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Original Research Article

A study on spectrum of fungal pathogens in an Indian multi-specialty hospital

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A B S T R A C T

Fungus can cause severe infection in immunocompromised patients and also in debilitating diseases. Numerous studies have identified risk factors for acquiring these infections, most of which are very common among hospitalized patients; some factors act primarily by inducing immunosuppression (e.g., corticosteroids, chemotherapy, malnutrition, malignancy, and neutropenia), while others primarily provide a route of infection (e.g., extensive burns, indwelling catheter) and some act in combination. These infections are called opportunistic infection. Across the world, the Fungal Epidemiology has created a space of greater importance in modern time because of Fluconazole Resistance strain and appearance of Candida non albicans. The authors in the present study has investigated the epidemiology of fungal pathogens in a Multi-Speciality Hospital of South Kolkata, India by using culture data; antifungal susceptibility testing with fluconazole, flucytosine amphotericin B, voriconazole, and itraconazole. During the study period, the Candida species showed the highest value (n=61) out of total (n=108) positive cases found. Candida non albicans showed 100% sensitivity only to amphotericin B and flucytosine. Here, the authors dwell on the spectrum of fungus found in the hospital and ponder on the techniques in mycology in this context. The knowledge would prove useful in selecting empirical antifungal therapy and formulating prophylactic and pre-emptive strategies.

Keywords

Fungus; Candida species; Epidemiology

Introduction

Advances in medical and surgical therapy have gradually changed the type of patients cared for in Indian hospitals. Newer technologies and therapies have become common at many medical centers, resulting in many immune-compromised individuals. Also, care in specialized units and the use of invasive monitoring devices, parenteral nutrition, broad spectrum antimicrobial agents, and assisted ventilation have helped to treat patients suffering from previously devastating or fatal diseases and have provided life, previously thought to be nonviable (Scott and William, 1996).

However, these successes have resulted in the proliferation of a severely ill, immuno-

compromised, hospitalized patient population. These immune-compromised patients are highly susceptible to infections caused by organisms such as fungi that were previously considered to be of low virulence or "nonpathogenic" (Bodey, 1988). Fungal infections in these patients are often severe, rapidly progressive and difficult to diagnose or treat (Edwards, 1991). 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. This paper is based on the Fungal Epidemiology of a hospital that focuses on investigative mycology techniques and drug sensitivity for major fungal pathogens.

Materials and Methods

Specimen Collection

Sputum was collected from the patients from a deep cough after rising in the morning. All respiratory specimens were collected into sterile, leak-proof, screw-top containers. Respiratory tract secretions including sputum, bronchio alveolar lavarge, and nasal swab were submitted for fungus cultures.

Skin and nail scrapings submitted for dermatophytes cultures were disinfected with 70% isopropanol before sampling. Skin samples were gently scraped from the outer edge of a surface lesion using a sterile scalpel. Nail specimens were collected as scrapings or cuttings. For these skin and nail specimen's potassium hydroxide (KOH) wet was mounted.

Blood from patients were cultured to detect wide variety of fungal pathogens and opportunistic saprophytes. A lysis-centrifugation system was employed that lyses red and white blood cells which may be harbouring fungi.

First-voided morning urine specimens were asked from the patients for urine culture as they are more concentrated. Body fluids like pus, tissue samples like corneal scraping and others like ear swab were collected in a sterile manner and transported to the lab for testing.

The fresh specimens appropriately and properly collected were next sent to the laboratory by labeling the volume and accompanied by pertinent critical patient information. All specimens for fungus culture transported to the microbiology laboratory was processed as soon as possible, since many pathogenic fungi grow slowly, any delay in processing will increase the possibility of overgrowth by rapidly growing contaminants and will decrease the probability of isolating the causative agent. The specimens submitted for fungal culture were respiratory tract secretions, skin, nails, tissue, blood and urine. Microscopic examination of specimens for fungal elements was combined with fungus culture to understand the specific and relevant mycology laboratory techniques and to focus on the wide spectrum of fungus found in the hospital. Finally by correlating the results based on the species of isolates, antifungal drug sensitivity was tested for analyzing the drug resistance.

