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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION, SPECIATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF CANDIDA CAUSING BLOODSTREAM INFECTION IN ICU PATIENTS

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ABSTRACT

Blood stream infections (BSI) caused by Candida species are a significant cause of morbidity and mortality in hospitalized patients. Although Candida albicans has been the most common Candida species isolated from BSI's, there is an increasing trend of isolation of non-albicans Candida from BSI. With the shift to non-albicans Candida species, antifungal resistance has become a major cause of concern in the management of candidemia. In the present study, a total of 100 patients who were admitted in ICU's of different departments of Victoria hospital, Bangalore Medical College and Research Institute who developed signs and symptoms of nosocomial BSI were screened. Two blood samples were collected and inoculated onto BHI broth, incubated and subcultured onto blood agar and MacConkey agar after 24 hrs incubation. Growth was subjected to Gram's stain and subcultured onto Sabouraud's dextrose agar. Speciation and drug susceptipility pattern of candida isolates was determined by BD Phoenix ™ Automated Microbiology System. Out of 100 samples, only 2 were positive for Candida. Both were Candida krusei species and found resistant to fluconazole. Increased use of fluconazole has been implicated in shift to non albicans Candida causing BSI and this study confirms it. This study illustrates the importance of Candida in causing BSI and change in species distribution from albicans to non-albicans Candida and their resistance pattern which helps in formulating antibiotic policy of the hospital.

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INTRODUCTION

Candida species are the most common cause of fungal infections worldwide. They cause a wide variety of infections, including simple, mucocutaneous infections, but they also cause severe invasive infections that can involve virtually any organ. Blood stream infections (BSI) caused by Candida species are a significant cause of morbidity and mortality in hospitalized patients. Nowadays, Candida spp. is known as the fourth most frequently isolated pathogen from the blood stream, among hospitalized patients in North American hospitals. (Wisplinghoff *et al*., 2004) The reasons for the increase in fungal infections are multi-factorial: better clinical evaluation and diagnosis, greater survival for patients with malignancies, chronic diseases, increasing number of transplants, complex surgical procedures, catheters, implants, and use of broad spectrum antibiotics. Although Candida albicans has been the most common Candida species isolated from BSIs, there is an increasing trend of isolation of non-albicans Candida from BSI.

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This shift has been attributed to the increased use of fluconazole. With the shift to non-albicans *Candida species*, antifungal resistance has become a major cause of concern in the management of candidemia. *C. glabrata* and *C. krusei* have been shown to be resistant to fluconazole and other triazoles. *C. tropicalis* and C. parapsilosis have been found to have variable susceptibility pattern to azoles. Few reports show Candida species being resistant to amphotericin B and echinocandins also (Giri *et al*., 2013). The shift of the species distribution of Candida causing BSI thus has important clinical implications. Data regarding their anti-fungal susceptibility will help improve outcome of patients. Since the speciation and anti-fungal susceptibility is not routinely performed, the results will guide empirical antifungal therapy. This study is carried out to determine the incidence of candidemia in ICU patients, speciation, susceptibility pattern and risk factors associated with it.

MATERIALS AND METHODS

This prospective longitudinal study was carried out in the months of August and September 2014 in the Department of

Microbiology, Victoria hospital, Bangalore. The study was conducted after obtaining necessary clearance from the Institutional Ethics Committee. Informed consent was taken from all the patients who participated in the study. One hundred patients admitted in the ICUs of different departments who developed signs and symptoms of nosocomial bloodstream infection (BSI) were screened for Candidemia. Patients already having Candidemia, patients on prophylactic antifungal therapy or patients with less than 48 hours of stay in hospital, and patients who were known to be HIV positive were excluded.

Medical history and probable risk factors such as underlying illness, presence of central venous catheters, total parenteral nutrition, prior use of antimicrobials, cancer chemotherapy, use of corticosteroids, diabetes mellitus, abdominal surgery, neutropenia, any invasive procedure or devices, and duration of ICU stay were recorded. 2 blood samples of 5 mL each were collected from each patient. The samples were collected under aseptic conditions. All the blood samples were inoculated into Brain Heart Infusion (BHI) Broth. Samples were transported immediately to the laboratory. The BHI broth was incubated for 7 days at 37^0C before declaring negative. After 24hours, it was sub cultured onto blood agar and MacConkey agar. Any growth on these media was subjected to Gram's stain. Gram stained smears positive for Candida were sub cultured onto Sabouraud's Dextrose Agar (SDA) with gentamicin and incubated at 37^0C. Candida isolates were then speciated with BD Phoenix™ Automated Microbiology System. Germ tube tests and sugar assimilation tests were also done. The drug susceptibility pattern of Candida isolates was determined using the BD Phoenix™ Automated Microbiology System. Standard ATCC strain (*C. albicans* ATCC 90028) was used as control.

