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Research Article

Characterization, Antifungal Susceptibility and Phenotypic Virulence Markers of Candida Isolates from Blood Stream Infection

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Abstract

Background: Candidemia is a blood stream infection which is caused by various Candida species in children and adults. About more than 200 species of Candida are identified and only about 10% of Candida species cause human infections. In the Blood Stream Infection (BSI), Candida is the 4th most common isolated organism in the hospitalized and critically ill patients. Identification of Candida species in blood stream infection and antifungal susceptible pattern is urgent need for diagnosis of Candidemia.

Objective: To study the spectrum of Candida species in blood stream infection and to study the antifungal susceptibility pattern and virulence factors.

Material and Method: A total of 1500 blood culture samples were collected in one year (2019-2020) at King George Medical University, Lucknow. Out of 1500 patients, 100 blood cultures were positive for Candidemia. Identification of Candida species was done by conventional technique and reconfirmed by MALDI-TOF. Antifungal susceptibility pattern was performed for accuracy of drug concentration which helps clinicians for treatment of patients.

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Results: We found that the *Candida tropicalis* 26% were more prevalent than *Candida albicans* (17%). The most common risk factor for development of Candidemia was ICU admission (55). Resistance of fluconazole for *Candida albicans* 3(17.65%) and *Candida tropicalis* 3(11.5%), *Candida utilis* 1(6.7%), *Candida parapsilosis* 6(50%), *Candida auris* 11(100%), *Candida glabrata* 3(60%), *Candida duobushaemulonii* 1(100%).

Conclusion: As there is a shift to non-albicans candida causing candidemia as well as increase resistance to fluconazole drug which causes high rate of morbidity and mortality in critically ill Candidemia patients. There is need for identification of Candida species and accurate drug for treatment of patients.

Keywords

Virulence Factors; Candida; MALDI-TOF

Abbreviations

BSI: Blood Stream Infection; MALDI TOF-MS: Matrix Assisted Laser Desorption/Ionization Time of Flight; ICU: Intensive Care Unit; CLSI: Clinical and Laboratory Standards Institute; SDA: Sabouraud's Dextrose Agar; NaCl₂: Sodium Chloride; CaCl₂: Calcium Chloride

Introduction

Candidemia is a blood stream infection which is caused by various Candida species in children and adults [1]. Candida is a commensal of skin and mucous membrane. About more than 200 species of Candida are identified and only about 10% of Candida species cause human infections [2]. In the Blood Stream Infection (BSI), Candida is the 4th most common isolated organism in the hospitalized and critically ill patients [3]. The global incidence of Candidemia estimates to be around 1 to 8 cases per 1,00,000 population [4].

In the US, 8-10% hospital acquired blood stream infection is caused by the Candida species and are responsible for fourth most common blood stream infection pathogen, and number three cause of blood stream in Intensive Care Unit (ICU), third leading cause of Central-Line-Associated Bloodstream Infections (CLABSI) and the second leading cause of catheter-associated urinary tract infections [5-20]. In the Europe, Candida species are responsible for only 2-3% of blood stream infection and accounts for 6th to 10th leading cause of blood stream infection [21].

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The incidence and prevalence of candidemia, is quite different from different part of the India. A study by Verma, et al., conducted in SGPGI Lucknow, state that the incidence of candidemia was 1.61 per 1000 patients who were admitted for Candidemia and ranked *Candida* species 8th among all isolate from blood stream infection [22]. A study in a New Delhi gave prevalence 18% of candida in blood culture [23]. A study in South India reported an incidence rate of 5.7% for candidemia among children with onco haematological malignancies [24]. Study from Rohtak, North India, reported an isolation rate of 8.1% for *Candida* species from cases of neonatal septicaemia [25]. Xess, et al., from AIIMS, New Delhi, found a prevalence rate of 6% for *Candida* species in a 5-year study (2001-2005) [26]. A study by Sahni, et al., from Maulana Azad Medical College, New Delhi, found an incidence rate of 6.9% for *Candida* species in BSI [27].

The incidence of Candidemia was reported from the patients admitted in the Intensive care unit (ICU) was 6.9 per 1000 in a recent study and about 7.5% of ICU patients receive antifungal therapy [5,6]. The genus *Candida* is composed of a heterogeneous group of organisms and more than 17 different *Candida* species are known to be the etiological agents of human infection, 90% of invasive infection are caused by *Albicans*, *C. tropicalis*, *C. glabrata*, *C. auris*, *C. parapsilosis* and *C. krusei* [7]. In the invasive *Candida* infection, *Candida albicans* accounts for nearly 50% of all species but there is increasing non- albicans candida species infection also [8].

Virulence factors are responsible to cause disease which depends on virulence factors and survival traits. They include growth of hemolytic activity, formation of biofilm, proteinase, phospholipase and esterase activity [10,17].

The aim of the present study is to identify the *Candida* species for blood culture isolates, study virulence factors and identify their sensitivity pattern to the available antifungal drugs by Broth microdilution method.