Results and Discussion

Out of 108 positive cases, 61 cases are Candida isolates, 30 cases are Aspergillus isolates, 3 case are Fusarium

isolates, 4 cases are Tricophyton isolates, 1 case is Mucor isolates, 1 case is Penicillium isolate, 2 case is Curvalaria isolate, 2 case is Cladosporium isolate, 1 case is Tricosporon isolate, 1 case is Scopulariopsia isolate, 1 case is Acremonium, 1 case is Microsporum. The study of Rumpa Saha et al. (2008), states that the Candida isolates as the most common pathogen especially in blood stream infection which supports our findings.

Out of 61 Candida species cases; 23 are Candida non albicans, 21 are *Candida albicans*, 10 are *Candida tropicalis*, 2 are *Candida parapsilosis*, 1 is *Candida fomata*, 1 is *Candida kruzi*, 1 is *Candida globrata*, 1 is *Candida Guilliermondi*, 1 is *Candida albujinia* isolates

Out of 30 Aspergillus isolates cases; 9 are *Aspergillus fumigatus*, 16 are *Aspergillus flavus*, 5 are *Aspergillus niger*. Baddley J. W. et al. (2003), Xess I. et al. (2004), in their study observed that *A.fumigatus* was the most common and predominant species isolated from respiratory specimens.

Our results showed only in the cases of corneal samples the incidence of bacterial keratitits was higher than that of the fungal keratitis. The common Bacteria were Coagulase Negative *Staphylococcus* (9.37%), *Pseudomonas aerginosa* (9.37%), *Nocardia* (9.37%), *Staphylococcus aureus* (15.62%) and *Streptococus pnemoniae* (18.75%). The common Fungus recovered were Curvalaria (6.25%), Fusarium (12.50%) and Asprgillus (18.75%). The study comes in line with the studies of Laspina F. et al. (2004) and Bashir G. et al. (2005).

The Candida species (n=61) showed to hold the major share among the total positive cases (n=108) found in the hospital. Among the Candida species, Candida non albicans (n=23) and *Candida albicans* (n=21) were the two major pathogens. Thus a comparative analysis between the two major pathogens in drug sensitivity is performed to evaluate and select antifungal therapy.

The number of *Candida albicans* showing drug sensitivity is 30. The 9 cases i.e. been taken here by the authors were all showing sensitivity to drug Fluconazole, Flucytosine, AmphotericinB, Voriconazole, Itraconazole. There is no S-DD (Intermediate) and resistance is being observed whereas the total number of Candida Non albicans showing drug sensitivity is 32. In all the cases Flucytosine and Amphotericin B is showing 100% sensitivity. The sensitivity of Fluconazole is 71%, 7% is S-DD(Intermediate), and 22% is resistance. The sensitivity of Voriconazole is 85%, 8% S-DD (Intermediate), and 7% is resistance. The sensitivity of Itraconazole is 50%, 29% is S-DD, (intermediate) and 21% is resistance. The study is in consistence with the results of the study of Rumpa Saha et al., 2008 that the emergence of Candida non-albicans, with often intrinsically resistance fluconazole pattern leads to difficulty in the management of septicemia.

After analysis the data collected from a Multi-Speciality Hospital of South Kolkata, India it is observed that routine mycological techniques are very useful in identifying the fungus isolates. It is also seen that the KOH preparation is better if only be used for tissue specimens such as

