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Spectrum of candidal species isolated from neonates admitted in an Intensive Care Unit of teaching hospital of Kashmir, North India

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Abstract:

BACKGROUND: Candidal infections are an important cause of morbidity and mortality in Neonatal Intensive Care Unit. Neonatal candidiasis is increasing in frequency, mainly because of increase in the survival of babies with low-birth weight, preterm births, advancement in medical field, life support systems, relative immunodeficiency, and extensive use of broad-spectrum antibiotics. Over the past few decades, there has been a progressive shift from the predominance of *Candida albicans* to nonalbicans *Candida* species.

AIMS AND OBJECTIVES: The objective of the current study was to know the prevalence of nonalbicans candidemia in neonates and their antifungal susceptibility pattern.

MATERIALS AND METHODS: In this study, a total of 424 samples from clinically diagnosed septicemic neonates were included. Identification of *Candida* isolates from these samples as well as their antifungal sensitivity testing was performed with Vitek 2 Compact (Biomérieux France) using Vitek 2 cards for identification of yeast and yeast-like organisms (ID-YST cards).

RESULTS: A total of 246/424 (58.01%) cases were blood culture positive. Out of these, 80/246 samples tested positive for candidemia (32.5%). *Candida tropicalis* (13.8%) was the predominant species isolated among the non-albicans *Candida* followed by *Candida krusei* (4.8%), *Candida parapsilosis* (3.2%), *Candida guilliermondii* (2.8%), and *Candida dubliniensis* (2.0%). We found an increase in the antifungal drug resistance, especially for the azole group of drugs, both in *C. albicans* and non-albicans *Candida* species. All the isolates were uniformly sensitive to micafungin, voriconazole, and caspofungin.

CONCLUSION: Candidemia in neonates is an ominous prognostic sign and is an important entity in our region. The present study highlights the mycological shift of *Candida* species in neonatal candidemia with a preponderance of nonalbicans *Candida* species.

Key words:

Antifungal susceptibility, *Candida albicans*, neonatal candidaemia, non-albicans *Candida*

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Introduction

The incidence and prevalence of candidemia are on a rise in many countries worldwide. According to National Nosocomial Infection Surveillance, USA in the 1990s, *Candida* species remained the fourth most common bloodstream pathogen

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accounting for 8% of all hospital-acquired bloodstream infections and is a cause of significant morbidity and mortality.^[1,2] The Asian scenario regarding the incidence of candidemia is, however, not very clear due to a lack of multicentric studies. A 13-year long study on candidemia from a tertiary care hospital in Thailand showed a prevalence of 6.14% for *Candida* species among blood culture isolates.^[3] In India, there are a few studies indicating the increasing trend

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of candidemia in some tertiary care hospitals.^[4] The mortality rate associated with candidemia worldwide is also high ranging from 10% to 49%.^[5]

Importance of *Candida* species in Neonatal Intensive Care Units (NICUs) is increasingly being recognized. *Candida* species accounts for 9%–13% of all blood isolates in NICUs.^[6] *C. albicans* is the most commonly isolated species and accounts for 50%–70% of cases of invasive candidiasis.^[2,7] However, the recent studies suggest that, with the introduction of fluconazole and itraconazole, there is an increase in the prevalence of nonalbicans candidial septicemia.^[8] *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis* are being increasingly isolated in patients of neonatal septicemia.^[9] *Candida* species can spread through vertical transmission from maternal flora or through horizontal transmission from hands of health-care workers. The risk factors associated with candidemia include the use of broad-spectrum antibiotics, low birth weight (LBW), prematurity, and intravenous catheter.^[10]

Over the past year, we noticed an increase in the isolation rate of nonalbicans *Candida* species from cases of neonatal septicemia, which prompted us to undertake the present study; to analyze and evaluate the change in the species distribution of *Candida* species in neonatal septicemia and determine their *in vitro* antifungal susceptibility and the risk factors associated with their acquisition. Further speciation and susceptibility testing of *Candida* sp. is still not routinely being done at most of the centers and as such no reliable data are available from our region regarding the estimation of antifungal use in hospitals. Furthermore, because of considerable regional variability, the local epidemiological knowledge is critical in terms of prevention and management of invasive *Candida* infections and can guide to initiate empirical antibiotic treatment which is essential for the management of the neonatal sepsis.

Materials and Methods

This prospective observational study was conducted in the Department of Microbiology, Government Medical College, Srinagar, Kashmir, over a period of 1 year (January 2016 to December 2016). This study was approved by the Ethical Committees of our institute and informed consent was obtained from each patient's next of kin. Candidemia was defined as at least one positive blood culture for *Candida* species in the presence of signs and symptoms of sepsis.

With all aseptic precautions, about 1–2 ml of blood is drawn from each neonate. Blood culture samples were incubated in BacTAlert 3D (Biomérieux, India[®]) automated blood culture system. One milliliter of blood

was inoculated into ready to use BacT/ALERT PF Plus culture bottles (yellow color coded) for pediatric use with all due precautions and shaken well. The culture bottles were loaded into the instrument after scanning the barcode of the bottle and incubated. Positive or negative culture bottles were determined by BacT/ALERT Microbial Detection System. Blood cultures were considered negative only after 7 days of incubation.

