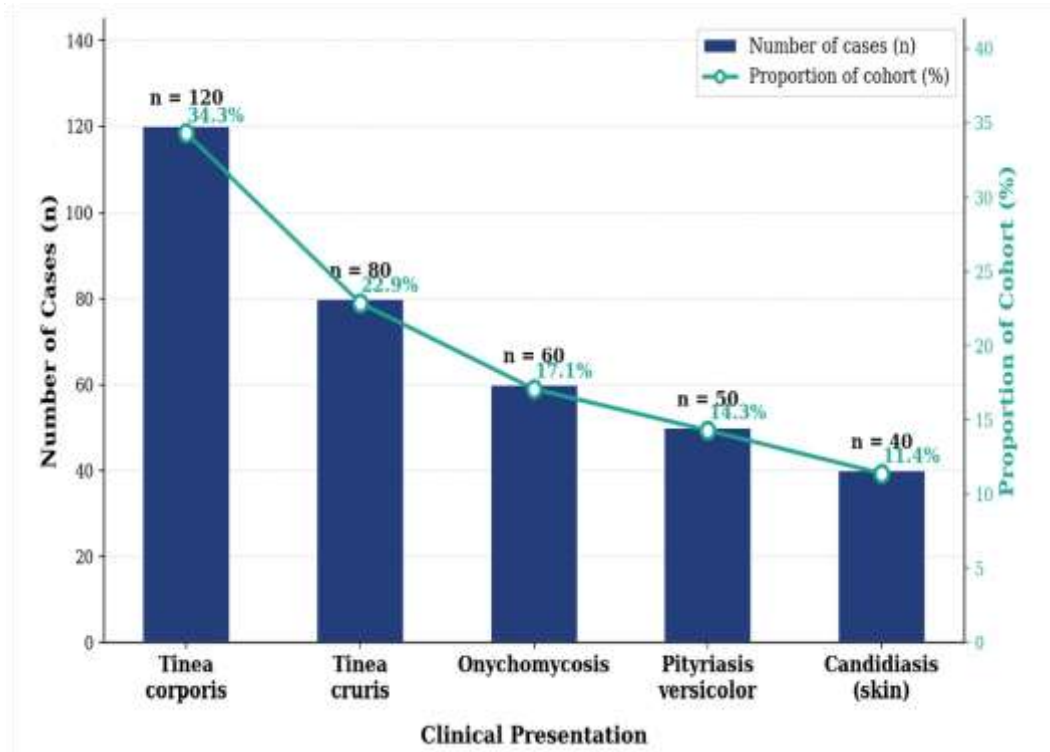


Figure 2. The clinical spectrum of superficial fungal infections in the study group is shown all cases observed in the study population ($N = 350$) were plotted on a bar chart with the number of cases identified clinically or by laboratory testing as confirmed (n , left y-axis) and the proportion of the total number identified by clinical and/or laboratory testing confirmed ($\%$, right y-axis). *Tinea corporis* was the most frequent presentation ($n = 120$, 34.3%), followed by *tinea cruris* ($n = 80$, 22.9%), *onychomycosis* ($n = 60$, 17.1%), *pityriasis versicolor* ($n = 50$, 14.3%), and *cutaneous candidiasis* ($n = 40$, 11.4%). The categories are listed from most to least frequent.

