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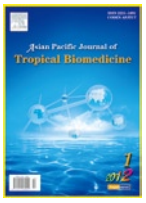

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# Fungal profile of clinical specimens from a tertiary care hospital

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ABSTRACT

**Objective:** To investigate the prevalence of some common fungal infections in relation to the site of involvement over one year period from a tertiary care hospital. **Methods:** Samples were collected from the patients presenting with clinically suspected fungal infections. Direct microscopy with KOH was done to visualize presence of any fungal element and gram staining was done for any suspected yeast infection. For fungal culture all samples were inoculated on two isolation media; one sabouraud's dextrose agar (SDA) and the other SDA with chloramphenicol and cycloheximide. **Results:** A total of 2 228 samples from various infections suspected of fungal etiology were received during the one year period of analysis, out of which nail was the most frequent. Dermatophytes were found to be most frequent fungal isolates. **Conclusions:** There are distinct patterns of geographical variation in the etiology of fungal infections and it is essential to determine the local etiology within a given region when planning a management strategy

## 1. Introduction

In recent years, yeasts and molds have emerged as important pathogens[1]. These fungi are a leading cause of morbidity and mortality in cancer, burn, and surgical patients as well as neonatal intensive care unit patients[2]. Advances in newer technologies in medical and surgical therapies, use of invasive monitoring devices and broad-spectrum antimicrobial agents over the past two decades have helped to treat patients suffering from previously devastating or fatal diseases, but they have however, changed the type of patients cared for in our hospitals. These successes have resulted in proliferation of many severely ill and immunocompromised individuals who are highly susceptible to infections caused by fungi that were previously considered to be of low virulence. Consequently, infections due to previously obscure fungi are being seen more commonly in hospitalized patients. Fungal infections in these patients are often severe, rapidly progressive, and difficult to diagnose or treat. Early initiation of antifungal therapy is critical in reducing the high mortality rate in these patients. Despite intensive efforts by many investigators, early and rapid diagnosis of systemic fungal infections remains limited. Culture detection of fungal species is often delayed because of slow or absent growth of fungal isolates from clinical specimens. Hence, continued epidemiologic and laboratory research is needed to better characterize these pathogens, allowing for improved diagnostic and therapeutic strategies in the future. With this aim in mind this retrospective analysis was undertaken to know the prevalence of some common fungal infections in relation to the site of involvement over one year period from a tertiary care hospital in east Delhi.

## 2. Materials and methods

### 2.1. Study design

This retrospective study comprised of samples received from all the patients suspected of fungal infections over a period of one year (April 2009 to April 2010) in the Microbiology Laboratory of University College of Medical Sciences (UCMS) which is attached to Guru Teg Bahadur Hospital (GTBH), a tertiary care hospital in east Delhi.

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### 2.2. Samples

The samples were collected from the patients presenting with clinically suspected fungal infections from various clinical departments in the mycology division of the Department of Microbiology and were kept in separate sterile containers. All samples were analyzed by direct microscopy and culture.

### 2.3. Microbiological lab diagnosis

For direct microscopic 40% KOH in nails and 10% KOH in other samples was used to visualize presence of any fungal element and for any suspected yeast infection gram staining was done to look for gram positive yeast cells. For fungal culture all samples were inoculated on two isolation media; one sabouraud's dextrose agar (SDA) and the other SDA with chloramphenicol and cycloheximide. All culture media and antibiotics were obtained from Hi-media Laboratories, Mumbai, India. The culture tubes were incubated at 25 ℃ and 37 ℃ and examined daily for six weeks.

The identification of fungi was done by macro and micro morphological evaluation by inspection of the cultures tubes and by slide culture technique respectively. The characteristics considered for fungus identification were macroscopic aspects of texture, colour, growth rate and microscopic aspects such as mycelium and conidium types, relationship between hyphae and fructification organs by lactophenol cotton blue mount. Micro culture on slides was the technique used for observation of filamentous fungi. The yeast isolates were identified by standard tests like germ tube, different spore production on corn meal agar (CMA) or rice starch agar (RCA), urease production and sugar fermentation and assimilation tests[3].