SAMPLE ID	SAMPLE	RESULT	SAMPLE ID	SAMPLE	Result
Puka56059	Sputum on SDA	*Aspergillus fumigatus*	Pana73359	Bal on SDA	Aspergillus fumigatus
Pana54374	Bal on SDA	*Aspergillus flavus*	Pana 78376	Bal on SDA	Microsporum
Pana56660	Sputum on SDA	*Aspergillus fumigatus*	Pana76902	Blood Culture	Candida krussi
Pana56470	Bal on SDA	*Aspergillus flavus*	Pana76911	Blood Culture	Non albicans candida
Pana57235	Skin Scrapings on SDA	*Tricophyton*	Pana83081	Blood Culture	Aspergillus fumigatus
Puka54830	Skin Scrapings on SDA	*Scopulariopsis*	Pana65557	Blood Culture	Candida albicans
Puka75908	Skin Scrapings on SDA	*Tricophyton*	Pana66505	Blood Culture	C.tropicalis
Puka55734	Skin Scrapings on SDA	*Tricophyton*	Pana83613	Blood Culture	C.tropicalis
Pana60240	E Tube on SDA	*Tricophyton*	Pana65938	Blood Culture	C.tropicalis
Pana60777	E Tube on SDA	*Non albicans candida*	Pana67149	Blood Culture	Candida albicans
Puka59003	Bal on SDA	*Aspergillus flavus*	Pana67351	Blood Culture	C.tropicalis
Pana60072	Suction on SDA	*Aspergillus flavus*	Pana76716	Blood Culture	C.guilliermondii
Pana62555	Corneal Button on SDA	*Non albicans candida*	Pana70020	Blood Culture	C.tropicalis
Pana81935	Sputum on SDA	*Aspergillus niger*	Pana70989	Blood Culture	C.tropicalis
Pana62486	Sputum on SDA	*Non albicans candida*	Pana70805	Blood Culture	C.parapsilosis
Pana65755	Sputum on SDA	*Non albicans candida*	Pana786122	Blood Culture	C.tropicalis
Pana61708	Bal on SDA	*Non albicans candida*	Pana70861	Blood Culture	C.famata
Puka57587	Nasal Swab on SDA	*Candida albicans*	Pana72899	Blood Culture	Candida albicans
Puka61429	Bal on SDA	*Aspergillus flavus*	Puka37685	Blood Culture	Candida albicans
Pana63983	Nasal Swab on SDA	*Aspergillus niger*	Pana74431	Blood Culture	Non albicans candida
Pana75308	Nasal Swab on SDA	*Aspergillus flavus*	Pana87214	Blood Culture	C.tropicalis
Puka72602	E Tube on SDA	*Candida albicans*	Pana76092	Blood Culture	Tricosporon
Pana79071	Bal on SDA	*Aspergillus flavus*	Pana 78779	Blood Culture	C.parapsilosis
Pana74294	E Tube on SDA	*Fusarium*	Pana 84407	Routine Culture Urine	Non albicans candida
Pana75503	E Tube on SDA	*Aspergillus flavus*	Pana69431	Routine Culture Urine	Candida albicans
Pana76348	Bal on SDA	*Penicillium*	Puka69651	Routine Culture Urine	Non albicans candida
Puka75292	Nail Scrapping on SDA	*Aspergillus fumigatus*	Pana77030	Routine Culture Urine	Candida albicans
Pana70805	Routine Culture Urine	*C.tropicalis*	Pana88962	Routine Culture Urine	Candida albicans

