EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 6
267-272
Year: 2024

IDENTIFICATION AND SUSCEPTIBILITY TESTING OF CANDIDA SPECIES FROM NEONATES ADMITTED IN ICU BY VITEK-2 SYSTEM

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Article Received on 17/04/2024 **Article Revised on 07/05/2024** **Article Accepted on 27/05/2024**

ABSTRACT

Background: In the Neonatal Intensive Care Unit (NICU), candidal infections constitute a significant contributor to morbidity and mortality. The incidence of neonatal candidiasis is rising, primarily due to increased survival rates for low-birth-weight newborns, premature deliveries, life support systems and widespread use of broad-spectrum antibiotics. The prevalence of Candida species has gradually shifted over the last few decades from *Candida albicans* to non-albicans Candida. **Methods:** A total of 68 cases of neonatal candidaemia were used in this study. Using Vitek 2 Compact (Biomerieux France), the identification of Candida isolates from these samples and their antifungal sensitivity testing were carried out. Vitek 2 ID-YST cards are used to identify yeast and organisms that resemble yeast. The study was conducted in the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka. **Result:** After 68 cases of newborn candidaemia were analyzed, the study found a 1.9:1 male preponderance. The majority of instances of candidemia were linked to low birth weight, 86.77% of the positive samples for candidemia were caused by non-albicans Candida, while *C. albicans* was the cause of 13.23% of the cases. Most of the *C albicans* exhibited resistance to widely used antifungal medications. The susceptibilities of each isolate to micafungin, voriconazole, and capsofungin were all the same. **Conclusions:** Around the world, candidaemia is becoming a more pressing issue for NICU patients. The etiological agent's antifungal susceptibility must be determined in conjunction with its identification due to changes in the epidemiology and pattern of antifungal susceptibility of Candida infections. With a prevalence of non-albicans Candida species in our location, our study highlights the clinical significance and mycological shift of Candida species in neonatal candidemia.

KEYWORDS: Antifungal susceptibility, *Candida albicans*, neonatal candidaemia, non-albicans candida, Vitek 2 ID-YST.

INTRODUCTION

Candida species endured the fourth most common bloodstream pathogen regarding as 8% of all hospital-acquired bloodstream infections and attributed a significant cause of morbidity and mortality.[1] The incidence and prevalence of candidemia are on a rise in many countries worldwide. The Asian scenario regarding the incidence of candidemia is, ever so not very clear owing to a lack of multicentric studies.[2] From a tertiary care hospital in Thailand a 13 years long study of candidemia showed a prevalence of 6.14% for Candida species among blood culture isolates.[3] Few studies indicate the increasing trend of candidemia in some tertiary care hospitals In India.[4] The mortality rate associated with candidemia worldwide is also high ranging from 10% to 49%.[5] In Neonatal Intensive Care Units (NICUs) importance of Candida species is increasingly being recognized. All blood isolates in NICUs shows 9%–13% of candida species.[6] Some current works shows an increase in the prevalence of non-albicans candidial septicemia after introduction of fluconazole and itraconazole.[7] Among neonatal septicemia another study shows that *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis* are being increasingly isolated.[8] Candida species can spread through vertical transmission from maternal flora or through horizontal transmission from hands of health-care workers. Candidaemia associated risk factors include the use of broad-spectrum antibiotics, low birth weight (LBW), prematurity, and intravenous catheter.[9]

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. Clinicians now depend on identification of Candida to the species level in order to optimize the selection of antifungal agents allowing them to provide the best possible patient care.[10] Therefore, there is a need for continuous surveillance to monitor trends in incidence, species distribution and antifungal drug susceptibility profiles of candidaemia.[11] Automated species identification and sensitivity of candida can be observed by the Vitek 2 system.[12]

In Bangladesh, little is known about the actual situation of infections caused by Candida species, though the considerable burden of candidaemia has been estimated.[13]

The present study was conducted to clarify the prevalence of individual Candida species and their susceptibility pattern by Vitek 2 automated system. Information regarding the prevalence of Candida species, based on accurate identification within this study, may provide advancement in the overview of candidaemia in neonatal ICU in a tertiary care hospital in Bangladesh for a better understanding of its etiology and antifungal treatment.