Methodology

The observational study was conducted in the Mycology Laboratory of Postgraduate Department of Microbiology, King George Medical University, Lucknow from August 2019 to July 2020.

This study was approved by Institutional Ethics committee in King George's Medical University, Lucknow, India.

Total 1500 patients were enrolled with suspected case of septicemia. Blood sample was collected from the peripheral veins after cleaning the skin with disinfectant and withdrawn 5-10 ml of blood were collected in the blood culture bottles and incubated in BacTAlert (Biomérieux) automated blood culture system. After getting positive alarm, direct Gram's stain

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was made from blood culture bottle to see the gram positive budding yeast cells and sub-culture was done on Sabouraud's dextrose agar (HiMedia, Mumbai, India) and blood agar plate. Identification of *Candida* species was done by conventional technique and reconfirmed by MALDI-TOF [13,14]. Antifungal susceptibility pattern (CLSI M27-A3 guidelines) was performed by Broth Micro-dilution method.

To study the virulence factor, biofilm formation was assessed by tube method as described by the Branchini, et al., [9]. We prepared a media in tube containing 10 ml Sabouraud's dextrose broth supplemented with 8% of glucose (HiMedia, Mumbai, India). Inoculate the loopful test strain of 24 hour fresh culture from SDA plate. The tubes were incubated at 37°C for 24 hours. After incubation, the broth was aspirated out with the sterile Pasteur pipette. After aspiration, wall of the tube was stained with 1% safranin, the tubes was kept for 7 minute, after that safranin was removed from the tubes. Biofilm production was graded visually as negative, weak positive (one plus), moderate positive (2+) and strongly positive (3+).

The phospholipase activity of *Candida* isolate was determined by Price, et al., with minor modification. Prepare a medium 200 ml egg yolk agar, with 13 gms of SDA, 11.7 gms NaCl, 0.111 gms CaCl₂ and 10% sterile egg yolk. All the reagents were mixed with 180 ml distilled water except egg yolk agar and autoclaved. 20 ml of egg yolk agar was mixed with sterilized reagents. The plate was prepared by the final mixture of all the reagents and store in a refrigerator. Inoculum was prepared from fresh sub-culture on SDA plate in sterile distilled water and match to the 0.5 McFarland turbidity standards. A volume of 10 µl of inoculum was inoculated onto the surface of egg yolk plate and incubated at 37°C for 7 days. After incubation the precipitation zone of diameter around the yeast growth was calculated. A ratio between the colony and zone of diameter was calculated as Pz score. If the Pz score was 1, considered as negative, ratio between 0.64 and 0.99 was taken as positive and the ratio was ≤0.63 taken as strong positive [10].

Results

A total 1500 blood isolates were collected during 1 year study (2019-2020). Out of 1500 blood culture samples, 100 were positive for *Candida* species (6.6% prevalence).

Out of 100 *Candida* isolates patients, 50 patients were male and 50 patients were female. 55 patients were admitted in Intensive Care Unit, 41 in Hospital Inpatient care (IPD), 2 in Obstetrics and Gynecology (O and G) and 2 patients were admitted in trauma center Table 1.

The spectrum and percentage of different *Candida* species in 100 isolates are given in Table 2 and Fig. 1 by MALDI-TOF. The species isolated were *Candida tropicalis* 26 (26%), *Candida albicans* 17 (17%), *Candida utilis* 15 (15%), *Candida parapsilosis* 12 (12%), *Candida auris* 11 (11%), *Candida glabrata* 5 (5%), *Kodamaea ohmeri* 5 (5%), *Candida krusei* 3 (3%),

Candida orthopsilosis 3 (3%), *Candida kefyr* 1 (1%), *Candida fermentati* 1 (1%), *Candida duobushaemulonii* 1 (1%). We observed that Candidemia was more frequently caused by non-albicans *Candida* species as compared to *Candida albicans*.

In patients age <3 year and 19 to 60 years age group *Candida* infection was mainly caused by non-albicans *Candida*, 36 (43.4%) and 31 (37.3%).

Resistance rates of fluconazole, amphotericin B, caspofungin, voriconazole and itraconazole in non *Candida albicans* were 28.92%, 48.19%, 42.17%, 18.07% and 22.89% respectively. Total 8 isolates (*Candida glabrata* and *Candida krusei*) were intrinsically resistance to Fluconazole.

100 *Candida* isolates were obtained from clinically suspected Candidemia patients were analysed for virulence factors. Biofilm activity was observed in 76 *Candida* isolates (76%) and phospholipase activity was observed in 21 *Candida* isolates (21%).

All strain of *Candida krusei* 3 (3%), *Candida auris* 11 (11%), *Candida tropicalis* 26 (26%), 14% strains of *Candida albicans*, 7% strains of *Candida parapsilosis*, 11% strains of *Candida utilis*, 4% strains of *Kodamaea ohmeri* were found to be positive for biofilm production [Table 1]. All strains of *Candida auris* 11 (11%) and 10% strains of *Candida albicans* were positive for phospholipase activity.

