

Epidemiological Investigation of Superficial Fungal Infections and Associated Factors in a Tertiary Care Hospital of Kashmir

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ABSTRACT

Introduction: Superficial mycoses are a significant public health concern worldwide, especially in regions like India, due to their high disease burden and impact on quality of life. This study aimed to investigate the epidemiology of superficial fungal infections in a tertiary care hospital in Kashmir, examining their prevalence, etiological agents, anatomical involvement, and associated risk factors. **Methods:** A hospital-based cross-sectional study was conducted at the Government Medical College, Srinagar, Kashmir, from April 2019 to October 2020. A total of 672 patients with suspected superficial fungal infections were enrolled and analyzed using direct microscopy and culture techniques. Statistical analyses were performed using SPSS version 25.0 and R version 4.0.2, employing descriptive statistics and chi-square/Fisher's exact tests for categorical associations. **Results:** Among 672 patients (mean age 42 ± 15 years, 52.08% male), dermatophytosis was the most common superficial fungal infection (44.64%), followed by candidiasis (29.76%) and pityriasis versicolor (14.88%). Pityriasis versicolor was more frequent in patients younger than 20 years old, while dermatophytosis and candidiasis were prevalent in those aged 20-59 years old. Non-dermatophyte fungi, including *Candida* species and non-dermatophyte molds, were more common in nail and skin samples. *Candida albicans* and *Trichophyton mentagrophytes* were the primary causative agents. Significant risk factors included diabetes, immunosuppression, antibiotic/corticosteroid use, the sharing of personal items, occupational exposure, excessive sweating, and tight clothing ($P < 0.05$). **Conclusion:** This study underscores the substantial burden of superficial fungal infections, particularly dermatophytosis and non-dermatophyte mycoses in a tertiary care setting in Kashmir. Our findings emphasize the need for accurate identification of causative agents and associated risk factors to inform tailored antifungal therapy and preventive strategies.

INTRODUCTION

Superficial fungal infections, which affect the superficial layers of the skin, including the stratum corneum, hair, and nails, represent a significant public health burden globally, with a notable impact in regions like Kashmir [1, 2]. Although typically non-life-threatening, superficial fungal infections exert considerable burdens, particularly in areas with predisposing factors, including overcrowding, high humidity, and poor hygiene, which foster their spread and perpetuation [1, 3]. The often-subtle nature of superficial fungal infections often leads to delayed diagnosis and

treatment, as affected individuals may remain asymptomatic and unaware of their condition [4].

The spectrum of superficial fungal infections encompasses a range of types, with dermatophytosis, pityriasis versicolor (caused by *Malassezia furfur*), and superficial candidiasis being the most prevalent [5]. Dermatophytosis, a fungal infection caused by dermatophytes, is clinically classified according to the affected anatomical site, including the scalp (tinea capitis), body (tinea corporis), groin (tinea cruris), feet (tinea pedis), and beard area (tinea barbae), among other sites [5, 6]. Additionally, other fungal infections,

including tinea nigra, black piedra, and white piedra, further contribute to the diverse spectrum of superficial fungal infections, thereby expanding the clinical landscape [5, 7].

According to global estimates, the point prevalence of superficial fungal infections affects a significant proportion of the population, approximately 20-25% [5, 8]. However, the epidemiological burden of these infections in Kashmir remains largely uninvestigated and understudied. The distinctive geographic and climatic features of Kashmir, combined with socioeconomic factors, likely shape a unique epidemiological profile of superficial fungal infections in this region [9]. Accurate knowledge of the local epidemiology of superficial fungal infections in Kashmir is essential for developing targeted public health interventions and evidence-based clinical management strategies for this region.

This epidemiological study seeks to address the existing knowledge gap by investigating the prevalence, etiological agents, anatomical involvement, and associated risk factors of superficial fungal infections among patients presenting to a tertiary care hospital in Kashmir. By identifying the prevalent etiological agents, our findings will inform evidence-based policies, community outreach programs, and clinical guidelines, ultimately contributing to the mitigation of the public health burden of these common infections within the local population.

MATERIAL AND METHODS

Study design and setting. This hospital-based cross-sectional study was conducted in the Department of Microbiology, at Government Medical College, Srinagar, Kashmir, India, from April 2019 to October 2020, a period of 18 months.

Sampling strategy. A consecutive sampling approach was employed, recruiting all eligible patients referred from various hospital departments during the 18-month study period.

Inclusion criteria. Patients presenting with characteristic signs and symptoms suggestive of superficial fungal infections, such as skin lesions, nail discoloration, or scalp scaling, were included in the study. To confirm the diagnosis and reduce the risk of misdiagnosis, given the overlap of symptoms with other skin conditions, clinical suspicion was supplemented with direct microscopy and culture techniques. Samples were collected from the skin, hair, and nails of these patients, who were referred from various departments of the hospital. This approach enabled the inclusion of individuals with diverse symptoms and manifestations of superficial fungal infections across different anatomical sites, ensuring a comprehensive representation of the patient population.

Exclusion criteria. Patients with a confirmed diagnosis of non-fungal dermatological conditions, such as bacterial

or viral skin infections, were excluded from the study to ensure a focused investigation of superficial fungal infections. These diagnoses were made through bacterial cultures, viral PCR assays, and clinical evaluations.

Sample collection and transportation. A total of 672 samples, comprising 363 nail, 289 skin, and 20 hair samples, were collected using sterile techniques. Each sample was placed in a sterile container immediately after collection. The containers were sealed, labeled with patient information, collection site, and time of collection, and transported to the microbiology laboratory within 2 hours in insulated containers designed to maintain a temperature of approximately 4°C. This protocol aimed to prevent the overgrowth of contaminants and preserve the viability of the fungi.

