Original Article

Epidemiology of *Candida* blood stream infections: experience of a tertiary care centre in North India

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Abstract

Introduction: Bloodstream infections due to *Candida* species are becoming a major cause of morbidity and mortality in hospitalized patients. The spectrum of candidemia has changed with the emergence of non-*albicans Candida* species, especially among critically ill patients.

Methodology: In a retrospective study (July 2009 to December 2009) on candidemia, various *Candida* species isolated from blood cultures were characterized and studied along with the determination of their antifungal susceptibility to amphotericin B, itraconazole, and fluconazole by Etest. Probable risk factors for patients in the intensive care unit (ICU) presenting with candidemia were also analyzed.

Results: During the study period, a total of 4651 samples were received, out of which 468 samples (10.06%) were positive for growth of organisms: 441 (94.20%) aerobic bacterial pathogens and 27 (5.79%) *Candida* species. The most common *Candida* spp. isolate was *C. tropicalis* (40.8%) followed by *C. albicans* (29.6%), *C. glabrata* (18.5%) and others (11.1%). Out of the 27 *Candida* strains, 24 (88.9%) were isolated from patients treated in the ICU. Among these, association of previous use of broad-spectrum antibiotics in 22 patients (91.6%) and central line catheter insertion in 20 patients (83.3%) were found to be statistically significant as compared to non-candidemia patients (p <0.05). Antifungal susceptibility testing of the isolates revealed a lower level of drug resistance to amphotericin B (18.5% of the isolates) versus 77.8% resistance to fluconazole.

Conclusion: Rapid changes in the rate of infection, potential risk factors, and emergence of non-*albicans Candida* demand continued surveillance of this serious bloodstream fungal infection.

Key words: candidemia; epidemiology; ICU; *Candida albicans*; non-*albicans Candida*

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Introduction

Candida species are the fourth most common cause of bloodstream infections and are the leading cause of invasive fungal infections among hospitalized patients [1]. Candidemia is a life-threatening fungal infection associated with a mortality rate of 38%; it also prolongs hospital stays by as much as 30 days and increases the cost of medical care [2]. The frequent use of antibiotics, central venous catheters and other invasive devices, abdominal surgery and stays in the intensive care unit (ICU) puts patients at a high risk of infection with *Candida*, which has been shown to have the shortest survival prospects of any bloodstream infection [3]. The spectrum of candidemia has changed with the emergence of non-*albicans Candida* (NAC) species, a strain with the threat of increased mortality and antifungal drug resistance, especially in immunocompromised and severely ill patients [4]. It is very important to identify *Candida* to the species level to optimize the selection of the antifungal agent. More importantly, intrinsic and emerging resistance to azoles represents a major challenge for empirical, therapeutic, and prophylactic strategies. The changing scenario has necessitated routine antifungal susceptibility testing since both *in vitro* resistance and toxicity issues must be considered when selecting an antifungal agent for therapy [5].

Methodology

The Government College Medical Hospital, Chandigarh, is a tertiary health-care centre with a 14-bed ICU (adult, combined medical, and surgical). The ICU is run by a full-fledged Department of Anaesthesia and Intensive Care with round-the-clock available faculty, residents, and paramedical staff. There is an institutional antibiotic policy in place and strict infection control practices are regularly followed.

The present retrospective study was conducted by the Department of Microbiology over a period of six months (from July 2009 to December 2009). During this time period, the department received a total of 4651 samples on which blood culture and sensitivity testing were performed. Blood for culture was collected under strict aseptic conditions. A total of 5 mL of blood was added to each of two bottles containing sodium polyanethole sulfonate (SPS) broth and bile broth (Hi-Media Laboratories, Mumbai, India). Both bottles were incubated aerobically at 37˚C for seven days. Routine subculturing was done on 5% sheep blood agar and MacConkey agar after 24 hours, 48 hours, and then at seven days. In between these points, subculturing was done only if there was visible turbidity. The aerobic bacterial isolates were further identified based on standard microbiological techniques [6].