## 3. Results

The frequency distribution of samples from clinically suspected fungal infections from various clinical departments was listed as follow: the most frequent sample received in the mycology division for fungal diagnosis was nail in around 35% of total samples followed by skin, hair, corneal scraping and urine in 32%, 20%, 4% and 3%; sputum and biopsy samples in 2% each; blood culture and CSF in 1% each and pus in 0.4% of the total samples respectively.

The detailed breakup of the age and sex distribution of the study group is shown in Table 1. Majority of the patients belonged to the adult age group (>14 years and < 60years) with an overall predominance of males over the females with the exception of pus and biopsy samples where females predominated.

As regards the proportion of various fungal isolates that were obtained from these sample during this one year period it was seen maximum positivity was seen in biopsy samples (55%) followed positivity of 46%, 38% and 34% in skin, blood and urine samples respectively. The detailed distribution of the frequency of isolation of the fungi in various other samples in given in Table 2. Among the various fungal isolates obtained during this study, Dermatophytes were found to be most frequent amounting to 422 out of the total of 612 fungal isolates (69%). The lesser frequent isolates were *Candia* spp. (112/612 = 18%) and *Aspergillus* spp. (42/612 = 7%).

When the correlation between direct microscopy and culture was done for various samples, it was found that highest correlation between microscopy and culture (100%) was found for hair and pus samples (Table 3).

## 4. Discussion

Fungi are opportunistic organisms which are ubiquitous in nature. The increased incidence of systemic fungal infections in the past two decades has been overwhelming. Earlier, it was pathogenic dimorphic fungi, which were

**Table 1**
Age and sex distribution in relation to site of involvement

| Clinical sample | Age group | | | | Total | Male to female ratio (M:F) |
|---|---|---|---|---|---|---|
| | Newborn | Pediatric (<= 14 years) | Adult (> 14 years <=60 years) | Geriatric (> 60 years) | | |
| Nail | 0 | 80 | 572 | 130 | 782 | 1.2:1 |
| CSF | 0 | 2 | 24 | 2 | 28 | 13:1 |
| Sputum | 0 | 10 | 22 | 4 | 36 | 3.5:1 |
| Blood culture | 12 | 4 | 0 | 0 | 16 | 1:1* |
| Pus | 0 | 8 | 0 | 2 | 10 | 2:3 |
| Biopsy | 0 | 4 | 32 | 8 | 44 | 5:6 |
| Corneal scraping | 0 | 4 | 72 | 20 | 96 | 5:3 |
| Urine | 10 | 16 | 34 | 4 | 64 | 1.46:1 |
| Hair | 2 | 307 | 119 | 20 | 448 | 1:1 |
| Skin | 6 | 99 | 571 | 28 | 704 | 2.2:1 |
| Total | 30 | 534 | 1444 | 218 | 2228 | 2228 |

*: excluding newborns.

**Table 2**
Relative proportions of fungal isolates obtained from clinical samples.

| Fungal isolate | Clinical sample | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nail | CSF | Sputum | Blood culture | Pus | Biopsy | Corneal scraping | Urine | Hair | Skin | Total (%) |
| Candida albicans | 16 | 0 | 8 | 6 | 0 | 8 | 0 | 10 | 10 | 19 | 77 (12.6) |
| Non albicans Candida | 18 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 9 | 35 (5.7) |
| Dermatophyte | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 94 | 296 | 422 (68.9) |
| Aspergillus spp. | 16 | 0 | 0 | 0 | 0 | 10 | 6 | 10 | 0 | 0 | 42 (6.9) |
| Acremonium | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 4 (0.6) |
| Geotricum | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (0.6) |
| Aureobasidium | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 6 (0.98) |
| Syncephalastrum | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.3) |
| Trichosporon | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.3) |
| Scedo | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.3) |
| Cladosporium | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.3) |
| Fusarium | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.3) |
| Cryptococcus | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (0.6) |
| Curvularia | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 (0.6) |
| Bipolaris | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 (0.6) |
| Pseudoallescheria | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 (0.3) |
| Total culture positivity (%) | 94 (12) | 4 (14) | 10 (28) | 6 (38) | 0 (0) | 24 (55) | 22 (23) | 22 (34) | 108 (24) | 324 (46) | 612 |

