ORIGINAL ARTICLE

Study of *Candida* Bloodstream Infections in Surgical Intensive Care Unit Patients and Susceptibility Profile of the Isolates

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Abstract:

Background: The increased incidence of fungal infections in the past two decades has been overwhelming. Despite the fact that invasive fungal infections are still under-diagnosed and under-reported, bloodstream infection due to *Candida* is now being recognized as an important public health problem especially in ICU patients with considerable morbidity, mortality and health care costs. *Objective:* To study the incidence, risk factors and antifungal susceptibility of *Candida* bloodstream infection in our hospital. *Material and Methods:* In the present study, the blood samples were collected from patients admitted in Surgical ICU. Samples were processed and antifungal susceptibility of the isolates was performed using standard protocol. *Results:* Out of total 93 patients, 14 (15.05%) were positive for candidemia with equal distribution of both *C. albicans* and non-*albicans Candida* spp. The risk factors associated with candidemia showing statistical significance were length of ICU stay > 7 days, mechanical ventilation, central venous catheters and uncontrolled diabetes. Among the patients with candidemia the mortality rate was 78.57 %. Resistance to Amphotericin B was seen in 33.33 % isolates of *C. tropicalis a*nd 100 % isolates of *C. rugosa*. 33.33 % of *C. tropicalis* and 50 % of *C. rugosa* showed dose dependent susceptibility to Fluconazole. *Conclusion:* Early diagnosis and antifungal susceptibility testing is very important in the treatment of candidemia for reducing the mortality rate.

Keywords: Antifungal Susceptibility Testing, Candidemia, Risk factors

Introduction:

Invasive Fungal Infections (IFIs) are the most common life-threatening conditions in Intensive Care Units (ICUs) and are often viewed as opportunistic infections. The isolation of fungi from blood culture should never be considered as a contaminant but it must be viewed with high suspicion especially if the sample is from patients known to be susceptible for invasive fungal infections. *Candida* is one of the most frequent cause of Bloodstream Infections (BSIs) leading to significant morbidity and mortality, especially in non-neutropenic critically ill patients [1].

Between 1995 and 2002, the frequency of nosocomial candidemia rose significantly from 8% to 12% of all reported bloodstream infections. Currently, more than half the cases of candidemia are found in medical and surgical ICUs due to immunocompromised critically ill patients who are more prone for nosocomial infections [1].

Several other identified risk factors such as indwelling catheters, use of broad spectrum antibiotics and corticosteroids, parenteral nutrition, prolonged uncontrolled diabetes mellitus, assisted ventilation, prior abdominal surgery, HIV/ AIDS contribute to immunosuppression by altering the host defences. Colonization with *Candida* species further aggravates the situation. These patients are highly susceptible to nosocomial infections mainly due to fungal pathogens leading to BSIs [2].

Candida albicans has been the predominant species causing candidemia [3]. Recently, shift from *C. albicans* to non-*albicans Candida* species mainly *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei*, etc. as dominant pathogens are reported. This shift in the *Candida* species causing BSIs is mainly attributed to use of azoles in the prophylactic treatment of immunocompromised patients. This in turn has led to reduced susceptibility of non-*albicans Candida* species to antifungal agents either due to intrinsic resistance to fluconazole, biofilm production or acquired resistance to azoles during therapy [4, 5]. The conventional blood culture is a reliable method for detecting fungal BSIs in the ICU patients.

However, delay in identification can prolong the initiation of appropriate treatment with an effective antifungal agent ultimately leading to poor outcome [3]. Thus, continued surveillance and knowledge of epidemiological trends of aetiologic agents and their susceptibility pattern is an important guide in patient management [5]. The present study was carried out to know the incidence and various risk factors of *Candida* BSIs in surgical ICU and to determine the antifungal susceptibility pattern of the isolates.

