

Original Research Article

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A Study on Prevalence and Clinico-Mycological Profile of Superficial Fungal Infections in a Tertiary Care Hospital

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ABSTRACT

The incidence of superficial mycoses has increased recently due to frequent usage of antibiotics and various immunosuppressive conditions. In the background of immunosuppression, detection of these agents becomes necessary for effective management and prevention of further invasions. The objective of the study was to determine the prevalence and causative agents of superficial mycoses in order to provide early and efficient treatment. A cross-sectional study was conducted for 3 months (June-August 2015) on patients with suspected superficial mycoses attending our Dermatology OPD. Skin, hair and nail samples were collected and transported aseptically to Microbiology laboratory for further processing. Out of the 394 cases that attended the dermatology OPD, 48 clinically suspected cases of superficial mycoses were studied. Prevalence of superficial mycoses was 10.4%. 41 cases showed positive culture, while only 37 cases were KOH positive. *T. rubrum* was the most common dermatophyte (48.27%) and the commonest causative agent (34.14%) while *Candida spp* were the most common non-dermatophyte (50%) and 3rd most common etiological agent (12.20%) isolated. We concluded that along with dermatophytes, dermatomycotic fungi are also emerging as an important cause of superficial mycoses. Though culture was found to be more sensitive than KOH mount, both techniques are important tools of diagnosis.

Keywords

Superficial mycoses, Dermatophytes, Tinea corporis, Onychomycosis, *T. rubrum*, *Candida spp*.

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Introduction

Fungal infections are worldwide in distribution of which superficial infections are the most common human infections (Brown *et al.*, 2012). These are rarely life threatening but they have their own negative effects on the patient's emotional, social and occupational

status (Langan *et al.*, 2010). In the tropical and subtropical countries like India superficial fungal infections are more prevalent due to the fact that heat and moist conditions are more suitable for the growth of fungi (Peerapur *et al.*, 2004). Superficial mycoses refer to the diseases of the skin and its appendages caused by fungi caused by Dermatophytes, candida

species and other non-dermatophytic moulds such as *Aspergillus spp*, *Fusarium spp* and *Acremonium* species. They have affinity for tissues that are keratin rich like skin, hair and nails, and thus they produce inflammatory response associated with clinical signs and symptoms such as itching etc, along with cosmetic defects. The three genera of dermatophytes that are recognised are *Epidermophyton*, *Microsporum* and *Trichophyton* (Grover and Roy, 2003)

Over the last decades, an increasing number of non – dermatophyte filamentous fungi have been recognized as agents of skin and nail infections in humans, producing lesions clinically similar to those caused by dermatophytes (Patel *et al.*, 2010).

Recently there has been an increase in the incidence of fungal infections. This increase may be a result of frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, lymphomas, leukemia and *Human Immunodeficiency Virus* (HIV) infections (Petmy *et al.*, 2004).

Previously these infections were considered as mere cosmetic problems but in recent years these have gained importance as major public health problems. This is because affected patients experience embarrassment in social and work situations, where they feel unclean, unwilling to allow their hands or feet to be seen. Patients may fear that they will transmit their infection to family members, friends, or co-workers, fears that can lead to diminished self-esteem and the avoidance of close relationship. In fact, many patients with fungal nail infections experience serious physical, psychosocial, and occupational effects as a result of this disease.

A correct diagnosis is important to initiate appropriate treatment and also essential for

epidemiological purposes. In the background of immunosuppression, detection of these agents becomes mandatory for the effective management of mycoses to prevent further invasions (Kannan *et al.*, 2006).

The present study was conducted to know the prevalence and possible etiological agents of superficial fungal infections in our area, in order to provide early and appropriate treatment to reduce the social burden.

The objective of the study is to determine the prevalence and causative agents of superficial fungal infections in order to provide early and efficient treatment and reduce the morbidity.

Materials and Methods

This is a cross-sectional study conducted for a period of 3 months (June- August 2015) and included patients of various age groups with suspected superficial mycoses attending the outpatient department (OPD) of Dermatology of our hospital. The institutional ethical committee clearance was obtained.

