

# Mycological Profile and Prevalence of Superficial Mycoses Agents: A Study from North India

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## Abstract

**Background:** Superficial fungal infections are one of the commonest human infections. Causative agents of such infections may vary from yeasts like *Candida species*, *Trichosporon species* to dermatophytes and non-dermatophyte moulds. Fungal culture therefore, holds importance in identification and characterization of a fungal isolate, so that proper diagnosis can be made and correct treatment is instituted. Our objective was to study the etiology of the superficial fungal infections in patients presenting to the dermatology department in a tertiary care hospital in New Delhi. **Materials and Methods:** A total of 340 skin and hair samples from patients clinically suspected to have superficial fungal infection of skin and hair were microscopically examined and cultured over a period of 2 years. The percentage and frequency distribution of etiological fungal agents was studied. Also the performance of the culture and microscopy as methods of detecting fungal agents was statistically compared using Kappa and proportions of positive and negative agreement as well as McNemar's Chi-squared value. Corresponding p-values were also calculated for both kappa and Chi-squared values. The analysis has been done using Epitools. **Results:** Of these, 57.6% were positive for fungal elements by microscopic examination and the overall positivity of fungal infection was 70%. Out of 238 culture positive samples, 72.7% grew dermatophytes and 27.3% grew non-dermatophytes (including 16.8% yeasts and 10.9% non-dermatophyte moulds. *Trichophyton mentagrophytes* was the commonest (60.7%) dermatophyte isolated, followed by *T. rubrum* (17.3%), *T. violaceum* (7.5%), *T. tonsurans* (7.5%), *T. verucosum* (2.9%), *Microsporium gypseum* (1.2%) and *M. canis* (0.6%). Among the isolated non-dermatophytes, *Candida species* was the commonest (50.8%) majority of which were *C. albicans*, other non-dermatophytes included moulds like *Fusarium spp.* (6.1%), *Aspergillus fumigatus* (4.6%), *A. flavus* (3.1%), *Alternaria spp.* (3.1%), *Acremonium spp.* (3.1%), *A. niger* (3.1%) etc. and yeasts like *Trichosporon spp.* (10.8%). **Conclusion:** Dermatophytosis still remains the most common type of fungal infection involving skin and its appendages but non-dermatophytes are also slowly emerging as the causative agents for these infections.

**Keywords:** Dermatophytes, Hair, Skin, Superficial Mycoses, Yeast

## 1. Introduction

Fungal infections are globally prevalent and can manifest as superficial, subcutaneous and deep mycoses. Of these, superficial infections are the commonest human infections<sup>1,2</sup>. In India, prevalence of superficial fungal infections is high owing to appropriate temperature and

moisture conditions that favor fungal growth<sup>3</sup>. Fungal infections that invade and parasitize horny layers of the skin and other keratin rich structures like nails and hair are referred to as superficial mycoses<sup>4</sup>. Causative agents of such infections vary from yeasts like *Candida species*, *Trichosporon species* to dermatophytes and non-dermatophyte moulds<sup>5</sup>. Signs and symptoms like dermal

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inflammatory response and intense itching are clinically identical for both dermatophytes and non-dermatophyte infections<sup>4</sup>. Fungal culture therefore, holds importance in identification and characterization of a fungal isolate, so that proper diagnosis can be made and correct treatment is instituted. This is specially the case with non-dermatophyte moulds which are mostly resistant to usual dosage of the therapy used for treating infections by dermatophytes<sup>6</sup>.

Recent decades have seen a rise in the prevalence of infections caused by Dermatophytes which are also the most common pathogens accountable for causing fungal infections worldwide. This may be because of increasing burden of immunocompromised patients, changes in lifestyle, increased human migration and tourism<sup>7</sup>. Though Dermatophyte infections are not life threatening but if not diagnosed and treated appropriately, may take a chronic and progressive course<sup>8</sup>. Also, because the clinical presentation of these superficial fungal infections might be mistaken for other non-infectious conditions or non-fungal infections, failure to achieve proper laboratory diagnosis may lead to inappropriate treatment; more so when the antifungal drugs with or without corticosteroids are freely available as over the counter drugs. Therefore, any clinical diagnosis needs to be supported by a confirmatory laboratory diagnosis which includes direct microscopic examination and culture for definitive identification of etiological fungal agent<sup>6</sup>.

