

Clinicomycological Study of Superficial Mycoses: A Changing Trend of Increasing Non-dermatophyte Mold Infection

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ABSTRACT

Introduction

Superficial mycoses refer to the diseases of skin and its appendages caused by fungi. The geographical distribution of fungi may change from time to time; hence this study was planned. To find out the prevalence of different clinical types of dermatomycoses and dermatomycotic mold infections and their etiological agents.

Methods

A total of 560 clinically diagnosed cases of superficial mycoses visiting tertiary care centre for a period of two years were included in the study. Specimens like skin scrapping, nail clipping, hair were collected and subjected to KOH mount and culture. Identification of species was done by macroscopic examination of culture, tease mount, slide culture and Germ tube test. Wood's lamp examination was also performed.

Results

Superficial mycoses were more common in the age group of 21-30 years (33.22%) and in males (71.96%). Tinea corporis 163/469 (34.75%) was the commonest clinical type in dermatophytosis followed by tinea cruris 145/469 (30.92%). 53.04% cases were positive by direct microscopy and 62.86% cases showed culture positive. Out of 352 culture positive cases, dermatophytes were isolated in 80.11% followed by yeasts (15.91%) and non-dermatophyte molds (NDM) (3.98%). The most common isolate among dermatophytosis was *Trichophyton rubrum* 156/282 (55.32%). An *Aspergillus* spp., *Hortaea werneckii*, *Cladophialophora carrionii* and *Exophiala jeanselmei* were some of NDM.

Conclusions

It was found that along with dermatophytes, non-dermatophytic fungi are also emerging as an important cause of superficial mycoses. A rare NDM like *Cladophialophora carrionii* and *Exophiala jeanselmei* can have possible causative role in the tinea corporis.

Keywords: superficial mycoses; dermatophytosis; trichophyton rubrum; non-dermatophyte molds (NDM).

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INTRODUCTION

Superficial mycoses refer to the disease of skin and its appendages caused by both of yeast and some filamentous fungi. These fungi have the capability to produce keratinase, which allows them to metabolize and live on human keratin like skin, nail and hair.¹ They can be further subdivided into pityriasis (tinea) versicolor, dermatophytosis, cutaneous candidiasis and non-dermatophytic fungi.

Dermatophytosis is the fungal infection of keratinized tissue. It is caused by one of three asexual genera of dermatophytes - trichophyton, epidermophyton and microsporum.^[2] They are filamentous, keratinophilic, pathogenic fungi and depending on their habitat, they can be categorized as geophilic, zoophilic and anthrophophilic.²

Infection of skin or nail can also be caused by non – deramtophytic and yeast – like fungi. Over the last decades, an increasing number of non – dermatophytic filamentous fungi have been recognized as agents of skin and nail infections in humans, producing lesions clinically similar to those caused by dermatophytes.³

Though several reports on dermatophytosis are available from different parts of the country, there are very few reports on non – dermatophytic fungi and yeast like fungi as causative agents of superficial mycoses along with dermatophytes from eastern region of India.

The prevalence of superficial mycotic infections has risen to such a level in the last decades that skin mycoses now affect more than 20–25% of the world's population, making them one of the most frequent forms of infections.⁴ The distribution of the dermatomycoses, their aetiological agents and the predominating anatomical infection patterns vary with geographical location and a wide range of environmental and cultural factors.^{4,5} Despite regional characteristics and

predispositions for dermatophyte infections, the spectrum of dermatophytes is not static. Booming mass tourism, international sports activities and increasing migration mean that less common or forgotten species are being imported and disseminated.⁶

As this study aims to isolate the causative species of dermatomycoses, it may help in identifying any yet unrecognized changing trend in this aspect of the disease. It can also provide information regarding the predominant clinical pattern and the common epidemiological factors influencing the occurrence of this disease. This knowledge will help in implementing better preventive measures for elimination of this common fungal infection.