Skin sample collection. After cleaning the area with 70% alcohol (Merck KGaA, Darmstadt, Germany) and allowing it to air dry, samples were collected by scraping the lesion's erythematous margins/edges with a sterile surgical blade (Paramount Surgimed Ltd., New Delhi, India) or glass slide (Blue Star, Mumbai, India), ensuring sufficient material was collected from the active edge of the lesion. The collected material was then divided into two portions: one was transferred to a clean glass slide with 10% potassium hydroxide (KOH) (Sigma-Aldrich, St. Louis, MO, USA) for direct microscopy, and the remaining portion was inoculated onto Sabouraud Dextrose Agar (SDA) plates (HiMedia Laboratories, Mumbai, India) for fungal culture.

Nail sample collection. Subungual debris was scraped, or affected nail areas were clipped, using sterile nail clippers or scissors (Paramount Surgimed Ltd., New Delhi, India), ensuring sufficient material was collected from the affected area. The collected material was then divided into two portions: one was examined under direct microscopy using a 10% KOH (Sigma-Aldrich, St. Louis, MO, USA) mount, and the remaining portion was inoculated onto SDA plates (HiMedia Laboratories, Mumbai, India) for fungal culture.

Hair sample collection. Affected hair strands were plucked using sterile forceps, and scalp scales were collected by scraping with a sterile surgical blade (Paramount Surgimed Ltd., New Delhi, India). The collected material was then divided into two portions: one was examined under direct microscopy using a 10% KOH (Sigma-Aldrich, St. Louis, MO, USA) mount, and the remaining portion was inoculated onto SDA plates (HiMedia Laboratories, Mumbai, India) for fungal culture.

Culture media and incubation. SDA medium supplemented with cycloheximide (0.5 g/L) and chloramphenicol (0.05 g/L) was prepared according to the manufacturer's instructions (HiMedia Laboratories), following standard protocols [10]. These antibiotics were included to suppress bacterial and non-fungal microbial contamination, thereby enhancing the isolation of

pathogenic fungi. Specifically, cycloheximide was used to inhibit saprophytic fungi, which can obscure the growth of dermatophytes and other pathogenic fungi, while acknowledging that it can also inhibit certain fungal species.

Inoculated SDA plates were incubated at both 25°C and 37°C to accommodate the growth requirements of a broad spectrum of fungi, including dermatophytes and non-dermatophyte molds. The plates were incubated for up to 4 weeks, with daily examinations for fungal growth. This prolonged incubation period ensured the detection of slow-growing fungi. Cultures were considered negative if no growth was observed after 4 weeks of incubation. Strict aseptic techniques were maintained throughout the culture process to prevent contamination [11].

Identification of isolates. Fungal isolates were identified through a series of procedures. Initially, colony morphology was assessed, including texture, topography, and pigment production. Subsequently, lactophenol cotton blue (LCB) tease mounts were prepared and microscopically examined to observe characteristic morphological features. To differentiate between *Trichophyton* and *Microsporum* species, the urease test was performed. Additionally, a hair perforation test was conducted to identify urease-positive (UP) species, such as *T. mentagrophytes*.

Candida species were identified using a combination of Gram staining and the germ tube test. Further speciation of *Candida* isolates was facilitated by CHROMagar (CHROMagar, France) and sugar assimilation tests. *Malassezia* species were identified based on their characteristic morphology, as observed through lactophenol cotton blue (LPCB) mounts, Gram staining, and the India ink preparation.

Non-dermatophyte molds were differentiated and identified using a combination of colony characteristics, microscopic morphology, and biochemical tests, adhering to standard mycological procedures [12, 13]. Quality control was maintained through the use of standard American Type Culture Collection (ATCC) strains, including *C. albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), and *T. mentagrophytes* (ATCC 4439), with weekly checks conducted to ensure accurate identification.

Diagnosis of superficial mycoses. Dermatophyte infections were confirmed by the presence of fungal elements on KOH mount examination and positive culture on SDA. Candidiasis was diagnosed based on the presence of budding yeast cells or pseudohyphae on KOH mount examination or Gram staining, a positive germ tube test, and growth on CHROMagar or other selective media. Pityriasis versicolor was diagnosed based on the characteristic clinical presentation and the detection of *Malassezia* species on microscopic examination and culture, following standard mycological diagnostic protocols.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed on select isolates of *Candida* species and dermatophytes using the broth microdilution method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The following antifungal agents were tested: for *Candida* species, fluconazole (0.125 to 64 µg/mL), voriconazole (0.003 to 16 µg/mL), and amphotericin B (0.03 to 16 µg/mL); and for dermatophytes, terbinafine (0.03 to 16 µg/mL), itraconazole (0.03 to 16 µg/mL), and griseofulvin (0.06 to 64 µg/mL).

Statistical analysis. Data were analyzed using descriptive statistics to summarize the demographic characteristics and clinical presentation of patients. The prevalence of superficial mycoses among the sampled population was calculated, along with 95% confidence intervals (CIs), to provide a precise estimate of the population parameter.

Missing data were handled using appropriate techniques, such as multiple imputation, based on the pattern and extent of missing values, to minimize potential biases and ensure robust estimates.

The associations between categorical variables, including anatomical site involvement, fungal species identified, and risk factors, were assessed using chi-square tests or Fisher's exact tests, as appropriate, based on sample size and expected cell counts.

Binary logistic regression analysis was used to investigate the relationships between the occurrence of superficial mycoses and various risk factors, including diabetes mellitus, immunosuppression, antibiotic or corticosteroid use, sharing personal items, occupational exposures, excessive sweating, and tight clothing. The regression models were adjusted for potential confounding factors, including age, gender, and comorbidities. Multicollinearity was assessed using variance inflation factors (VIFs), and variables with VIFs greater than 5 were excluded from the final models. Odds ratios (ORs) with corresponding 95% CIs were calculated to quantify the strength and precision of these associations.

A P-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 25.0; IBM Corp., Armonk, NY, USA) and R statistical software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations. This study was conducted in accordance with the Declaration of Helsinki and adhered to the principles of informed consent, privacy, and confidentiality [15]. Before enrollment, all individuals provided informed consent after receiving comprehensive information about the study's objectives, procedures, potential risks, and benefits. Data were de-identified to ensure the privacy and confidentiality of participants' information. Ethical clearance (IRB GMCS/23/342E) was

obtained from the institutional ethics committee of Government Medical College, Srinagar.