Our objective was to study the etiology of the superficial fungal infections in patients presenting to a dermatology department in a tertiary care hospital in New Delhi.

## 2. Materials and Methods

This study was carried out in the Department of Microbiology at a tertiary care hospital in Central Delhi, India over a period of 2 years starting from January 2017 to December 2018. It included clinical samples from patients of various age groups with suspected superficial mycoses attending Dermatology clinics in our hospital.

Samples received included plucked hair and skin scrapings transported in a sterile container or a paper envelope to Microbiology laboratory for laboratory diagnosis.

All the samples were first subjected to microscopic examination of their Potassium hydroxide (10% KOH) wet mount preparation.

All the samples were inoculated on Sabouraud's Dextrose Agar (SDA) with antibiotics (Chloramphenicol 0.05 mg/ml, Gentamicin 0.02 mg/ml) and Cycloheximide (0.5 mg/ml) and plain SDA without Cycloheximide.

Each sample was inoculated on 2 tubes of both the media and one tube from each set was incubated at 25°C and other at 37°C respectively and examined biweekly for a period of 6 weeks. Slopes showing no growth for 6 weeks were discarded. If growth was obtained on SDA, identification was made based on colony morphology, microscopic appearance and other relevant tests. The growth of moulds on SDA was observed to study the colony morphology, the color and texture of the surface, topography, pigment on reverse and the rate of growth. Microscopic examination of culture was done using lactophenol cotton blue (LPCB) preparation and slide culture was done in case the morphology was not distinct in LPCB preparation.

The growth of yeasts was confirmed by colony characteristics, LPCB mount and gram stain of the colony. Various tests like Germ tube test, colony color on Chrom agar (HiMedia), morphology on Corn meal agar with Tween 80, urease tests were done to speciate the isolated yeast.

## 3. Statistical Analysis

The percentage and frequency distribution of etiological fungal agents was calculated. To compare the performance of the two different diagnostic modalities i.e., culture and microscopy that were applied on the same sample, we calculated Kappa and proportions of positive and negative agreement as well as McNemar's Chi-squared value. Corresponding p-values were also calculated for both kappa and Chi-squared values. A p-value<0.05 was considered to be significant. The analysis was done using Epitools.

## 4. Result

A total of 340 samples from 295 patients clinically suspected (Figure 1, 2, 3) to have superficial fungal infection of skin and hair were tested over a period of 2 years. Majority of the samples sent were skin scrapings 86.4% (294/340) followed by hair 13.5% (46/340). The most common clinical presentation was Tinea corporis (158/295) with an overall incidence of 53.56%, followed

by *T. cruris* 24.41% (72/295), *T. capitis* 13.90% (41/295), *T. pedis* 6.10% (18/295), *T. manuum* 1.69% (5/295) and *T. barbae* 0.34% (1/295). Among the 295 patients, 159 (53.8%) were males and 136 (46.2%) were females. Most of the clinically suspected superficial mycoses samples were collected from patients aged 11 to 60 years, with majority of them seen in the age group between 21 to 40 years (Table 1).



**Figure 1.** Figure showing a case of Tinea corporis with erythematous scaly annular lesion.



**Figure 2.** Figure showing a case of Tinea manuum with scaly lesions on dorsum of hand.



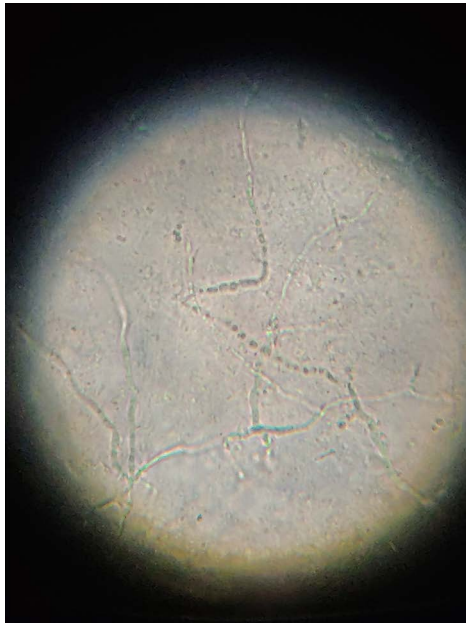
**Figure 3.** Figure showing a case of Tinea pedis with scaly lesions on dorsum of foot.