METHODS

It was cross sectional observational study conducted over two years in eastern India. The study population included 560 patients, diagnosed clinically as having superficial mycoses randomly selected from the Outpatient Department of Dermatology, Venereology and Leprosy, and then diagnosis was confirmed in collaboration with Department of Microbiology at a tertiary care hospital. The study was assessed and approved by an institutional ethics committee. A detailed clinical history was noted. Patients which had history or documentary evidence of anti-fungal treatment in last three months and patients unwilling to take part in the study were excluded. Before collection of the sample, patient was explained about the procedure & written informed consent was taken. The clinical specimens (like skin scrapping, infected hair taken by plucking, clipped nails) were collected on a small white paper envelop. Immediately after collection, 10% KOH mount examination was done and nail clippings were dipped in 40% KOH solution overnight for study on the next morning. Clinical

specimens including skin, nail and hair were also inoculated on Sabouraud's dextrose agar with chloramphenicol (50mg/L) and cycloheximide (500mg/L) with or without olive oil overlay. The cultures were incubated at 25°C & 37°C for a period of 1 month before giving negative result. If any growth was obtained, identification was made based on colony characteristics, pigment production; microscopic appearance on Lactophenol cotton blue (LPCB) teased mount, slide culture, urease test, hair perforation test and germ tube test. Also, repeat culture was done to rule out contamination when non-dermatophyte molds (NDMs) were detected.

Mean age and frequency distribution of clinical and mycological data was expressed in percentages. All responses were tabulated by using Microsoft Excel Software. Data analysis was done using descriptive statistics.

RESULTS

Among the 560 patients enrolled in our study, majority of patients were in the age group of 21-30 years (33.22%) and 71.96 % cases were male and rest were female. Male to female ratio was 2.6:1.

Out of 560 cases, 83.75% patients were diagnosed of having dermatophytosis, followed by pityriasis versicolor (8.03%), candidiasis (7.86%) and tinea nigra (0.36%) (Figure 1).



Figure 1. Tinea nigra - A1 to 2 mm sized multiple brown to black discrete macules without scaling on palm.

A majority of cutaneous mycoses cases were from urban area (81.96%) and middle economic class (83.21%). The history of contact with infected partner or person was present in 20% of cases. The literate patients (75.54%) were mostly affected than illiterate (24.46%). In occupation point of view, students (32.14%) were most commonly affected.

In this study, out of 469 total clinical diagnosis of dermatophytosis, 34.75% cases were *t. Corporis* followed by *t. cruris* (30.92%) and least were *t. manuum* (2.77%) and *t. faciei* (2.77%) followed by *t. barbae* (1.92%). The incidence of multiple site infection was 95 (20.26%) and most common was *t. corporis* with *t. cruris* 78 (82.10%).

In 44 cases of candidiasis, most common clinical type was monilial balanoposthitis (36.36%) and least were erosio interdigitalis blastomycetica (2.27%) and median rhomboid glossitis (2.27%). The KOH positivity was seen in 297 (53.04%) and fungal isolates were isolated in 352 (62.86%) superficial mycoses cases. A culture positivity was higher in dermatophytosis and candidiasis cases than pityriasis versicolor in comparison to KOH mount. Both cases of Tinea nigra showed growth on culture but were KOH negative. (Figure 2).

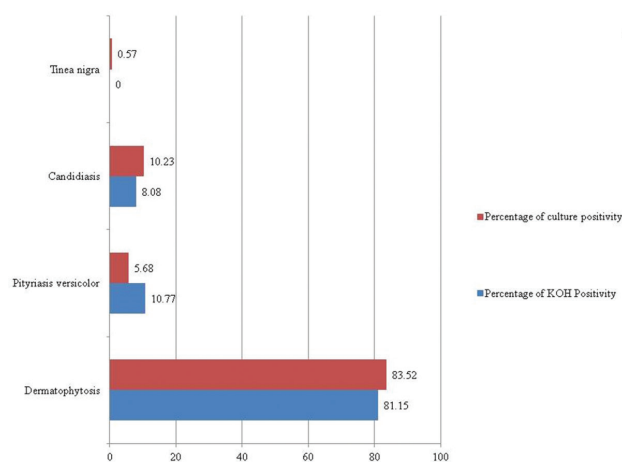


Figure 2. Comparison of KOH positivity with culture positivity in different types of superficial mycoses.

