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Incidence of *Candida* in patients admitted to ICU

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ABSTRACT

Profiling and antifungal susceptibility of Candida sp. in blood was conducted and the specimens were collected from Thanjavur Medical College, Thanjavur, India. A total of 31 isolates were selected for prospective analysis over a period of 6 months. Candidemia was diagnosed by positive blood culture bottles. Candida sp. were identified to species level by germ tube test, Hi- Chrome Candida agar, methyl blue SDA agar, rapid trehalose and maltose test, corn meal agar and sugar fermentation tests using standard techniques. All the Candida isolates were tested for antifungal susceptibility to amphotericin B and fluconazole by the disc diffusion method. Most frequent isolates were C. parapsilosis (66%), C. tropicalis (19%), C. albicans (6%), C. glabrata (6%) and C. guillermondii (3%). About 6.5% polymicrobial candidemia has been described. Most of the blood isolates were from the Intensive Care Units (ICU) (81%) especially neonatal (61%) and few from other wards (19%). 84% isolates were resistance to fluconazole, 56% isolates were resistant to voriconazole and 16% isolates were resistance to amphotericin B. Among the isolates C. parapsilosis was found to be predominant in occurrence. Most of the isolates were resistant to fluconazole.

Keywords: Candidemia, ICU, antifungal susceptibility.

INTRODUCTION

There has been an increase in the incidence of opportunistic infections in immunocompromised patients and children. Their incidence has greatly increased over the past several decades with the introduction of broad-spectrum antibiotics. *Candida* sp. has become the fourth most common organism responsible for bloodstream infection in the intensive care unit (ICU) [Eggimann *et al*., 2003]. *Candida* sp. account for 9-13% of all blood isolates in neonatal intensive care units NICUs [Baradkar *et al*., 2008]. Among them, the *Candida albicans* and several related *Candida* sp. are of foremost importance as opportunistic pathogens in immunocompromised hosts, which may cause life threatening infections. Although *C. albicans* is the species mainly isolated from patients but other species also cause nearly 40% of the infections. It is therefore very important to identify *C. albicans* as well as other *Candida* sp. rapidly yet reliably in routine clinical microbiology practice.

The primary isolation of yeasts from biological specimens is generally based on culture on Sabouraud dextrose agar, a medium that does not allow species identification; differential isolation media like chromogenic media that distinguish *Candida* sp. on the basis of the morphology and colour of the colonies on primary cultures were evaluated. A valuable and simple test for the rapid, presumptive identification of *C. albicans* is the germ tube test [Donald Sheppard *et al*, 2008].The resistance of non - *C. albicans* isolates to currently available antifungal drugs represents a major challenge for future empirical therapeutic and prophylactic strategies [Krcmery and Barnes, 2002]. Rapid and accurate species identification is thus necessary to optimize the choice of antifungal therapy. The

antifungal susceptibility testing of the yeast isolates are done by disc diffusion method by using amphotericin B, voriconzole and fluconazole.

The profile of *Candida* sp. differs from geographical locations. In many reports, identification of *Candida* sp. isolates has been based on the commercially available kits for their biochemical characteristics. In recent years *Candida* sp. infection has increased clinical awareness. Thus, the present work was aimed to culture and identify the *Candida* sp. from blood stream infections and to determine the distributions of species involved in these infections.

MATERIALS AND METHODS

Sample Collection

For a period of six months the clinical isolates of *Candida* sp. from blood were collected from 31 patients from Thanjavur Medical College, Thanjavur, India. The blood was usually drawn for from peripheral vein, e.g. vena cubitalis for isolation of the pathogen. Blood was collected with a sterile needle and syringe and evacuated in bottle of blood culture bottle. Thorough disinfection of the patient's skin was done by scrubbing the venipuncture site with 70% alcohol or iodine, to reduce the contamination rate.

Fungal blood cultures

The biphasic brain heart infusion (BHI) (Hi-Media, Mumbai, India) agar plus broth was used for fungal blood culture. 5 ml of blood was inoculated into the culture bottles, they were incubated in an upright position for 30 d at 37º C. Cultures were examined daily for visual evidence of growth, and each examination was followed by gentle mixing of the blood-broth mixture over the agar slants. Visual growth was stained and sub-cultured into appropriate media for identification [Glenn Roberts and John Washington II, 1975]. The growth was sub-cultured on SDA medium and then incubated at 37ºC. For morphological characterization Lacto phenol cotton blue test (LPCB) was done.

Test for germ tube formation

The suspected *Candida* cultures were inoculated into 0.5 ml of human serum in a small tube and incubated at 37º C for 2 h. After 2 h of incubation, a loop-full of culture was placed on a glass slide and overlaid with a cover-slip. The preparation was examined under bright field microscope for germ tube with no constriction at the point of attachment to the yeast cells which is noticed only in *C. albicans* [Donald Sheppard *et al*, 2008].

