Original Research Article

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An etiological and antifungal profile of candidemia in children

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ABSTRACT

Background: Candidemia causing increased mortality rates and emergence of antifungal drug resistance needs an urgent intervention to salvage immunocompromised and severely ill patients. This study aimed to isolate and identify *Candida* species and evaluate their antifungal susceptibility profile from blood stream infections in children.

Methods: Fungal cultures from blood recovered positive for yeasts were subcultured on Sabouraud dextrose agar. Suspected purified colonies of *Candida* were confirmed and identified upto species level by both conventional and automated techniques. Antifungal susceptibility testing of isolates was evaluated using agar based E-test method for fluconazole, voriconazole and caspofungin on Mueller-Hinton agar supplemented with 2% glucose.

Results: Total of 43 isolates of *Candida* species were recovered from blood samples. Non *albicans Candida* species accounted for 88.30% of cases; whereas 11.60% of cases were caused by *C. albicans*, *C. tropicalis* (39%) was the most frequent isolate recovered in candidemia patients followed by *C. parapsilosis* (18%), *C. albicans* (12%), *C. glabrata* (12%), *C. kefyr* (9%), *C. pelliculosa* (5%), and *C. krusei* (5%). Antifungal susceptibility results revealed Caspofungin demonstrated good activity against all *Candida* spp. *C. parapsilosis* followed by *C. tropicalis* and *C. glabrata* demonstrated high resistance to fluconazole. For voriconazole, maximum resistance was shown by *C. tropicalis* as compared to others.

Conclusions: Candidemia is a threatening prognostic sign in children and an important entity in our hospital. Identification of *Candida* species and antifungal sensitivity testing is a must to select a suitable and effective antifungal therapy to abrogate the emerging resistance to antifungals.

Keywords: Candida species, Antifungal agents, Antifungal susceptibility testing, Candidemia in children

INTRODUCTION

Candida species form a part of the normal flora of human skin and mucosa capable of causing a variety of infections and emerging as an important nosocomial pathogen. It is one of the common opportunistic pathogen. At present Candida species is the fourth most common cause of bloodstream infections causing invasive life threatening fungal infections among hospitalized patients. The global incidence of candidemia has increased more than fivefold in the last decade. The global incidence of candidemia has increased more than fivefold in the last decade.

It has been reported that frequent use of broad spectrum antibiotics, use of immunosuppressive agents, underlying malignant diseases, HIV infection, organ transplantation, prolonged stays in the intensive care unit (ICU), abdominal surgery and exposure to invasive procedures put patients at a high risk of infection with *Candida*. The crude mortality rate of candidemia is extremely high ranging from 36-63%; it prolongs hospital stays by as much as 30 days and increases the cost of medical care.

Till recent times, Candida albicans remained the most frequently isolated species, but now the spectrum of

candidemia has changed with the emergence of nonalbicans Candida (NAC) species causing increased mortality rates and emergence of antifungal drug resistance, especially in the immunocompromised and severely ill patients.⁶

Candidemia represents a major challenge among healthcare-related infections due to its difficult diagnostic and therapeutic management. Incidence of antifungal resistance to Candida sp. has been on an increasing trend over the past decade. It is important to identify *Candida* to the species level, to optimize the selection of appropriate antifungal agent. Studies on the prevailing rate of infections and antifungal susceptibility testing, can help in deciding the clinical strategies.8 More importantly, intrinsic and emerging resistance to azoles represent a major challenge for empirical, therapeutic, and prophylactic strategies. The changing scenario has made routine antifungal susceptibility testing imperative since both in vitro resistance and toxicity issues must be looked at when selecting an antifungal agent for therapy. Also, most studies focus on clinical and epidemiological data from adults, however there has been less work concerning the infections in children. So, this study was aimed to identify the various species of Candida causing blood stream infections in children and to investigate the susceptibility pattern of these species to antifungal agents using M27-S4 CLSI guidelines in order to select a suitable and effective antifungal therapy to abrogate the emerging resistance to antifungals.