The KOH positive cases with culture positivity was seen in 192 (34.29%) cases. KOH positive with culture negative was seen in 103 (18.39%). KOH negative with culture positive was seen in 161 (28.75%) as shown in Table 1.

Trichophyton (275/ 282 i.e. 97.52%) was the most frequently isolated genus in which *T. rubrum* (156/ 275 i.e. 56.73%) (Figure 3a, 3b, 3c) was the most common species, followed by the *T. Mentagrophytes* (98/275 i.e. 35.64%).

Table 1. Comparison of KOH mount with fungal culture. (N = 560)

	Culture, growth	Culture, no growth	Total
KOH positive	192 (34.29%)	105 (18.75%)	297 (53.04%)
KOH negative	160 (28.57%)	103 (18.39)	263 (46.96%)
Total	352 (62.86%)	208 (37.14%)	560 (100%)

Table 2. Frequency of various mycological isolates. (N = 352)

Mycological isolates	No. of isolates	Percentage (%)
DERMATOPHYTES	Out of 282	
282 (80.11%)		
<i>Trichophyton rubrum</i>	156	55.32
<i>Trichophyton mentagrophyte</i>	98	34.75
<i>Trichophyton violaceum</i>	12	4.26
<i>Microsporum audouinii</i>	7	2.48
<i>Trichophyton tonsurans</i>	5	1.77
<i>Trichophyton schoenleinii</i>	4	1.42
NON-DERMATOPHYTE YEASTS 56 (15.91%)	Out of 56	
Malassezia species:	20	5.68
Candida:	36	10.23
<i>Candida albicans</i>	30	83.33
Non-albicans candida	6	16.67
NON-DERMATOPHYTE MOULDS 14 (3.98%)	Out of 14	
<i>Aspergillus niger</i>	5	35.71
<i>Aspergillus flavus</i>	4	28.57
<i>Hortaea werneckii</i>	2	14.29
<i>Cladophialophora carrionii</i>	2	14.29
<i>Exophiala jeanselmei</i>	1	7.14

In 352 culture positive cases, dermatophytes were isolated in 80.11% followed by yeasts (15.91%) and non-dermatophyte molds (3.98%). (Table 2)

A 56 (i.e. 15.91%) specimens were positive for yeast isolates, amongst which *Candida* was 36 (10.23%) (Figure 4) and *Malassezia* 20 (5.68%). The commonest NDM isolated was *Aspergillus spp.*

in 9/352 (2.56%) (Figure 5a, 5b, 5c, 5d) followed by *Hortaea werneckii* (Figure 6a, 6b). The NDM including *Cladophialophora carrionii* and *Exophiala jeanselmei* (Figure 7a, 7b, 7c) were isolated from tinea corporis lesion.

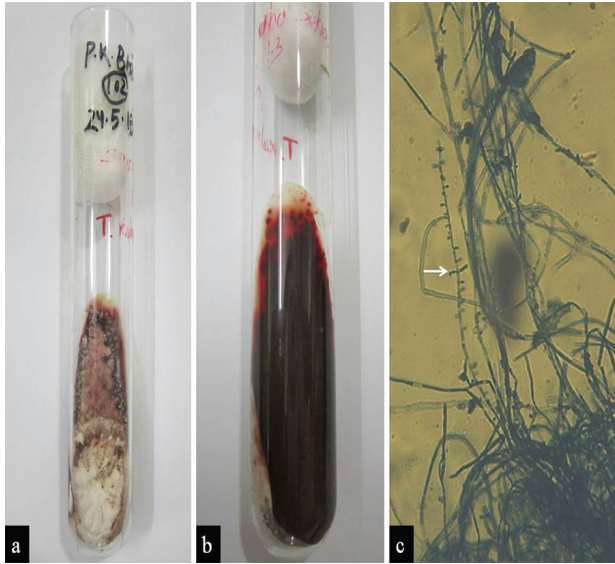


Figure 3. (a & b): *Trichophyton rubrum* - White downy and red colonies with reverse deep red pigmentation (SDA) (c) Small teardrop shaped microconidia [white arrow] arranged along the side of the hyphae (LPCB, x400).

