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Microbiology

# MYCOLOGICAL PROFILE OF FUNGAL INFECTIONS FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

KEY WORDS: fungi, Candida, Aspergillus

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**ABSTRACT**

In last few years, Fungi have emerged as an important agents causing human infections leading to increased morbidity and mortality. Hence, the present study was conducted out to study the spectrum of fungal infections in patients of a tertiary care hospital.

A total of 281 clinical specimens were included in the study. Among these, fungal growth was seen in 102 specimens (36.30%). All samples were studied by direct microscopy and culture as per standard microbiological procedures. Maximum number of fungal isolates were obtained from sputum 46 (58.92%), followed by urine 16(15.68). The various fungi isolated were *C. albicans, C.non albicans, A. fumigatus, A. flavus, A. niger, A. nidulans, Fusarium species, Mucor species, Curvularia species, Penicillium species, Acremonium species, Cladosporium species.*

In the era of automation and molecular methods, even conventional methods should be considered as good tools for the detection of fungal infections.

## INTRODUCTION

In recent times, there is an emergence of fungal infections as an important cause of morbidity and mortality especially in critically ill patients. Indiscriminate and improper use of broad spectrum antimicrobial agents in various diseases has contributed to the increased propensity for fungal infections caused by both yeasts and moulds.[1] Many factors predispose to opportunistic invasive fungal infections like prolonged hospitalization, anticancer therapy, AIDS and other immunocompromised conditions.[2,3]

Early initiation of antifungal therapy is critical in deducing the high mortality rate in these patients. Rapid diagnosis of systemic fungal infections remains limited and culture detection of fungal isolates is often delayed due to slow or absent growth of fungal isolates from clinical samples.[4]

Successful treatment of fungal infections requires high index of clinical suspicion, knowledge on various aetiological agents and their susceptibility to available antifungal agents and familiarity with measures that can be taken to reduce the chances of spread or re-infections.[5]

The isolation of these agents from clinical specimens may pose a challenge to the clinicians unless there is proper identification of the organisms. Hence, improved diagnostic methods and proper routine laboratory practices are needed for better isolation of these pathogens. So, this study was undertaken to find out the spectrum of fungal pathogens at our tertiary care hospital.

## MATERIAL AND METHODS

The prospective study was conducted in the Department of Microbiology of a tertiary care hospital for a period of 1 year (August 2018 to July 2019). Various clinical specimens received at Mycology laboratory were included in study. A total of 281 clinical specimens were studied. Case history of patients was recorded. All samples were studied by direct microscopy and culture as per standard microbiological procedures.[6,7,8]

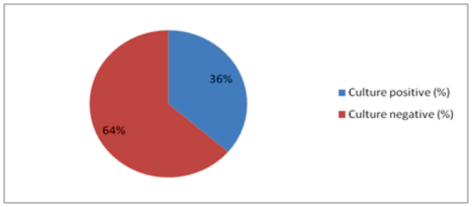
For direct microscopic examination, the samples were dissolved in KOH solution (10 %, for other specimens and 40 % for nail specimens) and observed under microscope for fungal elements. Gram staining was done to detect presence of yeast cells. Culture was done on Sabouraud's dextrose agar with antibiotics and also on Sabouraud's dextrose agar without antibiotics. The culture tubes were incubated at 25°C and 37°C and examined the growth for four weeks.

The identification of fungi was done by macroscopic examination of culture tubes. The texture, color and growth rate were taken into consideration. Microscopic examination was done by slide culture technique with Lactophenol cotton blue mount (LPCB mount) and charcteristics such as arrangement and type of hyphae, mycelium, conidium types were noted. The yeast isolates were identified by gram stain, Indian ink preparation germ tube test, chlamydospore formation on cornmeal agar (Dalmau technique), urease test, sugar fermentation and assimilation and colour production on CHROM agar.

## RESULTS

A total of 281 clinical specimens were included in the study. Among these, fungal growth was seen in 102 specimens (36.30%) while (63.30%) specimens were negative for fungal growth. (Figure 1)

**Figure 1 Distribution of total specimens (n=281)**




Maximum number of isolates were observed in age group of 46-60 years followed by age group of >60 years. Overall, Male preponderance was seen in the study (Male:Female 2.4). (Table 1)

**Table 1. Age and gender wise distribution of the samples**

| Age range | Male | Female | Total |
|---|---|---|---|
| <15 | 6 | 2 | 8 |

| | | | |
|---|---|---|---|
| 15-30 | 8 | 4 | 12 |
| 31-45 | 11 | 5 | 16 |
| 46-60 | 28 | 13 | 41 |
| >60 | 19 | 6 | 25 |
| Total | 72 | 30 | 102 |