Different Species	Total (n=100)	Percentage (%)
<i>Candida krusei</i>	3	3.00%
<i>Candida auris</i>	11	11.00%
<i>Candida albicans</i>	17	17.00%
<i>Candida duobushaemulonii</i>	1	1.00%
<i>Candida fermentati</i>	1	1.00%
<i>Candida glabrata</i>	5	5.00%
<i>Candida kefyr</i>	1	1.00%
<i>Candida orthopsilosis</i>	3	3.00%
<i>Candida parapsilosis</i>	12	12.00%
<i>Candida tropicalis</i>	26	26.00%
<i>Candida utilis</i>	15	15.00%
<i>Kodamaeaohmeri</i>	5	5.00%

Table 1: Spectrum of *Candida* isolates by MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight).

		Total [n=100]	<i>Candida albicans</i> [n=17]		Non-albicans <i>Candida</i> [n=83]	
			Total	(%)	Total	(%)
Fluconazole	R	27	3	17.65%	24	28.92%
	S	54	10	58.82%	44	53.01%
	I	11	4	23.53%	7	8.43%
Amphotericin B	R	48	8	47.06%	40	48.19%
	S	52	9	52.94%	43	51.81%
	R	45	10	58.82%	35	42.17%
Caspofungin	S	47	7	41.18%	40	48.19%
	I	8	0	0.00%	8	9.64%
	R	16	1	5.88%	15	18.07%
Voriconazole	S	77	14	82.35%	63	75.90%
	I	7	2	11.76%	5	6.02%
Itraconazole	R	20	1	5.88%	19	22.89%
	S	58	10	58.82%	48	57.83%
	I	22	6	35.29%	16	19.28%

Resistance- R; Sensitive- S; Intermediate- I

Table 2: Antifungal susceptibility pattern of *Candida* isolates by Broth Microdilution.

Figure 1: Diagram shows percentage of *Candida* species in Blood Stream Infection (BSI).

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Discussion

Candidemia is a blood stream infection which is caused by various *Candida* species in children and adults [1]. The burden of candidemia increases in last few decades in the tertiary care hospitals, representing more than 80% of nosocomial infection caused by *Candida* species [11].

There is geographical variation in the distribution *Candida* species in blood stream also. *Candida albicans* was the predominant species found in blood but over the past few years, the distribution changed towards non *albicans* *Candida* species in blood. In our study non *albicans* *Candida* species were more common than *Candida albicans*. There was a similar prevalence of non-*Candida albicans* species was predominate in blood stream infection found in different countries, like Asia, South Europe and America [12].

In our study the predominant species was *Candida tropicalis* (26%) followed by *Candida albicans* (17%), 3rd species was *Candida utilis* (15%) and 4th emerging species was *Candida auris* was (11%). A similar to our study was conducted in New Delhi, India by Oberoi JK, Wattal C, et al., showed that the *Candida tropicalis* (29.2%) was predominant species followed by *Candida albicans* (16.8%) [13]. A another similar study conducted in New Delhi, India in different trauma centers by Mathur P, Hasan F, et al., found that *Candida tropicalis* (39.4%) than *Candida albicans* (14%) [14].

In our study the most common risk factor was patients admitted in ICU 55% and most prevalent species was non *Candida albicans* 45 (54.2%). A study by Alkharashi N, Aljohani S, et al., found that 60.2% ICU patients developed Candidemia [15].

Resistance rates of fluconazole for *Candida albicans* and non-*Candida albicans* were 17.65% and 28.92%. According to Rizvi, et al., 2011. In our study we found that Fluconazole resistance in *Candida albicans* was 19.8% which is similar to 28.92% present study.

We studied in this study virulence factors of *Candida* species in blood stream infection to understand pathogenesis like, biofilm formation, and phospholipase activity. Expression of these virulence factors of *Candida* species escapes from host defence mechanism. In our study biofilm formation, and phospholipase activity was 76%, and 21%. A study by Subramanya SH, Baral BP, et al., found 62% phospholipase activity in *Candida* isolates and biofilm formation was 74.07% [16].

Biofilm production was more found in non-*Candida albicans* (62%) than *Candida albicans* (14%), similar results was observed by many others [17]. All strains of *Candida tropicalis* were positive for biofilm production. Highest grade (+3) was found in *Candida tropicalis* and *Candida utilis*. Phospholipase activity was found in *Candida auris* 11 (11%) and *Candida albicans* 10 (10%). A study by De Luca C, Guglielminetti M, et al., found 48% phospholipase activity was seen in *Candida albicans* and no any activity was found in non-*Candida albicans* [18].

Conclusion

As there is a shift to non-albicans candida causing candidemia as well as increase resistance to fluconazole drug which are the first line treatment modality in critically ill Candidemia patients there is need for identification of Candida to species level in routine practice to achieve better feedback to clinicians.

Conflicts of Interest

The authors declare that have no competing interest and not any conflict of interest.

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