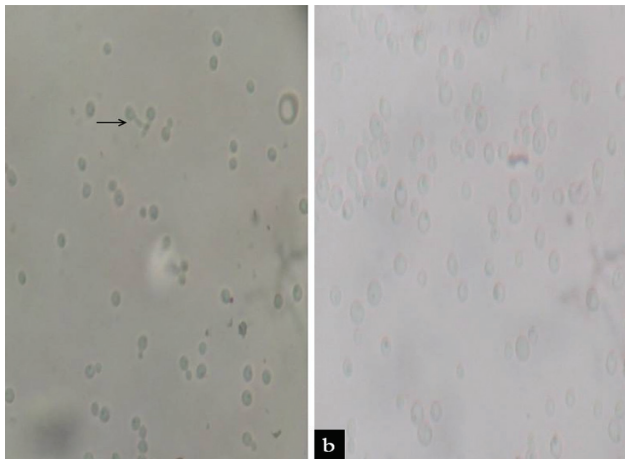


Figure 4. Germ tube test - Germ tube seen in *candida albicans* [a-black arrow] and absent in non-*albicans candida* [b] (x400).

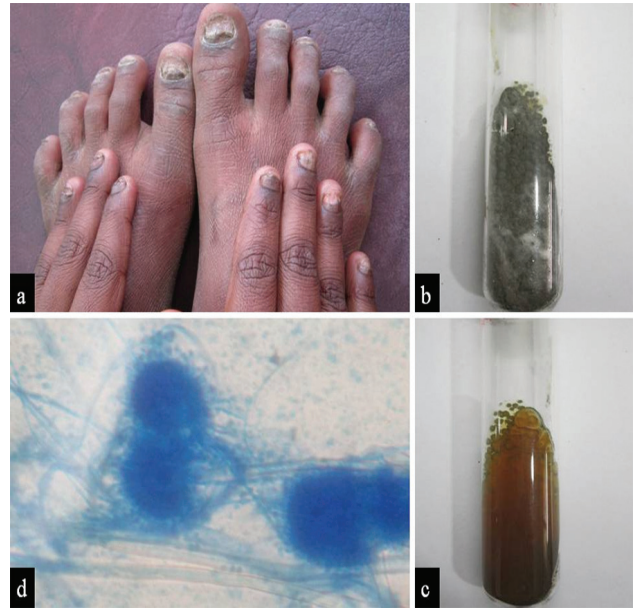


Figure 5. a. Nail dystrophy with periungual hyperpigmentation. (b & c) *Aspergillus flavus* - matlike rugose colonies with surface yellow-green and reverse pinkish pigmentation. (SDA). (d) Globose vesicles with conidial heads that radiate and are loosely formed rather than compact. (LPCB, x400).

