INTRODUCTION

Candida species are ubiquitous yeasts but opportunistically invasive in individuals whose cell mediated immunity is decreased due to any diseased condition or by any iatrogenic intervention, mostly observed in intensive care unit, oncology department, organ transplant department and dialysis unit. Candida bloodstream infections (BSI) have become a major problem in tertiary-care hospitals worldwide (1,2). Candidemia has been observed particularly among patients hospitalized for long periods, who have been exposed to antibiotics, immunosuppressive therapy, parenteral nutrition, and multiple invasive medical procedures (2). Over the last two decades, both the incidence of nosocomial candidemia and the proportion of blood stream infection due to Candida spp. other than Candida albicans have increased (3). Non-albicans Candida spp (NAC) are of special concern, since some are highly virulent and are associated with treatment failure due to reduced susceptibility to antifungal agents (4). Candida strains possess a number of virulence factors which enable...
the organism to cause hematogenously disseminated infections in susceptible hosts. One amongst them is biofilm production. The ability to form extensive biofilms on the catheters and other prosthetic devices also contribute to the prevalence of intravascular nosocomial infection and associated with high level of antimicrobial resistance (5).

The aims and objectives of this study were: a) to identify the various species of Candida causing bloodstream infection; b) to detect the production of biofilm by Candida as a virulence marker and c) to analyse the risk factors associated with Candida infection.

MATERIALS AND METHODS

This study was conducted at Kasturba Medical College and hospital, Mangalore which is an 840 bedded tertiary care hospital, over a period of one year. All blood isolates received from patients during this period were screened for candidemia prospectively. All hospitalized patients with a positive blood culture for Candida were included in this study. Children and adults were included in the study if Candida organisms were isolated from a single blood culture. One or two blood samples were collected at the same time from the patient after all aseptic precautions. For all adult study patients, the following characteristics were recorded: age, gender, the presence of underlying malignancy, neutropenia, seropositivity for the human immunodeficiency virus antibody, diabetes mellitus, bone marrow or solid-organ transplant, abdominal or cardiothoracic surgery, underlying medical disorders, any ongoing treatment, the duration of hospitalisation and presence of biomedical devices. For neonates along with the other data presence of prematurity and low birth weight were also recorded.

These characteristics were determined at the time that the initial blood sample that was obtained for culture and from which a fungal pathogen was isolated. Blood culture was performed using the automated blood culture system. Subcultures were done from all positive BacT/Alert blood culture bottles onto 5% sheep blood agar, Sabouraud’s dextrose agar (SDA) with antibiotic and MacConkey’s agar. The plates were examined after overnight incubation at 37°C. Candida spp. identified by colony morphology, Gram stain of the colonies and further speciation was done by germ tube formation, chlamydospore formation, sugar containing HiCandida™ identification kit, and chrom agar test.

The chromogenic medium, HiMedia CHROM agar® (HiMedia, Mumbai, India), has chromogenic substances which helps in the rapid identification of the Candida species, based on the reactions between the specific enzymes of the different species and the chromogenic substances. As per the colour code which is provided with the chromogenic media, C. albicans produces blue-green colonies, C. tropicalis produces dark blue to blue grey colonies, C. glabrata produces white to cream coloured colonies and C. krusei produces pale pink to purple, rough colonies.

HiCandida™ identification kit is a colorimetric identification system utilizing twelve conventional biochemical sugars. The tests are based on the principle of pH change and sugars utilization. On incubation, organisms undergo metabolic changes which are indicated by a spontaneous colour change in the media. 18 Candida spp. can be identified by this method.

Procedure. This kit cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. So isolates of Candida obtained from blood culture were subcultured onto SDA agar. Inoculum was prepared by picking 2-4 well isolated colonies and making a homogenous suspension in 2-3 ml sterile saline. The density of the suspension was adjusted to 0.50 OD at 620 nm. Then sugar containing each well of the kit was inoculated with 50 microlitres of the above inoculum by surface inoculation method and the kit was incubated at 22.5°C for 24-48 hours. Identification of different species was done as per the standards given in the identification index of the provided kit manual.