MATERIALS AND METHODS

This prospective observational study took place at the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, for a duration of two years (January 2022 to December 2023). Each patient's guardian provided informed consent. Candidaemia was determined by the presence of at least one positive blood culture for Candida species along with 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 (Biomerieux, 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.

Identification of the organism was confirmed with automated Vitek 2 compact 60 system (BioMerieux India®) using Vitek 2 cards. 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 2020.

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.

RESULT

During the Two-years study period cases selected from NICU of BSMMU. On analyzing 68 cases of neonatal candidemia, the sex distribution of selected cases was found to be male 45 (66.17%) and female 23 (33.83%). There was a male preponderance in this study with a ratio 1.9:1 [Figure1].

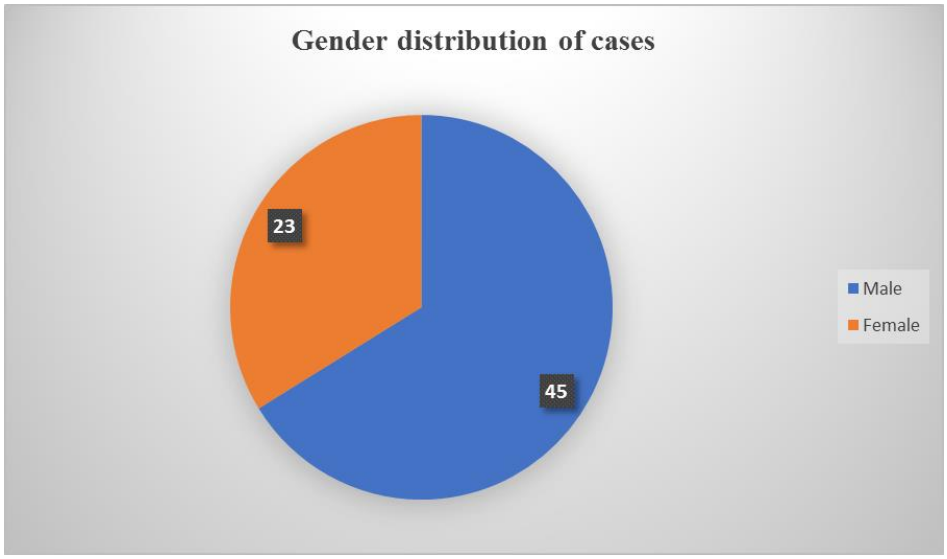

Fig. 1: Gender distribution of cases.

Table 1: Risk factors among selected cases n=68.

Risk factor	No of cases of candidemia	P value
Low birth wt.	55	0.001
Respiratory distress	53	
Broad spectrum antibiotics >7days	54	
Preterm	52	

Table 2: Species level distribution of Candida species among Blood Sample in NICU n=68.

Name. Of Species	No. of Isolates	Percentage
C.tropicalis	21	30.08
C.ciferri	15	22.05
C.guilliermondii	14	20.58
C.albicans	09	13.23
C.parapsilosis	05	7.35
C.auris	04	5.88
Total Positive	68	100

Table 3: Antifungal susceptibility profile of Candida isolates n=68.

Candida Species (n)	Resistance (%) to antifungal agents					
	Fluconazole n(%)	Flucytosin n(%)	Amphotericin B n(%)	Caspofungin n(%)	Micafungin n(%)	Voriconazole n(%)
C.tropicalis (21)	10(47.61)	9(42.85)	2(9.52)	0	0	0
C.ciferri (15)	8(53.33)	7(46.6)	3(20.0)	0	0	0
C.guilliermondii (14)	2(14.28)	2(14.28)	1(7.14)	0	0	0
C.albicans (9)	4(44.4)	2(22.2)	1(11.1)	0	0	0
C.parapsilosis (5)	3(60.0)	2(40.0)	1(20.0)	0	0	0
C.auris (4)	2(50.0)	2(50.0)	1(25.0)	0	0	0

Table 4: MIC distribution of antifungal drugs against most common Candida species.