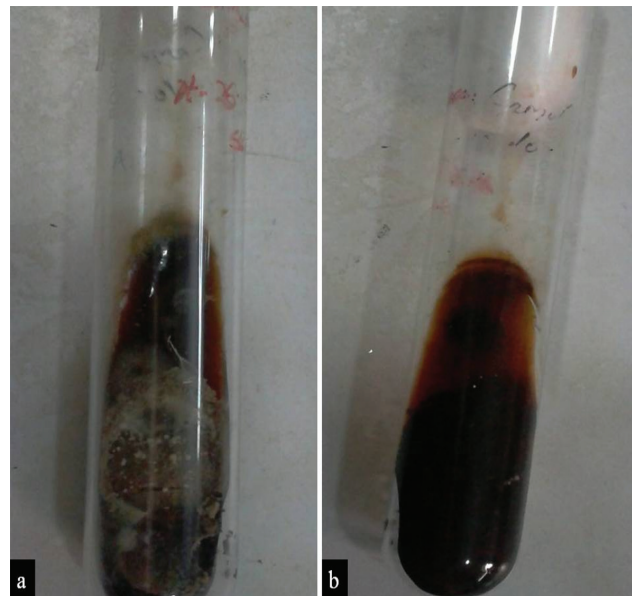


Figure 6 (a & b). *Hortaea werneckii* - velvety olive-green to black colonies and reverse of colony is jet black. (SDA).

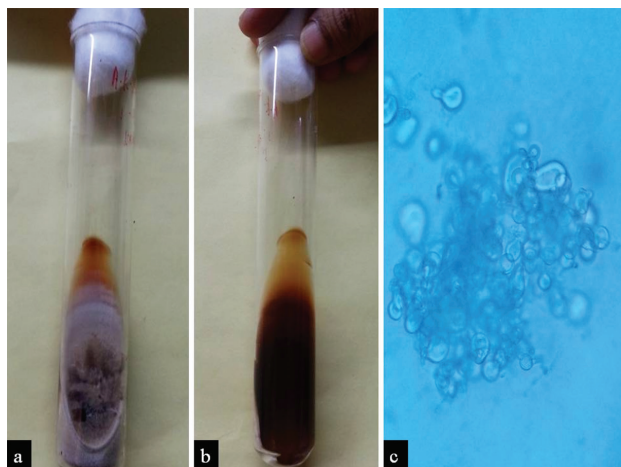


Figure 7 (a & b). *Exophiala jeanselmei* - Velvety colonies, surface pigment is olive gray to brownish black with jet black reverse. The topography is typically dome shaped, folded, and spreading. (SDA). (c) Pigmented annellides and anneloconidia. (LPCB, x400).

As is evident by Table 3, in present study the most common isolate was *T. rubrum* (55.17%) mainly isolated from t. corporis, t. cruris and t. unguium cases. The second common isolate was

T. mentagrophytes (34.77%) which was isolated most commonly from t. cruris and t. corporis followed by t. capitis and onychomycosis. Nine (2.59%) isolates of *Microsporum spp.* were grown from t. corporis, t. cruris, t. pedis and t. capitis.

In Wood's lamp examination, 44.44% cases of Pityriasis versicolor cases showed fluorescence in which yellow fluorescence (26.67%) was most common.

DISCUSSION

Superficial mycoses form a large group of patients attending the dermatology OPD of our tertiary care hospital. The higher temperature as well as body sweating facilitates fungal growth. Identification of fungal agent is importance for epidemiology and also for therapy.

In present study, males were more affected than females. The male to female ratio was 2.6:1. Prior studies^[7,8] have similar observations except a study in Egypt the ratio of the girls was more than the males.^[9]

Clinical types	Isolates of dermatophyte						Total
	<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>T. schoenleinii</i>	<i>T. violaceum</i>	<i>T. tonsurans</i>	<i>M. audouinii</i>	
T. corporis	72	41	1	6	3	4	127
T. cruris	74	47	1	5	3	3	133
T. pedis	5	6	1	1	0	1	14
T. manuum	6	3	0	1	0	0	10
T. faciei	5	2	1	0	0	0	8
T. capitis	2	7	0	1	0	1	11
T. unguium	24	13	0	1	1	0	39
T. barbae	4	2	0	0	0	0	6
Total	192 (55.17%)	121 (34.77%)	4 (1.15%)	15 (4.31%)	7 (2.01%)	9 (2.59%)	348 (100%)

In our study, 21-30 years of age group (33.22%) was most commonly affected followed by adult age group. This finding is well correlated with prior studies.^{4,7,8,10} Majority of the patients were from urban area (81.96%) than rural area (18.04%). This could be due to more humid environment in urban than rural place and also due to easy accessibility of our study centre to urban.

