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Corresponding Author

Amith Joshi,
SDM College of Medical Sciences
and Hospital, Dharwad, Karnataka,
India

Author Designation

^{1,2}Assistant Professor

³Associate Professor

⁴Professor

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Emerging Candidemia in Neonatal Intensive Care Unit and its Implication on Choice of Treatment

¹Amith Joshi, ²Sughosh Kulkarni, ³D. Anushka, and ⁴R.D. Kulkarni

^{1,2,4}SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India

³S Nijalingappa Medical College, Bagalkot, Karnataka, India

ABSTRACT

Candida is one of the most frequent organisms to be isolated from blood of hospitalized individuals, especially children. Candidemia is often difficult to diagnose clinically the bio-markers of sepsis are not very reliable. Complete laboratory diagnosis of Candidemia up to identification of species takes several days in routine diagnostic laboratories. There is a need for quicker identification. The emergence of non-albicans Candida species as predominant cause has made speciation and antifungal susceptibility testing imperative on diagnostic laboratories. Along with this, there is increase in resistance to routinely used antifungal agents by several species of Candida. To estimate the burden of Candidemia and identify the species of Candida causing blood stream infections in neonates admitted to intensive care unit. To determine antifungal susceptibility patterns of the isolates. To understand various risk factors associated with Candidemia in neonates admitted to hospital. Blood samples sent from NICU were processed in automated blood culture system. Samples with budding yeast cell growth were subcultured on blood agar and Sabouraud's dextrose agar. Preliminary test like germ tube identification and morphological identification by Delmau's method were performed following which the isolates were tested for sugar assimilation by modified Wickerham and Burton's method (tube with indicator method). The isolates were tested against fluconazole and voriconazole by modified disk diffusion method. Other data on cases were retrospectively collected from case sheets. Out of 258 blood samples collected for culture, 55 had growth of Candida in them (21.31%). C. krusei was the most predominant species (78%), followed by C. tropicalis (18%). There was just one isolate of C. albicans. Majority of C. krusei isolates were resistant to fluconazole (83%), while all 55 isolates were susceptible to voriconazole. Data analysis revealed that 6 neonates succumbed during their stay, while 7 were discharged against medical advice. Catheterization (98.1%), preterm and meconium aspiration (69.1%) were found to be predominant risk factors associated in the cases of Candidemia. All neonates received parenteral fluconazole therapy on preliminary diagnosis of Candidemia. Non-albicans Candida species (C. krusei, in present study) are the predominant causes of Candidemia. Mortality rate was 10.9%. Fluconazole is recommended as drug of choice on laboratory confirmation of Candidemia. However, predominance of C. krusei (which is known to be resistant to fluconazole) in this study calls for alternative drugs like voriconazole to be used for treating Candidemia. It also highlights the importance of quick speciation of Candida and determining the antifungal susceptibility, which will aid in the evidence-based treatment of such cases.

INTRODUCTION

Candida is the predominant cause of invasive fungal infections^[1]. *Candida* is the most 4th common organism isolated from blood in many western countries^[2]. In India too, *Candida* is one of the important causes of invasive mycosis, often causing increase in mortality and hospitalization. Incidence varies from 1-12 per thousand admissions across various hospitals in the country^[3,4]. Candidemia is particularly more common in intensive care units of the hospital. Most studies from India identify prolonged hospitalization, ICU care, total parenteral nutrition, major abdominal surgeries, immunosuppression mechanical ventilation as common predisposing factors for nosocomial Candidiasis^[3]. In Children, the other predominant risk factors are very low birth weight, prematurity, use of broad-spectrum antibiotics etc. If rising incidences of Candidemia is one part of the problem, the second part is emergence of non-albicans *Candida* spp. (NAC). Various studies from India have demonstrated that NAC constitutes anywhere from 50-80% of the total *Candida* isolates in cases of Candidemia^[5,6]. Important species of *Candida* causing candidemia are *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, etc. The biggest impact of emergence of NAC has been in the treatment of the cases. It is known that some of the NAC are resistant to commonly used antifungal agents. Particularly, *C. krusei*, which is known to be intrinsically resistant to fluconazole. A study from Singh *et al*^[7]. India demonstrated pan-azole resistance is observed in up to 6% of the isolates. It is a sign of huge worry that drug resistance is now being observed against Echinocandins, the most potent drug against *Candida*. Therefore, it has become imperative on diagnostic laboratories to speciate *Candida* isolates and provide their antifungal resistance pattern to the treating doctors. This study was therefore conducted to understand the predominant species of *Candida* causing Candidemia in Neonatal ICU's and their antifungal resistance.

