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Original Research Paper

Clinical Microbiology

FUNGAL PROFILE OF CLINICAL SPECIMENS FROM A TERTIARY CARE HOSPITAL – A RETROSPECTIVE STUDY.

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ABSTRACT

Introduction- Fungal infections are emerging as one of the most dreaded groups of infectious diseases with serious morbidity and mortality. Advanced therapeutics, the use of invasive devices, irrational use of broad spectrum antimicrobials and other environmental changes have resulted in an alarming increase in fungal diseases. Studies related to epidemiology, and distribution patterns across various geographical areas are distinct and different and the actual picture is still unclear. Hence, understanding the spectrum of fungal isolates recovered from clinical specimens will aid in effective management and control of these infections, ultimately improving patient outcomes and reducing the burden of fungal diseases in hospital settings. **Materials and Methods**- A retrospective study was conducted in the Microbiology Department of Government Medical College, Surat from January 2023 to December 2023. All the samples that were received in the microbiology laboratory for fungal culture and sensitivity testing was processed using standard microbiological guidelines for isolation of yeast and mold. Susceptibility testing of different yeast species to available antifungal agents was performed by using standard guidelines by disc diffusion method as per standard guidelines. **Results** – Out of a total of 2767 samples received during study period, 221 fungal isolates were grown in the laboratory after standard processing. Non-candida albicans were most predominant (48%) which constituted species like Candida tropicalis, Candida krusei, Candida parapsilosis, etc. Invasive fungal infections were caused by Non-candida albicans, especially Candida tropicalis followed by Candida krusei. Higher resistance to tested azole agents was seen among yeast isolates retrieved from Intensive care units. **Conclusion** -Since fungal diseases exhibit significant geographical variations, it is critical to ascertain the local pattern of etiology and other essential parameters of a specific place or institute to aid in future management strategies.

KEYWORDS : Yeast, Mold, Non candida albicans, azole

INTRODUCTION

Fungal infections are rapidly emerged and evolved in the past three decades and the impact on human health are undeniably horrifying. With the advent of mucormycosis during the covid-19 pandemic, people all over the world have faced the deadly claws of a single fungal agent and similar to this,thousands of them are rapidly evolving in terms of their pathogenicity as well as diversity . 13 million fungal infections and 1.5 million mortalities occur globally every year with a noteworthy population among them being immunocompromised. [2] Approximately 5.5 crore people who forms 4% of India's population, suffer from a fungal disease each year.[3] Several factors can be attributed to this alarming emergence and spread of fungal infections globally. These include enormous amount of use of broad-spectrum antimicrobial agents, increase in immunocompromised patients, increased use of medical devices including invasive devices , improper diagnosis and management, rather poor knowledge of the fungal spectrum ,global warming and environmental changes, etc. Invasive procedures (catheter, dialysis, aspiration), burns, and HIV patients are more susceptible to fungal infection. Colonisation of mucosal surfaces and catheters are the main associated risk factors in these patients. [4] Fungi, being ubiquitously present globally, they are now seen to equally contribute to the causation of hospital acquired infections besides community-acquired infection profile.[1] Among the various commonly encountered fungal pathogens, non candida albicans have shown a striking emergence rate with higher prevalence and association with invasive diseases. [6,7] One such instance is that of Candida auris, a quickly spreading multidrug-resistant strain , especially encountered in critical care unit settings. [6] Also, Non candida albicans are associated with a higher degree of drug resistance to prominent antifungals like azoles compared to non-candida albicans. [7]Other then common fungal etiologies like candida and Aspergillus, filamentous fungi are also gaining significant emergence; examples being penicillium, zygomycetes, fusarium, etc. [8] The most predominantly encountered pathogenic Molds include certain hyaline hyphomycetes, endemic fungi, the *Mucorales*, and some dematiaceous fungi.[9] World Health organization have acknowledged fungal diseases, especially invasive infections an s cause of health concern globally [1] Because of their dimorphic nature, biofilm forming properties and rapidly acquiring resistance to antifungal agents, it is becoming difficult to control them without extensive and precise knowledge regarding epidemiology, microbiology and about possible targets of elimination. Diagnosis of fungal agents are challenging. This is because of a lack of clinical suspicion and appropriate sampling, slow or late growth of fungal pathogens on culture, lack of advanced diagnosis at peripheral level healthcare facilities, prior misuse of antifungal agents, etc. [4,5] In the context of the growing rate of antifungal resistance, knowledge of local prevalence and susceptibility patterns are indispensable for treating clinicians. Hence continuous epidemiological and laboratory-level researches are required to tackle these infections, characterize the pathogens well, and aid in developing better diagnostic and management strategies in the future.[10] With this aim, the current study was conducted to know and analyse the ongoing fungal pathogenic spectrum, their site of involvement, distribution in critical care units and the susceptibility rates to available antifungal agents in a tertiary care hospital level.