Material and Methods:

This longitudinal hospital-based observational study was carried out in the Department of Microbiology, Krishna Institute of Medical Sciences Deemed University, Karad. After taking informed consent from the patient's relatives, blood samples were collected from patients admitted under Surgical ICU of Krishna Hospital and Medical Research Centre, Karad during a period of two years January 2010 to December 2011. All clinically suspected critically ill patients after 48 hours of admission in Surgical ICU were included in the study. Patients diagnosed as candidemia before 48 hours of ICU admission and neutropenic patients were excluded from the study. Detailed history of any intervention within 2 weeks before initial positive culture like use of broad spectrum antibiotic therapy, steroids, CVC catheter, mechanical ventilation, total parenteral nutrition, indwelling catheters, major surgery and antifungal prophylaxis was taken. History of duration of ICU stay, previous diabetes mellitus with blood glucose level > 180mg/dl before the onset of candidemia was also recorded.

5 to 10ml venous blood was collected under strict aseptic precautions in biphasic medium containing brain heart infusion agar and brain heart infusion broth. Blood-broth ratio 1:10 was maintained. The biphasic culture bottle was kept vented and was tilted after 48 hrs, 5 days and 7 days incubation to allow broth to flow over the whole agar surface and kept in that position for 1 hour. These cultures were carefully checked daily for growth. Culture bottles were incubated until growth appeared or for 6 weeks before discarding them as negative. The growth was confirmed to be yeast by Gram stain and further identification of *Candida* species was carried out as per the standard protocol using germ tube test, corn meal agar, sugar assimilation and fermentation tests [6].

Antifungal susceptibility testing of the isolated *Candida* species was carried out by broth microdilution method, a reference method for Broth Dilution Antifungal Susceptibility Testing of Yeasts [7]. The antifungal powders as pure salts were obtained from Sigma-Aldrich.

The antifungal agents tested against the isolates were Amphotericin B, Fluconazole, Itraconazole, Voriconazole and 5-flurocytosine.

Antifungal stock solutions were prepared at concentration of at least 1280 µg/mL, or ten times the highest concentration to be tested, whichever is greater. The drug concentration ranges used were: amphotericin B, itraconazole and voriconazole : 0.0313 to 16 µg/mL and flucytosine and fluconazole : 0.125 to 64 µg/mL.

Statistical Analysis:

Data entry and analysis was done, chi-square test and p value were calculated to determine significant association between risk factors and candidemia. P value < 0.05 was considered statistically significant. Analysis of risk factor was calculated by SPSS software package.

Results:

During the study period, out of a total 93 patients, 14 (15.05%) were positive for candidemia.

Out of 14 candidemic patients, 5 (35.71%) were above the age group of 70 years with male predominance i.e. 92.85%.

The distribution of both *C. albicans* and non-*albicans Candida* spp. were equal. Out of 14 candidemia patients, *C. albicans* was most common species isolated in 50% cases followed by *C. tropicalis* in 3(21.42%) cases. *C. rugosa* was isolated in 2 (14.28%) cases followed by 1 (7.14%) isolate each of *C. glabrata* and *C. lusitaniae* (Table 1).

Table 1: Species distribution of *Candida* Blood stream isolates in Surgical ICU

Species	SICU (%)
C. albicans	7 (50)
C. tropicalis	3 (21.42)
C. rugosa	2 (14.28)
C. glabrata	1 (7.14)
C. lusitaniae	1 (7.14)
Total	**14**

In the present study, the risk factors associated with candidemia showing statistical significance were length of ICU stay > 7 days, mechanical ventilation, central venous catheters and uncontrolled diabetes. Factors such as use of broad spectrum antibiotics, steroids, total parenteral nutrition, urinary catheter and prior surgery were not statistically significant (Table 2). Among 14 candidemic patients, death was observed in 11 (78.57%) patients.

Table 2: Risk factors associated with Candidemia in Surgical ICU patients

Sr. No.	Risk Factors	With Candidemia (N=14)	Without Candidemia (N=79)	Chi-square (X^2)	P value
1	**Length of ICU stay (>7days)**	13	48	4.100	0.0429*
2	**Use of Broad spectrum antibiotics**	14	78	0.179	0.6721
3	**Use of steroids**	08	50	0.019	0.8899
4	**Total Parenteral Nutrition**	14	78	0.179	0.6721
5	**Mechanical Ventilation**	12	41	4.252	0.0392*
6	**Central Venous Catheter**	11	36	3.945	0.0470*
7	**Urinary Catheter**	14	69	0.885	0.3467
8	**Uncontrolled Diabetes Mellitus**	04	05	4.427	0.0354*
9	**Prior Surgery (Abdominal/Neuro)**	06	33	0.005	0.9396