Samples such as skin scrapings, nail clippings, subungual scrapings and hair were collected under aseptic conditions after obtaining informed consent from the patients.

Specimen collection (Larone, 2011)

Scrapings of skin were taken from the active, peripheral edge of the lesion with a scalpel or the end of microscope slide, after it had been cleansed with 70% alcohol. Hair was plucked out from the root using sterile forceps. Brushings were taken from the area of scaly scalp. Infected nails were cleansed with an alcohol wipe and then scraped deeply enough (subungual scrapings) to obtain recently invaded nail tissue, using blunt end of the scalpel. The initial scrapings were discarded as they are usually contaminated. The samples

so collected were transported in a sterile container or a black paper envelope to Microbiology laboratory for further processing.

Specimen processing (Larone, 2011)

The samples received in the Microbiology laboratory were processed as follows:

Microscopy/ Potassium hydroxide (KOH) wet mount preparation

Portion of sample is placed on a labelled slide to which few drops of 10% KOH (for skin & hair samples) solution is added. Cover slip is placed over it and the slide is gently heated over flame without boiling. The slide is then carefully examined microscopically to detect presence of fungal elements. The nail samples were submerged in 20% KOH (Flores JM *et al.*, 2009) overnight for complete softening and clearing, in order to afford good visibility.

Culture

Media used for fungal culture were Sabouraud's Dextrose Agar (SDA) with antibiotics and cycloheximide (for dermatophytes) and plain SDA without cycloheximide (for candida and non-dermatophyte). Samples were inoculated on both the media, incubated at 30°C and examined daily for growth.

Confirmation of isolates

The growth of moulds was confirmed by

Colony characteristics

Tease Mount with Lactophenol Cotton Blue (LPCB) to detect the morphology of fungus

Slide culture technique

The growth of yeasts was confirmed by

Colony characteristics

Gram stain of the colony

Germ tube test

Chrom agar

Tease mount (Larone, 2011)

Place a drop of LPCB on a clean glass slide. With a sterile bent dissecting needle or sterile loop, remove a small portion of the colony from the agar surface and place it in the drop of LPCB. With two dissecting needles, gently tease apart the mycelial mass of the colony on the slide, cover with a coverslip, and observe under the microscope with low power (10X) and high-dry (40X) objective lenses.

Slide culture technique (Larone, 2011)

It is done to study the undisturbed morphology of fungi which helps to identify the fungal species. A microscopic slide is placed on a bent glass rod at the bottom of the petri dish with a filter paper. A piece of 1 cm block of SDA is put on the slide. The fungal growth obtained is inoculated at four sides of the agar block and covered with a sterile cover slip. Few drops of water are added on the filter paper to avoid drying of the agar. The lid of the petri dish is closed, the preparation is left at room temperature. When the growth appears approximately after 7-14 days (dermatophytes) or 3-4 days (non-dermatophytic molds) a drop of lactophenol cotton blue (LCB) is placed on the slide and the cover slip from the block is placed on it. This slide is examined microscopically for the structural arrangement of the fungi.

Gram stain (Allen *et al.*, 2005)

Smear from the yeast-like colonies were prepared on a clean glass slide and subjected to Gram stain (according to standard procedure). They were then observed under microscope for the presence of Gram positive budding yeast-like cells.

Germ tube test (Larone, 2011)

The culture showing yeast like dry and pasty colonies are treated with mammalian (foetal, bovine, sheep or normal human) serum and incubated at 37⁰C for 2 to 4hours. A drop of this suspension is placed on the slide, covered with a cover slip and examined microscopically for germ tubes which are seen as long tube like projections extending from the yeast cells.