METHODS

The present study was undertaken at Lady Hardinge Medical College and associated Kalawati Saran children hospital from October 2015 to March 2017. During this period, all patients from 0 to 10 years of age diagnosed as having blood stream infections were included in the study. Consecutive blood samples collected aseptically from patients were received routinely in the microbiology laboratory for culture and sensitivity testing. Blood culture was done using automated blood culture system. Under strict aseptic precautions, blood samples were collected in BactAlert culture bottles and incubated at 37°C for 1 week. Subcultures were performed from all BacT or alert blood culture bottles that detected a positive signal, onto 5% sheep blood agar and Sabouraud's dextrose agar (SDA) with antibiotic. The plates were examined after 24-48 hours incubation at 37°C. Preliminary gram stain was done to recover cultures positive for yeasts.

Typical *Candida* colonies, characterized by smooth, creamy and pasty appearance on SDA, were further subjected to speciation by both conventional and automated techniques. ¹⁰ Conventional identification was done by applying standard tests such as germ tube test, by observing pigmentation colour on HiChrome *Candida* differential agar, by studying growth morphology on corn meal agar and by performing sugar assimilation test

(HiMedia Candida Identification Kit KB006). Automated identification of *Candida* was done using VITEK 2 YST ID colorimetric cards.

Antifungal susceptibility testing

The antifungal susceptibility pattern of the isolates was evaluated using agar based E-test method for fluconazole, voriconazole and caspofungin on Mueller-Hinton agar supplemented with 2% glucose. The inoculum suspension was made by adjusting it to 0.5 McFarland standard i.e., 10⁶ cells/ml. Sterile swab was dipped into the inoculum suspension and the agar plates were inoculated by streaking it along the agar surface in all directions spreading it as a lawn culture. Before applying the E-test strips the agar plates were dried for 10-15 minutes. Plates were then incubated at 35°C for 24-48 hours or until visible growth was seen. After incubation, the value of MIC (Minimum inhibitory concentration) was read at the point of intersection between the halo and the E-test strip. 11 In case of azoles, significant inhibition, 80% decrease in growth density was required to visually select the end point. The organisms were categorized into susceptible (S), intermediate (I) and resistant (R) based on the MIC reading. Interpretation and comparison was done as per CLSI M27-S4 document guidelines. 12 The standard strains used were C. parapsilosis ATCC 22019, (Hi-Media Laboratories, Mumbai, India) and C. albicans ATCC 90028 (Hi-Media Laboratories, Mumbai, India).

RESULTS

During these 18 months of study period from October 2015 to March 2017, a total of 713 blood samples from children's blood were received in the department of microbiology for processing. *Candida* species were isolated from 43 (6.03%) blood samples. Out of 43 isolates, Non-albicans Candida accounted for 38 isolates (88.30%) whereas *Candida albicans* accounted for only 5 isolates (11.60%) (Figure 1).

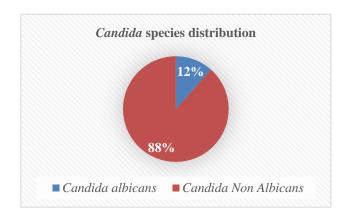


Figure 1: Distribution of *Candida* and non-albicans Candida from blood.

All isolates exhibited gram positive budding yeast forms with pseudohyphae except *C. glabrata* (budding yeast

forms only). The germ tube test potentially differentiated *C. albicans* from non-albicans Candida. The CHROM agar inoculation showed differently pigmented colonies depending on the species. The sugar assimilation test and cornmeal agar with Tween-80 inoculation effectively differentiated between different Candida species. All the tests were in accordance with each other. Automated identification also showed correlation with other tests. *C. tropicalis* (39%) was the commonest isolate recovered followed by C. parapsilosis (18%), C. albicans (12%), C. glabrata (12%), C. kefyr (9%), C. pelliculosa (5%) and C. krusei (5%) (Table 1).

Table 1: Distribution of *Candida* species isolated from blood.