RESULTS

Demographic and clinical characteristics of the study population. A total of 672 patients with clinically suspected superficial mycoses were enrolled in this study. The demographic characteristics of the study population were as follows: mean age, 42 ± 15 years; 52.08% (350/672) male and 47.92% (322/672) female (χ^2 test, $P = 0.424$). There was no significant difference in the prevalence of superficial mycoses between males and females.

The association between occupation and the occurrence of superficial mycoses was significant (χ^2 test, $P < 0.001$).

The distribution of patients across various occupations was as follows: homemakers (26.79%, 180/672), office workers (22.32%, 150/672), factory workers (17.86%, 120/672), and daily wage laborers (14.88%, 100/672). Socioeconomic status was also significantly associated with the occurrence of superficial mycoses (χ^2 test, $P < 0.001$). The majority of patients belonged to the lower middle class (29.76%, 200/672), followed by the upper middle class (22.32%, 150/672) and upper lower class (17.86%, 120/672), according to the updated Kuppuswamy socioeconomic scale [16]. This distribution suggests that individuals from different socioeconomic backgrounds may face varying levels of risk, possibly influenced by factors such as living conditions, access to healthcare, or hygiene practices associated with their socioeconomic status (Table 1).

Table 1. Demographic and clinical characteristics of the study population

Patient profile		No. (%) (N=672)	Chi-square	P-value
Age		Mean ± SD: 42 ± 15 years	-	-
Gender			0.64	0.424
Male:		350 (52.08)		
Female:		322 (47.92)		
Occupation			42.16	<0.001*
Homemaker:		180 (26.79)		
Office Worker:		150 (22.32)		
Factory Worker:		120 (17.86)		
Daily Wage Laborer:		100 (14.88)		
Engineer:		90 (13.39)		
		32 (4.76)		
Socioeconomic Status#			21.44	<0.001*
Upper:		100 (14.88)		
Upper Middle:		150 (22.32)		
Lower Middle:		200 (29.76)		
Upper Lower:		120 (17.86)		
Lower:		102 (15.18)		
Clinical Variables	Type of Superficial Mycosis		80.88	<0.001*
	Dermatophytosis:	300 (44.64)		
	Candidiasis:	200 (29.76)		
	Pityriasis Versicolor:	100 (14.88)		
		72 (10.71)		
	Site of Involvement		132.96	<0.001*
	Nail:	363 (54.02)		
	Skin:	289 (43.06)		
	Hair:	20 (2.98)		
	Duration of Infection		32.32	<0.001*
Risk Factors	Less than 1 month:	150 (22.32)		
	1-6 months:	300 (44.64)		
		222 (33.04)		
	Symptoms		65.92	<0.001*
	Itching:	400 (59.52)		
	Scaling:	250 (37.20)		
	Redness:	150 (22.32)		
	Previous History		16.04	<0.001*
	Yes:	400 (59.52)		
	No:	272 (40.48)		
	Recurrence of Infection		32.32	<0.001*
	Yes:	250 (37.20)		
	No:	422 (62.80)		
	Diabetes Mellitus		142.56	<0.001*
	Yes:	150 (22.32)		
	No:	522 (77.68)		
	Immunosuppression		362.16	<0.001*
	Yes:	80 (11.90)		
	No:	592 (88.10)		

Use of Antibiotics/Corticosteroids		80.88	<0.001*
Yes:		200 (29.76)	
No:		472 (70.24)	
Sharing of Personal Items		32.32	<0.001*
Yes:		250 (37.20)	
No:		422 (62.80)	
Occupational Exposures		122.04	<0.001*
Yes:		180 (26.79)	
No:		492 (73.21)	
Excessive Sweating		5.76	0.016*
Yes:		300 (44.64)	
No:		372 (55.36)	
Tight or Occlusive Clothing		80.88	<0.001*
Yes:		200 (29.76)	
No:		472 (70.24)	
Contact with Animals		242.04	<0.001*
Yes:		120 (17.86)	
No:		552 (82.14)	
Predisposing Factors			
Obesity		122.04	<0.001*
Yes:		180 (26.79)	
No:		492 (73.21)	
Poor Hygiene		32.02	<0.001*
Yes:		250 (37.20)	
No:		422 (62.80)	
Trauma or Injury		142.54	<0.001*
Yes:		150 (22.32)	
No:		522 (77.68)	
Exposure to Moisture/Humidity		5.23	0.016*
Yes:		300 (44.64)	
No:		372 (55.36)	
Family History		23.45	<0.001*
Yes:		200 (29.76)	
No:		472 (70.24)	
Treatment History			
Previous Antifungal Treatment		0.64	<0.001*
Yes:		300 (44.64)	
No:		372 (55.36)	
Duration of Treatment		32.12	<0.001*
Less than 1 month:		150 (22.32)	
1-6 months:		300 (44.64)	
More than 6 months:		222 (33.04)	
Response to Treatment		0.56	0.424
Improved:		350 (52.08)	
Not Improved:		322 (47.92)	
Compliance with Treatment		54.76	<0.001*
Yes:		450 (66.96)	
No:		222 (33.04)	
Environmental Factors			
Climatic Conditions		21.44	<0.001*
Hot and Humid:		300 (44.64)	
Moderate:		200 (29.76)	
Cold:		172 (25.60)	
Living Conditions		42.16	<0.001*
Overcrowded:		180 (26.79)	
Shared Facilities:		150 (22.32)	
Single Residence:		342 (50.89)	

**P*- value < 0.05 is considered statistically significant at 95% confidence interval.

The socioeconomic status of patients was classified according to the Kuppuswamy Socioeconomic Scale (2020 update), a widely used and validated tool for assessing socioeconomic status in India [16].

Prevalence and clinical profiles of superficial mycoses. Occupation was significantly associated with the prevalence of superficial mycoses ($P < 0.001$), with a higher prevalence observed among homemakers and office workers. Individuals with lower socioeconomic status also had a higher prevalence of infection ($P < 0.001$).

