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Original Article

Emergence of Nonalbicans *Candida* in Neonatal Septicemia and Antifungal Susceptibility: Experience from a Tertiary Care Center

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ABSTRACT

Aims: To know the distribution and antifungal susceptibility pattern of *Candida* species in neonatal septicemia cases.

Materials and Methods: In a prospective analysis blood samples from 825 clinically suspected cases of neonatal septicemia, collected aseptically, were cultured to look for the role of *Candida* spp. in septicemia. *Candida* isolates were speciated by germ tube test, Hi-CHROME agar, sugar fermentation, and sugar assimilation tests using standard protocol. All the *Candida* isolates were tested for antifungal susceptibility to fluconazole by the Disk Diffusion (DD) method and broth micro dilution-minimum inhibitory concentration (BMD-MIC) method using NCCLS guidelines.

Results: Isolation rate of *Candida* from neonatal septicemia cases was 8.1%. Most common isolate was *C. tropicalis* (61.19%), followed by *C. albicans* (19.40%), *C. glabrata* (11.94%), *C. parapsilosis* (5.97%) and *C. guillermondii* (1.49%). Low birth weight and previous antibiotic prophylaxis was found in 100% cases. Crude mortality rate was 50.1%. By DD method, 95.53% of the *Candida* isolates were sensitive to fluconazole. A discrepancy between DD method and BMD-MIC method was noted in 4.47% strains. One isolates each of *C. tropicalis*, *C. albicans*, *and C. glabrata* showed discrepancy.

Conclusion: Nonalbicans *Candida* has emerged as an important cause of neonatal septicemia. Routine susceptibility testing of *Candida* isolates by DD method should be confirmed by BMD-MIC method. Fluconazole can be used as empirical therapy for neonatal candidemia at our center.

Keywords: Antifungal sensitivity, nonalbicans *Candida*, neonatal septicemia

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INTRODUCTION

Importance of *Candida* species in nursery and intensive care units (ICUs) is increasingly being recognized. *Candida* species account for 9-13% of all blood isolates in neonatal intensive care units (NICUs).[1] Although *C. albicans* has historically been the most frequently isolated species, infections caused by the nonalbicans *Candida* have been diagnosed with increasing frequency in recent years, notably *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. Common use of broad-spectrum antibiotics, low birth weight (LBW), prematurity, and intravenous catheter, etc., makes neonates prone to candidemia.[2] The incidence and associated mortality due to candidemia can be influenced by several factors including characteristic of the population at risk, standard of the health care facilities available, distribution of *Candida* species, and prevalence of antifungal resistance.[3] These factors may vary from one geographical region to other. The increased isolation rates of nonalbicans *Candida* species and a gradual shift in the antifungal susceptibility profile have underlined the need to monitor laboratory data for possible emergence of resistance and to select most appropriate antifungal agent for therapy. We have noticed an increased isolation rate of nonalbicans *Candida* from neonatal septicemia cases over last few months. So we are presenting our findings that were observed while investigating the causes of candidemia in a neonatal ICU.

MATERIALS AND METHODS

A total of 825 clinically suspected cases of neonatal septicemia were included in the study from June 2008-December 2008. Demographic and

clinical data such as age, sex, birth weight, antibiotic prophylaxis, presence of CVC, and clinical outcome of the neonates were noted from clinical records. Approximately 1 to 2 ml of blood was collected under aseptic precautions and inoculated in biphasic brain heart infusion medium. Subculture was done on third, fifth, and seventh day. All the *Candida* isolates were subjected to germ tube test using normal human serum. Colonies were identified up to the species level on the basis of morphology on Corn meal agar, growth on Hi-CHROME *Candida* agar, carbohydrate fermentation, and assimilation patterns.[4] All the isolates were screened for antifungal susceptibility testing by the Disk Diffusion (DD) method using fluconazole (25 μg) (Hi- media, Mumbai) on Mueller-Hinton agar (MHA) supplemented with 2% glucose and methylene blue (GMB) 5 μg/ml. Zone diameters were interpreted as per the approved NCCLS (M44-A) guidelines.[5] The broth micro dilution-minimum inhibitory concentration (BMD-MIC) of the isolates was performed to the fluconazole using RPMI medium and MOPS buffer. MIC results were interpreted as per NCCLS (M27-A2) guidelines.[6] Isolates showing MIC ≤ 8 μg/ml were regarded as susceptible, 16-32 μg/ml as dose-dependent susceptible and ≥ 64 μg/ml as resistant. The quality control test was performed by using *C. parapsilosis (*ATCC 22019), *C. krusei (*ATCC 6258), and *C. albicans (*ATCC 90028).