Chrom agar (Chander, 2009)

It is selective and differential chromogenic medium used for identification of various candida species. It is based on direct detection of specific enzymatic activities by adding multiple chemical dyes i.e substrates of fluorochrome to media. Yeast like colonies obtained from SDA are inoculated on this agar, incubated at room temperature (30⁰C) for 48-72 hours and looked for following colours of colonies:-

- C.albicans* -Light green
- C.dubliniensis* - Dark green
- C.glabrata* - Pink to Purple
- C.krusei* - Pink
- C.parapsilosis* - Cream to Pale pink
- C.tropicalis* - Blue with Pink halo

All the materials required for culture were obtained from HiMedia Laboratories Pvt Ltd, Mumbai, India.

Statistical analysis

The results were expressed as percentages for the analysis of various data. Microsoft excel was used for the interpretation of these results.

Results and Discussion

A total of 394 patients attended the dermatology OPD during the study period.

Out of these, 48 clinically suspected cases of superficial fungal infections were studied. Out of the 48 clinical cases 62.5% (30) were males and 37.5% (18) were females with a male to female ratio of 1.67:1. Prevalence of superficial fungal infections in our study was 10.4% (41/394). The most common age group affected was between 31-45 years (33.33%), followed by 16-30 years (27.08%) (Table 1). Out of the 48 clinical samples, 39.58% (19/48) were skin scrapings, 31.25% (15/48) were hair samples and 29.16% (14/48) were nail clippings [Chart 1]. Out of the 48 cases, 85.41% (41) were culture positive, of which 77.08% (37) were both KOH and culture positive, 8.33% (4) were KOH negative and culture positive. 14.58% (7) were both KOH and culture negative. There were no cases where KOH was positive and culture negative (Table 2).

Out of the 41 positive cultures obtained, 70.73% (29) were dermatophytes and 29.26% (12) were non-dermatophytes. Among the dermatophytes, *T.rubrum* 48.27% (14/29) was the most common isolate and among non-dermatophytes *candida species* 50% (6/12) were the commonest isolates.

Overall, *T.rubrum* 34.14% (14/41) was the commonest isolate, followed by *T.mentagrophytes* 19.51% (8/41), *C.albicans* 12.20% (5/41) and *M.gypseum* 9.76% (4/41). Other isolates were *T.verrucosum*, *Curvularia*, *A.fumigatus* (4.89% each, i.e 2 each) and *T.tonsurans*, *C.parapsilosis*, *Bipolaris* and *Alternaria spp* (22.43% each i.e. 1 each) [Chart 2].

There were no cases of mixed infections, *Malassezia* infections and infections due to *Epidermophyton spp* in this study.

Different types of Superficial mycoses cases which were studied are described in Table 3. It was observed that Onychomycoses (29.17%),

Tinea corporis (25%) and *Tinea capitis* (20.83%) were the commonest types of Superficial mycoses in our study (Fig. 1 and 2).

Significance of the results related to research work

Superficial mycoses form a large group of patients attending the Dermatology OPD of our tertiary care hospital. Apart from the clinical symptoms superficial fungal infections can cause debilitating effects on a person's quality of life. Although rarely life threatening they may in some circumstances spread to other individuals or become invasive. Most superficial fungal infections are easily diagnosed and readily amenable to treatment (Abida Malik *et al.*, 2014).

Prevalence of superficial fungal infections in our study was 10.4%. This was in concordance with other studies which showed similar prevalence rate of 12.61% (Flores *et al.*, 2009) and 9.16% (Eftekharjo *et al.*, 2015).

In the present study, males were more affected than female with a male to female ratio of 1.67:1. Other studies done in India, showed similar observation with male to female ratios of 1.79:1, 4.26:1, 1.8:1 and 1.63:1 respectively (Grover *et al.*, 2003; Kannan *et al.*, 2006; Nawal *et al.*, 2012 and Surendran *et al.*, 2014). But a study done by Dulla *et al.*, 2015, showed that females were more affected than males with female to male ratio of 1.1:1. Increased incidence in males can be attributed to their greater outdoor exposure and physical activity.