Detection of biofilm production was done by a method proposed by Brachini et al. (6). According to the method, a loopful of organisms from the surface of a Sabouraud dextrose agar plate was inoculated into a tube containing 10 ml of Sabouraud broth supplemented with glucose (final concentration, 8%). The tubes were incubated at 35°C for 24 h, after which the broth was aspirated out and the walls of the tubes were stained with safranin and they were examined for the presence of a viscid slime layer. Each isolate was read independently by two different observers.
RESULTS

Incidence and patient demographics. A total of 80 cases of candidemia were identified. Among these 20 neonates, 13 children, 28 adults and 19 patients were above 60 years of age group. Male predominance was noted in 71.25% (n=57).

| Co-morbid conditions/Risk factors | (%) | Number |
|----------------------------------|-----|--------|
| Vascular access devices          | 95  | 19     |
| Prematurity                      | 70  | 14     |
| Low birth weight                 | 65  | 13     |
| Prior antibacterial therapy       | 25  | 5      |
| Use of corticosteroids           | 20  | 4      |
| Nervous system disorders         | 10  | 2      |
| Gastrointestinal disorders       | 10  | 2      |
| Recent surgeries                 | 10  | 2      |
| Respiratory disorders            | 5   | 1      |

Concomitant conditions and risk factors for candidemia. At the time of the diagnosis of candidemia, 65 (81.25%) patients were admitted in the intensive care unit (ICU) (including the neonatal intensive care unit). Different co-morbid conditions and risk factors were identified among the recruited patients. Among the neonates, most important risk

Table 1. Co-morbid conditions/Risk factors in neonates with candidemia (n=20)

| Co-morbid conditions/Risk factors | (%) | Number |
|----------------------------------|-----|--------|
| Vascular access devices          | 95  | 19     |
| Prematurity                      | 70  | 14     |
| Low birth weight                 | 65  | 13     |
| Prior antibacterial therapy       | 25  | 5      |
| Use of corticosteroids           | 20  | 4      |
| Nervous system disorders         | 10  | 2      |
| Gastrointestinal disorders       | 10  | 2      |
| Recent surgeries                 | 10  | 2      |
| Respiratory disorders            | 5   | 1      |

Table 2. Co-morbid conditions/Risk factors in patients with candidemia (excluding neonates) (n = 60)

| Co-morbid conditions/Risk factors | (%) | Number |
|----------------------------------|-----|--------|
| Implantation of intravenous catheter | 96.67 | 58   |
| Prior antibacterial therapy       | 70  | 42     |
| Renal insufficiency               | 46.67 | 28   |
| Urinary catheter                  | 45  | 27     |
| Hepatic disorder                  | 31.67 | 19  |
| Central venous catheter           | 30  | 18     |
| Diabetes                          | 28.33 | 17  |
| H/O alcoholism                    | 26.67 | 16  |
| On dialysis                       | 21.67 | 13  |
| Respiratory disorders             | 18.33 | 11  |
| Malignancy                        | 18.33 | 11  |
| Mechanical ventilation            | 15  | 9      |
| Recent surgeries                  | 15  | 9      |
| Use of corticosteroids            | 11.67 | 7   |
| Neurological disorders            | 10  | 6      |
| Neutropenia                       | 10  | 6      |
| Cardiovascular disorders          | 8.33 | 5     |
| HIV/AIDS                          | 1.67 | 1     |
factor was placement of vascular access devices (n=19; 95%) followed by prematurity (n=14; 70%) and low birth weight (n=13; 65%). Use of intravenous canulae (n=58, 96.67%) and prior use of antibiotics (n=42, 70%) were found to be associated with the occurrence of candidemia in other age group of patients. Table 1 and Table 2 summarize the distribution of underlying concomitant conditions and risk factors within the patients with candidemia.