OBSERVATION AND RESULTS

Out of the 100 ICU patient samples tested, 21 samples were found to be culture positive. Of the culture positive samples, Klebsiella species was the most common among the bacteria. Among the fungi, only Candida was isolated from 2 samples. Germ tube was not produced by either of the Candida isolates.

Table 1. Organisms isolated in 100 suspected nosocomial septicaemia cases

S.No	Isolate	No (%)
1	Klebsiella	10 (10%)
2	E.coli	6 (6%)
3	Proteus	3 (3%)
4	Candida	2 (2%)
	Total	21 (21%)

Table 2. Antifungal susceptibility pattern of Candida isolates

Drug	C.krusei 1	C.krusei 2
Fluconazole	Resistant	Resistant
Flucytosine	Sensitive	Sensitive
Amphotericin	Sensitive	Sensitive
Caspofungin	Sensitive	Sensitive
Micafungin	Sensitive	Sensitive

Glycerol and succinic acid were assimilated by both the isolates on performing the sugar assimilation test. Both the isolates were identified to be Candida krusei. One isolate was found to be resistant to fluconazole, moderately sensitive to flucytosine and sensitive to voriconazole, amphotericin B, caspofungin and micafungin. The other isolate was found to be resistant to fluconazole and sensitive to flucytosine, voriconazole, amphotericin B, caspofungin and micafungin. Both the isolates were samples of neonates admitted in the Neonatal Intensive Care Unit (NICU). Low birth weight was the only risk factor recorded in both the cases.

DISCUSSION

The present study emphasizes the importance of candidemia in ICU patients. The incidence of candidemia in ICU patients as reported by studies varies widely. A study in a tertiary care center in south India has reported a prevalence of 0.65%. (Giri *et al*., 2013) Another study reported a prevalence of 11.2%. (Chowta *et al*., 2007) In this study, the incidence was found to be 2%. Candida was the fourth most commonly isolated pathogen from blood in this study. Increased use of fluconazole has been implicated in the shift towards non-albicans Candida causing Blood Stream Infection. This shift has been reported by many studies. (Giri *et al*., 2013; Shivaprakasha *et al*., 2007) However, some studies have reported that C. albicans remains the most common species. (Chowta *et al*., 2007; Deepak Arora and Neerja Anand, 2011) This study confirms this changing trend with the isolation of C. krusei from 2 cases. In the study by Giri *et al*, Candida krusei constituted 5.13% of all candida isolates from blood. (Giri *et al*., 2013) 3.3% of candida isolates are krusei as reported by Xess *et al*. (2007) Both the C. krusei isolates of this study were found to be resistant to fluconazole. The intrinsic resistance of C. krusei to fluconazole is well known. In the strains of C. krusei studied by Alison *et al*. fluconazole resistance was largely the result of a decreased susceptibility of 14α-demethylase to the inhibitory effects of fluconazole. (Alison *et al*., 1998) Increased fluconazole use is implicated in the emergence of resistant species, especially C. krusei and C. glabrata. A significant percentage of Candida isolates from blood are fluconazole resistant as reported by various studies. Giri *et al* found 30.8% of Candida isolates to be fluconazole resistant while Xess *et al* found 11.7% isolates to be fluconazole resistant. (Giri *et al*., 2013; Xess *et al*., 2007) Low birth weight was the only risk factor in both the cases of candidemia in this study. Prematurity, low birth weight, broad spectrum antibiotics, total parenteral nutrition and indwelling catheters are reported to be common risk factors for neonatal candidemia. (Sardana *et al*., 2012; Juyal *et al*., 2013)

Conclusion

Candida is found to be the fourth most common cause of Blood Stream Infection in this study. The emergence of non- albicans Candida causing Blood Stream Infection has been shown. The known intrinsic resistance of C. krusei to fluconazole will guide antifungal therapy. Studies with a larger sample size and longer study duration will provide a larger picture about the mycological profile of candidemia.

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