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CURRENT PROFILE OF FUNGAL PATHOGENS IN MYCOLOGICAL INFECTIONS IN A RURAL TEACHING HOSPITAL AREA.

Pavani G Associate Professor, Department of microbiology, AIMSR, Hyderabad. - Corresponding author

Anuradha M. Professor, Department of microbiology, AIMSR, Hyderabad.

ABSTRACT

Objectives: To detect the fungal pathogens in various clinical specimens.To know the current profile of fungal pathogens in various mycological infections.

Material and methods: Various samples were collected from patients with clinically suspected fungal infections. KOH mount ,gram staing and fungal culture was done for all the samples. Germ tube test was done to speciate Candidal isolates into Candida albicans and Nonalbicans candida.

Results : 1267 samples were processed over a period of one year. In nail samples the major pathogens were Candida species . Candida was the sole pathogen in blood stream mycosis. There is increased prevalence of cryptococcal meningitis , Urinary tract aspergillosis, keratitis due to filamentous fungi.

Conclusion : Candida is emerging as the most common pathogen in onychmycosis. Candidal infections are still an important cause of neonatal sepsis. Increased prevalence of opportunistic infections like cryptococcal meningitisand urinary tract aspergillosis suggests a rising population of immunocompromised patients.

KEYWORDS : Fungal pathogens, Mycological infections, Candida.

Introduction:

Fungi have emerged as important pathogens in the recent years[1]. They are a leading cause of morbidity and mortality in cancer, burn, and surgical patients as well as in neonatal intensive care unit patients[2]. Over the past two decades, advances in medical and surgical therapies and use of invasive monitoring devices and broad-spectrum antimicrobial agents 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.[3]. Furthermore, the aetiology of these infections has changed. In the 1980s, yeasts like Candida albicans were the most common causative agents of invasive mycoses. In recent years, moulds like Aspergillus spp. have become frequent in certain groups of patients, such as patients with solid organ transplantations, cancer or prolonged neutropenia.[4,5] Hence, continued epidemiologic and laboratory research is needed to study these pathogens, allowing for improved diagnostic and therapeutic strategies in the future.

Previous research show that there are distinct patterns of geographical variation in the etiology of fungal infections and sometimes, 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 medication by patients before seeking medical attention which may effect the culture reports.[3] In such a scenario ,it is useful to have indepth knowledge of the the local etiology within a given region when planning a management strategy. Hence this analysis was done to know the aetiological agents in the common fungal infections and to know the current prevalence of these pathogens in our hospital area .

Materials and Methods:

Samples were collected from all the patients with clinically suspected fungal infections over a period of one year from july 2010 to july 2011 in the central lab of Mediciti institute of medical sciences. A total of 1267 samples were collected in separate sterile containers which included Nail , CSF ,respiratory samples Blood , pus, corneal scraping s, urine, hair clippings and skin scrapings. All the samples were subjected to direct microscopy and fungal culture. For direct microscopy 40% KOH for nails and 10% KOH for other samples was used to visualize presence of any fungal elements and for any suspected yeast infection, gram staining was additionally done to look for gram positive yeast cells. Then all the samples are inoculated on two isolation media i.e sabouraud's dextrose agar and sabouraud's dextrose agar with chloramphenicol and cycloheximide. All culture media and antibiotics were obtained from Hi-media Laboratories, Mumbai, India. The inoculated culture tubes were incubated at 25 ^{o}C and 37 ^{o}C and examined daily for six weeks.

The identification of fungi was done by macroscopic and microscopic examination of the growth in the cultures tubes . The characteristics considered for fungus identification under macroscopic aspects were texture, colour and growth rate .Microscopic aspects such as types of mycelia and conidia, relationship between hyphae and conidia were observed by performing a lactophenol cotton blue mount. A germ tube test was performed for differentiating Candia albicans from non albicans candida .

Results:

A total of 1267 specimens from clinically suspected fungal infections were processed out of which only 101 (8%) specimens were positive for the presence of fungi while remaining 1166 (92 %) specimens were negative for the presence of fungi. In these 1166 specimens, 240 specimens showed no growth of organisms while remaining 926 specimens showed the growth of pathogenic bacteria.