**Table 1.** Frequency table showing age group of patients with suspected superficial fungal infections

Age groups	No. of patients	Percentage %
0 to 10	2	0.68%
11 to 20	13	4.41%
21 to 30	74	25.08%
31 to 40	84	28.47%
41 to 50	53	17.97%
51 to 60	42	14.24%
61 to 70	21	7.12%
>70	6	2.03%
Grand Total	295	100%

**Table 2.** Agreement statistics between the two diagnostic methods used for detection of fungal infections

	Microscopy Positive	Microscopic Negative	Total
CULTURE POSITIVE	149 (43.8%)	89 (26.2%)	238 (70%)
CULTURE NEGATIVE	47 (13.8%)	55 (16.2%)	102 (30%)
Total	196 (57.6%)	144 (42.4%)	340 (100%)



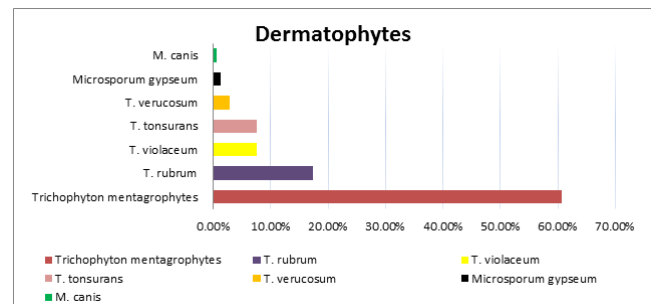
**Figure 4.** KOH mount microscopy showing thin hyaline septate branching hyphae with arthroconidia.

Of the 340 samples from suspected cases of superficial mycoses involving skin (n=294) and hair (n=46), 57.6% (196/340 includes 183 skin & 13 hair samples) were confirmed by KOH microscopic examination (Figure 4), out of which 43.8% (149/340) samples were both KOH and culture positive while 13.8% (47/340) of microscopy positive samples were culture negative (Table 2). Eighty nine samples (26.2%) were negative on microscopic examination but were culture positive whereas 16.2% (55/340) samples were negative to both microscopy and culture. For the samples that were culture positive and negative on microscopic examination and when non-dermatophyte moulds were isolated, repeated samples from these cases were taken and only if they grew the same fungus, were considered positive. Thus, the positivity of fungal infection was 70% (238/340).

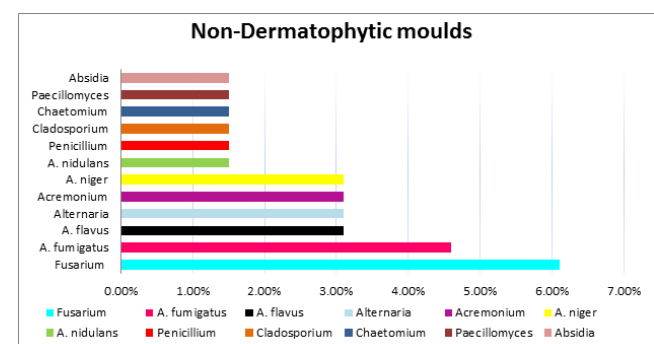
The percentage of positive agreement and negative agreement between the two diagnostic methods i.e. Microscopy and culture was found to be 68.66 and 44.72 respectively. The overall agreement between the two methods was found to be 60%. McNemar’s Chi square test was applied to check whether there is a significant difference between agreements of the two methods. We found that there is a significant difference between the agreement of the two methods (McNemar’s  $\chi^2 = 12.36$ , p-value= 0.0004). The Kappa statistics along with 95% C.I. was also calculated to check the proportion of agreement of the tests and it was found to be 0.148(0.045-0.251) with

$Z=2.83$  & p-value= 0.002, suggesting significant low level of agreement between these tests. Considering Culture as gold standard for the diagnosis of fungal infection, the sensitivity and specificity of microscopy is found to be 62.6 (56.1-68.8) % and 53.9 (43.7-63.8%) respectively.