Table.1 List of fungus found in the hospital

uka70320	Routine Culture Urine	Candida albicans	Pana70989	Routine Culture Urine	Non albicans candida
Pana74285	Routine Culture Urine	Candida albujinia	Pana74276	Routine Culture Urine	Non albicans candida
Pana76792	Routine Culture Urine	Non albicans candida	Pana77912	Routine Culture Urine	Non albicans candida
Pana77344	Routine Culture Urine	Non albicans candida	Puka49672	Routine Culture Urine	Non albicans candida
Pana76916	Routine Culture Urine	Candida albicans	Pana79088	Routine Culture Urine	Non albicans candida
Pana77464	Routine Culture Urine	Non albicans candida	Pana76301	Routine Culture Pus	Aspergillus flavus
Pana73264	Routine Culture Urine	Non albicans candida	Puka35949	Corneal Scrapings	Aspergillus fumigatus
Pana73835	Routine Culture Urine	Non albicans candida	Puka32670	Corneal Scrapings	Aspergillus flavus
Pana76566	Routine Culture Urine	Candida albicans	Puka11018	Corneal Scrapings	Fusarium
Puka86733	Routine Culture Urine	Non albicans candida	Puka43082	Corneal Scrapings	Fusarium
Pana74715	Routine Culture Urine	Candida albicans	Puka38067	Corneal Scrapings	Aspergillus fumigatus
Pana74791	Routine Culture Urine	Candida albicans	Pana57410	Corneal Scrapings	Aspergillus flavus
Pana75005	Routine Culture Urine	Candida albicans	Pana54217	Corneal Scrapings	Aspergillus flavus
Pana87089	Routine Culture Urine	Candida albicans	Pana58263	Corneal Scrapings	Aspergillus flavus
Pana87384	Routine Culture Urine	Non albicans candida	Pana58701	Corneal Scrapings	Mucor
Pana75404	Routine Culture Urine	Candida albicans	Pana22253	Corneal Scrapings	Aspergillus flavus
Pana75587	Routine Culture Ear Swab	Aspergillus fumigatus	Pana70998	Corneal Scrapings	Aspergillus niger
Pana75843	Routine Culture Pus	Aspergillus fumigatus	Pana58706	Corneal Scrapings	Acremonium
Pana75918	Routine Culture Urine	Non albicans candida	Pana87099	Corneal Scrapings	Aspergillus niger
Pana75239	Routine Culture Urine	Candidaglobreta	Pana639875	Corneal Scrapings	Aspergillus flavus
Pana75972	Routine Culture Urine	Candida albicans	Pana76898	Corneal Scrapings	Cladospotium
Pana75638	Routine Culture Urine	Candida albicans	Pana58465	Corneal Scrapings	Curvalaria
Pana75455	Routine Culture Urine	Candida albicans	Pana87099	Corneal Scrapings	Aspergillus niger
Pana76195	Routine Culture Urine	C.tropicalis	Pana639875	Corneal Scrapings	Aspergillus flavus
Pana77292	Routine Culture Urine	Non albicans candida	Pana76898	Corneal Scrapings	Cladospotium
Pana76830	Routine Culture Urine	Candida albicans	Pana58465	Corneal Scrapings	Curvalaria