MATERIALS AND METHODS

This was a prospective study conducted at a tertiary care teaching hospital in north Karnataka for six months. Consecutive isolates of *Candida* received for routine culture and sensitivity were collected and subjected for analysis. Blood samples sent in the automated pediatric blood culture bottles from NICU were subjected for processing in BACTAlert3D. Blood culture flagging positive for growth were gram stained first and then on identification of budding yeast cells, were sub-cultured on Blood agar and Sabouraud's agar for 24-48 hours. On growth of yeast like colonies on media, germ tube test was done to differentiate *C. albicans* from NAC. Later, the *Candida* colonies were

subjected to speciation firstly following the Dalmau's method of morphological identification and secondly by sugar assimilation by modified Wickerham and Burton's method^[8]. This method identifies assimilation patterns of various species of *Candida*. The test takes a minimum of 3-4 days and the isolates have to be incubated for up-15 days for final identification. Whenever there was discrepancy in the two methods of speciation, a repeat testing was performed. On further observation of discrepancy, the Wickerham and Burton's method was taken as standard.

Antifungal susceptibility testing was done by Kirby-Bauer disk diffusion method following the CLSI (M44-A2) guidelines using modified Muller Hinton agar (MHA with 5% glucose and Methylene blue indicator) and commercially available antifungal disks (Fluconazole-25 µg and Voriconazole-1 µg). The demographic data was obtained retrospectively from the medical records after obtaining necessary consent and getting approval from ethical committee. Standard statistical analysis was performed while analysing the data.

RESULTS AND DISCUSSIONS

A total of 258 blood samples were collected from Neonatal Intensive Care Unit (NICU) for culture and sensitivity during the study period. The monthly average was 43 samples. Of the 258 samples, 55 (21.3%) samples showed growth of *Candida* spp. Majority of the isolates were from males 32 (58.2%). The predominant risk factor amongst cases of Candidemia was Preterm delivery with meconium aspiration 38 (69%), followed by very low birth weight 13 (23.6%) term delivery with meconium aspiration 8 (14.5%). It is important to note that, 54/55 cases had indwelling catheter. Two cases in the study had multiple risk factors (Table-1). The CRP (C-reactive protein) level was estimated in all 55 neonates and it was noted that 31/55 (56.3%) cases had CRP levels higher than 10 mg/L, in 10/55 (18.1%) cases it was

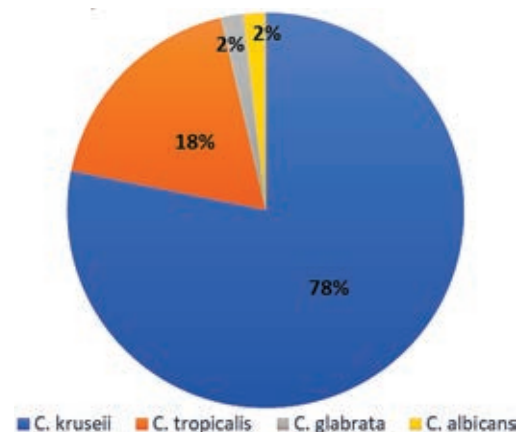


Fig. 1: Speciation of *Candida* isolates

Table 1: List of risk factors observed in the study

Risk Factors	Observation
Catheterization	98.1%
Preterm with me conium aspiration	69.1%
Low birth weight	23.6%
Term neonates with me conium aspiration	14.5%