**Table 2. Distribution of fungal isolates**

| Type of specimen | Number of fungal isolates (%) |
|---|---|
| Sputum | 43(42.16) |
| Urine | 16(15.68) |
| Blood | 11(10.78) |
| Pus/swab | 10(9.80) |
| BAL | 10(9.80) |
| Corneal scrapping | 8(7.84) |
| Nail | 4(3.92) |
| Total | 102 |

Maximum number of fungal isolates were obtained from sputum 46 (58.92%), followed by urine 16(15.68) and Blood 11(10.78).Pus/swab, BAL, Corneal scrapping accounted for 10(9.80%), 10(9.80%), 8(7.84), isolates respectively. Isolation from nail specimens was seen in 4(3.92%) of isolates.(Table 2) Of the total 102 fungal isolates that were culture positive, 72(70.59%) were yeast like fungi, and 30(29.41%) were moulds.(Table 3) Among the yeast like fungi, *Candida albicans* was isolated from 32(31.37%) cases, while *Candida non albicans* was isolated from 40(39.22%) cases. (Table 3)

The species distribution of Candida species is depicted in Figure 2. Among the 40 *Candida non albicans* isolates, *C.tropicalis* constituted for 16 isolates, *C.glabrata* for 11 isolates, *C.krusei* for 9 isolates. Six isolates belonged to *C. parapsilosis.*

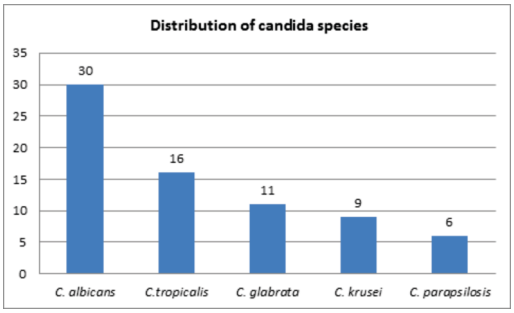
Among the 30 moulds, *Aspergillus species* were isolated from 20(19.61%) cases. Most common Aspergillus species isolated was *A.fumigatus* 9(8.82%) and majority of these isolates were obtained from sputum specimen. *A. flavus* and *A. niger* accounted for 6(5.88%) and 4(3.92%) cases. A single isolate of *A.nidulans* was obtained from a case of corneal ulcer in corneal scraping specimen. (Table 3)

Among the other fungal isolates obtained during the study, *Fusarium species* was isolated in 2 cases of corneal ulcer from corneal scraping specimen. Two isolates of Dermatophytes were obtained from nail clipping specimen. One isolate was identified as *Trichophyton mentagrophtye* and other as *Trichophyton rubrum*. *Mucor species* accounted for two isolates. While *Penicillium species*, *Curvularia species*, *Acremonium species, Cladosporium species* accounted for single isolate each. (Table 3)

**Table 3. Spectrum of isolates among various samples**

| Organism | Blood | sputum | BAL | Pus/ Swab | Urine | Corneal scrapping | Nail | Total(%) |
|---|---|---|---|---|---|---|---|---|
| *C. albicans* | 4 | 10 | 2 | 2 | 12 | | 2 | 32(31.37) |
| *C.non albicans* | 7 | 17 | 7 | 5 | 4 | | | 40(39.22) |
| *A. fumigatus* | | 8 | 1 | | | | | 9(8.82) |
| *A. flavus* | | 5 | | | | 1 | | 6(5.88) |
| *A. niger* | | 2 | | 2 | | | | 4(3.92) |
| *A. nidulans* | | | | | | 1 | | 1(0.98) |
| *Fusarium species* | | | | | | 2 | | 2(1.96) |
| *Penicillium species* | | 1 | | | | | | 1(0.98) |
| *Curvularia species* | | | | | | 1 | | 1(0.98) |
| *Trichophyton mentagrophtye* | | | | | | | 1 | 1(0.98) |
| *Trichophyton rubrum* | | | | | | | 1 | 1(0.98) |
| *Acremonium species* | | | | | | 1 | | 1(0.98) |
| *Mucor species* | | | | 1 | | 1 | | 2(1.96) |
| *Cladosporium species* | | | | | | 1 | | 1(0.98) |
| Total | 11 | 43 | 10 | 10 | 16 | 8 | 4 | 102 |

**Figure 2. Distribution of candida species**




## DISCUSSION

Fungal infections are often insidious in development and their diagnosis is often delayed because of the co-existing illnesses. The emergence of these infections has created a challenge in their diagnosis and this makes the management difficult.[4] A total of 281 clinical specimens were received during the study period of one year. Out of these, fungal growth was seen in 102 specimens. Maximum number of isolates were observed in age group of 46-60 years followed by age group of > 60 years. It may be due to the increased outdoor activities, and greater physical exertion in this group and also because there is waning of immunity as the age advances. This finding correlates with the study of Narayan et al[5] who also showed similar finding.