Species distribution. Candida albicans accounted for 22 isolates (27.5%) whereas non-albicans Candida spp. accounted for 58 isolates (72.5%). C. glabrata (n=22; 37.93%) was the commonest isolate recovered among the non-albicans Candida spp. Multiple Candida species were not recovered from any patient. Distribution of Candida spp. isolated from blood is shown in Table 3. C. krusei was the most common pathogen isolated among neonates. Common isolated species among children were Candida glabrata and C. tropicalis. Candida albicans and Candida glabrata were also commonly isolated species among the adults and >60 years of age group.

Biofilm production. Amongst the 80 strains of Candida spp. isolated from blood, biofilm production was found in 31 strains (38.75%). Biofilm production was seen more in non-albicans Candida spp. (83.87%) especially in C. tropicalis (66.67%, 8 of 12). The results of biofilm production are shown in Table 4.

Mortality. Overall mortality was 20% (n=16/80). Three patients died before instituting antifungal therapy and 13 patients died after instituting antifungal therapy. Out of 16 patients died, C. albicans isolated only in six patients (37.5%) where as non-albicans spp. isolated in rest of the 10 patients (62.5%). Biofilm producing Candida strain were isolated only in 5 (31.25%) out of 16 patients died.

DISCUSSION

Over the past 30 years, numerous investigators have reported that the frequency of severe infections caused by yeasts, especially Candida spp., has increased dramatically (7). Emerging non-albicans Candida spp. showed increased virulence, increased mortality and resistance to common antifungal drugs (8). Hence, species identification is very important.

The epidemiology of candidemia may vary depending on geographic locations, even between

### Table 3. Distribution of Candida spp. isolated from blood

| Candida species | Number of isolates (%) |
|-----------------|------------------------|
| C. albicans     | 22 (27.5)              |
| C. glabrata     | 22 (27.5)              |
| C. krusei       | 13 (16.25)             |
| C. tropicalis   | 12 (15)                |
| C. stellatoide  | 04 (5)                 |
| C. guilliermondii | 03 (3.5)             |
| C. parapsilosis | 02 (2.5)               |
| C. dubliniensis | 02 (2.5)               |
| Total           | 80                     |

### Table 4. Biofilm production by Candida species isolated from blood

| Candida spp. | Number of isolates (%) | Biofilm production Number (%) |
|--------------|------------------------|-------------------------------|
| C. albicans  | 22 (27.5)              | 5 (22.73)                     |
| C. tropicalis| 12 (15)                | 8 (66.67)                     |
| C. glabrata  | 22 (27.5)              | 9 (40.91)                     |
| C. krusei    | 13 (16.25)             | 7 (53.84)                     |
| Other non-albicans spp. | 13 (16.25) | 2 (18.18) |
units in the same hospital. Although *C. albicans* has been the predominantly isolated species, there has been a shift in the distribution to the non-albicans *Candida* species as reported previously (8) and confirmed in our study as the proportion of non-albicans *Candida* species was 72.5%. This increased prominence of non-albicans spp. causing BSI has been extensively noted in other Indian studies also (9). *C. glabrata* was the most frequently recovered non-albicans isolate in our study similar to other worldwide reports (10, 11).

The high incidence of *Candida* bloodstream infections among patients at the extremes of the age spectrum is consistent with previous studies; neonates and age group >45 years accounted for highest number of isolates in our study. In recent years, there is a marked shift in isolation rates of non-albicans *Candida* species compared to *Candida albicans* in cases of neonatal sepsis. Kossoff et al. (12) showed significant shift from *Candida albicans* to non-albicans *Candida* spp. Chakrabarti et al. in a five years (1990-1995) report said that isolation of *C. tropicalis* and *C. guilliermondii* were common in early 90’s but later there was increase in isolation rate of *C. krusei* and *C. glabrata* (13). In our study *C. albicans* was isolated as the most prevalent isolate among adults and older age groups. This could be due to prophylactic use of fluconazole is not a standard practice in our hospital as suggested by Bassetti et al. (8).