On the basis of culture characteristics, out of 238 culture positive samples, 173 (72.7%) grew dermatophytes and 66 (27.3%) grew non-dermatophytes (including 16.8% yeasts and 10.9% non-dermatophyte moulds). Among the isolated dermatophytes, *Trichophyton mentagrophytes* was the commonest (60.7%), followed by *T. rubrum* (17.3%), *T. violaceum* (7.5%), *T. tonsurans* (7.5%), *T. verucosum* (2.9%), *Microsporum gypseum* (1.2%) and *M. canis* (0.6%). Among the isolated non-dermatophytes, *Candida* species was the commonest (50.8% of non-dermatophytes), majority of which were *Candida albicans* (55%) followed by *C. tropicalis* (25%), *C. glabrata* (10%), *C. parapsilosis* (5%) and *C. krusei* (5%) and *Trichosporon spp.* (10.8% of non-dermatophytes) was the other yeast isolated. Other non-dermatophytes included moulds like *Fusarium spp.* (6.1%), *Aspergillus fumigatus* (4.6%), *A. flavus* (3.1%), *Alternaria spp.* (3.1%), *Acremonium spp.* (3.1%), *A. niger* (3.1%), *A. nidulans* (1.5%), *Penicillium spp.* (1.5%), *Cladosporium spp.* (1.5%), *Chaetomium spp.* (1.5%), *Paecilomyces spp.* (1.5%) and *Absidia spp.* (1.5%).(Figure 5, 6).



**Figure 5.** Percentage of Dermatophyte moulds isolated in culture from skin and hair samples.



**Figure 6.** Percentage of Non-dermatophyte moulds isolated in culture from skin and hair samples.

**Table 3.** Comparing the percentage of Dermatophyte moulds as etiological agents of superficial fungal infections reported in various studies

	Present study	Vasudha CL <i>et al.</i> , 2019, Telangana <sup>11</sup>	Dulla <i>et al.</i> , 2015, Vijayawada <sup>23</sup>	Bhatia <i>et al.</i> , 2014, Himachal Pradesh <sup>20</sup>	Khadka <i>et al.</i> , 2016, Nepal <sup>21</sup>	Pakshir <i>et al.</i> , 2009, Iran <sup>22</sup>
<i>T. mentagrophytes</i>	60.7%	19.51%	27.3%	64.9%	39.6%	32.5%
<i>T. rubrum</i>	17.3%	34.14%	36.4%	35.1%	11.7%	20%
<i>T. violaceum</i>	7.5%	-	-	-	-	10%
<i>T. tonsurans</i>	7.5%	2.43%	7.3%	-	5.4%	5%
<i>T. verrucosum</i>	2.9%	4.88%	3.6%	-	-	5%
<i>M. gypseum</i>	1.2%	9.5%	3.6%	1.35%	-	7.5%
<i>M. canis</i>	0.6%	-	-	-	5.4%	-

## 5. Discussion

Rising trends in superficial fungal infections warrant their surveillance to accurately identify their burden and geographical distribution. Superficial mycoses are majorly caused by dermatophytes, however, yeasts and non-dermatophyte moulds hold equal importance as pathogens for cutaneous fungal infections<sup>9</sup>. The present study was conducted to assess prevalence of the various fungal pathogens causing superficial mycoses in skin and hair.

Incidence of clinically suspected superficial mycoses was high in males and male to female ratio was 1.16:1 which is similar to other studies<sup>10,11,12</sup>. This male predominance may be attributed to increased outdoor activities leading to increased perspiration which predisposes to development of fungal infections. Lower incidence of such infections in women may also be because of under reporting of female patients to hospitals, especially the ones from rural areas due to social stigma and limited accessibility to healthcare services<sup>13</sup>. Our study reports high incidence of clinically suspected superficial fungal infections in population aged 21-40 years, which is comparable with other studies conducted worldwide<sup>11,14,15</sup>. The reason of the increased susceptibility of this age group to develop these infections may be due to more physical activity, increased chance for exposure, and changes in hormonal pattern<sup>16</sup>. The commonest clinical type seen in our study was *T. corporis* (53.56%) followed by *T. cruris* (24.41%) which collaborates well with other studies like Bindu V *et al.* (54.6%) and Kaur R (32%)<sup>12,19</sup>.

