Original Research Paper

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Clinical Microbiology

CANDIDA ALBICANS- A SILENT KILLER

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ABSTRACT **Background:** Over the past few years there has been a dramatic rise in the incidence of invasive candidiasis which can be attributed to broad spectrum antimicrobial use, increased number of immunocompromised patients, advanced life support systems and long term use of immunosuppressive drugs. **Aims and Objectives:** The present study was carried out to study the speciation and antifungal susceptibility pattern of *Candida* isolates from various clinical samples. **Materials and Methods:** A retrospective laboratory based study was carried out in the microbiology section, department of laboratory services of a tertiary care hospital from May 2021 to May 2023. *Candida* isolates obtained in various clinical samples were identified by Gram stain, KOH wet mount, colony characteristics on Sabouraud's dextrose agar (SDA) and automated VITEK® 2 compact system (Biomerieux). Antifungal susceptibility testing (AFST) was done by using YS08 cards and interpreted as per CLSI guidelines. **Result:** *Candida ablicans* (81.2%) was the most common species isolated in our study followed by *Candida tropicalis* (11.2%), *Candida* species (3.4%), *Candida parapsilosis* (2.3%), *Candida glabrata* (1.5%) and *Candida famata* (0.4%). *Candida* isolates showed 39.7 % resistance to voriconazole, 35.5% to flucytosine, 34.7%, to fluconazole, 32% to amphotericin B, 21.6% to micafungin and 20.4% to caspofungin. **Conclusion:** *C.albicans* was the most common isolate in our study. Maximum resistance was observed for voriconazole. Antifungal susceptibility testing should be adopted as a routine diagnostic practice before commencing antifungal therapy to decrease antimicrobial resistance and promote stewardship.

KEYWORDS : Antifungal susceptibility testing (AFST), Azoles, Candida albicans, Immunocompromised, Non albicans Candida (NAC), Nosocomial, Stewardship

INTRODUCTION

Fungi are ubiquitous in nature with *Candida* species (spp) being the most common fungal pathogens to infect humans. Over the past couple of decades there has been a dramatic increase in the incidence of mycotic infections especially among the hospitalized patients. This multifactoral causation can be attributed to the use of invasive medical devices, travel to endemic areas, immunosuppressive therapies, advanced age, HIV-AIDS, and broad spectrum antifungal use amongst a few.[1]

Advances in medical technologies have enabled treatment of fatal and life threatening diseases increasing the longevity of immuno-compromised patients. However, these patients continue to remain susceptible to common fungal agents developing mycotic infections which often mimic bacterial or viral illnesses making their diagnosis difficult and delayed.[2] Rampant and indiscriminate use of broad spectrum antifungal agents such as azoles has further accelerated the global issue of antifungal resistance with hardly any newer agents in the pipeline for future generations.

Candida albicans is one of the most common fungal agents to cause nosocomial infections. However, widespread use of azoles has led to a shift towards Non albicans *Candida spp* (NAC) being the dominant isolate. *Candida glabrata, Candida krusei, Candida dubliniensis, Candida kefyr, Candida tropicalis,* and *Candida parapsilosis* are some of the prevalent NAC species associated with human infections.[3]

The last few years have witnessed a changing epidemiology of fungal infections with isolates that were previously considered contaminants turn into pathogenic fungi that not only infect the immuno-compromised but also the immunocompetent.[4] In the light of the above, this retrospective study was undertaken to determine the microbiological spectrum of *Candida* infections and to determine their antifungal susceptibility pattern.

MATERIALS AND METHODS

A retrospective laboratory based study was carried out in the microbiology section, department of laboratory services of a tertiary care hospital from May 2021 to May 2023. Various clinical samples such as urine, blood, bronchoalveolar lavage (BAL), central venous catheter tip, endotracheal (ET) secretions, high vaginal swab (HVS), pus, sputum, stool, and nail were collected from the patients for fungal culture and sensitivity upon clinical suspicion.

Sample processing was carried out by using standard microbiological techniques.[5] Immediately after receipt of samples, KOH wet mounts were performed and examined by microscopy for presence of round to oval budding yeast cells with or without pseudohyphae. For fungal culture, samples were inoculated into two tubes of Sabouraud's dextrose agar (SDA) with and without cyclohexamide (HiMedia®) and incubated aerobically at 37◦C and 25◦C respectively for 21 days. Readings were taken every 2 days and tubes were discarded after 3 weeks in the absence of any growth. Culture identification was done on the basis on colony morphology, growth rate, temperature, and texture. For blood steam infections, blood culture bottles were incubated in BacT Alert 3D system (Biomeriuex) aerobically for 21 days and those who were flashed positive by the system were subcultured on SDA. Smears for gram staining from positive culture bottles were also prepared. On gram staining, *Candida spp* appear as gram positive budding yeast cells with or without pseudohyphae.