The culture bottles that tested positive for yeasts were subcultured on Sabouraud dextrose agar and the yeasts were identified by standard mycological techniques, namely germ-tube test, morphology on cornmeal agar, color and colony characteristics on CHROMAgar Candida medium (Hi-Media Laboratories, Mumbai, India), carbohydrate assimilation and fermentation tests (Discs, media procured from Hi-Media Laboratories, Pvt. Ltd. Mumbai, India). The antifungal susceptibility pattern of the isolates was evaluated by the Etest (AB Biodisk, Solna, Sweden) for amphotericin B, fluconazole and itraconazole, on Mueller-Hinton agar supplemented with 2% glucose. The zone diameter endpoints were read at the complete inhibition for amphotericin B. In the case of azoles, the inhibition zone usually has a diffuse zone edge; therefore, the reading was taken at a point showing significant inhibition or marked decrease in growth intensity, usually corresponding with about 80% inhibition of growth. MIC breakpoints ≤ 8 mg/L for fluconazole and ≤ 0.12 mg/L for itraconazole were interpreted as susceptible; 16-32 mg/L and 0.25-0.5 mg/L as dose-dependent susceptible; and ≥ 64 mg/L and ≥1 mg/L as resistant, respectively. For amphotericin B, strains were considered susceptible if MIC was ≤ 1 mg/L and resistant if ≥ 2 mg/L based on the criteria of the Clinical Laboratory Standards Institute [7]. The standard strains used were *C. parapsilosis* (ATCC 22019, (Hi-Media Laboratories, Mumbai, India) and *C. albicans* ATCC 90028 (Hi-Media Laboratories, Mumbai, India).

An episode of candidemia was identified when the *Candida* was isolated from the blood culture of the patient; the second episode was defined if it occurred at least 30 days after the first episode. Clinical data of all candidemia patients were recorded on standard forms and analyzed. 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 also recorded. Chi square test was used to determine the association of various risk factors in candidemia patients and non-candidemia patients. The *p* value was calculated and a *p* value of less than 0.05 was considered statistically significant.

Results

During the study period, a total of 4651 samples were received in the department for processing, of which 468 samples (10.06%) appeared to be culture positive. Among these 468 isolates, 441 (94.20%) were aerobic bacterial pathogens and 27 (5.79%) were organisms belonging to *Candida* species. Among aerobic bacterial pathogens, the most common isolates by morphology, Gram staining, culture characteristics and biochemical reactions were *Acinetobacter calcoaceticus baumanii complex* (n = 115; 26.07%) followed by *Staphylococcus aureus* (n = 92; 20.86%), *Klebsiella pneumoniae* (n = 82; 18.59%), *Enterococcus* spp. (n = 51; 11.56%), *Salmonella Typhi* (n = 37; 8.39%), *Pseudomonas aeruginosa* (n = 25; 5.66%), *Escherichia coli* (n = 11; 2.49%), *Citrobacter* spp. (n = 9; 2.04%), and others. Among *Candida*, *C. tropicalis* was the most frequently isolated species (n = 11; 40.74%), followed by *C. albicans* (n = 8; 29.62%), *C. glabrata* (n = 5; 18.51%), *C. parapsilosis* (n = 1; 3.70%), and other species which that could not be identified (n = 2; 7.40%). Concomitant candidemia and bacteremia was observed in five patients (two with *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus-baumannii complex* and one with *Klebsiella pneumoniae)*.

All episodes of candidemia were nosocomial and occurred 48 hours after admission to the hospital. Of these, 24 (88.9%) occurred in patients admitted to the ICU, while only 3 episodes (11.1%) occurred in patients in other hospital wards. As most of the cases were from the ICU, the presence of various risk factors between those presenting with candidemia and those without candidemia during the same time period were compared (Table 1). The most common factors associated with candidemia were the use of broad-

Table 1. Association of various risk factors for candidemia in ICU patients (n = 205)