Persons of all ages were susceptible but maximum cases of fungal infection occurred between the age group of 31-45 years (33.33%) followed by 16-30 years (27.08%). This was in concordance with study done by Dulla *et al.*, (2015), which showed a higher prevalence in the age group 31-40 (26.4%) years.

The culture positivity rate was 85.41%, which was in concordance with study done in Iran (Eftekharjo *et al.*, 2015), which showed 84.1% culture positivity. But other studies (Surendran *et al.*, 2014; Dulla *et al.*, 2015 and Prasad *et al.*, 2013) done in India showed lesser culture positivity rate of 39%, 57.6% and 69.51% respectively.

The KOH positivity rate was 77.08%, and comparable with studies done in various parts of India 70.4% (Dulla *et al.*, 2015) and 75.57% (Prasad *et al.*, 2013). But lesser when compared to the study done by Surendran *et al.*, (2014) which showed a positivity of 96%.

In our study, 8.33% were KOH negative and culture positive. Culture was found to be superior to KOH wet mount. Though KOH wet mount is rapid, but at times gives false negative results which misleads the clinician. Hence all the KOH negative samples need to be cultured to confirm the diagnosis and to identify the undetected cases. The drawback of fungal culture is that it is time consuming.

The different clinical types of superficial mycoses and the various fungi isolated in this study are compared with other Indian studies in table 3, 4, 5, 6 and 7. Onychomycoses (29.17%) was the most common type of superficial mycoses in our study whereas *Tinea corporis* was the most common type of clinical presentation in other studies (30.19%, Bhatia *et al.*, and 44.3%, Surendran *et al.*). Dermatophytes, especially *T.rubrum* was the commonest isolate in most of the studies (Dulla *et al.*, 2015; Prasad *et al.*, 2013 and Abida Malik *et al.*, 2014) except for the study done by Bhatia *et al.*, where *T. mentagrophytes* (64.9%) was the most common dermatophyte isolated. Among the non-dermatophytes, *Candida species* (14.63%) were the most common isolate in our study which was similar to study done by Surendran *et al.*, (2014) (67.5%).

Table.1 Age distribution of suspected clinical cases

Age group of patients (in years)	Number of patients	Percentage (%) n=48
5 - 15	6	12.5
16 - 30	13	27.08
31 - 45	16	33.34
46 - 60	8	16.67
> 60	5	10.41
Total	48	100

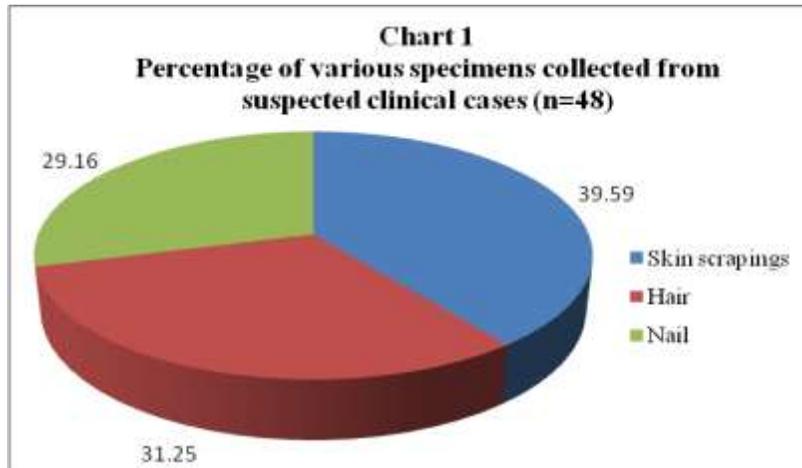


Table.2 Frequency of positive and negative results after specimen examinations (Direct microscopy Vs Culture)

Diagnostic test	KOH positive	KOH negative	Total (n=48)
Fungal culture positive	37 (77.08%)	4 (8.33%)	41 (85.41%)
Fungal culture negative	0 (0)	7 (14.59%)	7 (14.59%)
Total	37 (77.08%)	11 (22.92%)	48 (100%)