MATERIALS AND METHODS-

Study design - This is a retrospective study conducted in the Department of Microbiology, Government Medical College and New Civil Hospital, Surat for a period of one year from January 2023 to December 2023.

Study Samples -The samples of patients suspected of having fungal infections were received in the Mycology laboratory of the Department of Microbiology from various clinical departments. Demographic data and other related sample specifications like the site of collection, time of collection, etc

were also documented.

Microbiological processing –Direct microscopic examination were done with help of the wet mount technique using 40% Potassium hydroxide for nails and 10%potassium hydroxide (KOH) for another sample. Gram staining was also done to look for fungal elements like hyphae, yeast cells, or spores. Following that, culture inoculation of samples were done in two sets of Sabourauds dextrose agar (SDA) and were incubated at 25 C and 37 C, for mold and yeast isolation respectively. Subsequent examination of cultures were observed for any growth weekly up to 4-6 weeks before discarding. Once colonies are grown, macroscopic and microscopic evaluations are employed for further identification of the fungus. For identification of Mold, macroscopic features are examined like obverse and reverse appearance of mold colonies, texture, colour, growth rate and microscopic aspects such as mycelium and conidium types, relationship between hyphae and fructification organs by lactophenol cotton blue mount. The yeast isolates were identified by standard tests like germ tube, different spore production on corn meal agar (CMA), urease production and growth on Chrome differential agar. Antibiotic susceptibility of candida isolates are done using kirby bauer disc diffusion method using discs of fluconazole and voriconazole.

RESULTS

A total of 2767 samples were received in Mycology laboratory of Department of Microbiology from various clinical departments from patients suspected of having fungal infections. Samples constituted sputum , urine, blood cultures, wound swabs, pus aspirates , skin and appendages, corneal scraping , tissue specimens, etc. Upon direct microscopy of the specimen by KOH wet mount examination and gram staining, 245(8.8%)showed fungal elements . On culture , 221 (7.9%) fungal isolates were found.

Out of total of 221 fungal isolates, 43% were from sputum, 31% were from urine, 12% were from blood, 5% from wound swab and pus aspirates, 4 % were from skin, hair, and nail samples, 3.5% from corneal samples and 3% from a tissue sample. Figure 1 shows the sample-wise distribution of fungal isolates. Non-candida albicans were more in number compared to Candida albicans as shown in Figure 2. 42% of total isolates were Candida tropicalis, 33% were Candida albicans, 8.5 % were Candida krusei, 6% were Candida glabrata , 1.3% were Aspergillus niger 1.8%, 1.3 % were Aspergillus flavus , 1.8 % were Mucor , 0.9 % were fusarium and 0.97% were trichophyton. Majority of fungal isolates were from sputum (43%), followed by urine (31%) and blood (12%). No growth was seen in any of the Cerebrospinal fluid (CSF) and bronchoalveolar lavage (BAL) samples. Among the isolates , a significant proportion (38%) were grown from samples from the Intensive care units (ICU) of various department like medicine, surgery etc. Analysis of demographic parameters have shown a male predominance (67%) over female patients , whose samples yielded positive culture. And mostly patients belong to elderly age group. Antifungal Susceptibility testing were done for all Candida isolates only using kirby bauer disc diffusion method using fluconazole and voriconazole drug discs . 36% of the Candida isolates were susceptible to both fluconazole and voriconazole and among the Non-candida albicans (NCA),only 33% were susceptible to both drugs .