Risk Factors	Number of patients with candidemia in ICU (24)	Number of patients without candidemia in ICU (181)	*p* value
Use of antimicrobials (in prior 2 weeks)	22	115	$p < 0.05$
Central Venous Catheters	20	104	$p < 0.05$
Total parenteral nutrition	16	90	ns
Candida colonization in urine	14	88	ns
Major Surgical Procedure (in prior 30 days)	10	74	ns
Use of prophylactic antifungal agents	7	28	ns
Neutropenia	4	21	ns
Use of corticosteroids/ Immunosuppressive agents	6	39	ns
Diabetes mellitus	3	40	ns
Chronic renal failure	2	24	ns
Length of stay in ICU (> 15 days)	22	60	$p < 0.05$

spectrum antimicrobial agents in 22 (91.6%) and central line catheters in 20 (83.3%). The association was found to be statistically significant with $p < 0.05$. Candidemia was also found to be associated with the increased duration of ICU stay in these patients, with most of the patients staying for more than 15 days in the candidemia group ($p < 0.05$ as compared to non-candidemia patients).

The association of various risk factors among *C. albicans* and non-*albicans Candida* group were also compared. Results are given in Table 2.

Antifungal susceptibility testing revealed a lower level of resistance to amphotericin B: 5 (18.5%) in comparison to 17 (62.9%), and 21 (77.8%) of the strains were found to be resistant to itraconazole and fluconazole, respectively. Notably, all strains of *C. glabrata* were found to be resistant to fluconazole and only 2 (40.0%) strains were susceptible to amphotericin B, while all strains of *C. albicans* were susceptible to amphotericin B and 5 (62.5%) were resistant to fluconazole. Among *C. tropicalis*, 9 (81.8%) strains were resistant to fluconazole and 8 (72.7%) were resistant to itraconazole. The antifungal resistance pattern of the isolates is shown in Table 3.

Of the 27 episodes, 15 were treated with antifungal agents; 12 episodes were not treated with antifungals because patients were transferred to other hospitals or died before a final diagnosis could be made, or no reason was documented in their files.

The mortality was found to be 40.7% (11/27). Of the patients who died, ten were from the ICU and one was from a pediatric emergency ward. Out of 11, 7 (63.6%) patients had candidemia due to non-*albicans Candida* spp. The direct correlation of candidemia as the cause of death in any of the patients could not be ascertained due to multiple underlying pathologies.

Discussion

The spectrum of candidemia has changed with the emergence of non-*albicans Candida* species and with acquired antifungal resistance assisted by an increase in the high-risk population. This study highlights the prevalence of candidemia among the hospitalized patients and its correlation to the well-known risk factors. Moreover, the predominance of non-*albicans Candida* species over *C. albicans* was a notable feature as more than 70% of bloodstream infections were caused by non-*albicans Candida.* In this study, *C. tropicalis* was the most common isolate (40.74%) followed by *C. albicans* (29.6%) and *C. glabrata* (18.5%). Earlier data clearly shows that both the incidence of nosocomial candidemia and the proportion of bloodstream infections due to non-*albicans Candida*, particularly *C. tropicalis, C. glabrata* and *C. parapsilosis,* have increased [8-10]. This change in pattern has been partly attributed to increased immune suppression resulting in higher numbers of susceptible immunocompromised patients and also to the prophylactic use of antifungal agents in critically ill patients [11,12].

Hospitalization (especially in the ICU), placement of central venous catheters, and previous antimicrobial therapies played significant roles in the development of candidemia in this setting (Table 1).

Table 2. Comparison of risk factors between *C. albicans* and Non-*albicans Candida* (NAC) species