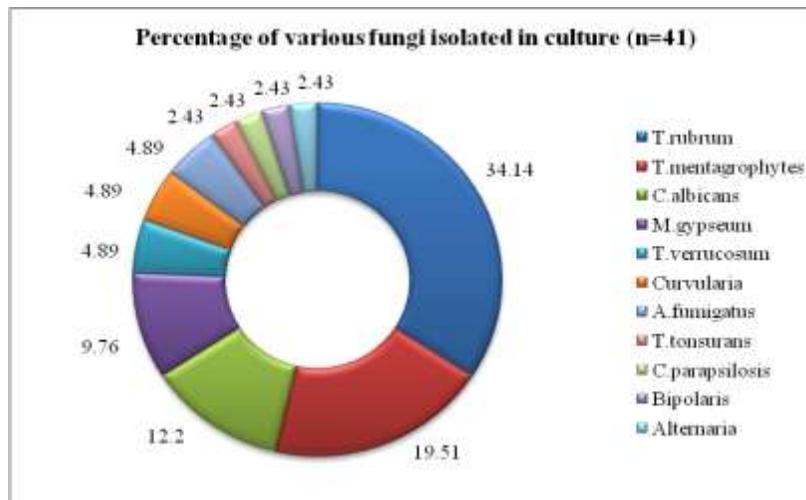


Table.3 different types of superficial mycoses cases isolated in our study

Provisional clinical diagnosis	Number of cases	Percentage of cases (n=48)	KOH mount positive	Fungal Culture positive
<i>Tinea corporis</i>	12	25%	10	12
<i>Tinea capitis</i>	10	20.83%	6	6
<i>Tinea barbae</i>	5	10.42%	3	4
<i>Tinea cruris</i>	5	10.42%	4	5
<i>Tinea pedis</i>	1	2.08%	1	1
<i>Tinea manuum</i>	1	2.08%	1	1
Onychomycosis	6	29.17%	12	12
Total	48	100	37	41

Table.4 Comparison of clinical cases isolated with other studies

Provisional clinical diagnosis	Present study	Karnataka (Surendran <i>et al</i>)	Himachal Pradesh (Bhatia <i>et al</i>)
<i>Tinea corporis</i>	25%	44.3%	30.19%
<i>Tinea capitis</i>	20.83%	0%	3.96%
<i>Tinea barbae</i>	10.42%	2.1%	0.49%
<i>Tinea cruris</i>	10.42%	38.2%	17.32%
<i>Tinea pedis</i>	2.08%	2.7%	16.83%
<i>Tinea manuum</i>	2.08%	3.3%	3.96%
Onychomycosis	29.17%	8.1%	23.26%

Table.5 Fungi isolated from superficial fungal infections: A comparison with different studies conducted in India

	Present study	Vijayawada (Dulla <i>et al</i>)	Aligarh study (Abida Malik <i>et al</i>)	Cuttack study (Prasad <i>et al</i>)
Dermatophytes	70.37%	68.7%	72%	52.53%
Non-dermatophytes	29.26%	31.3%	18.8%	4.38%

Table.6 Distribution of Dermatophytic isolates in comparison with other studies

	Present study	Dulla et al	Bhatia et al	Prasad et al	Surendran et al	Abida Malik et al
<i>T.rubrum</i>	34.14%	36.4%	35.1%	50.92%	67.5%	58.5%
<i>T.mentagrophytes</i>	19.51%	27.3%	64.9%	27.77%	20%	21.1%
<i>Microsporum gypseum</i>	9.5%	3.6%	1.35%	Nil	Nil	4.1%
<i>T.verrucosum</i>	4.88%	3.6%	Nil	Nil	Nil	Nil
<i>T.tonsurans</i>	2.43%	7.3%	Nil	Nil	Nil	Nil

Table.7 Distribution of Non-dermatophytic fungal isolates in comparison with other studies