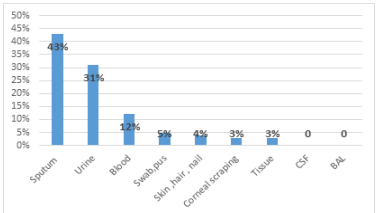

Figure 1: Distribution of fungal culture isolates from various specimens

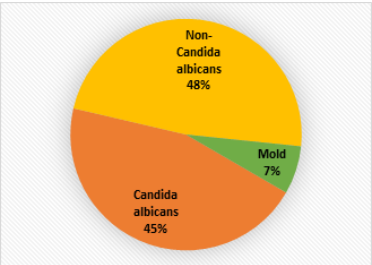

Figure 2: Distribution of types of fungal isolates

Table 1: Spectrum of fungal isolates from various clinical specimens

Fungal Isolate	Urine	Sputum	Blood	Tissue	Pus/swab	Corneal	Skin/hair/nail	Total (%)
Candida albicans	11	14.2	2	0	2.5	0	3.6	33.2
Candida tropicalis	17	19.7	5	0	0.5	0	0	42.2
C. Parapsilosis	0	3	0	0	0	0	0	3
Candida krusei	3	1	3	0	1.5	0	0	8.5
Candida glabrata	0	5	1	0	0	0	0	6
Aspergillus niger	0	0	0	0.9	0	0.9	0	1.8
Aspergillus flavus	0	0	0	0.4	0	0.9	0	1.3
Mucor sp	0	0	0	1.8	0	0	0	1.8
Fusarium sp.	0	0	0	0	0	0.9	0	0.9
Trichophyton	0	0	0	0	0	0	0.97	0.97
Total (%)	31%	43%	12%	3%	5%	3%	4%	100

DISCUSSION

Fungal infections have significantly emerged over the last few decades. They are ubiquitously present and show geographical variation and specific local epidemiological spectrum. The present study was conducted in a tertiary care hospital in south Gujarat region of western India. In the present study, out of 2767 samples received over one year, ,221(8%) samples were positive for fungal growth. This is similar to studies conducted by Hitesh Ahir et.al (6.7%) [15], conducted in east-central Gujarat. Whereas culture positivity rates were much higher in studies conducted by Alim A. et al (24%) [16] and Kashyap B. et al (27%) [5] conducted in northern parts of India. This shows the diversity of epidemiology according to geographical and climatic areas and emphasizes the need of more regional studies. Majority of fungal isolates were obtained from sputum(42%) followed by urine (31%) and blood (12%) in present study . These findings were consistent with Nageshwari et al. [16] who also had fungal growth maximally from sputum (58.92%), followed by body fluids (13.98%).

In our study isolates of Non-candida albicans (48%) were more than Candida albicans (45%) , which correlates with other studies conducted in India , Kuwait and Europe [11,12,13]. Non-candida albicans, especially Candida tropicalis, were predominantly found to cause candidemia , consistent with other Indian studies [14,15]. Higher fluconazole and voriconazole resistance in our study were consistent with findings of study conducted by Ahir H.R et al [15]. This indicates emergence of Non candida albicans species like Candida tropicalis, Candida krusei, Candida parapsilosis as a significant multidrug resistant group of fungal pathogen associated with invasive infections and in critical care unit setup, thus warranting strict surveillance and

infection control practices in hospitals.

Limitations

Study population only included samples of patients visiting tertiary care hospital. Study period was only one year and hence not suitable for a comprehensive analysis of the trend of fungal disease.

CONCLUSION

Because of various climatic conditions, socio-economic differences, environmental variations in various parts of India, fungal spectrum varies a lot throughout the country. However an increasing trend of both prevalence and severity is noted globally. Hence the study of local epidemiological and microbiological factors including resistance patterns of fungal pathogens will aid in strengthening preexisting knowledge to tackle these rapidly growing deadly organisms, identify various lacunae in the diagnostic methods, and further act as guidance for implementing proper diagnostic and antifungal stewardship at institution and regional level.

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