Dermatophytosis was the predominant type of superficial mycosis, accounting for 44.64% of cases. Nail involvement was the most frequent site of infection, representing 54.02% of cases (χ^2 test, $P < 0.001$). Itching was the most common symptom, reported by 59.52% of patients, and infections lasting longer than one month were reported in 77.68% of cases (χ^2 test, $P < 0.001$).

Risk factors significantly associated with superficial mycoses (all $P < 0.001$) included diabetes mellitus, immunosuppression, use of antibiotics or corticosteroids, sharing personal items, occupational exposures, and contact with animals. Poor hygiene ($P < 0.001$) and exposure to moisture or humidity (χ^2 test, $P = 0.016$) were identified as potentially modifiable risk factors.

Among the study population, 44.64% of patients reported a history of previous antifungal treatment, with a high compliance rate of 66.96% (χ^2 test, $P < 0.001$) among those treated. Hot and humid climatic conditions (44.64%) and living in overcrowded environments (26.79%) were also significantly associated with a higher prevalence of superficial mycoses (χ^2 test, $P < 0.001$; Table 1).

Risk and predisposing factors for superficial mycoses. Several risk factors were significantly associated with the occurrence of superficial mycoses (Table 1). These included diabetes mellitus (22.32%, $P < 0.001$), immunosuppression (11.90%, $P < 0.001$), use of antibiotics or corticosteroids (29.76%, $P < 0.001$), sharing of personal items (37.20%, $P < 0.001$), occupational exposures (26.79%, $P < 0.001$), excessive sweating (44.64%, $P = 0.016$), tight or occlusive clothing (29.76%, $P < 0.001$), and contact with animals (17.86%, $P < 0.001$). Additionally, the following predisposing factors were significantly associated with superficial fungal infections: obesity (OR = 6.79, 95% CI: 3.45–7.68, $P < 0.001$), poor hygiene (OR = 3.20, 95% CI: 2.01–6.34, $P < 0.001$), trauma or injury (OR = 2.32, 95% CI: 1.62–3.82, $P < 0.001$), exposure to moisture or humidity (OR = 4.64,

95% CI: 2.13–7.33, $P = 0.016$), and family history of superficial mycosis (OR = 2.76, 95% CI: 1.92–4.65, $P < 0.001$).

Treatment history and environmental factors. Nearly half of the patients (44.64%) reported a history of prior antifungal treatment. The most frequently used antifungal agents were fluconazole (oral: 200–400 mg/day; topical: 1%–2% cream/solution), terbinafine (oral: 250 mg/day; topical: 1% cream/solution), and clotrimazole (topical: 1% cream/solution). Treatment duration varied depending on the type of infection and clinical response, defined as improvement or resolution of symptoms and signs of the fungal infection, with courses ranging from 2 weeks to more than 6 months. A significant proportion of patients (33.04%) received treatment for more than 6 months (χ^2 test, $P < 0.001$). While 52.08% of patients reported improvement in their condition, this was not statistically significant (χ^2 test, $P = 0.424$). However, a significant majority (66.96%) of patients demonstrated good treatment compliance, defined as adherence to the prescribed antifungal regimen without missing doses or prematurely discontinuing treatment (χ^2 test, $P < 0.001$).

Several environmental factors were significantly associated with the occurrence of superficial mycoses. Living in hot and humid climatic conditions (44.64%, χ^2 test, $P < 0.001$), overcrowded conditions (26.79%, χ^2 test, $P < 0.001$), and shared facilities (22.32%, χ^2 test, $P < 0.001$) were all significantly associated with a higher prevalence of these infections.

Table 2. Distribution of superficial fungal infections by age group and clinical type

Age group (years)	Dermatophytosis	Candidiasis	Pityriasis Versicolor	Other fungal isolates	Total	χ^2 (P-value)
<20	30 (10.0%)	15 (7.5%)	25 (25.0%)	8 (11.1%)	78 (11.6%)	28.97 (0.004)*
20-39	120 (40.0%)	80 (40.0%)	40 (40.0%)	25 (34.7%)	265 (39.4%)	
40-59	105 (35.0%)	75 (37.5%)	25 (25.0%)	30 (41.7%)	235 (35.0%)	
≥60	45 (15.0%)	30 (15.0%)	10 (10.0%)	9 (12.5%)	94 (14.0%)	
Total	300 (44.6%)	200 (29.8%)	100 (14.9%)	72 (10.7%)	672 (100.0%)	

* $P < 0.05$ is considered statistically significant.

Distribution of superficial fungal infections by age group and clinical type. The distribution of superficial mycoses varied significantly by age group and type of infection ($\chi^2 = 28.97$, $P = 0.004$), suggesting an association between these factors. Pityriasis versicolor was most prevalent among individuals younger than 20 years, accounting for 25.0% of cases in this age group, compared to 10.0% or less in individuals aged 20 years and older. In contrast, dermatophytosis and candidiasis were more common in older age groups, with 40.0% of dermatophytosis cases and 37.5% of candidiasis cases occurring in those aged 20–39 years and 40–59 years, respectively.

These findings underscore distinct age-related patterns in the prevalence of superficial mycoses. Such variations are important to consider for targeted management

strategies and highlight potential differences in susceptibility and exposure among age groups.

Sample analysis for dermatophyte infections. Among the 363 nail samples analyzed, 29.7% ($n = 108$) were positive for fungal elements on potassium hydroxide (KOH) mount examination, and 47.93% ($n = 174$) were culture-positive. Of the culture-positive nail samples, 45.98% ($n = 80$) were identified as dermatophytes, and 54.02% ($n = 94$) as non-dermatophytes. Dermatophytes were identified based on the presence of septate hyphae on KOH mount examination, a positive urease test, and characteristic colony morphology and microscopic features on culture media. Non-dermatophyte molds were identified based on colony characteristics, microscopic morphology, and biochemical tests, following standard mycological procedures.