Differential identification of *Candida*

Hichrome *Candida* agar

Overnight colonies were inoculated on Hichrome *Candida* agar (Hi-Media, Mumbai, India) and incubated at 37ºC for 48 h. The colonies were identified by their colour [Baradkar *et al.*, 2010]

Methyl Blue-Sabouraud Agar

This fluorometric test for rapid identification of *C. albicans* was performed according to the methods of Roggentin *et al*. [1999]. Overnight colonies were transferred to petri dishes containing methyl blue- sabrouraud media. Petri dishes were incubated at 37ºC and the results were evaluated after 48 h of incubation. The colonies were evaluated under UV lamp (wavelength 365 nm) for brightly fluoresced colonies [Mine *et al*., 2001].

Test for chlamydospore formation

The suspected *Candida* cultures were streaked on the Corn Meal Agar (CMA) (Hi-Media, Mumbai, India) plate. A heavy inoculum of yeast was streaked across a plate containing the medium and a cover slip is placed over it. The streak was projected beyond the cover slip. Then the plates were incubated at 25º C for 48 - 72 h. The edge of the cover slip was examined under low power and high power objective for chlamydospores, blastospores and pseudohyphae [Staib and Morschhauser, 2007].

Carbohydrate fermentation tests

Fermentative yeasts recovered from clinical specimen's produces carbon dioxide and alcohol. Production of gas rather than a pH shift is indicative of fermentation. The test organism was inoculated in the sugar medium and incubated at 37º C for 48-72 h. The results were interpreted by colour change from yellow to pink and gas production in the Durham's tube.

Rapid Trehalose and Maltose test

The test is based on the capacity of *C. glabrata* to hydrolyze trehalose (but not maltose) into two glucose molecules in less than one minute. Three URS-1 G (Teco diagnostics, U.S.A) urine reagent strips were used for each test. The detection pad of the first strip was hydrated with one drop of 0.01% trehalose solution; the second strip was hydrated with one drop of 0.01% maltose solution, and the third with one drop of sterile distilled water. One or two yeast colonies with an identical appearance were picked from the agar plates and smeared directly on to each of the three reagent pads. Care was taken to discard all excess liquid. A positive test on any strip was indicated by comparing the corresponding colour chart within 1 minute. [Willinger B., 2005].

Anti Fungal Susceptibility Test (AFST)

All the clinical isolates were screened for antifungal susceptibility testing by the disc diffusion method using amphotericin-B (100 units), voriconazole (1µg) and fluconazole (25µg) (Hi-Media, Mumbai, India), on Muller Hinton agar (Hi-Media, Mumbai, India) (MHA) supplemented with 2% glucose and methylene blue dye 0.5µg/ml. The test isolates were streaked over the entire agar surface of the plate three times, turning the plate at 60º angle between each streaking and allowed the inoculum to dry for 5-15 minutes with lid in place. The anti- fungal discs such as amphotericin-B and fluconazole were placed aseptically by sterile forceps at the centre at least 24 mm apart. Then the plates were incubated at 37ºC for 48 h. Zone diameters were interpreted as per the approved NCCLS (M44-A) guidelines [Clinical and Laboratory Standards Institute. 2004].

RESULTS

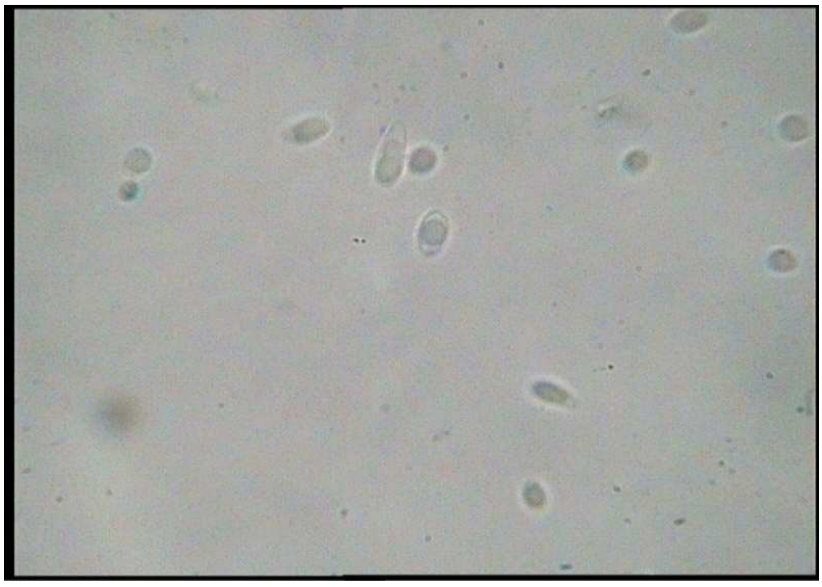
Sample Collection

In the total number of specimens collected 58% of isolates were from female patients and 42% of isolates were from male patients, 61% isolates were from age ranging below 10, 13% isolates were from age ranging 50 and above, 7% isolates were isolated from age ranging 21-30 and 41-50 and 3% from age 31-40.

Fungal blood cultures

Fungal blood culture bottles showed remarkable growth in most of the bottles and the fungal colonies were identified as *Candida* sp. by LPCB staining (Fig. 1).