Table 2: CRP levels in the cases studied

CRP level-range	Total cases (n = 55)
>10 mg/dl	31 (56.3%)
5-10 mg/dl	10 (18.1%)
<5 mg/dl	14 (25.4%)

Table 3: Antifungal susceptibility pattern among the Candida isolates

Candida species	Fluconazole (n=55)		Voriconazole (n=55)	
	Sensitive	Resistant	Sensitive	Resistant
C. krusei (n=43)	07	36	43	0
C. tropicalis (n=10)	10	0	10	0
C. glabrata (n=01)	01	0	01	0
C. albicans (n=01)	01	0	01	0

between 5-10mg/L and in the remainder of the cases (25.4%) it was less than 5 mg/L. A majority of the neonates 33 (60%) had received broad spectrum antimicrobial therapy. The average time of stay of the neonates at the time of positive blood culture was 7.5 days. The species distribution amongst the isolates has been demonstrated in (Fig. 1). *C. krusei* was the most predominant species 43 (78%). It was followed by *C. tropicalis* 10 (18%). There was one isolate, each identified as *C. albicans* and *C. glabrata*. The sensitivity pattern of various species against the two antifungal agents has been described in (Table.3). All isolates were sensitive to voriconazole, whereas resistance to fluconazole was seen in majority of the *C. krusei* isolates 36/43 (83.7%). On following up of the cases further, 6 (10.9%) neonates died during their stay in the NICU, while 7 were discharged against medical advice. 42 (76.3%) neonates conditions improved and were discharged from hospital later.

Candidemia is a serious clinical condition with the potential to cause higher mortality and morbidity in neonates. In our study we noted that 21.3% of the blood samples sent from suspected cases of sepsis were positive for *Candida* spp. *Candida* was the most predominant non-bacterial isolate from blood. The prevalence rate of 21.3% in the study was higher to Chaudhary *et al.*^[9] and some other single centre studies have demonstrated higher prevalence than ours^[10]. C-reactive protein (CRP) is considered to be one of the sensitive indicators of septicaemia/blood stream infections. There has been no clear guidelines on the cut-off level of CRP. Several studies done in the past have suggested that the sensitivity of detected septicaemia improves when the CRP level is set anywhere between 5-10mg/L. In our study 56.3% of the cases had CRP levels higher than 10 mg/L suggesting a higher likelihood of septicaemia. Some studies have shown that CRP levels can be used to

predict Candidemia^[11], whereas few other studies have suggested that the CRP levels are unreliable especially in cases of fungal blood stream infections^[12]. Speciation of isolates revealed that a majority (83.7%) of the isolates were *C. krusei*. The second most common isolate was *C. tropicalis* 10 (18%). This is in consonant with results of few studies which demonstrated predominance of *C. krusei* amongst neonatal blood isolates^[13]. Though there is no clear evidence of the reason behind predominance of *C. krusei* in blood, but some studies have noted that this particular species is the most hydrophobic amongst all^[14]. This explains one of our observations in the study, as majority of our isolates were derived from cases which had indwelling catheter in them. Predominance of NAC in blood isolates has been noted in many studies^[15]. But in our study, only one isolate (1.8%) was identified as *C. albicans*. *C. tropicalis* is noted to be one of the most common NAC (Non-albicans *Candida*) species to be isolated amongst various clinical samples especially from tropical countries, hence the name^[16]. Some studies in India on Candidemia have attributed *C. tropicalis* to be the most predominant isolate^[17,18].