Candida isolates grow rapidly on SDA at 37◦C in 24-72 hours. *Candida spp* appear as white to creamy, smooth/ rough, shiny, pasty colonies. On blood agar, extensions commonly called as 'feet' develop at the border of the colony.[6] Once they are identified by colony morphology and gram staining, further speciation and AFST was carried out by automated VITEK® 2 compact instrument (Biomerieux) using YST and AST YS08 cards respectively as per manufacturer's instructions. This instrument uses spectrophotometric readings to calculate the MIC (Minimum inhibitory concentration) values for clinically important Candida spp. Fluconazole, Voriconazole, Flucytosine, Amphotericin B, Caspofungin and Micafungin are tested in this AST card.[3] For identification, *Candida albicans* ATCC 14053 was used and for AFST, *Candida parapsilosis* ATCC 22019 was used as an internal quality control. Results were interpreted as per CLSI (Clinical and Laboratory Standard Institute) guidelines.[7] Statistical analysis was performed using MS excel 2007 software.

RESULTS

A total of 1000 samples from various clinical departments were received in the microbiology laboratory for fungal culture and sensitivity from May 2021 to May 2023. Out of these, fungal pathogens were grown in 45% samples. *Candida spp* account for 259 of the total fungal culture positives. Figure 1 shows the demographic details of the *Candida* isolates (N=259). Maximum isolates were from geriatric population (age >60years), male gender and inpatient (IPD) location.

Table 1 shows the distribution of *Candida* isolates in clinical samples. Maximum isolates were obtained from urine (35.1%) followed by respiratory samples (29.3%) and then blood (25.8%).

Candida ablicans (81.2%) was the most common species isolated in our study followed by *Candida tropicalis* (11.2%), miscellaneous *Candida spp* (3.4%), *Candida parapsilosis* (2.3%), *Candida glabrata* (1.5%) and *Candida famata* (0.4%).

Overall, *Candida* isolates showed 39.7 % resistance to voriconazole,

35.5% to flucytosine, 34.7% to fluconazole, 32% to amphotericin B, 21.6% to micafungin and 20.4% to caspofungin.

AFST pattern of *Candida albicans* is shown in Figure 2 (N=210) while that of NAC is shown in Figure 3 (N=49). *Candida albicans* have demontrated maximum resistance to azoles (47.1% to fluconazole and 37.6% to voriconazole) while NAC showed maximum resistance to Flucytosine (32.6%).

DISCUSSION

The reputation of *Candida spp* as an innocent bystander and commensal organism has been questioned over the last couple of years over its involvement in most nosocomial infections.[8] According to the National Nosocomial Infection Survey (NNIS), *Candida spp* are the fourth leading cause of health-care associated bloodstream infections.[9, 10] Albeit the increased frequency of isolating NAC as the dominant organism in most studies, *C. albicans* continues to be the most commonly identified pathogenic member.[11]

A high index of clinical suspicion is required for diagnosis of fungal infections.[12] A thorough understanding of various *Candida spp*, their virulence pattern, and antifungal resistance profile is required for an appropriate clinical diagnosis and early selection of an adequate antifungal agent.

A sound diagnosis of fungal pathogens is now possible due to the increased availability and accessibility of microbiological resources in developing countries.[13] Despite the advances in molecular diagnostic techniques and rapid diagnostic methods, fungal culture remains the gold standard for diagnosis of *Candida* infections irrespective of their sampling site.[11]

Our study reported *C. albicans* as the most common fungal isolate which is in corcordance with many other studies.[2-4, 14-15] However, a changing epidemiology of *Candida spp* with progressive shift towards NAC as the predominant isolate is also seen in certain studies.[9,16-17]

No single test can distinguish commensalism, colonization, contamination or pathogenicity of this organism. Hence, *Candida spp* must always be reported with its clinical and microbiological correlation.[11]

Similar to our study, Hitesh R. Ahir et al and Lekshmi Balaraman et al also reported maximum isolates from urine samples.[16,18] Aijaz N et al and Manmeet Gill et al reported maximum isolates from blood samples while Sailaja BSG et al reported highest isolates from sputum samples.[3,14,19] A large number of *Candida spp* were also reported from respiratory samples in our study. *Candida* pneumonia is a rare sighting and its occurrence is often debatable with definitive diagnosis only by histopathological examination (HPE). It arises either due to aspiration of oropharyngeal secretions or hematogenous seedling of the lungs.[11] Both the IDSA and ESCMID discourage antifungal therapy in the absence of a HPE evidence.[20]

C. albicans can colonize mucous membranes of different anatomical sites in high numbers due to its ability to flourish in a wide range of environmental conditions. These include high and low pH, aerobic and anaerobic atmosphere as well as low and high glucose levels. This silent killer is able to evade the host immune defenses by shielding its core cell wall component, (1, 3)-D-glucan with glycoproteins thus escaping detection by the macrophage dectin-1 receptor.[13]

In our study, maximum species were isolated from geriatric population, male patients, and from in-patient areas (Figure 1). Similar findings have also been reported by Hitesh R. Ahir et al, Aijaz N et al, and Sailaja BSG et al in their studies.[1,3,14] *Candida spp* invade the host by formation of biofilms, hyphal forms, adherence properties, secretion of hydrolytic enzymes, and quorum sensing mechanisms.[17] Prominent risk factors include hospitalization, advanced age, immunosuppression, use of invasive devices and exposure to antibiotics.[11] Due to the aforementioned reasons it is rightly called 'the disease of the diseased'.[18] Males are the dominant gender affected as they are prone to physical labour with increased sweating, overcrowding, and poor personal hygiene which subsequently exposes them to fungal pathogens.[21]