Figure.1 Percentage of various types of fungus found in all types of sample

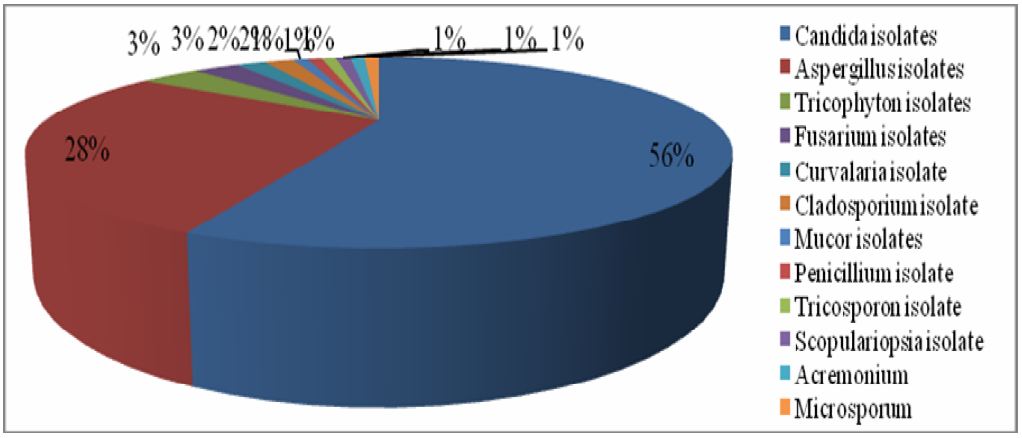


Figure.2 Percentage of Candida isolates found in all samples

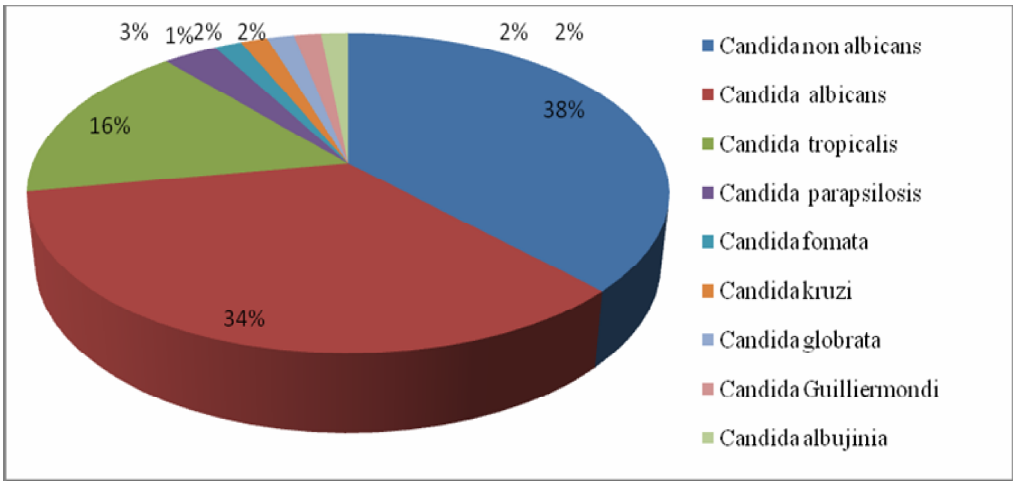


Figure.3 Percentage of Aspergillus isolates found in all samples

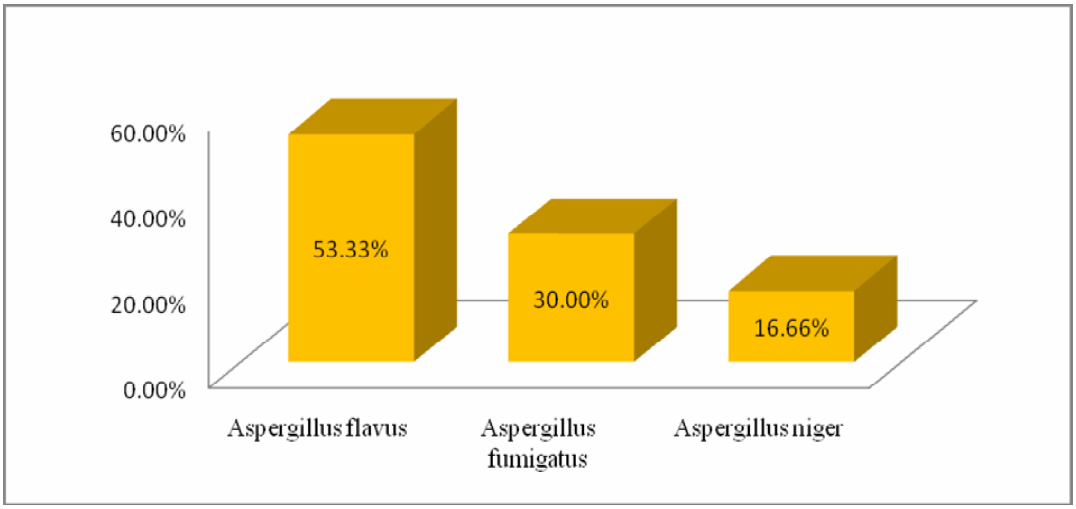


Figure.4 Percentage of drug sensitivity of *Candida albicans*

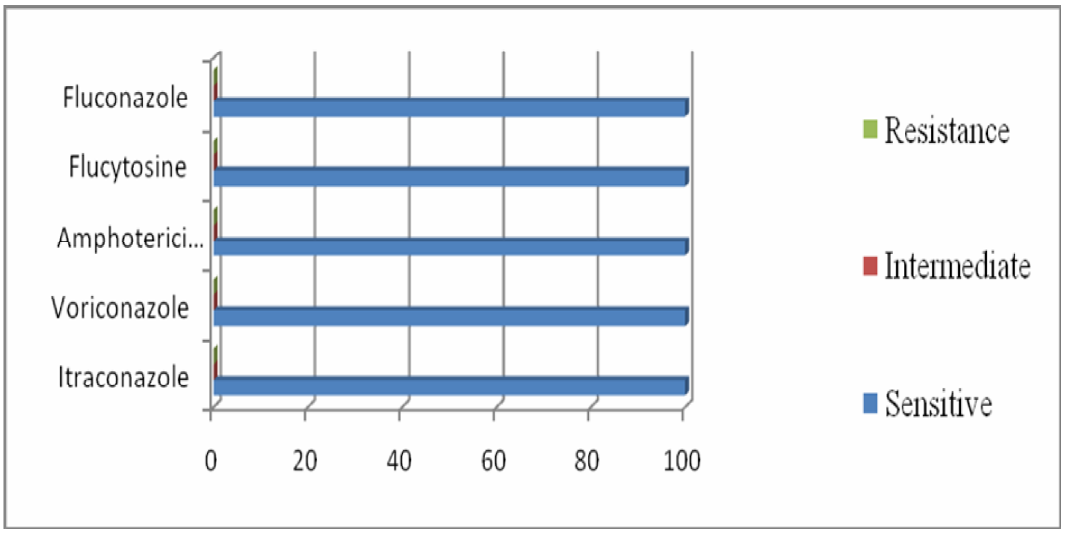
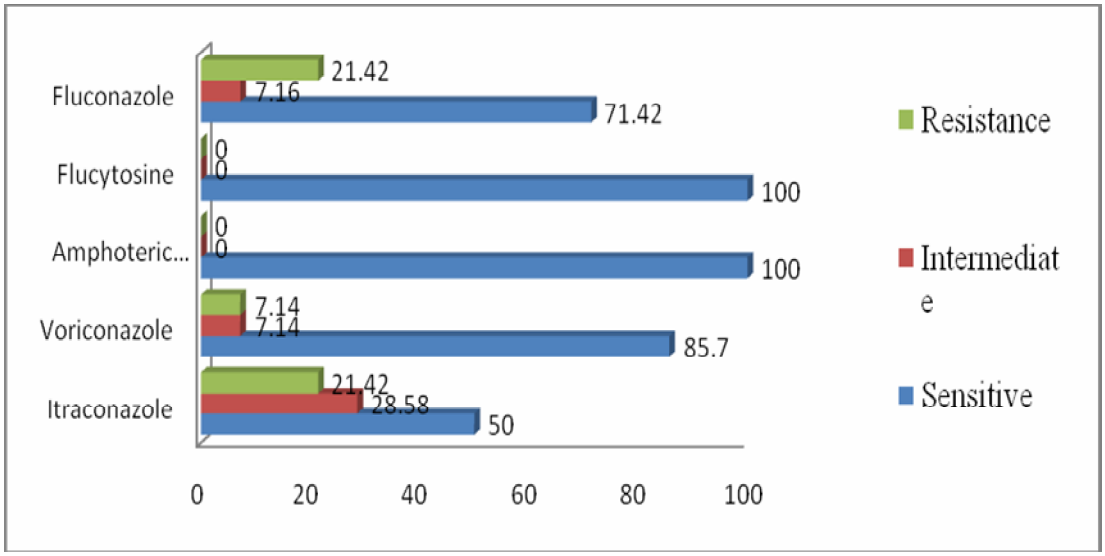


Figure.5 Percentage of drug sensitivity of Candida non albicans



skin scrapings but methods like Lactophenol Cotton Blue, Indian Ink, Germ Tube test, Slide culture technique when used were seen to give best results, as they easily identified the fungus morphology. Studies of Thomas P.A.et al. (1991) and Cardenes et al. (2004) support our findings.

It is apparent that fungal infections are becoming more prominent. There are an increasing number of immune-compromised patients and patients receiving a broader range of antifungal agents in Indian hospitals today. Consequently, infections due to obscure fungi are being seen more commonly in hospitalized patients. Although diagnostic and therapeutic modalities for candidiasis are improving, however this paper may

help to understand the threats in a multi-specialty hospital and consequent efforts to prevent them. In addition, standards for susceptibility testing are further needed to develop that would help, guide clinicians and hospital epidemiologists in the management of fungal infections. However further surveillance, continued epidemiologic and laboratory research is needed to better characterize the fungal pathogens found in multi-specialty hospitals and allow for improved diagnostic and therapeutic strategies. Studies of risk factors and the development of new methods for rapid diagnosis and monitoring should help decrease the morbidity and mortality associated with fungal infections

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