Amphotericin B	MIC Range								
	≤0.06	≤0.12	≤0.25	0.5	≤1	2	4	8	>8
C.tropicalis		1	3	8	7				2
C.ciferri			1	7	4				3
C.guilliermondii		2	6	5					1
C.albicans		3	3	2					1
C.parapsilosis		1	1	2					1
C auris			2	1					1
Caspofungin									
C.tropicalis	12	4	3	2					
C.ciferri	8	3	2	1		1			
C.guilliermondii	9	2	1	1			1		
C.albicans	4	2	3						
C.parapsilosis	3	1	1						
C auris			2	2					
Flucytosin									
C.tropicalis			1	3	8				9
C.ciferri			1	5	2				7
C.guilliermondii		1	2	6	3				2
C.albicans		1	1	1	4				2
C.parapsilosis				1	1	1			2
C auris					1	1			2
Fluconazol									
C.tropicalis			4	1	6				10
C.ciferri		1	3	2	1				8
C.guilliermondii				5	6	1			2
C.albicans		2	2	1					4
C.parapsilosis		1	1						3
C auris					1	1			2

Micafungin									
C.tropicalis	15	3	3						
C.ciferri	10	1	2	1		1			
C.guilliermondii	9	2	1	1			1		
C.albicans	5	1	3						
C.parapsilosis	3	1	1						
C auris			2	2					
Voriconazol									
C.tropicalis	13	6	2						
C.ciferri	11	2	2						
C.guilliermondii	5	9							
C.albicans	6	2	1						
C.parapsilosis	2	1	2						
C auris		2	2						

Majority of Candidaemia cases were associated with Low Birth Weight followed by Broad spectrum antibiotics use, Respiratory distress and Preterm delivery [Table 1].

Among the positive samples for candidaemia, non‑albicans Candida were responsible for 86.77% cases, whereas 13.23% cases were due to *C albicans*. *C. tropicalis* (30.88%) was the predominant species isolated among the non-albicans Candida followed by *C ciferri* (22.05%), *C guilliermondii* (20.58%), *C parapsilosis* (7.35%), and *C auris* (5.88%) [Table 2].

The majority of the *C albicans* were resistant to commonly used antifungal drugs such as fluconazole (44.4%), flucytosine (22.2%), and amphotericin B (11.1%). Among the non‑albicans Candida sp., *C. tropicalis* showed 47.6% resistance to fluconazole, 42.8% resistance to flucytosine, and 9.5% resistance to amphotericin B. *C Ciferri* and *C. parapsolosis* were more resistant to fluconazole, flucytosine and amphotericin B, than *C. albicans*. *C guilliermondii* showed 14.2% resistance to both fluconazole and flucytosine and 7.14% resistant to amphotericin B. *C auris* showed 50.0% resistance to fluconazole, 50.0% resistance to flucytosine, and 25.0% resistance to amphotericin B. All the isolates were uniformly sensitive to micafungin, voriconazole, and caspofungin [Table 3].

DISCUSSION

One of the leading causes of death in NICU is septicemia.[14] During the past decade candidaemia is developing a common occurrence in patients admitted in ICU.[15] Emergence of non-albicans Candida (86.77%) was a major cause of neonatal candidaemia which is a remarkable feature of our study.