Amongst the NAC species, *C.tropicalis* is the most common isolate in our study (Table 1) which is in concordance with other studies.[9,14-16,18-19]

As seen in Figures 2 and 3, *C.albicans* showed 37.6% resistance to Voriconazole and NAC species reported 32.6% resistance to Flucytosine respectively. Hitesh R. Ahir et al showed 26% resistance to Voriconazole for *C.albicans* but only 4% NAC resistance to Flucytosine in their study.[16] Aijaz N et al also showed maximum resistance to fluconazole (37.5%) followed by voriconazole (33.33%) for *C.albicans* in their study.[3] The constant rise in resistance to azoles is particularly concerning as they are the most common agents being used for antifungal prophylaxis as well as treatment. Although NAC species are not dominant in our study but a high resistance to azoles has been reported. Oberoi K Jaswinder et al have reported a statistically significant relation between fluconazole use and the emergence of NAC strains.[22] The relevance of performing AFST before commencing antifungal agents cannot be emphasized enough. Hence, it is imperative that we limit the empirical use of fluconazole only for emergency cases. A constant vigil in the form of an active antimicrobial stewardship committee and periodic audits by the infection control team is profoundly required to fight this menace of azole resistance.

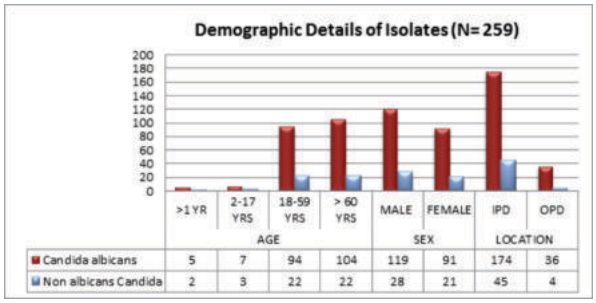

Figure 1: Showing Demographic Details Of The *Candida* Isolates (n=259)

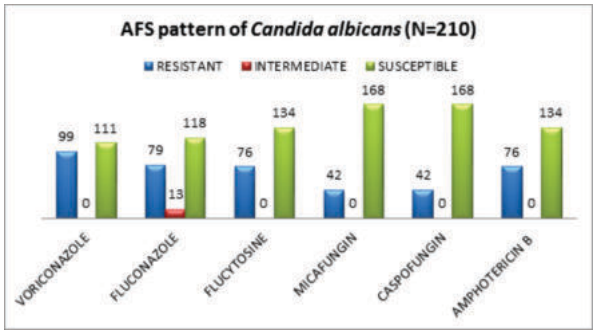

Figure 2: Showing AFST Pattern Of *Candida Albicans* (n= 210)

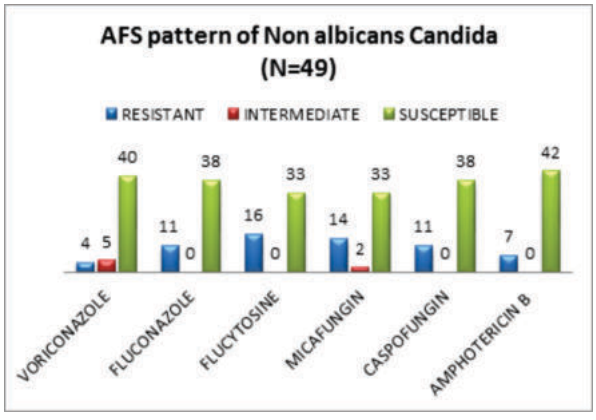

Figure 3: Showing AFST Pattern Of Non Albicans *Candida Spp* (NAC) (n=49)

Table 1: Showing Distribution Of *Candida* Isolates In Clinical Samples (n=259)

Organism	Blood	U-rine	Respi-ratory sample*	Body Fluids	S wabs**	Nail	Pus/ wound swab	Cather tip	Total
C. albicans	41	77	71	0	9	1	9	2	210
C. tropicalis	15	5	5	1	2	0	1	0	29

Candida spp	3	6	0	0	0	0	0	0	9
C. parapsilosis	4	2	0	0	0	0	0	0	6
C. glabrata	4	0	0	0	0	0	0	0	4
C. famata	0	1	0	0	0	0	0	0	1
Total	67	91	76	1	11	1	10	2	259

*Respiratory samples include sputum, ET secretions, BAL, throat swab
**Swabs include high vaginal swab and oral swabs

CONCLUSION

Despite a widespread shift towards NAC in most hospital settings, our set up continues to isolate *Candida albicans* as the predominant species. Enhanced surveillance activities and sustainable infection control practices in the form of AFST testing and appropriate disinfection protocols can aid in effective antimicrobial stewardship. Preventing emergence of antifungal resistance strains is the need of the hour for often neglected but life threatening mycotic infections.

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Conflict of Interest: None

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