**Table 3**
Correlation between direct microscopy and culture based diagnosis in various clinical samples.

| Clinical sample | Direct microscopy positive | | Direct microscopy negative | | Agreement between direct microscopy and culture (%) |
|---|---|---|---|---|---|
| | Culture positive | Culture negative | Culture positive | Culture negative | |
| Nail | 85 | 126 | 9 | 562 | 83 |
| CSF | 3 | 4 | 1 | 20 | 82 |
| Sputum | 8 | 4 | 2 | 22 | 83 |
| Pus | 0 | 0 | 0 | 10 | 100 |
| Biopsy | 14 | 2 | 10 | 18 | 73 |
| Corneal scraping | 21 | 6 | 1 | 68 | 93 |
| Urine | 14 | 6 | 8 | 36 | 78 |
| Hair | 108 | 0 | 0 | 340 | 100 |
| Skin | 324 | 26 | 0 | 354 | 96 |

known to cause systemic infections. However, more recently, newer and less common fungal agents started causing more number of infections. Awareness of fungal infections has increased in clinical practice with the increased survival of patients having immunocompromised states[4]. These infections are often insidious and their diagnosis is usually delayed because of the coexisting illnesses[5]. Numerous studies have identified common risk factors for patients developing fungal infections. Most of these risk factors are very common in all hospitalized patients, and it is therefore difficult to determine which patients are at greatest risk for developing fungal infections. In general, two populations have been at risk for acquiring invasive fungal infections. The first are persons at increased susceptibility of infection because of their geographic location. The second population includes persons with increased host susceptibility (i.e., severely ill, immunocompromised, or malnourished individuals) who develop opportunistic infections. Although diagnostic and therapeutic modalities for some fungi are improving, there is still much to learn about many of the other fungi that are diagnosed in our hospitals nowadays. Fungi are eukaryotic cells; they are more complex than bacteria. A thorough appreciation and understanding of fungal infections, including diagnostic and therapeutic modalities, are needed among clinicians and microbiologists to provide better patient care.

A total of 2228 samples from various infections suspected of fungal etiology were received in our mycology division during the one year period of analysis out of which nail was the most frequent (35%). Previous studies[6] have reported that onychomycosis is the commonest nail disorder encountered in clinical practice constituting 20-40 % of all diseases of nails[7] and 30 % of superficial mycotic infections[8]. In our study nail infections amounted to approximately 15% of total fungal infections. Various Indian workers have reported incidence to be 0.5 to 5% in the general Indian population[9]. This has been related to a variety of etiological factors, including a rise in immunocompromised patients, an aging worldwide population and a rise in environmental risk factors secondary to life style changes. Dermatophytes, especially Trichophyton rubrum are the most frequently implicated causative agents in onychomycosis. Previously regarded as contaminants, yeasts are now increasingly recognized as pathogens in finger nail infections, as are some

moulds[10]. Another recent study from north India reported that the most common fungal isolates in onychomycosis were dermatophytes (49.5%), followed by Candida spp. (40.4%) and nondermatophyte molds (10.1%)[11]. In our study candida species accounted for 34/94 (36%) {candida albicans-16; non albicans candida-18} of fungal nail infections followed by dermatophytes in 30/94 (32%) cases. Several nail disorders that may mimic fungal nail infections, like psoriasis, lichen planus, bacterial infections, contact dermatitis, traumatic onychodystrophies, idiopathic onycholysis, paronychia congenital etc must be differentiated from one another[12]. Compared to other superficial mycoses, this condition is persistent, intractable and poses serious concern to the clinicians as it often becomes a chronic source of recurrent superficial mycotic skin infections, besides causing considerable disfigurement.

Fungal infections of the central nervous system (CNS) are relatively uncommon neuroinfections that are increasingly being recognized these days. This is mainly due to the increased awareness of clinicians about these conditions, the growing pool of immunocompromised host, the advances in imaging, and the availability of microbiological techniques to confirm the diagnosis from body fluids and specimens. Given the non specific disease pattern of invasive CNS fungal infection, the pursuit of a specific diagnosis is important and by defining the specific infecting organism, targeted antifungal therapy can be deployed. Potential pathogens include yeasts, Aspergillus spp., other moulds of an increasing variety, and a range of dimorphic fungi, often associated with particular geographical locations. In a recent study from south India, during a period of 7 years there were a total of 453 cases with neuroinfections and only 2.7% of them satisfied their criteria for CNS mycosis.13 Similarly fungal infection of the CNS was relatively rare in our experience (0.6% of all fungal infections). Cryptococcal meningitis is the most common clinical fungal infection of the CNS[13,14]. In our study also cryptococcus was isolated in 100% of CSF samples that came for fungal diagnosis.