Positive samples were examined by microscopy of Gram-stained preparations and subcultured on blood agar plate, MacConkey agar plates, and Sabouraud dextrose agar slant with antibiotics but without cycloheximide (Hi-Media Pvt. Ltd., Mumbai, India) in aerobic atmosphere. The preliminary identification was done by colony morphology on SDA, chromogenic media (HiChrome, Himedia, Pvt., Ltd.), growth at 45°C, germ tube test, and by carbohydrate fermentation and assimilation tests.^[11]

Identification of the organism was confirmed with automated Vitek 2 compact 60 system (BioMérieux India[®]) using Vitek 2 cards. The Vitek ID and AST cards were chosen according to the results of the Gram staining and used according to the manufacturer's instructions. The Vitek-2 ID and AST cards were logged and loaded into the Vitek-2 Compact system. Antifungal sensitivity was performed against amphotericin B, 5 flucytosine, fluconazole, caspofungin, voriconazole, and micafungin. The MICs obtained were resolved into the three clinical categories (susceptible, intermediate, and resistant), according to the interpretative criteria provided by the automated systems' recommendations (CLSI guidelines (M100-S25) version 2015).

For the identified cases, we collected the demographic data, underlying disease, and the presence of risk factors.

Data were maintained in Microsoft Office Excel, and tests of proportions were used for analysis. Results were recorded in percentages.

Results

A total of 424 samples from clinically diagnosed septicemic neonates were received in the Department of Microbiology during the 1-year study period. A total of 246/424 (58.01%) cases were blood culture positive. Out of these, 80/246 samples tested positive for candidemia (32.5%).

Among the positive samples for candidemia, non-albicans *Candida* were responsible for 82.85% cases, whereas 17.5% cases were due to *Candida albicans*. *C. tropicalis* (13.8%) was the predominant species isolated among the nonalbicans *Candida* followed by *Candida krusei* (4.8%),

C. parapsilosis (3.2%), *Candida guilliermondii* (2.8%), and *Candida dubliniensis* (2.0%) [Table 1].

Once we correlated our results of candidemia with the clinical presentation of the neonates and associated risk factors, it was observed in the present study that prematurity and LBW were the most common risk factors followed by prolonged antibiotic therapy, ventilator support, and prolonged central venous line [Table 2].

It was observed in our study that the most common clinical presentation was respiratory distress, followed by failure to thrive, lethargy, and convulsions [Table 3].

The majority of the *C. albicans* were resistant to commonly used antifungal drugs such as fluconazole (42%),

flucytosine (28%), and amphotericin B (14%). Among the non-albicans *Candida sp.*, *C. tropicalis* showed 49% resistance to fluconazole, 42% resistance to flucytosine, and 10% resistance to amphotericin B. *C. krusei* and *C. parapsilosis* were more resistant to azoles, particularly fluconazole, than *C. albicans*. All the isolates were uniformly sensitive to micafungin, voriconazole, and caspofungin [Table 4].

Discussion

Sepsis remains the main cause of morbidity and mortality in NICUs,^[12] and candidemia is becoming a common occurrence in patients admitted in ICUs, especially during the past decade.^[13]

In our study, isolation rate of *Candida* from neonatal septicemia cases was 18.86%, which is similar to several other reports showing the frequency of isolation from 13.6% to 19.6% cases.^[10] A notable feature of our study was the emergence of nonalbicans *Candida* (82.85%) as a major cause of neonatal candidemia. Our findings are supported by other studies from different regions of India that have documented predominance of nonalbicans *Candida* over *C. albicans* in neonatal septicemia.^[6,14] A shift toward nonalbicans *Candida* was also noted by Kapila, Mendiratta *et al.*, and Pfaller *et al.*^[15-17]

The increased incidence of nonalbicans *Candida* is probably due to the greater use of invasive devices, broad-spectrum antibacterial agents, more extensive surgical procedures, and use of advance life support on various transplant patients and selection of less susceptible species by the pressure of antifungal agent such as fluconazole.^[18]

In our study, *C. tropicalis* (13.8%) was the most common species followed by *C. albicans* (5.6%), *Candida krusei* (4.8%), *C. parapsilosis* (3.2%), *C. guilliermondii* (2.8%), and *C. dubliniensis* (2.0%).

Our study is comparable to the epidemiological studies carried out in different parts of our country indicating *C. tropicalis* as the common cause of nosocomial candidemia.^[17,19,20]

C. tropicalis as a cause of fungemia in neonatal ICU have been linked to the presence of the fungus on the hands of the hospital personnel. The ability of this organism to produce clusters is one of its major virulence factors. Once introduced into the immunocompromised host, *C. tropicalis* may be more virulent than *C. albicans* and can rapidly progress from colonization to invasion.

A number of risk factors are responsible for the emergence of neonatal candidemia in the recent years.