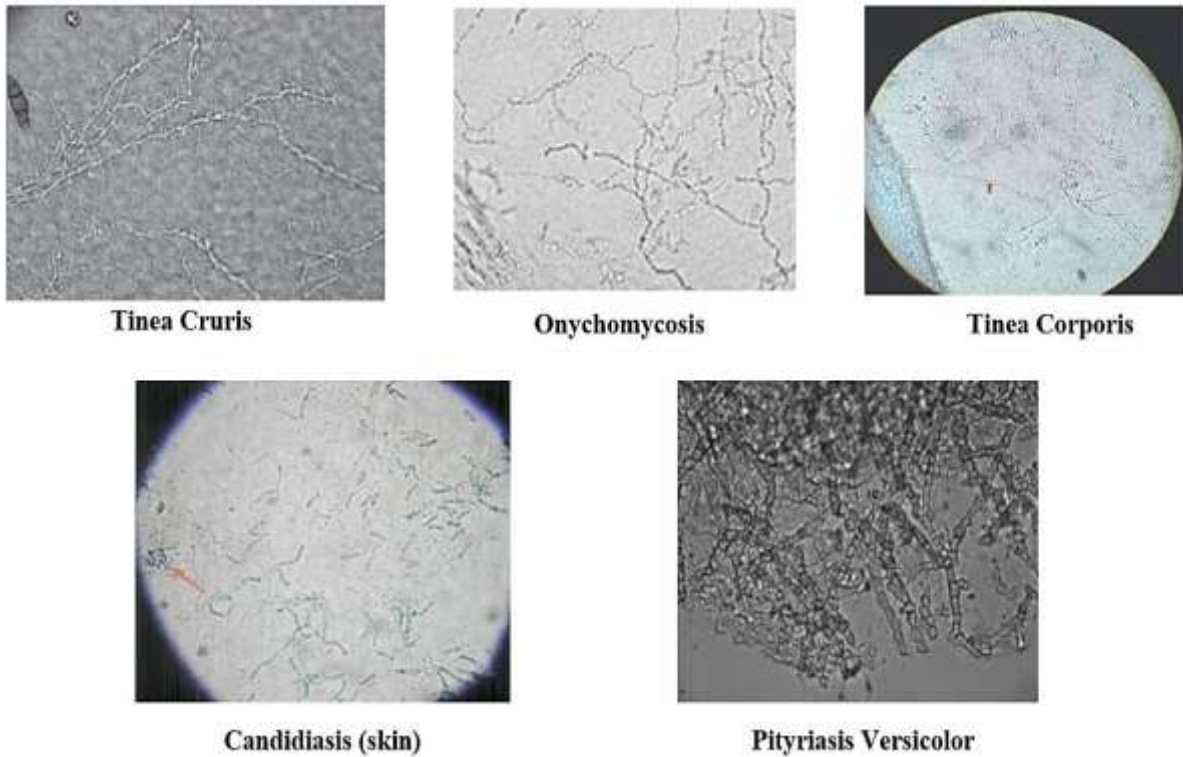


Figure 3. Representative microscopic findings of superficial fungal infections. Representative microscopic fields showing typical fungal elements observed in clinical specimens from patients with *tinea cruris*, *onychomycosis*, *tinea corporis*, *cutaneous candidiasis*, and *pityriasis versicolor*.