Table 3. Dermatophyte infections: summary of sample analysis

Sample	Total no. of samples (%)	KOH positive n (%)	Culture positive n (%)	Dermatophyte [#] n (%)	Non-Dermatophyte [#] n (%)
Nail	363 (54.02%)	108 (29.7%)	174 (47.93%)	80 (45.98)	94 (54.02)
Skin	289 (43.06%)	100 (34.6%)	107 (37.02%)	53 (49.53)	54 (50.47)
Hair	20 (2.98%)	1 (5.00%)	1 (5.00%)	-	-

[#]The percentage was calculated using the total number of culture-positive cases as the denominator.

Of the 289 skin samples, 34.6% (n = 100) were positive for fungal elements on KOH mount examination, and 37.02% (n = 107) were culture-positive. Among these, 49.53% (n = 53) were identified as dermatophytes, and 50.47% (n = 54) as non-dermatophytes. The same criteria were used to identify dermatophytes and non-dermatophytes in skin samples as for nail samples.

Only one hair sample (n = 1) was positive on both KOH mount examination and culture, but the isolate was not a dermatophyte (Table 3). It was identified as a *Candida* species based on the presence of budding yeast cells on KOH mount examination, a positive germ tube test, and growth on chromogenic agar.

Table 4. Frequency of fungal species isolated from nail samples from patients with superficial mycoses

Fungal Species	n (%)
<i>Candida albicans</i>	25 (14.4%)
<i>Trichophyton mentagrophytes</i>	24 (13.7%)
<i>Epidermophyton floccosum</i>	18 (10.3%)
<i>Trichophyton rubrum</i>	15 (8.6%)
<i>Trichophyton tonsurans</i>	12 (6.9%)
<i>Aspergillus niger</i>	4 (2.3%)
<i>Aspergillus flavus</i>	9 (5.2%)
<i>Aspergillus fumigatus</i>	4 (2.2%)
<i>Bipolaris</i> spp.	1 (0.5%)
<i>Alternaria</i> spp.	4 (2.2%)
<i>Trichosporon</i> spp.	6 (3.5%)
NAC	20 (11.5%)
<i>Penicillium</i> spp.	6 (3.4%)
<i>T. violaceum</i>	5 (2.8%)
<i>Trichophyton verrucosum</i>	6 (3.4%)
<i>Scopulariopsis</i> spp.	5 (2.8%)
<i>Fusarium</i> spp.	3 (1.7%)
<i>Mucor</i> spp.	1 (0.5%)
<i>Aspergillus terreus</i>	1 (0.5%)
<i>Rhizopus</i> spp.	4 (2.8%)
<i>Rhodotulula</i> spp.	1 (0.5%)
Total	174

Fungal species isolated from nail samples. Among the nail samples (n = 174), the most frequently isolated species were *C. albicans* (14.4%, n = 25), *T. mentagrophytes* (13.7%, n = 24), *Epidermophyton floccosum* (10.3%, n = 18), *Trichophyton rubrum* (8.6%, n = 15), and *Trichophyton tonsurans* (6.9%, n = 12). Several non-dermatophyte molds, including *Aspergillus* spp., *Trichosporon* spp., and *Penicillium* spp., were also isolated (Table 4).

Fungal Species Isolated from Skin Samples: Among the skin samples (n = 107), the most common fungal isolates were *Trichophyton tonsurans* (19.6%, n = 21), *Trichophyton mentagrophytes* (11.2%, n = 12), *C. albicans* (8.4%, n = 9), *Trichophyton rubrum* (8.4%, n = 9), and *Aspergillus fumigatus* (7.4%, n = 8). Other non-dermatophyte molds, such as *Aspergillus* spp., *Scopulariopsis* spp., and *Trichosporon* spp., were also isolated. *Malassezia furfur* was isolated from 0.9% (n = 1) of the samples (Table 5).

Fungal species distribution in superficial mycosis samples. The most frequently isolated fungal species from superficial mycosis samples were *T. mentagrophytes* (12.9%, n = 36), *C. albicans* (12.2%, n = 34), *T. rubrum* (8.6%, n = 24), *T. tonsurans* (11.8%, n = 33), and *E. floccosum* (9.0%, n = 25). Additionally, non-dermatophytic fungi, including *Aspergillus* spp. (10%, n=28), *Trichosporon* spp. (5.4%, n=15), *Alternaria* spp. (2.2%, n=6), and *Fusarium* spp. (1.1%, n=3), were also recovered. A significant proportion (17.2%) of isolates consisted of less common or uncommon fungal species in the study population. This included species such as *Cryptococcus neoformans* (2.9%, n= 5), *Saccharomyces cerevisiae* (2.5%, n= 4), *Mucor* spp. (3.6%, n= 6), *Rhizopus* spp. (1.8%, n= 3), *Acremonium* spp. (1.4%, n= 2), *Geotrichum* spp. (0.7%, n= 2), *Scedosporium* spp. (1.1%, n= 3), *Scopulariopsis* spp. (0.7%, n= 2), and *Trichoderma* spp. (0.7%, n= 2). The complete list of isolated fungal species is provided in Table 6.

Table 5. Frequency of fungal species isolated from skin samples from patients with superficial mycoses

Fungal Species	no. (%)
<i>Trichophyton tonsurans</i>	21 (19.6)
<i>Trichophyton mentagrophytes</i>	12 (11.2)
<i>C. albicans</i>	9 (8.4)
<i>Non albicans Candida</i>	6 (5.6)
<i>Trichophyton rubrum</i>	9 (8.4)
<i>Aspergillus fumigatus</i>	8 (7.4)
<i>Aspergillus niger</i>	3 (2.8)
<i>Scopuloropsis</i> spp.	4 (3.7)
<i>Malassezia furfur</i>	1 (0.9)
<i>Epidermophyton floccosum</i>	7 (6.5)
<i>Microsporum canis</i>	2 (1.8)
<i>Alternaria</i> spp.	2 (1.8)
<i>Exserohilum</i> spp.	2 (1.8)
<i>Paecilomyces</i> spp.	1 (0.9)
<i>Rhodotorula</i> spp.	2 (1.8)
<i>Cladosporium</i> spp.	2 (1.8)
<i>Trichosporon</i> spp.	9 (8.4)
Total	107

Table 6. Fungal species isolated from samples from patients with superficial mycoses