RESULTS

A total of 67 (8.1%) *Candida* isolates were recovered from 825 specimens. Male to female ratio was 2:1. The mean age of neonates was 3.8 days (SD ± 5.9). All the neonates (100%) were low birth weight (between 1.0-1.5 kg), and had received prophylactic antibiotics. Other associated findings were presence of central venous line in 71%, prematurity 40%, perinatal asphyxia 31.2%, jaundice 27.8%, and meconium aspiration in 10.8% of neonates; crude mortality rate was 50.1%. One isolate was from HIV positive neonate. Majority of isolates were *C. tropicalis* (61.19%), followed by *C. albicans* (19.40%), *C. glabrata* (11.94%), *C. parapsilosis* (5.97%), and *C. guillermondii* (1.49%). The result of *in vitro* susceptibility testing of *Candida* isolates to fluconazole is shown in Table 1. By DD method, 89.55% of *Candida* isolates were sensitive to fluconazole. The results of antifungal susceptibility testing by DD and BMD-MIC were compared for fluconazole. The DD testing performed in accordance with NCCLS M 44-A guidelines was comparable in 95.53% strains. A discrepancy between DD method and BMD-MIC test result for susceptibility to fluconazole was noted in 4.47% strains.

Table 1: Comparison of antifungal susceptibility testing of *Candida* isolates by DD and BMD-MIC method

Species/No. of isolates n = 67	Test method	Fluconazole (%)		
		S	*SDD*	*R*
C. tropicalis (41)	DD	87.80 (36)	9.75 (4)	2.43 (1)
	MIC	92.68 (38)	7.31 (3)	0.00
C. albicans (13)	DD	92.3 (12)	0.00	0.00 1
	MIC	100 (13)	0.00	0.00
C. glabrata (8)	DD	25 (2)	12.5 (1)	62.5 (5)
	MIC	50 (4)	0.00	50 (4)
C. parapsilosis (4)	DD	100 (4)	0.00	0.00
	MIC	100 (4)	0.00	0.00
C. guillermondii (1)	DD	100 (1)	0.00	0.00
	MIC	100 (1)	0.00	0.00

Numbers are in parenthesis; S - Sensitive; SDD - Susceptible dose dependant; R - Resistant

DISCUSSION

In our study, isolation rate of *Candida* from neonatal septicemia cases was 8.1%, which was lower than several other reports showing frequency of isolation in 13.6-19.6% cases.[2] *C. albicans* was the predominant organism in the earliest population based study conducted during 1992-1993 by CDC USA.[7] But a notable feature of our study was the emergence of nonalbicans *Candida* (80.59%) as a major cause of neonatal candidemia. *C. tropicalis* (61.19%) was the most common species followed by *C. albicans* (19.40%), and *C. glabrata* (11.94%). Our findings are supported by other studies from the same geographical area that have documented predominance of nonalbicans *Candida* over the *C. albicans in* neonatal septicemia.[8,9]

Antifungal susceptibility testing in our study revealed that all the *Candida* isolates except *C. glabrata* were 100% sensitive to fluconazole, though, three isolates of *C. tropicalis* were found to be in the Susceptible Dose Dependant (SDD) range with MIC 16 μg/ml, when tested by broth micro-dilution MIC method. In contrast to our study, several other Indian studies have reported a high (18.75-24%) fluconazole resistance against all the *Candida* spp.[10] The results of DD susceptibility testing to fluconazole were compared by BMD-MIC method. Overall, four strains of *C. tropicalis* were SDD by DD method that was reduced to three with BMD-MIC method. One strain of *C. tropicalis* that was resistant by DD method was found to be SDD with MIC 16 μg/ml. This result of our study emphasizes that DD method alone gives false resistance. Several risk factors have been cited as predisposing to candidemia in neonates including underlying illness, LBW, broad-spectrum antibiotic, asphyxia neonatorum, hyperalimentation, and total parentral nutrition.[2] In our study, LBW and broad spectrum antibiotic were the most common associated findings present in 100% neonates with candidemia.

CONCLUSION

Our study shows that nonalbicans *Candida* has emerged as a major cause of neonatal candidemia. LBW and broad spectrum antibiotics are the most common predisposing factors. Routine susceptibility testing of *Candida* isolates by DD method should be confirmed by BMD-MIC method. Fluconazole can be used as empirical therapy for neonatal candidemia at our center.

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