Out of 560 patients 20% cases showed positive history of contact with infected partner or other infected persons.

The commonest clinical types of superficial mycoses in our study were dermatophytosis (83.75%) followed by pityriasis versicolor (8.03%) and candidiasis (7.86%) and two cases of tinea nigra (0.36%). This is similar to studies done by^{11,8} and Kannan *et al.*¹² in Tamilnadu. But in study done by Bharbuiya *et al.*¹³ in Kolkata candidiasis was second most clinical type after dermatophytosis and in study done by Mishra *et al.*¹⁴ from western Odisha, pityriasis versicolor was most common. Only Kannan *et al.*¹² study had one tinea nigra case as present in our study (2 cases).

Our study showed, in dermatophytosis most common clinical type was tinea corporis (34.75%), followed by tinea cruris (30.92%), which concurs with reports from other parts of India.^{4,7,8, 11,15} The incidence of tinea capitis was 3.01% in present study which is comparable with reports from other workers.^{16,17} Tinea capitis is less common in India than other countries.^{16,18,19} This may be attributable to the use of hair oils which are customarily used by Indians and had been shown to have an inhibitory effect on dermatophytes in Vitro.^{20,21} The incidence of onychomycosis is 15.25% in present study which is comparable to study²² in Peru (24%) and more than the study¹³ in Kolkata (8.11%). The reported incidence of tinea pedis varies from 26.4% in

Pune²³ to 0.4% in Ahmedabad²⁴. It is towards higher side in Western country. In present study, it was 5.67%. This could be because of the regular use of shoes and socks, predisposing to perspiration and maceration. In our study multiple site infection was 20.26% which is near similar to the study conducted by Grover *et al.*(17.3%) in Calcutta¹⁰ but less as compared to study conducted by Mishra *et al.*¹⁴ (25%). Tinea cruris associated with tinea corporis was most common and found in 13.93% (78/560 cases) which is higher than study conducted in desert district of Rajasthan (10.4%).²⁵

In Dermatophytosis, groin was most common and beard area, neck were least common sites affected. This could be due to occlusive clothing in groin area.

In present study, in candidiasis most common clinical type was balanoposthitis (36.36%), followed by intertrigo (34.09%). Comparing this, the intertrigo had most common in Barbhuiya *et al.*¹³ from Kolkata and oral candidiasis in Kannan *et al.*¹² study from Tamilnadu.

Among various methods, the KOH preparation has shown good sensitivity in comparison with culture. In our study, KOH positivity rate was 53.04% and culture positivity rate was 62.86%. The study is in lines with the other studies^{10,15} done across various parts of India. However, high KOH positivity rate are depicted in other studies^{4,14,26} The incidence of culture positivity of our study is comparable with study¹² of Tamilnadu. A variations in KOH and culture may be due to the results of various contributory factors involved in collection, transport, inoculation and incubation of specimen. The small sample material, absence of the infectious agent in the sample and difference in pH and sensitivity of media used may be some of responsible factors. In present study, culture positivity is more than KOH positivity. This could be due to the fungal

hyphae being missed in KOH smear. There were 160 cases in which KOH was negative but culture was positive (28.57%). Similar finding is also noted by other investigators.^{4,10} This can be explained by drying out procedure.¹⁰