Male preponderance was seen in the present study with male to female ratio of 2.4. Similar male preponderance was seen in other Indian studies by Alim et al[9], Nawal et al[10] who also had similar observation.

Sputum was the most common specimen from which maximum number (58.92%) of isolates were obtained. Nageshwari et al[11] also observed sputum to be the most common specimen. Most of the patients showed underlying lung conditions and were immunocompromised patients and this might be the reason from maximum positivity from sputum specimen. Hence, proper history and clinical correlation are important tools that guide towards proper diagnosis.

In the present study, *Candida albicans* was isolated from 32(31.37%) cases, while *Candida non albicans* was isolated from 40(39.22%) cases. However, according to various studies, *Candida non albicans* species have more isolation rate than *C.albicans* suggesting that there is an emergence of Candida non-albicans species as important pathogens.[12,13]

Whereas Nageshwari et al[12], isolated majority of *Candida albicans* followed by *Candida non albicans*.

*Candida albicans* species was most commonly associated with urinary tract infection ie. 12 cases. Among these 12 cases, 5 patients were diabetics and 7 patients were catheterized patients. In diabetics, susceptibility to Candida infection increases probably because of increased antibiotic use, associated illnesses and hyperglycaemia.[14] Catheterisation is a risk factor for urinary tract infection because it allows migration of organisms into the bladder from external peri-urethral surface.[15] Among the *Candida non albicans* species, *C.tropicalis* was the common species. This also correlates with the study of Nageshwari et al.[11]

Speciation of Candida species by CHROMagar on the basis of colour differentiation is a rapid, convenient and reliable method for identification of clinically important Candida species when compared with cumbersome traditional techniques. In developing countries, CHROMagar can be taken as a simple phenotypic test alternative to molecular based assay. CHROMagar has high sensitivity as well as specificity for the identification of Candida species.[16,17]

Among the *Aspergillus species*, *A. fumigatus* was the most common species 9(8.82%).these were more commonly obtained from sputum specimens in patients of chronic pulmonary diseases.

In the present study, 8 corneal scrapings were found to be culture positive. These were also positive in direct microscopy. In the present study, *Fusarium species* was the most common isolates with two isolates of *Fusarium species* 2(1.96%). Narayan et al[9] also observed *Fusarium species* to be the commonest isolate in keratomycosis cases. These patients had a history of trauma that further contributed to fungal infection. One isolate of *Curvularia species was* isolated from a patient who had a history of trauma and foreign body in the eye. In the present study, a single *Aspergillus nidulans* was isolated in a patient who also had history of trauma in the eye. One isolate of *Cladosporium species* was also isolated from a case of fungal keratitis. Ulcerating keratitis due to *Acremonium species* was seen in a single case. So, this shows a variety of fungi that are involved in causing fungal infections of the eye. Processing of corneal scrapings has importance of direct microscopy and bedside inoculation of specimen as a useful diagnostic tool which needs vigilance.

Nail is known as a common site for Dermatophytic infection and Onychomycosis.[7] In the present study, *Candida albicans* accunted for 2 cases of nail infections and Dermatophyes were isolated from 2 cases. The nail clipping specimen revealed positive KOH findings in all these cases.

In the present study, one isolate of *Penicillium species* was isolated from sputum specimen. The possibility of this fungus as contaminant was ruled out by its repeated isolation.

So, the pattern of fungal infections varies from one geographic region to other. Studies regarding isolation of fungi from various specimens are needed be carried out to find out the common prevalent fungal agents in a geographic location. This study depicts various isolates obtained from the various specimens to know the causative agents causing the infections. This would help clinicians to know the epidemiology in their areas and serve as guide in management of the cases.

## CONCLUSION

There is an increase in incidence of fungal infections in past few years and more commonly immunocompromised patients are involved. These infections can be life threatening if not diagnosed and treated. Early laboratory diagnosis would help in reducing the morbidity and mortality associated with these fungal infections. Proper diagnosis can prevent delay in initiating proper treatment therapy. Here comes the role of the role of diagnostic mycology laboratory in management of fungal infections. Conventional methods like KOH examination and fungal culture should be considered as good tools for the detection of fungal elements from the clinical samples and with the proper clinical correlation, these methods help in proper diagnosis and treatment.

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