Risk Factors	**Number of episodes of candidemia (%)**	*C. albicans (8)*	Non-*albicans Candida* (19)			
			C. tropicalis (11)	*C. glabrata (5)*	Others (3)	Total NAC
Age > 55 years	5 (18.5)	2	0	3	0	3
Stay in ICU/ other ward	24 (88.9)	7/1	9/2	5	3	17/2
Use of antimicrobials (in prior 2 weeks)	25 (92.5)	6	11	5	3	19
Central Venous Catheters	22 (81.4)	6	9	5	2	16
Total parenteral nutrition	16 (59.2)	5	7	3	1	11
Candida colonization in urine	15 (55.5)	6	6	3	0	9
Major Surgical Procedure (in prior 30 days)	11 (40.7)	4	4	2	1	7
Use of prophylactic antifungal agents	9 (33.3)	1	3	5	0	8
Neutropenia	6 (29.6)	2	1	3	0	4
Use of corticosteroids/ Immunosuppressive agents	6 (22.2)	1	3	0	2	5
Others (Diabetes mellitus, Chronic renal failure, Malignancy)	6 (22.2)	1	5	0	0	5
Expired	11 (40.7)	3	5	2	1	8

Table 3. Antifungal resistance pattern of *Candida* isolates by Etest

Antifungal agent	***C. tropicalis* (*n* = 11)**	***C. albicans* (*n* = 8)**	***C. glabrata* (*n* = 5)**	***C. parapsilosis* (*n* = 1)**	***Candida* spp. (*n* = 2)**
Amphotericin B	2	0	3	0	0
Itraconazole	8	3	4	1	1
Fluconazole	9	5	5	1	1

Candidemia was associated with a prolonged stay (more than 15 days) in the ICU in these patients. The intravenous lines were withdrawn from candidemia patients; failure to remove catheters has been found to be associated with poor outcome, which could simply reflect a high probability of death [13,14]. Another important factor was prior administration of antimicrobials leading to impaired gut flora and impaired gut motility with subsequent overgrowth of yeast contributing towards candidemia [15,16]. Multiple predisposing and underlying factors were probably responsible. Frequently, early treatment or prophylactic intervention with antifungal drugs is given to reduce the risk for subsequent candidemia in ICU patients [17]. As with the widespread use of broad-spectrum antimicrobials, the selective pressure exerted by the frequent use of antifungals also encourages the proliferation of drug-resistant *Candida* spp. posing a new challenge in the effective management of nosocomial candidemia [18].

Risk factors that were identified in patients with non-*albicans* candidemia were prior use of antimicrobials and use of central venous catheters, with both being present more frequently in patients with non-*albicans* candidemia than *albicans* candidemia (Table 2). All these factors are well known and the current findings are in concordance with the reports of previous researchers [19].

This study again focuses attention on non-*albicans Candida* isolates being resistant to fluconazole. This aspect has been highlighted by the isolation of *C. glabrata* spp. in five cases out of a total

of 27 patients in the study. Resistance to amphotericin B is attributable to the reduction in ergosterol in resistant mutants of *Candida.* Such cases have been reported from immunocompromised patients who have received extensive antifungal agents and broad-spectrum antimicrobials. The susceptibility data are similar to those of other studies published in recent years [20]. Kothari *et al.* have previously reported 83% of *C. tropicalis* and 42% of *C. albicans* to be resistant to fluconazole [21]. Patients with non-*albicans Candida* are more likely to require a greater dosage of fluconazole to be cured clinically. There is need, therefore, to identify patients at risk of non-*albicans Candida* candidemia to initiate empirical therapy.

In the ICU, there is usually a failure of host defense mechanisms and also complications associated with the patient's underlying disease. Therefore, mortality is not solely related to the pathogenicity of the *Candida* species. In this study, mortality in patients presenting with candidemia was high, but direct correlation of candidemia and mortality could not be ascertained. However, in previous publications, mortality in patients with candidemia has been reported to be up to 80% [18,19]. Barberino *et al*. suggest that invasive candidiasis is a better marker for disease severity than an independent risk factor for mortality during the course of infection [22].

Based on the present results, it is clear that routine screening of *Candida* isolates to the species level followed by confirmation of resistant strains by antifungal susceptibility testing is essential. Clinicians must be aware of the wide breadth of debilitating conditions in which candidemia can arise, as removal of central lines, withdrawal of broad-spectrum antimicrobials, and treating the underlying diseases are all important initial steps in the management of systemic fungal infections. Moreover, continued surveillance of candidemia is important to document changes in its epidemiological features and antifungal susceptibilities.

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