In present study most common dermatophytes were Trichophyton species (97.52%), in which *T. rubrum* was commonest followed by *T. mentagrophye*. Trichophyton species were more commonly isolated than Epidermophyton and Microsporum with *T. rubrum* is the main dermatophyte reported from India and other countries.²⁷ Some studies^{7,26} from India showed *T. mentagrophye* as commonest dermatophyte isolate followed by *T. rubrum*. In our study out of the total isolates of dermatophyte *T. rubrum* was 55.32%, but in a study¹⁴ in western Odisha in 1995-1996, *T. rubrum* isolation rate was 76%. Many other species of dermatophytes like *T. schoenleinii*, *T. verrucosum*, *T. ferrugineum*, *T. concentricum* and *M. audouinii* have been isolated by other workers^{28,29}, but we could isolate only *T. rubrum*, *T. mentagrophytes*, *T. violaceum*, *T. schoenleinii*, *T. tonsurans* and *M. audouinii*. Tinea corporis, tinea cruris and tinea unguium were mainly caused by *T. rubrum* and the main causative agent of tinea pedis, tinea capitis was *T. mentagrophyte* in present study whereas tinea cruris is mainly caused by *E. floccosum* and tinea capitis by *T. violaceum* in other study.²⁷ It could be explained on the basis of different climatic conditions and geographic distribution.¹⁰

In present study, in pityriasis versicolor cases KOH positivity was 71.11% and culture isolation rate was 44.44% which is less than the study done by Kannan *et al.*¹² from Tamilnadu (56.41%). We observed that culture positivity was increased after adding olive oil in culture medium than any other oil or without oil.

We found 54.55% positivity in KOH mount

and 81.82% positivity in culture in candidiasis cases. *Candida albicans* (83.33%) was the most common isolate in all candidiasis cases which is similar to the study¹³ from Kolkata but in a study¹² from Tamilnadu non-albicans candida species were more common.

We could isolate 3.98% non-dermatophyte molds in total isolates, in which most common was *Aspergillus niger* and rest were *Aspergillus flavus*, *Hortae werneckii*, *Cladophialophora carrionii* and *Exophiala jeanselmei*. Our isolation rate and species of NDM are compared with other studies^{8,10,30,31} in table 4.

Study	NDM isolation rate	Commonest NDM isolate
Hazarika <i>et al.</i> ⁸	14.51% (n=130)	<i>Aspergillus</i> and <i>Fusarium</i>
Grover <i>et al.</i> ¹⁰	34% (n=146)	<i>Cladosporium</i> spp.
Sarma <i>et al.</i> ³⁰	11.5% (n=100)	<i>Curvularia lunata</i> and <i>Fusarium</i>
Kumar <i>et al.</i> ³¹	6.6% (n=122)	<i>Exophiala</i> spp.
Present study	3.98% (n=560)	<i>Aspergillus</i> spp.

An *Aspergillus* species were isolated from onychomycosis cases in which periungual hyperpigmentation was the most common clinical feature as described by previous study.²⁷ *Exophiala* species isolated from tinea corporis lesion in which papulonodular lesions were at border with less scaling and *Cladophialophora* species isolated from tinea corporis lesion which was more scaly with crusts at places. It is suggested that this subgroup may have a direct causative role as it fulfills the criteria of a pathogen (proposed initially for nails) viz. isolation in pure culture, KOH positive and absence of dermatophytes in the same culture.³²

But their primary pathogenic role in cutaneous fungal infections cannot be proven with certainty yet.

The higher incidence of superficial mycoses is seen in months of march to august due to summer season and humid atmosphere which is also correlating well with other studies.⁴

In present study, Wood's lamp examination of all pityriasis versicolor cases, 44.44% cases showed fluorescences and yellow fluorescence was most common.

CONCLUSIONS

The present study shows that the fungal infections are still one of the common prevalent skin diseases and this involvement is considerable in third decade and generally in the males, and from the involvement point of view, groin is the

most common site for dermatophytosis. Close attention must be paid to the type of clothing and hygiene maintenance of body. Fungal culture is shown to be more sensitive than KOH examination. The superficial mycosis is caused by not only dermatophytes but also non-dermatophytic molds. Some non-dermatophytic molds like *Cladophialophora carrionii* and *Exophiala jeanselmei* can have possible causative role in the tinea corporis. Thus, species identification is very important to initiate prompt and appropriate antifungal therapy.

Limitations: Patients who had history of immunosuppressive conditions or on any immunosuppressive medications and who were presented with severe, widespread dermatophytosis were not included in the study; hence this study is deprived of clinicomycological status of all those kind of patients.

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