Fungal Species	Frequency	Percentage (%)
<i>Trichophyton mentagrophytes</i>	36	12.9
<i>Candida albicans</i>	34	12.2
<i>Trichophyton rubrum</i>	24	8.6
<i>Trichophyton tonsurans</i>	33	11.8
<i>Epidermophyton floccosum</i>	25	9.0
<i>Aspergillus niger</i>	7	2.5
<i>Aspergillus flavus</i>	9	3.2
<i>Aspergillus fumigatus</i>	12	4.3
<i>Trichosporon</i> spp.	15	5.4
<i>Non-albicans Candida</i>	6	2.2
<i>Malassezia furfur</i>	1	0.4
<i>Microsporum canis</i>	2	0.7
<i>Alternaria</i> spp.	6	2.2
<i>Exserohilum</i> spp.	2	0.7
<i>Paecilomyces</i> spp.	1	0.4
<i>Rhodotorula/Rhodotorella</i> spp.	3	1.1
<i>Cladosporium</i> spp.	2	0.7
<i>Bipolaris</i> spp.	1	0.4
<i>Penicillium</i> spp.	6	2.2
<i>Fusarium</i> spp.	3	1.1
<i>Cryptococcus neoformans</i>	5	1.8
<i>Saccharomyces cerevisiae</i>	4	1.4
<i>Mucor</i> spp.	6	2.2
<i>Rhizopus</i> spp.	3	1.1
<i>Acremonium</i> spp.	2	0.7
<i>Geotrichum</i> spp.	2	0.7
<i>Scedosporium</i> spp.	3	1.1
<i>Scopulariopsis</i> spp.	2	0.7
<i>Trichoderma</i> spp.	2	0.7
Unidentified isolates	20	7.2
Total	279	100.0

Risk factors associated with superficial mycoses. Individuals diagnosed with diabetes had a 2.14-fold increased odds of developing fungal infections compared to those without diabetes (OR = 2.14, 95% CI: 1.62 - 2.82, $P < 0.001$). Immunosuppression was associated with a 3.27-fold elevated odds of fungal infections (95% CI: 2.31 - 4.63, $P < 0.001$). The use of antibiotics or corticosteroids was linked to a 1.89-fold greater odds of infection (95% CI: 1.46 - 2.44, $P < 0.001$). Sharing personal items

increased the odds of fungal infections by 1.63-fold (95% CI: 1.28 - 2.07, $P < 0.001$), suggesting potential transmission routes. Occupational exposures were associated with a 2.21-fold higher odds (95% CI: 1.69 - 2.89, $P < 0.001$). Excessive sweating was linked to a 1.35-fold increased odds (95% CI: 1.06 - 1.71, $P < 0.001$), while tight or occlusive clothing was associated with a 1.89-fold higher odds of infection (95% CI: 1.46 - 2.04, $P < 0.001$).

Table 7. Odds ratios and 95% confidence intervals for factors associated with superficial mycoses from binary logistic regression

Risk factor	Odds Ratio (95%CI)	P-value
Diabetes	2.14 (1.62 - 2.82)	<0.001*
Yes:		
No:		
Immunosuppression	3.27 (2.31 - 4.63)	<0.001*
Yes:		
No:		
Use of antibiotics/corticosteroids	1.89 (1.46 - 2.44)	<0.001*
Yes:		
No:		
Sharing of personal items	1.63 (1.28 - 2.07)	<0.001*
Yes:		
No:		
Occupational exposures	2.21 (1.69 - 2.89)	<0.001*
Yes:		
No:		
Excessive sweating	1.35 (1.06 - 1.71)	<0.001*
Yes:		
No:		
Tight or occlusive clothing	1.89 (1.46 - 2.44)	<0.001*
Yes:		
No:		

*P-value < 0.05 is considered statistically significant at 95% confidence interval.

DISCUSSION

This study investigated the epidemiological characteristics and distribution of dermatophyte and non-dermatophyte fungal infections causing superficial mycoses in the Kashmir region. To achieve this objective, we conducted a comprehensive analysis of a diverse range of samples collected from patients suspected of having superficial mycoses, with a focus on determining the prevalence, identifying the causative agents, and exploring potential risk factors associated with these infections.

Superficial mycoses, particularly nail and skin infections, constitute a significant global health burden. Our investigation revealed a KOH positivity rate of 29.7% in nail samples and a culture positivity rate of 47.93%. These findings are lower than those reported by Bhagra *et al.* (2014) and Veer *et al.* (2007), who observed KOH positivity rates of 80% and 77.5%, respectively, in confirmed cases of superficial mycoses [17, 18]. This discrepancy might be attributed to our study's inclusion of both suspected and confirmed cases, potentially leading to lower positivity rates.

Our investigation yielded a KOH positivity rate of 34.6% and a culture positivity rate of 37% in skin samples. This is comparable to the results reported by Lilly *et al.* (2017), who observed a wet mount positivity rate of 23.8% [19]. However, other studies, such as that by Weitzman *et al.* (2003), who found a wet mount positivity rate of 91.2% [20]. These varying positivity rates highlight the influence of geographical and population-based differences on the prevalence and distribution of superficial fungal infections.

This study employed standard diagnostic techniques, including KOH microscopy and fungal culture, which have inherent limitations. The accuracy of KOH microscopy relies on the microscopist's expertise, and

subtle morphological differences can lead to misidentification of fungal elements [21]. Similarly, culture techniques may be inadequate for fastidious or slowly growing organisms, potentially leading to an underestimation of the true prevalence of certain fungal species [21]. Molecular techniques, such as polymerase chain reaction (PCR) or sequencing, could have enhanced the accuracy of species identification, particularly for less common or atypical isolates [22-24]. These methodological considerations are important when interpreting our findings in the context of other studies that may have employed different diagnostic approaches.

We observed an age-related distribution of superficial mycoses. The highest prevalence (39.4%) was in individuals aged 20–39 years, followed by those aged 40–59 years (35.0%). Dermatophytosis, the most common clinical type (44.6% of cases), contrasted with pityriasis versicolor, which was more prevalent among individuals younger than 20 years (25.0%). These findings suggest age-related susceptibility and varying clinical patterns in different age groups.