On analysing the demographic data, it was noted that with an exception of one case, almost all of the isolates were from cases which had one common risk factor amongst all of them, i.e., indwelling catheter. Presence of indwelling catheter has always been identified as risk factor amongst various studies^[19]. *Candida* dwelling in the skin and mucous membranes are pushed deeper into the body during the process of catheterisation and they may survive in deeper areas if proper disinfection process is not followed. But, in our study, it was worth noting that majority of the cases had at least one more risk factor in addition to indwelling catheter. Approximately 69% were pre term, which in itself is an independent risk factor for Candidemia. Therefore it was difficult to identify a single risk factor responsible for Candidemia in these cases.

Antifungal susceptibility testing revealed that all isolates were equally susceptible to voriconazole. But a majority of the isolates 36/55 (65.4%) were resistant to fluconazole. Amongst the fluconazole resistant isolates, all isolates were *C. krusei*. It is well known fact that *C. krusei* is intrinsically resistant to fluconazole^[20]. In our study too, invitro resistance to fluconazole was profound. Pfaller *et al.* and Samaranayake *et al.* reported breakthrough infections due to *C. krusei* among patients receiving fluconazole or amphotericin B^[21,14]. In a correspondence, Persons (1991) opined switching over to alternate drugs like amphotericin B in case of *C. krusei* candidemia. Many clinicians tend to start

the patient on fluconazole therapy once preliminary report of Candida is available. In our study we noted that 50/55 (90.9%) cases were put on fluconazole therapy as soon as the preliminary report of "budding yeast like cells" was released from the laboratory. It is understandable as it would take several days for the final identification of Candida to species level is available and waiting for so long to initiate anti-fungal therapy might further deteriorate the condition of the patients. IDSA guidelines (2009) for Candidiasis also opine that fluconazole is good choice for use as empirical therapy for neonatal candidiasis including candidemia and alternate drug would be amphotericin B^[22]. Considering all the previous studies, guidelines and opinions, it would be prudent to say that fluconazole can indeed be a drug of choice in case of Candidemia. But treating physicians should be aware of the pre dominant species causing infection in their intensive care units. This calls for good communication to be between laboratory and treating doctors. They should consider shifting to amphotericin B or other drugs in centres where *C. krusei* is widely prevalent. This makes speciation of Candida even more imperative for all diagnostic laboratories. In our study we also noted that 6/55 neonates succumbed to their condition. The mortality rate of 10.9% is high. As this was not a case control study, we could only estimate the crude mortality rate. Several studies have demonstrated significant variation in the mortality rate due to Candidemia. In a study by Tak V *et al*^[23] the crude mortality rate was 43% in a large multicentric study in India by Chakrabarti *et al*^[24] the crude mortality rate was estimated to be 43.7%. Internationally, EPIC 2 international series demonstrated crude mortality to be around 42%^[25]. Though we could not accurately ascertain that the cause of mortality to be due to Candidemia. The neonates had multiple serious co morbidities. Therefore such high mortality rates definitely calls for laboratories to develop methods that can speciate Candida with a short turnaround time and to perform anti-fungal susceptibility testing for all isolates of Candida in the laboratory.

Limitations of the Study: The study was done on routine samples which were collected just once from each of the neonates. It has been suggested in multiple studies that sampling blood more than once at different times increases the probability of detection of true infection and reduces the instances of picking up transient organisms in the blood. As predictor of septicemia, we focused on CRP levels with the data taken retrospectively, which has its own limitations. A prospective collection of CRP level

data along with follow up levels would have brought in more clarity and credibility for comparison and analysis.

CONCLUSION

Candidemia is an important and emerging clinical condition in hospitals, particularly in intensive care units involving children. Non Albicans Candida species are now the predominant cause of it. Though fluconazole is usually the drug of choice for treating Candidemia, the awareness of drug resistance amongst various NAC species is important. Further, it is important to know which species of Candida is predominant in the hospital settings and then taper antifungal treatment according to it. The study has demonstrated the importance of speciation of Candida and its impact on choice of antifungal treatment.

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