Fungal bloodstream infections are associated with significant patient mortality and health care costs. Invasive fungal infection is a severe clinical complication in immunocompromised patients, such as neutropenic patients, recipients of bone marrow or solid organ transplants, cancer patients receiving chemotherapy, and HIV-infected patients. However, during the past two decades, with advances in diagnostic and therapeutic interventions, critically ill patients with lesser degrees of immunocompromise, especially those in surgical and neonatal intensive care units (ICUs), have emerged as another population at high risk for fungal bloodstream infections[15]. The inability to diagnose many invasive fungal infections in a timely manner continues to be a significant problem, and improved diagnostic methods are needed to permit early detection of infection. In our analysis out of the total 16 blood culture samples submitted with a suspected fungal etiology, candida grew in 6 samples. Candida bloodstream infections are a common cause of late-onset sepsis in the NICU and are associated with significant mortality and neurodevelopmental impairment as evident in our case also where 12/16 cases were from the neonates[16].

Scarring of the cornea as a result of suppurative keratitis is an important cause of preventable blindness after unoperated cataract in some developing countries in the tropics[17]. The associated morbidity is the result of several factors and is directly affected by difficulties in patient management because of a lack of diagnostic facilities and appropriate treatment. Medical therapy remains empiric, but understanding of the aetiology of these infections can help guide selection of effective treatment. Fungi are identified as the principal etiological agent of corneal ulceration in as many as 2/3rd of these cases: a recent study from Ghana identified 44% fungal etiology of suppurative keratitis in 800 patients of south India[18]. Reported incidences of fungal keratitis from different regions of India vary from 5% to 40%[19]. More than 105 species of fungi, classified in 56 genera, have been reported to cause oculomycosis[20]. However, species of Fusarium, Aspergillus, and other hyaline hyphomycetes, and species of Curvularia and other dematiaceous hyphomycetes, are the usual isolates from patients with filamentous fungal keratitis, while Candida albicans is the most frequent cause of keratitis due to yeast-like and related fungi[18,19,21]. Filamentous fungi are responsible for a larger proportion of these corneal infections in tropical climates than in temperate climate as found in our analysis also.

Previous research show that there are distinct patterns of geographical variation in the etiology of fungal infections and it is essential to determine the local etiology within a given region when planning a management strategy. Knowledge of the risk factors for fungal infections and the clinical characteristics are important to enable prompt diagnosis and appropriate care. The recognition of the changing patterns of different types of fungal infections in an area will aid in the therapeutic approach and the implementation of control measures. Traditional diagnostic laboratory methods may be negative despite a clear clinical presentation due to difficulty in obtaining sufficient clinical material or due to self administration of antibiotics by patients before seeking medical attention which has been thought to affect the recovery of organisms in culture. It is imperative that the quality and quantity of specimen is optimal for accurate laboratory diagnosis. It is usually not possible to determine the significance of fungus observed by microscopy alone. In this study 95% of fungal infections could have been diagnosed based on the findings from microscopy alone and there was good agreement ranging from 80-100% between direct microscopy and culture (except biopsy samples with agreement of 73%). This is an important conclusion, since the majority of rural based clinics in areas where fungal infections are common do not have culture

facilities but may be able to perform simple microscopy.

The data on burden of mycotic infections in India are not clear though the climatic diversity in this country is suited for a wide variety of fungal infections. However, a definite rising trend has been noted. Hence, this analysis emphasizes the fact that the clinical mycology laboratory must be able to recognize this increasingly large group of potential pathogens. Organisms once thought to be contaminants are now confirmed pathogens causing systemic infection in immunocompromised patients. Hence, continued epidemiologic and laboratory research is needed to better characterize these pathogens, allowing for improved diagnostic and therapeutic strategies in the future.

## Conflict of interest statement

We declare that we have no conflict of interest.

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