Male predominance (71.25%) which was observed in the present study is similar to that found in previous reports by Chowta et al and Kao et al (14). Many risk factors and comorbid conditions have been reported in the development of candidemia in our study and also by other authors worldwide (15). *Candida* infection is not solely related to the pathogenicity of the *Candida* spp., but also to a failure of host-defence mechanisms and to complications associated with the patient’s underlying disease (16). The more severely ill patients are at higher risk for *Candida* infection and have a worse prognosis. This is particularly evident in ICU patients who require indwelling catheters and broad-spectrum antibiotic therapy, which place them at risk for *Candida* colonization and subsequent infection (17-20). In our study also, high proportions of cases (75%) were admitted in ICUs with severe illness. Among both children and adults, the most prominent risk factors were presence of intravascular catheters and prior bacterial infections/use of antibacterial agents, both of which are well documented independent risk factors for candidemia (21). In our study, all patients had at least one identifiable risk factor. Furthermore, previous antibiotic use especially with broad-spectrum antibacterial activity is known to affect the protection of bacterial gut flora against the fungal overgrowth (22). Most of the patients in our study had received broad-spectrum antibiotics before the onset of candidemia.

The most important risk factors for *C. albicans* were old age and procedures associated with intensive care. Similar observations were reported by Cheng et al. (23). *C. tropicalis* was most frequently isolated in adult age group and most of the patients were taking broad spectrum antibiotics at the time of diagnosis of candidemia as reported by other study (24).

The high incidence of bloodstream infections due to *Candida* among neonates is consistent with previous studies, in which risk factors identified for candidemia in neonates included low birth weight, the use of intravascular catheters and prematurity (25).

Since *Candida* biofilms have been considered as a virulence factor contributing to infection associated with various medical devices, a reliable method for their diagnosis is necessary. The biofilm positivity rates obtained in our study (38.75%) were considered to be an important finding because of the fact that biofilm production is a special feature of *Candida* pathogenicity. Our data provide evidence that the majority of non-albicans *Candida* species (83.87%) recovered from the blood have the capacity to produce significant amount of biofilm when grown in high-glucose medium. *Candida albicans* isolates recovered from blood demonstrated lower percentage (22.73%) of biofilm positivity. Biofilm positivity occurred most frequently in isolates of *C. tropicalis* (66.67%) followed by *C. krusei* (53.84%), *C. glabrata* (40.91%) and other non-albicans *Candida* spp. (18.18%). This result suggests that biofilm production is more important for non-*Candida albicans* strains and *C.albicans* possess mechanisms other than biofilm production to establish bloodstream infections, which is similar to those observed by others (5). The production of biofilm is also associated with high level of antifungal resistance. In our study biofilm producing *Candida* spp. isolated in 5 out of 16 patients died. Those five patients died after instituting the antifungal therapy. So, here
REFERENCES

the reason could be Candida spp. isolated in those patients were resistant to antifungal agents due to biofilm production.

Candida blood stream infection is an important cause of morbidity and mortality. We reported 20% mortality rate. Mortality attributable to candidemia reported from other studies is 12-38%. The overall mortality rate observed in our study was more with non-albicans Candida spp. which is similar to other studies. Staying in the intensive care unit increased the mortality rate by 3.6 times which was observed in our study. Severity of the underlying disease was a better risk factor of mortality, also mentioned by Barberino et al. (26) study. However, the excess mortality observed in the non-albicans group could be attributed to inappropriate treatment or a delayed therapy caused by a slower growth of non-albicans spp (20). In our study also 3 patients died due to delay in initiation of antifungal therapy.

CONCLUSION

Candidemia remains a major cause of morbidity and mortality in the health care setting, and the epidemiology of Candida infection is changing. Candidemia extends the duration of hospital stay and increases the cost of medical care. So, continued & careful surveillance of candidemia will be important to track trends of this serious infection and to document changes in its epidemiological features. Proper antibiotic and infection control policies will help to control the nosocomial infections in areas of hospitals which require special attention like the ICUs. Therefore, we consider that constant monitoring of patients who develop candidemia is essential, in order to be able to detect the epidemiological changes and resistance patterns in each hospital, as well as to identify the risk factors that allow early detecting of these infections and thus improving the prognosis of the patients.

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