**P value < 0.05 = Statistically significant*

Among the antifungal susceptibility testing, all the isolates of *C. albicans*, *C. glabrata* and *C. lusitaniae* showed 100% susceptibility to amphotericin B, 5-flurocytosine, fluconazole, itraconazole and voriconazole. Amphotericin B resistant was 100% among isolates of *C. rugosa* and 1/3(33.33%) isolates of *C. tropicalis*. *C. tropicalis* showed 100% susceptibility to 5-flurocytosine, fluconazole and itraconazole. *C. rugosa* showed 50% susceptibility to fluconazole and itraconazole. Voriconazole susceptibility was found to be 100% among the *Candida* species isolated.

Discussion:

The frequency of invasive mycoses has increased dramatically during the past two decades owing to rapid advances in the diagnostic and therapeutic modalities. The critically ill patients in the ICU are particularly at risk of nosocomial fungal infections mainly fungal BSI.

In the present study, among 14 patients with candidemia, 5 (35.71%) cases were observed above the age group of 70 years with male predominance (92.85%). These results were consistent with Klingspor *et al* [8] and Deorukhkar *et al* [9] have also reported maximum cases of candidemia at this age group with male preponderance. The incidence of nosocomial candidemia in Surgical ICU patients in the present study was 15.05% which is very high and is consistent with a study carried out by Kothari *et al* [10] in New Delhi who found 18% cases of nosocomial BSIs due to *Candida* spp. Sahni *et al* [11] from India, and Soub *et al* [12] from Qatar reported an incidence ranging from 5-7% which is comparatively low. In the past few decades, Non-*Albicans Candida* (NAC) species have been isolated from 35-65% of all candidemias among the patients admitted in hospital [13]. This increase in non-*albicans Candida* species is correlated with an increasing use of azoles for prophylaxis or empirical treatment [5]. Out of total 14 *Candida* spp. isolated, there was equal distribution of the *C. albicans* (50%) and non-*albicans Candida* spp. (50%) in the present study. This finding was inconsistent with studies carried out by Kothari *et al* [10], Singh *et al* [3] and Adhikari *et al* [14] who showed NAC spp. to be the predominant isolates among BSIs. However, Fraser *et al* [15], Chowta *et al* [16 and Arora *et al* [3] reported *C. albicans* to be the predominant spp. which was not the case in the present study. For management of candidemia speciation is very important, since certain species like *C. krusei* are known to be intrinsically resistant while *C. glabrata* shows reduced susceptibility to fluconazole. In the present study, *C. albicans* was most common species isolated in 50% cases followed by *C. tropicalis* in 3(21.42%) cases. *C. rugosa* was isolated in 2 (14.28%) cases followed by 1 (7.14%) isolate each of *C. glabrata* and *C. lusitaniae.* In studies carried out in Asian countries [4, 17] and also among few of the studies carried out in India [3, 10], *C. tropicalis* was the most frequent NAC spp. to be isolated. These findings were in consistent with the present study where *C. tropicalis* is the commonest NAC spp. isolated from 3/14 (21.42%) cases. *C. glabrata* is seen more often in older adults, potentially resistant with overall mortality rate higher than other NAC spp. [18]. Among a number of European studies [17], *C. glabrata* was the most common NAC spp. isolated from 10-25% of the total candidemia cases followed by *C. parapsilosis* and *C. tropicalis* [8]. This is in contrast to the present study where only 1/14 (7.14%) case of *C. glabrata* were isolated. In the present study 2 (14.28%) cases *C. rugosa* were isolated which is emerging cause of candidemia in recent years. Chakrabarti *et al* [19], in his 6 month study in ICUs of 25 hospitals in India found 5.8%

and Singh *et al* [3] reported 18.4% of *C. rugosa* which was not consistent with the present study.