Candida species	No. of isolates N (%)
Candida tropicalis	17 (39)
Candida parapsilosis	8 (18)
Candida albicans	5 (12)
Candida glabrata	5 (12)
Candida kefyr	4 (9)
Candida pelliculosa	2 (5)
Candida krusei	2 (5)
Total	43 (100)

Most of the patients showing bloodstream infections were within the age group of 2-4 years. Candidemia was seen to be more prevalent in female with 60.47% of cases than male with 39.53%. Out of total 43 patients having isolates of *C. albicans*, 4 (80%) were female and 1 (20%) was male. Whereas, among the patients having NAC

isolates, 22 (57.89%) were female and 16 (42.10%) were male (Table 2).

Table 2: Age and gender wise distribution of *Candida* isolates.

Age (in years)	Candida albicans		Non-albicans Candida		Total	
	Male	Female	Male	Female		
<2	0	1	5	8	14	
2-<4	1	2	8	6	17	
4-<6	0	1	2	2	5	
6-<8	0	0	0	2	2	
8-10	0	0	1	4	5	
Total	1	4	16	22	43	

The table 3 shows susceptibility pattern of Candida sp. to fluconazole, voriconazole, and caspofungin. Results of antifungal susceptibility showed 81.08% isolates sensitive to caspofungin, 56.75% to fluconazole, and only 43.24% to voriconazole. C. krusei showed 100% sensitivity to caspofungin whereas C. albicans showed 100% sensitivity to voriconazole and resistance to fluconazole and caspofungin was detected in 20% and 40% of the cases, respectively. However, all other Candida species demonstrated less than half of the sensitivity to voriconazole. Resistance to fluconazole, voriconazole, and caspofungin was identified in 37.83%, 40.54% and 13.51% of the cases, respectively. Nonalbicans Candida species, especially C. parapsilosis and C. tropicalis were more resistant to azoles, than C. albicans.

Table 3: Antifungal susceptibility profile of *Candida* isolates.

	Antifun	gals tested							
Organism	Fluconazole		Voriconazole			Caspofungin			
	S	I	R	S	I	R	S	I	R
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
C. tropicalis (n=17)	11 (64.70)	0	6 (35.29)	5 (29.41)	0	12 (70.58)	13 (76.47)	4 (23.52)	0
C. parapsilosis (n=8)	3 (37.5)	0	5 (62.50)	5 (62.50)	0	3 (37.50)	6 (75)	0	2 (25)
C. albicans (n=5)	4 (80)	0	1 (20)	5 (100)	0	0	3 (60)	0	2 (40)
C. glabrata (n=5)	-	3 (60)	2 (40)	-	0	0	2 (40)	2 (40)	1 (20)
C. krusei (n=2)	-	0	0	2 (100)	0	0	2 (100)	0	0
Total (n=37)	21 (56.75)	3 (8.10)	14 (37.83)	16 (43.24)	0 (0)	15 (40.54)	30 (81.08)	6 (16.21)	5 (13.51)

(S: Susceptible; I: Intermediate; R: Resistant).

DISCUSSION

Infection is regarded as a recurring problem among the patients admitted to tertiary care hospitals. Frequency of severe blood stream infections caused by yeasts,

especially *Candida* sp., has increased dramatically in the last few decades, particularly those caused by the nonalbicans *Candida*, showing the significance of laboratory diagnosis to enable correct species identification important for initiation of timely and adequate treatment and to avoid unnecessary antifungal treatment causing resistance. 13

The epidemiology of candidemia infection highly depends on geographical variations, even between different units within same hospital. Although *C. albicans* has been the predominantly isolated species till now, shift in the species distribution to non-*albicans* Candida has been noted in several major Indian hospitals as reported in previous studies. ¹⁴⁻¹⁶ In our study also the incidence rate of candidemia caused due to non-*albicans* Candida (84%) outnumbered Candida albicans (16%), thus establishing the significance of non-*albicans* Candida in causing blood stream infections. This increase may be allocated to intensive, long term use of antifungals which may in turn confer to their high level of resistance. ¹⁷⁻¹⁹