Table 1: Relative proportion of fungal isolates obtained from clinical samples

Fungal isolate	Clinical sample respiratory									
	Nail	CSF	samples	Blood culture	pus	corneal scraping	urine	hair	skin	total (%)
Candida albicans	6	0	18	18	0	0	8	0	6	56 (55.4 %)

Non albicans candida	2	0	0	0	0	0	0	0	2	4 (4.95%)
Dermatophytes	5	0	0	0	0	0	0	3	10	18(17.8%)
Aspergillus	4	0	0	0	0	3	3	2	0	12(11.9%)
Cryptococcus	0	2	0	0	0	0	0	0	0	2(1.98%)
Curvularia	0	0	0	0	0	1	0	0	0	1(0.99%)
Fusarium	2	0	0	0	0	2	0	0	0	4(3.96%)
Acremonium	1	0	0	0	0	0	0	0	0	1(0.99%)
Bipolaris	0	0	0	0	0	1	0	0	0	1(0.99%)
Aureobasidium	0	0	0	0	0	2	0	0	0	2(1.98%)
Total culture positives(%)	20 (19.8%)	2 (1.98%)	18 (17.8%)	18 (17.8%)	0 (0%)	9 (8.9%)	11 (10.8%)	9 (4.9%)	18 (17.8%)	101

Discussion :

There has been an increase in the incidence of fungal infections in the recent years. These infections are ubiquitous. In the past only dimorphic fungi were implicated in the causation of systemic fungal infections. But at present less common fungal agents are increasingly found as causative agents in these infections. Although diagnostic modalities for some fungi are improving, there is still much to learn about many of the less common fungal agents that are being isolated these days.[6] Fungi being more complex than bacteria, a thorough understanding of fungal infections is needed among clinicians and microbiologists to provide better care to the patients.

A total of1267 samples from various clinically suspected fungal infections were received in the central laboratory of our hospital. Nail was the most frequent sample processed. 19.8% of total fungal infections in our study were nail infections. Some authors have reported 0.5 to 5% prevalence of nail infections [7] and others have reported 20-40% prevalence in their studies.[8]. In the nail samples ,dermatophytes are the most frequently implicated agents in onychomycosis. But in the present day scenario ,yeasts and other moulds are being increasingly recognized as pathogens [9]. In our study also, isolation rate of Candida albicans and Non albicans candida was 40%. After candida the next common isolates were dermatophytes (25%). Onychomycosis poses a serious problem to the clinicians as it becomes chronic and causes disfigurement. Candida is emerging as the most common pathogen in onychomycosis. The knowledge of changed scenario of etiological agents in nail infections enables the clinician to deliver better treatment to the patients.

Bloodstream infections caused by fungi are costlier to treat and cause significant patient mortality . Critically ill patients with lesser degrees of immunocompromise especially those in surgical and neonatal intensive care units have also emerged as a risk group for these infections apart from immunocompromised patients. [10] . In our study, out of the 20 blood culture samples studied for fungal etiology ,18 samples (90%) grew candida .No other fungus was isolated. And all these samples were from the NICU. In another study also candida was the only fungal isoate in blood.[3] It is well known that Candidal infections are a common cause of sepsis in the NICU associated with significant mortality . Our study establishes the fact that candida are still an important cause of neonatal sepsis.

Central nervous system infections associated with fungi were uncommon infections but are increasingly being seen in the recent years. Increased availability of microbiological techniques to confirm fungal diagnosis from body fluids has led to their increased detection. Fungal infections of CNS have a nonspecific disease pattern. Potential pathogens are yeasts, aspergillus and dimorphic fungi depending on the various geographical areas. Cryptococcus is usually the most common yeast in CNS mycosis.[11,12] In our study also, cryptococcus was isolated in 66.6 % of the samples processed. Moreover Cryptococcus was the only isolate. However other studies from south India revealed only 2.7% isolation of cryptococcus in CNS infections .[11]. This suggests that there is increased prevalence of cryptococcal meningitis in our hospital area. Three successive studies spanning over a period of 12 years in AIIMS revealed that parallel to increase in number of HIV cases; HIV cryptococcosis co-infection also increased from 20% to 50 % in the recent years [13].It can be inferred that increased prevalence of cryptococcal meningitis in our hospital area could be due to increased immunocompromised conditions like HIV in our hospital area.