Hospitalization in the ICU setting has led to high risk for acquiring candidemia as a result of their underlying medical conditions and several risk factors. In the present study, the risk factors associated with candidemia showing statistical significance were length of ICU stay for more than 7 days, mechanical ventilation and central venous catheters and these reports were consistent with studies carried out by Sahni *et al* [11] and Michalopoulos *et al* [20]. However, in a study conducted by Yap *et al* [21] invasive candidemia occurred early during the ICU stay mostly within the first week. Because of skin colonization any break in the epithelial barrier act as a portal for invasive of *Candida* species. These species avidly adhere to materials used in IV catheters and provide a potential nidus for infection [18]. Though antibiotic use is believed to be a major risk factor predisposing to invasive candidiasis, yet this factor was not found to be significantly associated with candidemia in our ICUs which parallelled the observations reported by Yap *et al* [21]. However, contrary to this few studies [22, 11] showed that major risk factor for candidemia was usage of broad spectrum antibiotics. Uncontrolled diabetes mellitus was a significant risk factor leading to *Candida* BSI in the present study. However, Singh *et al* [3] did not find any significant association between hyperglycemia and development of candidemia. In the present study, prior surgery was not a significant risk factor for candidemia and this was consistent with the findings of Bassetti *et al* [5]. However, few other studies [15, 23-24] have suggested abdominal surgery as one of the important risk factor for candidemia which indicates *Candida* as an endogenous source.

Candidemia leads to significant morbidity and mortality especially in non-neutropenic critically ill patients. In the present study, 11 out of 14 candidemic patients died thus showing a high crude mortality rate of 78.57% which is consistent with a study conducted in United States with crude mortality rate ranging from 38-75% [25]. As more and more *Candida* species are reported to be showing drug resistance, drug susceptibility testing is mandatory. Among the 14 *Candida* isolates from SICU patients, 11 (78.57%) isolates were susceptible and 3(21.42%) isolates showed resistance to Amphotericin B after both 24 and 48 hours of incubation. Singh *et al* [3] found that the *Candida* spp. isolated from BSIs showed no resistance to Amphotericin B which was also noted in the present study where *C. albicans, C. lusitaniae* and *C. glabrata* showed 100% susceptibility to Amphotericin B. Singh *et al* [3] noted that *C. rugosa* showed 100% susceptibility to Amphotericin B which was not the case in the present study where 100% resistance was seen among *C. rugosa* isolates. One isolate (33.33%) of *C. tropicalis* showed resistance to amphotericin B. Thus, the resistance of NAC spp. to amphotericin B is more as compared to *C.albicans*. All the isolates except *C. rugosa* showed 100% susceptibility to 5-flurocytosine which was consistent with a study carried out by Adhikary *et al* [14]. Among 14 *Candida* isolates, no resistance was noted against fluconazole, but 50% isolate of *C. rugosa* showed dose dependent susceptibility against this drug, while Singh *et al* [3] observed increased resistance to fluconazole (MIC > 64μg/ml) in *C. rugosa*. Mokaddas *et al* from Kuwait [26] noted fluconazole resistance in 3.8% isolates of *C. albicans* which is in contrast to the present study where no such resistance was noted among *C. albicans* isolates. In the present study, out of 14 *Candida* isolates, 13 (92.85%) were susceptible while only 1 isolate showed dose dependent susceptibility to itraconazole which has been consistent with Estrella *et al* [27].

However, in a study carried out by Gonzalez *et al* [28] 4.60% of all *Candida* isolates from BSIs were resistant to itraconazole.

There was no resistance seen against voriconazole in the present study which was consistent with studies carried out by Mokaddas *et al* from Kuwait [26]. In contrast, Pfaller *et al* [29] reported 6.4% of *C. glabrata* isolates to be resistant to voriconazole.

Conclusion:

In conclusion, we report a considerably high incidence of candidemia and also the associated risk factors in Surgical ICU patients. Our study highlights the truth that the epidemiology of candidemia in India differs from that in Western countries. Hence, it should be mandatory to develop local guidelines on treatment of invasive candidiasis due to changing trends in the species distribution and antifungal resistance pattern among the isolates.

An increased level of suspicion and early initiation of appropriate systemically antifungal therapy actually improves the outcome of patients with candidemia.

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