Among the non-albicans *Candida*, *C. tropicalis* was the most frequently recovered isolate in our study similar to other worldwide reports, followed by *C. parapsilosis* and *C. albicans*. *C. tropicalis* and *C. parapsilosis* are emerging causes of candidemia in India, as has also been confirmed by other reports. ¹⁴ Studies done previously by Chakrabarti et al, Xess et al and Singh et al in North India are also in concordance with our study, and it emerges that *C. tropicalis* is the predominant species causing candidemia in North India. ²⁰⁻²² Wormwood et al also found *C. tropicalis* as the predominant species causing candidemia in Chile. ²³ This finding is also consistent with a study where highest proportion of *C. tropicalis* was isolated in Eastern Asia and Argentina. ²⁴

We identified less prevalence of *C. kefyr* (9%), *C. pelliculosa* (5%) and *C. krusei* (5%) in our study as seen in other settings and geographical regions. ^{24,25} However, *C. glabrata* (12%) was found to be causing infection to some extent in our patients. Role of this pathogen has only been recognized in the past few decades. A remarkable increase in the incidence rate of *C. glabrata* isolation from blood stream infection patients was studied by Trick et al and there is concern regarding increase in azole resistance among *C. glabrata* strains. ^{14,26}

In this study most of the patients showing bloodstream infections were within the age group of 2-4 years, especially neonates who accounted for the highest number of isolates in this study with patients admitted in both the wards and ICUs. In the neonatal wards and ICUs nosocomial fungal infection is an increasing problem because of advances in medical and surgical management. 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, prematurity, decreased immunity and their host response to *Candida* which may contribute to mortality. Non-albicans Candida accounted for most of the cases of neonatal candidemia in the present study, whereas *C. albicans* was responsible

for less no. of cases. This corroborates well with the results of other authors where non-albicans Candida accounted for 77% to 87.76% cases of neonatal candidemia, whereas *C. albicans* accounted for 12.24% to 23% cases. ^{21,29-32}

Candidemia was seen to be more prevalent in female with 60.47% of infected cases than male with 39.53%. It corroborates with another study on neonatal candidemia where 55.30% patients infected were female and 44.70% were male.²⁹ However, this observation is not much in agreement with other studies reporting male predominance.^{33,34}

In the present study, fluconazole resistance rate was 37.83% for *Candida sp*, which is in agreement with other Indian studies reported by Bhattacharjee et al, Kothari and Kumar. 25,32,35 Candida albicans showed 20% resistance to Fluconazole and non-albicans Candida exhibited 34.21% resistance. Fluconazole resistance is a matter of interest as it is one of the most common azoles that are used for treatment of disseminated candidiasis involving candidemia. Study shown by Adhikari et al in Southern India exhibited a prohibitive resistance to fluconazole for all Candida isolates, however they are found to be susceptible to polyenes, azoles, and echinocandins as indicated by western data.36 Because fluconazole is extensively used in various clinical states it is the prime reason of dominance of non-albicans Candida species over C. albicans.³⁷

C. albicans and C. krusei exhibited 100% activity against voriconazole and C. parapsilosis showed 62.50% activity. Voriconazole was found to be an effective azole among all the Candida isolates tested. Interestingly in our study, Candida isolates displaying resistance to fluconazole were susceptible to voriconazole which beyond doubt proves it to be an effective azole. Such finding implicits that voriconazole due to its vast species coverage, can be utilized in the treatment of candidemia caused by fluconazole resistant strains. These findings are in accordance with previous studies done by Madhavan et al, Helmi et al and Balaram et al. 38-40

The present study demonstrated 81.08% sensitivity pattern by caspofungin to *Candida* and non-albicans *Candida* species, which may be helpful to healthcare professional for treating the infections caused by non-albicans *Candida*. With increase in resistance of non-albicans *Candida* species causing candidemia, it seems mandatory to evaluate antifungal susceptibility testing and report their therapeutic outcome for effective management of patients. Despite the fact that newer and effective types of antifungals are available in the market, they are quite expensive and not easily affordable by all the patients.

Candidemia continues to be a considerable cause of morbidity and mortality in patients infected with blood stream infections. The shift in epidemiology from *C*.

albicans to non-albicans Candida and increasing resistance to some of the drugs emphasizes the need for constant monitoring of Candida species distribution and antifungal susceptibility testing for diagnosing emergence of antifungal resistance and optimum management of these patients helping in decreasing the overall morbidity and mortality in these patients.

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Institutional Ethics Committee

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