Our findings are reinforced by other studies from different regions of India that have documented predominance of non-albicans Candida over *C albicans* in neonatal septicemia.[6,16] Conveyance to non-albicans Candida was also noted by Kapila, Mendiratta et al., and Pfaller et al.[17,19]

The inappropriate use of invasive devices, broad‑spectrum antibacterial agents, more extensive surgical procedures, use of advance life support and selection of less susceptible species by the pressure of antifungal agent such as fluconazole probably are the reason for increased incidence of non-albicans Candida.[20]

Male predominance (64.6%) was noted in our study with a ratio of 1.9:1, which concordance with the study of Maharashtra by S. Tatte & R. Bhise.[21] Similar results were found by Caggiano et al with a ratio of 1.8:1.[22]

According to our study species dispersion shows *C tropicalis* (30.88%) as the most common species followed by *C ciferri* (22.05%), *C guilliermondii* (20.58%), *C parapsilosis* (7.35%), and *C auris* (5.88%). These results were comparable to the epidemiological studies carried out in India designating *C tropicalis* as the common cause of nosocomial candidaemia.[19,23] *C tropicalis* as a cause of fungemia in neonatal ICU has been linked to the presence of the fungus on the hands of the hospital personnel. One of its major virulence factors is the ability of this organism to produce clusters. *C tropicalis* may be more virulent than *C albicans* and can rapidly progress from colonization to invasion among immunocompromised host.[24]

Advent of neonatal candidaemia can be attributable to a number of risk factors including widespread use of broad‑spectrum antibiotics, preterm birth, LBW, and use of mechanical ventilation.[25,27] In our study, preterm birth (76.5%) and respiratory distress (77.9%) were the most common associated findings present in neonates with candidaemia which concordance with the results other studies.[9,10,28,29] Low birth weight (80.8%) was observed the most common clinical presentation followed by broad spectrum antibiotics used more than seven days. These findings analogues the study reported by Narains et al.[10]

In our study, 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.

Antifungal susceptibility testing by Vitek-2 system revealed that most of the *C. albicans* species were resistant to fluconazole (44.4%), flucytosine (22.2%), and amphotericin B (11.1%) respectively. Among the non-albicans Candida sp., *C. tropicalis* showed 47.6% resistance to fluconazole, 42.8% resistance to flucytosine, and 9.52% resistance to amphotericin B. Such type of increasing trends of fluconazole resistance were reported by Gupta et al and Kothari et al by 45.5% and 46% respectively.[26,30]

A variable range of resistance from 30% to 37% to flucytosine was reported by Bhatt et al and Pahwa et al consecutively.[31,32] In the present study, resistance to amphotericin B among all Candida isolates were found ranging from 10% to 25% which were similar to the results reported by Bhatt et al.[31] 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. Four cases of *C. auris* were isolated from blood culture and were resistant to fluconazole (50.0%), flucytosine (50.0%), and amphotericin B (25.0%). Micafungin, voriconazole, and caspofungin found sensitive in a consistent manner among all the isolates which accordance with the study done by Nazia et al.[33]

CONCLUSION

In the midst of NICU patients Candidaemia is springing up as a compelling problem worldwide. The change in epidemiology and pattern of antifungal susceptibility of Candida infection necessitates identification of etiological agent compulsory along with its antifungal susceptibility.

Our study accentuates the clinical importance and mycological shift of Candida species in neonatal candidaemia with a predominance of non-albicans Candida species in our region. From the results of our study it is noticeable that routine identification of Candida isolates to the species level, and the detection of resistant strains by antifungal susceptibility test is essential.

Vitek-2 automated system is considered as a reliable technique for antifungal susceptibility of yeast species with added advantage of being more rapid and easier than the alternative procedure developed by CLSI like broth microdilution method which is cumbersome and expensive. So, a fast and accurate technique for yeast identification is very important for microbiological laboratories. Accordingly, Vitek-2 automated system can be applied for early identification and antifungal susceptibility testing which is a paramount part for microbiological laboratories.

Furthermore, ongoing surveillance is required for candidaemia in order to track alterations in the epidemiological characteristics and antifungal susceptibility, as well as to carefully develop and evaluate a preventative approach. Moreover, the speciation of Candida in neonatal candidaemia and the steps taken to minimize risk factors can lower the neonates' morbidity, mortality, and need for antifungal medications.

ACKNOWLEDGEMENTS

Authors would like to thank the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University for their support.

ETHICAL MATTERS

Ethical approval was not required to carry out this work as the bacterial isolates were collected as part of routine patient care investigation in the hospital.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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