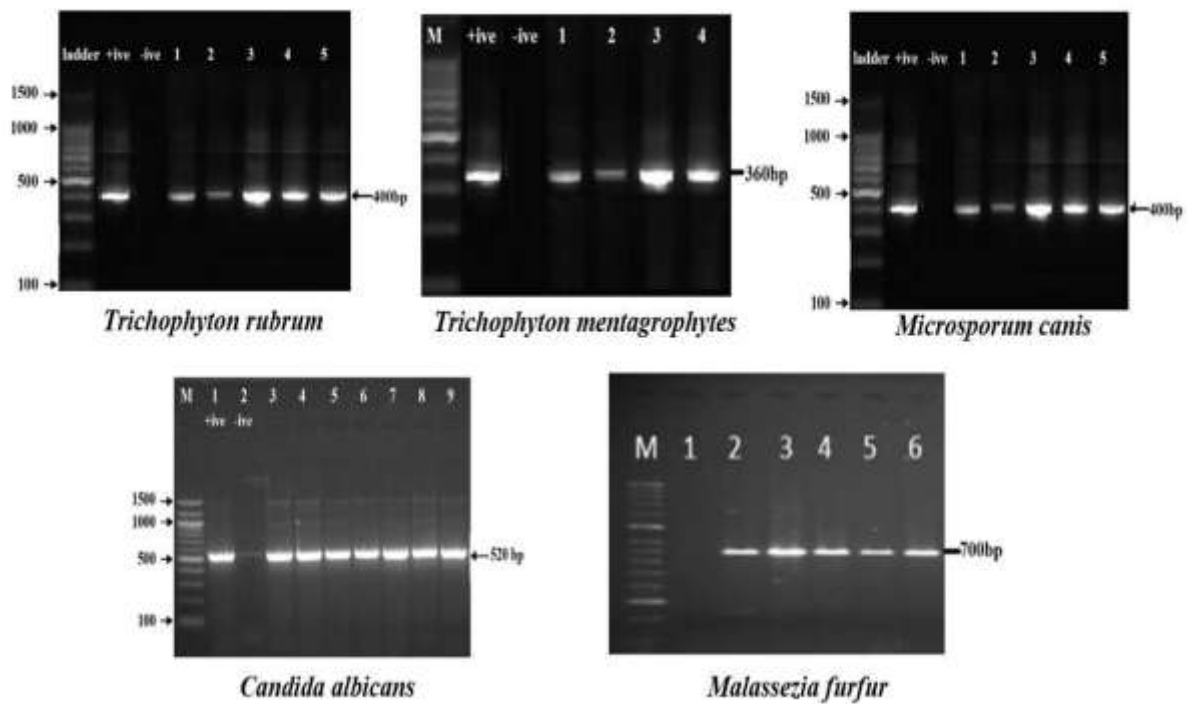


Figure 4. Representative microscopic and molecular findings of superficial fungal infections. Panel A. shows wet-mounted preparations stained with Lactophenol Cotton Blue (LPCB), demonstrating representative fungal elements from the clinical specimens examined at 40× magnification. Panel B shows representative agarose gel electrophoresis profiles of ITS-PCR products from the identified fungal isolates: *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Candida albicans*, and *Malassezia furfur*. The corresponding amplified products are approximately 400 bp, 360 bp, 400 bp, 520 bp, and 700 bp, respectively.

Table 1. Consolidated Demographic and Socioeconomic Factors Influencing Fungal Infection Prevalence

	Sub-category	Total (n)	Positive (n)	Prevalence (%)	p-value
Gender	Male	210	170	81.0	0.012
	Female	140	100	71.4	
Age (Years)	1–15	70	45	64.3	0.001**
	16–30	140	120	85.7	
	31–45	90	75	83.3	
	46+	50	30	60.0	
Education	Illiterate	120	110	91.7	<0.001**
	Primary	80	60	75.0	
	Secondary	90	70	77.8	
	Higher	60	30	50.0	
Income	Low	190	170	89.5	0.007*
	Middle	120	80	66.7	
	High	40	20	50.0	

Table 2: Independent predictors of fungal skin infection identified using multivariate binary logistic regression analysis.

Variable	Comparison	Adjusted OR	95% CI	p-value
Gender	Male vs Female	1.70	1.03–2.81	0.038*
Age	16–30 yrs vs 46+ yrs	4.15	2.12–8.10	<0.001**
Education	Illiterate vs Higher education	11.0	4.84–25.02	<0.001**
Income	Low vs High income	8.50	3.92–18.43	<0.001**

Statistically significant (*) at $p < 0.05$ and highly significant (**) at $p < 0.001$. aOR: Adjusted Odds Ratio; CI: Confidence Interval.

Table 3. Prevalence and amplicon size of fungal species identified by ITS-PCR (n = 270 positive cases).

<i>Fungal Species</i>	Positive Cases (n)	Prevalence (%)	Amplicon Size (bp)	p-value
<i>Trichophyton rubrum</i>	90	25.7	400	0.001**
<i>Trichophyton mentagrophytes</i>	70	20.0	360	0.005*
<i>Microsporum canis</i>	40	11.4	400	0.030*
<i>Candida albicans</i>	30	8.6	520	0.120
<i>Malassezia furfur</i>	20	5.7	700	0.250
<i>Total Positive</i>	270	77.1	--	--

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