Table 1: Distribution of microorganisms isolated from cases of neonatal septicemia (n=246)

	Number of isolates (%)
<i>Candida</i> species isolated (n=80)	
<i>Candida tropicalis</i>	34 (13.8)
<i>Candida albicans</i>	14 (5.6)
<i>Candida krusei</i>	12 (4.8)
<i>Candida parapsilosis</i>	8 (3.2)
<i>Candida guilliermondii</i>	7 (2.8)
<i>Candida dubliniensis</i>	5 (2.0)
Gram-negative bacilli (n=96)	
<i>Klebsiella</i> spp.	40 (16.2)
<i>Acinetobacter</i> spp.	28 (11.3)
<i>Escherichia coli</i>	14 (5.6)
<i>Enterobacter</i> spp.	8 (4.8)
Others	6 (6.6)
Gram-positive bacilli (n=70)	
Coagulase-negative <i>Staphylococcus</i>	41 (16.6)
<i>Staphylococcus aureus</i>	19 (7.7)
<i>Enterococcus</i> spp.	10 (4.0)

Table 2: Risk factors associated with cases of neonatal candidemia (n=80)

Underlying clinical feature	n (%)
Preterm	63 (78.5)
Low birth weight	59 (73.7)
Prolonged IV antibiotics	58 (72.5)
Ventilator support	45 (56.2)
Prolonged central venous line	40 (50)

IV = Intravenous

Table 3: Clinical presentations observed in cases of neonatal candidemia (n=80)

Sign/symptom	Number of cases
Respiratory distress	62 (77.5)
Failure to thrive	60 (75)
Feed intolerance	54 (67.5)
Lethargy	52 (65)
Convulsions	34 (42.5)
Bleeding tendency	21 (26.2)

Table 4: Antifungal susceptibility profile of Candidal isolates

<i>Candida species</i>	Resistance (%) to antifungals tested				
	Fluconazole, n (%)	Flucytosine, n (%)	Amphotericin B, n (%)	Voriconazole, n (%)	Caspofungin, n (%)
<i>Candida tropicalis</i>	16 (49)	14 (42)	10 (10)	0	0
<i>Candida albicans</i>	5 (42)	4 (28)	2 (14)	0	0
<i>Issatchenkia orientalis</i>	6 (50)	4 (35)	2 (16)	0	0
<i>Candida parapsilosis</i>	1 (12)	1 (12)	1 (12)	0	0
<i>Meyerozyma guilliermondii</i>	1 (14)	1 (14)	0	0	0

These include widespread use of broad-spectrum antibiotics, loss of mucosal immunity, colonization, LBW, and length of hospital stay.^[21-23] In our study, preterm birth (78.5%) and LBW (73.5%) were the most common associated findings present in neonates with candidemia. This is in concordance with the results of studies of other authors.^[10,24-26]

It was observed in the present study that respiratory distress (77.5%) was the most common clinical presentation, followed by failure to thrive (60%), feed intolerance (54%), and lethargy (57%). Convulsion (34%) and bleeding tendency (21%) (43.64%) were the less common clinical features seen in our cases. Similar findings have been reported by other authors also.^[26]

In our study, we found an increase in the antifungal drug resistance, especially for the azole group of drugs, both in *C. albicans* and nonalbicans *Candida* species. Antifungal susceptibility testing revealed that most of the *C. albicans* species were resistant to commonly used antifungal drugs such as fluconazole (42%), flucytosine (28%), and amphotericin B (14%). Among the nonalbicans *Candida* sp., *C. tropicalis* showed 49% resistance to fluconazole, 42% resistance to flucytosine, and 10% resistance to amphotericin B.

Many authors have also noted increasing trends of fluconazole resistance as reported 37.5% by Gupta *et al.*,^[22] 36% by Kothari and Sagar^[27] and 11.7% by Xess *et al.*^[14] *C. krusei* is innately resistant to fluconazole.

In other studies also, a variable range of resistance to flucytosine was reported ranging from 37% by Bhatt *et al.*^[28] to 0% by Pahwa *et al.*^[29]

In the present study, resistance to amphotericin B among all *Candida* isolates ranged from 10% to 14%. Similar results were reported by Bhatt *et al.* 8%.^[28] Although amphotericin B has a rapid cidal action against most strains of *Candida* species (especially, *C. albicans*), it is not the first choice due to associated nephrotoxicity and the newer lipid formulation having a better cidal effect profile.

All the isolates were uniformly sensitive to micafungin, voriconazole, and caspofungin.

Conclusion

The present study stresses the clinical importance and mycological shift of *Candida* species in neonatal candidemia with a predominance of nonalbicans *Candida* species in our region. It is evident from the results of our study that routine identification of *Candida* isolates to the species level, and the detection of resistant strains by antifungal susceptibility test is essential. Furthermore, there is a continued need for surveillance of candidemia to monitor changes in the epidemiological features and antifungal susceptibility and also to develop and evaluate prevention strategies. Furthermore, speciation of *Candida* in neonatal candidemia and measures taken to reduce the risk factors wherever possible and can reduce morbidity, mortality, and antifungal drug pressure in the neonates.

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Conflicts of interest

There are no conflicts of interest.

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