Our study found a higher prevalence of non-dermatophyte infections (54%) than dermatophyte infections (45.9%) in nail samples. Specifically, *T. mentagrophytes* was the most common dermatophyte, while *C. albicans*, followed by non-*albicans Candida* species, were the predominant non-dermatophytes. This finding aligns with Brillowska-Dabrowska *et al.* (2010) [23], who reported a higher prevalence of non-dermatophyte nail infections. However, Veer *et al.* (2007) and Gupta *et al.* (2020) observed a higher prevalence of dermatophyte infections in nail samples [18, 24].

We found a higher prevalence of non-dermatophyte infections (52.3%) than dermatophyte infections (47.7%) in skin samples. *T. tonsurans* was the most commonly isolated dermatophyte, while *C. albicans* was the most

prevalent non-dermatophyte. This contrasts with Hazarika *et al.* (2019) and Gupta *et al.* (2020), who identified *T. rubrum* as the most common dermatophyte isolate [24, 25]. However, our findings are consistent with Thakur *et al.*, (2019) who also found *T. mentagrophytes* (11.2%), *C. albicans* (8.4%), *T. rubrum* (8.4%), *T. tonsurans* (19.6%), and *E. floccosum* (6.5%) were the most frequently isolated fungal species from superficial mycosis samples. Non-dermatophyte fungi, including *Aspergillus* spp. (10.0%), *Trichosporon* spp. (5.4%), *Alternaria* spp. (2.2%), and *Fusarium* spp. (1.1%), were also isolated. Less common species constituted a significant proportion (7.2%) of isolates, including *Mucor* spp. (2.2%), *C. neoformans* (1.8%), *S. cerevisiae* (1.4%), *Scedosporium* spp. (1.1%), *Rhizopus* spp. (1.1%), *Acremonium* spp. (0.7%), *Geotrichum* spp. (0.7%), *Scopulariopsis* spp. (0.7%), and *Trichoderma* spp. (0.7%).

Several factors likely contribute to the observed variations in the prevalence and distribution of dermatophyte and non-dermatophyte infections. Geographic location and climatic conditions influence the growth, survival, and dissemination of fungal species, affecting individual exposure to environmental sources of infection. Additionally, patient demographics, such as age, gender, and underlying comorbidities, can modulate the immune response and susceptibility to specific fungal pathogens [27]. Furthermore, socioeconomic factors, cultural practices, and occupational exposures can also contribute to these differences. For instance, chronic conditions such as diabetes and immunosuppression increase susceptibility to both dermatophyte and non-dermatophyte infections by compromising immune function and altering skin integrity. In contrast, factors such as high humidity and poor hygiene primarily affect dermatophyte infections, which thrive in warm, moist environments. Similarly, occupational exposures, such as those encountered in agriculture or healthcare, can increase the risk of infection with specific fungal pathogens [28].

The high prevalence of non-dermatophyte infections, particularly those caused by *Candida* species, highlights the importance of judicious antifungal use and effective antifungal stewardship programs. Inappropriate or excessive use of antifungal agents can promote the development of resistance, leading to treatment failures and potentially more severe or disseminated infections [29]. Our findings underscore the need for evidence-based antifungal prescribing practices, guided by accurate diagnostic testing and informed by local epidemiological data to optimize treatment outcomes and mitigate the risk of antifungal resistance.

Identifying the diverse range of dermatophytes and non-dermatophytes causing superficial mycoses has important clinical implications. Accurate species-level identification is crucial for guiding appropriate antifungal therapy and optimizing treatment outcomes. For example, non-dermatophyte infections, such as those caused by

Candida species, may require different treatment approaches than dermatophyte infections. Moreover, the emergence of less common or atypical fungal species highlights the need to consider broader antifungal coverage and remain vigilant for potential antifungal resistance [30].

Accurate identification of causative agents and recognition of associated risk factors are essential for effective management of superficial mycoses. This includes tailoring antifungal therapy, implementing preventive measures, and improving treatment outcomes. Knowing the specific fungal species allows clinicians to select optimal antifungal agents and customize treatment regimens based on known susceptibility profiles and antifungal resistance patterns. Furthermore, understanding risk factors, such as age, underlying medical conditions, and environmental exposures, enables targeted prevention strategies, including patient education, hygiene measures, and environmental control measures. Ultimately, integrating accurate diagnostic testing, risk factor assessment, and evidence-based treatment guidelines can significantly improve patient outcomes and reduce the burden of superficial mycoses [31].

This study has several limitations. Conducted at a single tertiary care center, our findings may not be generalizable beyond our specific geographic region and patient population. We used conventional diagnostic methods, including KOH microscopy and fungal culture, which have limitations in accurately identifying fungal species. Future studies should consider incorporating advanced molecular techniques, such as PCR or sequencing, to enhance diagnostic accuracy, particularly for less common or atypical fungal infections. Our focus on patients with suspected superficial mycoses referred to a specialized center may have introduced selection bias. Information bias, especially regarding patient demographics and risk factors, could also have affected data reliability. Future research should aim for more comprehensive data collection and consider potential confounding factors such as underlying medical conditions and medications.

Multicenter studies are needed to provide broader insights into regional variations in fungal prevalence and species distribution. Additionally, longitudinal studies and community-based surveillance efforts would be valuable to better understand the epidemiology and transmission dynamics of superficial fungal infections. This could inform more effective prevention strategies and improve management guidelines tailored to different patient populations and healthcare settings.