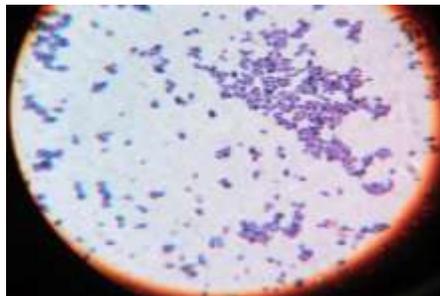
	Present study	Vijayawada (Dulla.et al)	Karnataka (Surendran et al)	Aligarh (Abida Malik et al)
<i>Candida species</i>	14.63%	12%	67.5%	3.5%
<i>A.fumigatus</i>	4.88%	4%	20%	15.6%
<i>Curvalaria</i>	4.88%	12%	Nil	Nil
<i>Alternaria</i>	2.43%	20%	Nil	6.25%

Fig.1 Identification of yeasts

Culture on SDA



Gram stain of the colony



Germ tube test



Chrom agar Candida



Fig.2 Identification of moulds

Ectothrix

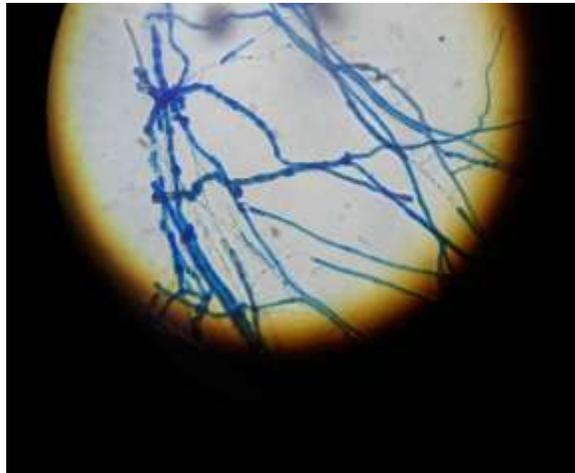
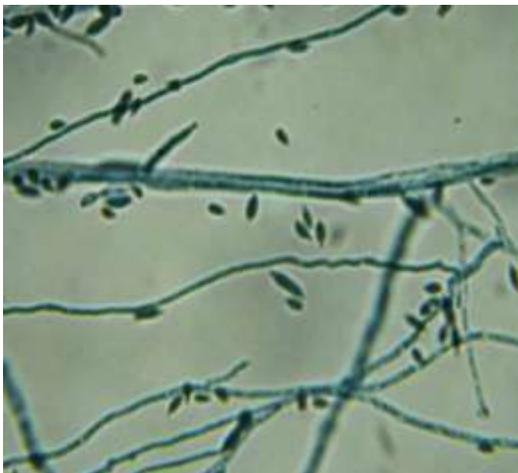


T.rubrum culture

Bipolaris in KOH mount (40x)



T. rubrum and *T. verrucosum* respectively (LPCB Mount after slide culture)



However, two studies (Dulla *et al.*, 2015 and Abida Malik *et al.*, 2014) done in Vijayawada and Aligarh showed that *Alternaria* (20%) and *A. fumigatus* (15.6%) were the most common non-dermatophyte species isolated respectively. From the above comparisons, it is clear that dermatophytes still continue to be the commonest causative agents in many parts of India. However, non-dermatophytic molds are also emerging as important causative agents and can no longer be neglected. The climatic conditions, overcrowding, unhygienic habits, occupational conditions and ignorance of common people have led to the persistence of these infections even though many of these infections are easily treatable.

In this study, *Bipolaris* was the rare fungal agent to be isolated and not many studies in India have reported it. This suggests that even rare fungal agents are prevalent in certain parts of our country and more studies are required from such regions in this regard.

It is concluded that along with dermatophytes, dermatomycotic fungi are also emerging as an important cause of superficial mycoses.

Though culture was found to be more sensitive than KOH mount in our study, both direct microscopy and cultures are important tools of diagnosis for the superficial fungal infections.

Good hygiene, sanitation and proper hand washes are effective methods for prevention of such infections.

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