Positive rates of culture in our study was 70% which was comparable to the rates found in other studies (60.67%, 63%, 56.8%)<sup>10,17,18</sup>. Similar to other studies<sup>10,11</sup>,

culture was found to be superior to microscopy which was positive for fungal elements in 57.6% cases of our study. However, culture is more sensitive in detecting these infections but there are some disadvantages associated with it. Culture in case of fungal pathogens takes weeks to come positive, also culture can give false negative results in patients on antifungal drugs. Microscopy on the other hand gives rapid results and can give positive results even in a person taking antifungal therapy<sup>11</sup>.

The present study shows that out of the 283 culture positive samples, 72.7% grew dermatophytes, 16.8% grew yeasts while non-dermatophyte moulds were seen in only 10.5% samples. The percentage isolation of dermatophytes (72.7%) in current study is more as compared to other studies by where isolation of dermatophytes was 56.8%, 45%, 60.67% and 63% respectively<sup>10,17,18,19</sup>. Majority of the samples processed in our study were skin samples (86.4%) which is comparable to other studies (40%-77%)<sup>10,11</sup>.

Amongst dermatophytes, *T. mentagrophytes* (60.7%) was the commonest to be isolated, followed by *T. rubrum* (17.3%), *T. violaceum* (7.5%), *T. tonsurans* (7.5%), *T. verrucosum* (2.9%), *Microsporum gypseum* (1.2%) and *M. canis* (0.6%). Majority of the studies from Asia have reported *T. rubrum* as the commonest isolate (Table 3), but our study along with others from Himachal Pradesh<sup>20</sup>, Nepal<sup>21</sup> and Iran<sup>22</sup> have reported *T. mentagrophytes* as the most common dermatophyte species isolated (as described in Table 3). This results from varying environmental conditions in different geographical terrains that is responsible for diverse distribution of fungal species<sup>5</sup>.

Other than dermatophytes, *Candida spp.* was the commonest isolate (50.8% of all non-dermatophytes), majority being *Candida albicans* (55%) followed by *C.*

*tropicalis* (25%), *C. glabrata* (10%), *C. parapsilosis* (5%) and *C. krusei* (5%). *Trichosporon spp.* (10.8% of all non-dermatophytes) was the other yeast isolated. Many studies have reported *Candida* as their most common non-dermatophyte isolate with percentages varying from 14.63-67.5%<sup>11,21,24</sup>.

Non dermatophyte moulds included mainly *Aspergillus spp.*, majority of which belong to species *Aspergillus fumigatus* (6.1%) followed *A. flavus*, *A. niger* and *A. nidulans*. Khadka S *et al.* have also reported *Aspergillus spp.* as the commonest (14.4%) non-dermatophyte mould isolated from superficial mycoses samples<sup>21</sup>. Other common isolates were *Fusarium spp.*, *Alternaria spp.* and *Acremonium spp.* Some moulds that were rarely isolated included *Penicillium spp.*, *Cladosporium spp.*, *Chaetomium spp.*, *Paecilomyces spp.* and *Absidia spp.* These findings collaborate well with various other studies which have also reported isolation of similar non-dermatophyte moulds<sup>10,21,24</sup>.

## 6. Conclusion

Dermatophytosis still remains the most common type of fungal infection involving skin and its appendages but there is a change in isolation pattern of dermatophytes and non-dermatophytes are slowly emerging as an important cause of superficial mycoses. These infections are prevalent in our country due to various factors including humid climate, poor hygiene and occupational exposure etc. The similarity in clinical presentation of these superficial fungal infections with non- fungal infections or non-infectious cause related conditions makes the matter worse with further increase in the incidence due to the use of rampantly sold over the counter antifungal agents and corticosteroids without proper laboratory diagnosis. And emergence of antifungal drug resistance highlights the importance of timely and accurate laboratory diagnosis of these infections which is a must for decreasing the disease burden and controlling the infection epidemic as a whole.

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