Infections of the cornea are a preventable cause of blindness in the tropics[14]. Medical therapy is usually empiric for these infections.Incidence of fungal keratitis in different parts of our country vary from 5-40% [15] . Candida Fusarium, Aspergillus, Curvularia and dematiaceous fungi are the usual isolates. [16]. Our study reveals filamentous fungi as the main isolates in keratitis. *Aspergillus* species (33.3%) and *Fusarium* species (22.2%)) were the most frequently isolated filamentous fungal pathogens from cases of mycotic keratitis .Our study was in accordance with the study of Hind et al whose isolation rate of *Aspergillus* species and *Fusarium* species was 27.6% & 17.2% respectively. [17] Other studies revealed Candida albicans as the most frequent cause of keratitis [18,19,20] However filamentous fungi are responsible for a larger proportion of corneal infections in tropical climates than in temperate climate .

In our study, Candida was the only fungus isolated from the respiratory samples. The isolation of Candida from culture of sputum, endotracheal aspirates, bronchoscopic samples, percutaneous lung needle aspirates and even from lung tissue may only represent colonization of the trachea-bronchial tree [21]. Despite the debate about the diagnosis of pulmonary candidiasis, the definitive diagnosis is still resting on histological demonstration of the yeast in lung tissue with associated inflammatory changes at autopsy [22,23].

There were no fungi reported in pus in the present study. All these pus samples were collected from infected wounds of post-operative patients where pyogenic bacteria are the usual pathogens.

In the hair samples,though the dematophytes were prevalent ,aspergillus was also a major isolate. The presence of non-dermatophytic moulds particularly Aspergillus and Penicillium species in hair may be due to the ubiquitous nature of their spores in our environment, carried transiently on healthy skin.[24,25]

Urine samples for fungal isolation revealed candida and aspergillus as predominant pathogens in our study. In many instances a report from the clinical laboratory indicating candiduria represents colonization or contamination of the specimen while collection and not invasive candidiasis. Even if infection of the urinary tract by *Candida* species can be confirmed, antifungal therapy is not always warranted. Further investigation may reveal predisposing factors, which if corrected or treated, result in the resolution of the infection. For those with symptomatic urinary tract infections , the choice of antifungal agent should depend upon the clinical status of the patient, the site of infection, and the pharmacokinetics and pharmacodynamics of the agent. [26]. Urinary tract aspergillosis is uncommon even in the era of increased frequency of invasive mycoses except for a few isolated case reports and rare case series or reviews. Previous research studies reveals that the majority of cases involve transplant recipients predominantly following renal transplantation but is also reported in other immunocompromised states such as AIDS and uncontrolled diabetes mellitus.[27]. This suggests that there is increased prevalence of Urinary tract aspergillosis in our hospital area.

Apart from dermatophytes, Candida sp. were seen in the skin scrapings . Candida is found in soil, on inanimate objects, in food and in hospital settings. Many Candida spp. tend to be commensal flora and can be recovered from numerous sources in and on sick patients.[28]. However, Candida spp. may become opportunistic and can produce a wide variety of infections causing difficulty in distinguishing between normal colonization and infection. The

isolation of Candida from "dirty" specimens such as wounds, skin, urine, sputum, or stool is not necessarily diagnostic or indicative of disease; Candida isolated from sterile sites such as CSF only should be considered diagnostic of infection.

The reasons for the increased isolation rate of *Candida* albicans and nonalbicans species of candida among the total samples processed can be attributed to the increase in the opportunistic pathogens of different species due to the environmental changes and weakened immune functions in the human body. Though in some sites candida may not be a significant pathogen, it should still be considered an important microbial species considering its detection rate i.e 60.4% of the total detection rate of fungal pathogens and hence requires continuous monitoring and support of additional clinical studies.

Conclusion :

Candida is emerging as the most common pathogen in onychmycosis and as a sole pathogen in blood stream mycosis. Candidal infections are still an important cause of neonatal sepsis . Filamentous fungi are the major pathogens in mycotic keratitis.. Increased prevalence of cryptococcal meningitis, urinary tract aspergillosis and increased overall isolation of candidal species in clinical samples is due to an increase in the immunocompromised patients in the recent years.

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