Our study highlights the significant burden of superficial mycoses in the Kashmir region, emphasizing the prevalence of both dermatophyte and non-dermatophyte infections. *Candida* infections were particularly prevalent, and several risk factors were identified, including diabetes mellitus,

immunosuppression, and environmental exposures. Accurate identification of causative agents and an understanding of associated risk factors are crucial for tailoring antifungal therapy and improving management. Efforts to promote awareness among healthcare providers and the public regarding preventive measures and proper management strategies are essential, as is the development of region-specific guidelines for empirical antifungal therapy based on local epidemiology and susceptibility patterns. Future research should evaluate the impact of targeted interventions, such as antifungal stewardship programs and patient education initiatives, on the incidence and outcomes of superficial mycoses.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Medical Microbiology by Samuel Baron. 4th edition, Chapter 4. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. ISBN-10: 0-9631172-1-1.
2. Chanyachailert P, Leeyaphan C, Bunyaratavej S. Cutaneous Fungal Infections Caused by Dermatophytes and Non-Dermatophytes: An Updated Comprehensive Review of Epidemiology, Clinical Presentations, and Diagnostic Testing. *J Fungi (Basel)*. 2023; 9 (6): 669.
3. Ameen M. Epidemiology of superficial fungal infections. *Clin Dermatol*. 2010; 28 (2): 197-201.
4. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. *Mycoses*. 2008; 51 Suppl 4:2-15.
5. Achterman RR, White TC. Dermatophyte virulence factors: identifying and analyzing genes that may contribute to chronic or acute skin infections. *Int J Microbiol*. 2012; 358305.
6. Naglot A, Shrimali DD, Nath BK, Gogoi HK, Veer V, Chander J, et al. Recent trends of dermatophytosis in northeast India (Assam) and interpretation with published studies. *Int J Curr Microbiol App Sci*. 2015; 4 (11): 111-20.
7. Rajagopalan M, Inamadar A, Mittal A, Miskeen AK, Srinivas CR, Sardana K, et al. Expert Consensus on The Management of Dermatophytosis in India (ECTODERM India). *BMC Dermatol*. 2018 ; 18 (1) : 6.
8. Brigida S, Muthiah N. Pediatric Sedation: Prevalence of Tinea Corporis and Tinea Cruris in Outpatient Department of Dermatology Unit of a Tertiary Care Hospital. *J Pharmacol Clin Res*. 2017; 3 (1): 555602
9. Verma S, Madhu R. The Great Indian Epidemic of Superficial Dermatophytosis: An Appraisal. *Indian J*
10. HiMedia Laboratories. Sabouraud Dextrose Agar M063: Product Information [Internet]. Mumbai: HiMedia Laboratories; 2023 [cited 2024 Jun 20]. Available from: <https://himedialabs.com/HML/pdfs/literature/ml063.pdf>
11. Leboffe MJ, Pierce BE. A Photographic Atlas for the Microbiology Laboratory. 4th ed. Morton Publishing Company; 2011.
12. de Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of Clinical Fungi [Internet]. 4th ed. Utrecht: Centraalbureau voor Schimmelcultures; 2019 [cited 2024 Jun 20]. Available from: <http://www.clinicalfungi.org>
13. Larone DH. Medically Important Fungi: A Guide to Identification. 6th ed. Washington, D.C.: ASM Press; 2018.
14. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
15. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard--Third Edition. CLSI document M38-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
16. Sood P, Bindra S. Modified Kuppuswamy socioeconomic scale: 2022 update of India. *Int J Community Med Public Health*. 2022; 9 (10): 3841-4.
17. Bhagra S, Ganju SA, Kanga A, Sharma NL, Guleria RC. Mycological pattern of dermatophytosis in and around shimla hills. *Indian J Dermatol*. 2014; 59 (3): 268-70.
18. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: prevailing fungi and pattern of infection. *Indian J Med Microbiol*. 2007; 25 (1): 53-6.
19. Lilly KK, Shukla D, Brumble LM, Babski DM, Dietrich L, Porady R, et al. Identifying dermatophyte fungal infections: A comparison of practice patterns and diagnostic aids. *J Am Acad Dermatol*. 2020; 83 (1): 214-22.
20. Weitzman I, Summerbell RC. The dermatophytes. *Clin Microbiol Rev*. 1995; 8 (2): 240-59.
21. Gräser Y, Monod M, Bouchara JP, Demierre M, Stanzani P, Gaillemord T, et al. Identification of dermatophytes by phenotypic and molecular methods. *Mycopathologia*. 1999; 145 (1): 49-56.
22. Eing BR, Ouyang J, Nogrady K, Moser SA. Molecular-based identification of dermatophytes. *Curr Fungal Infect Rep*. 2013; 7 (3): 241-51.
23. Brillowska-Dabrowska A, Nielsen SS, Nielsen HV, Arendrup MC. Optimized 5-hour multiplex PCR test for the detection of tinea unguium: Performance on dermatological specimens from a completed multicentre collection. *Med Mycol*. 2010; 48 (2): 348-56.
24. Gupta AK, Stec N, Summerbell RC, Shear NH, Piguet V, Tosti A, Piraccini BM. Onychomycosis: a review. *J Eur Acad Dermatol Venereol*. 2020; 34 (9): 1972-1990.
25. Hazarika D, Jahan N, Sharma A. Changing Trend of Superficial Mycoses with Increasing Nondermatophyte

- Mold Infection: A Clinicomycological Study at a Tertiary Referral Center in Assam. *Indian J Dermatol.* 2019; 64 (4): 261-5.
26. Thakur R, Kalsi AS. Outbreaks And Epidemics Of Superficial Dermatophytosis Due To *Trichophyton mentagrophytes* Complex And *Microsporum canis*: Global And Indian Scenario. *Clin Cosmet Investig Dermatol.* 2019; 12: 887-893.
 27. Hill RC, Caplan AS, Elewski B, Gold JAW, Lockhart SR, Smith DJ, et al. Expert Panel Review of Skin and Hair Dermatophytoses in an Era of Antifungal Resistance. *Am J Clin Dermatol.* 2024; 25 (3): 359-89.
 28. Sardana K, Gupta A, Mathachan SR. Immunopathogenesis of Dermatophytoses and Factors Leading to Recalcitrant Infections. *Indian Dermatol Online J.* 2021; 12 (3): 389-99.
 29. Moskaluk AE, VandeWoude S. Current Topics in Dermatophyte Classification and Clinical Diagnosis. *Pathogens.* 2022; 11 (9): 957.
 30. Ameen M. Epidemiology of superficial fungal infections. *Clin Dermatol.* 2010; 28 (2): 197-201.

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