Fig. 1 LPCB staining of *Candida sp.*



Test for germ tube formation

There was only two species of *Candida* isolated. Both of these strains were germ tube test positive. Most of the laboratories uses germ tube test for the identification of *Candida* sp. because it is easier to perform and interpret.

Differential identification of *Candida*

Both Corn meal agar and Hi – Chrome agar were useful in identifying the two isolates the species identified produced fluorescence under UV light in methyl blue SDA agar. In sugar fermentation test two strains which were identified were *C. tropicalis* and *C. parapsilosis*. For analysis these strains were considered as *C. parapsilosis*. *C. parapsilosis* is the second most common species found in patients with candidemia. The carbohydrate fermentation tests for *Candida* sp. are presented in table 1. The rapid trehalose and maltose test were positive for the two C. *glabrata* isolates. The limited sugars used in sugar fermentation test, produces same results for *C. glabrata* and *C. parapsilosis.*

Table 1. Sugar Fermentation Test

Blood isolates	Sugar					
	Glucose	Sucrose	Lactose	Maltose	Trehalose	Film
1	+	-	-	-	-	-
2	+	-	-	-	-	-
3	+	-	-	-	-	-
4	+	-	-	-	-	-
5	+	-	-	-	-	-
6	+	-	-	-	-	-
7	+	-	-	-	-	-
8	+	-	-	-	-	-
9	+	+	-	+	+	narrow
10	+	-	-	-	-	-
11	+	-	-	-	-	-
12	+	+	-	+	+	narrow
13	+	-	-	-	-	-
14	+	-	-	-	-	-
15	+	-	-	-	-	-
16	+	+	-	+	+	narrow
17	+	-	-	-	-	-
18	+	+	-	+	+	narrow
19	+	-	-	-	-	-
20	+	+	-	+	+	narrow
21	+	-	-	-	-	-
22	+	-	-	-	-	-
23	+	+	-	+	+	narrow
24	+	-	-	+	-	-
25	+	-	-	-	-	-
26	+	+	-	+	+	narrow
27	+	+	-	+	+	narrow
28	+	-	-	-	-	-
29	+	-	-	-	-	-
30	+	-	-	-	-	-
31	+	-	-	+	-	-

Antifungal susceptibility test

Out of the total number of isolates 83.87% strains were sensitive for amphotericin B, 43.55% were sensitive to voriconazole and 19.35% strains were sensitive for fluconazole. Some *Candida* species like *C. tropicalis, C. albicans* and *C. guillermondii* showed 100% resistance to fluconazole, whereas *C. parapsilosis* and *C. glabrata* showed 80% and 50% resistance to fluconazole respectively. Even 50% strains of *C. tropicalis* and *C. glabrata* were resistant to amphotericin B and 5% strains of *C. parapsilosis* were resistant to Amphotericin B. All strains of *C. albicans* and *C. guillermondii* were sensitive to amphotericin B.

DISCUSSION

Fungemia (Candidemia) is the presence of fungi or yeasts in the blood. It is most commonly seen in immunosuppressed or immunocompromised patients. The two most important risk factors are the use of broad-spectrum antibiotics and colonization by fungi. Other risk factors are dialysis, diabetes, lowered intestinal flora, suppressed immune system, high severity of illness, multiple abdominal surgeries, use of steroids and burns. Candidemia showed high mortality and morbidity. The most common pathogen is *Candida albicans*, causing roughly 70% of fungemia. In our study *Candida* isolates were predominantly isolated from female patients (58 %).

Further candidemia was frequently associated with infants (61%) and age group ≥50 (13%) which was nearly correlated with studies by Shivaprakasha *et al.,* [2007] of Kerala. They observed candidemia isolates more frequently from infants 44% and ≥ 40 year age group 23.7%.

Due to its ease in performing and result interpretation, germ tube method is generally used for identification of *C. albicans* but some *C. albicans* are germ tube negative, methyl blue SDA medium can be adopted to identify these germ tube negative *C. albicans*. In Hi-chrome *Candida* media the *Candida* sp. were identified by their colony colour and in corn meal agar the corresponding morphology for each species were observed. There was 100% correlation among species identification by corn meal agar and Hi – chrome agar. The rapid trehalose and maltose test requires low inoculum. It differentiates *C. glabrata* from *C. parapsilosis* by fermenting the trehalose but not maltose within 5 minutes. Thus it is a rapid and cost effective test for the identification of *C. glabrata*.

The Antifungal susceptibility of the isolates was determined by disc diffusion method. There was a dramatic increase in resistance with *C. tropicalis, C. parapsilosis, C. albicans and C. guillermondii*. This may be due to the difficulty associated with the differences in growth rates and the end point interpretation in disc diffusion method leads to low reproducibility. Most of the *Candida* sp. was susceptible to amphotericin B and the incidence of amphotericin resistant C*andida* sp. in our study was 16%.

CONCLUSION

Candidemia is considered as a major problem with regard to fungal infections in hospitals. The emergence of resistance against amphotericin B and an increase in the resistance against flucanazole was observed during the study. Further continuous surveillance programme are needed in order to identify the possible changes in the species distribution and antifungal susceptibility patterns of *Candida* isolates.

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