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Original Research Article

Study of risk factors and biofilm formation among non *albicans candida* species in neonatal *Candidemia*

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Abstract

Background: The changes in virulence factors associated with NAC species in neonatal *candidemia* were the subject of this study. The study was conducted in a rural tertiary referral teaching hospital of northern Maharashtra, India.

Materials and Methods: The research took place over the course of a year from January to December 2022, with all data collected and analyzed in real time. Blood samples were taken aseptically from newborns admitted to the neonatal intensive care unit (NICU) who showed symptoms that could indicate sepsis. Samples were inoculated in Brain Heart infusion broth, incubated at 37^0C for overnight incubation & subcultured on blood agar and SDA slant. Fungi was then further characterized through microscopic examination, biochemical assays, and observation of its growth patterns on selective media. These strains exhibited virulence factors were identified through the study of biofilm formation and other characteristics.

Result: Out of 383 neonates, Blood culture was positive in 269 (70.23%) cases. 87 were positive for *Candida species* (32.34%). NAC species were 68 (78.16%) and *C. albicans* 19 (21.84%). *C. tropicalis* was the most frequent non albicans species then by *C. glabrata* and *C. krusei*. Premature birth and low birth weight were the primary risk factors. Hemolysin production was frequent virulence factor then by Biofilm & Proteases production among all candida isolates.

Conclusion: When NAC species are detected in neonatal *candidemia*, a mycological shift in direction indicates a changed tendency and the escalating issue of anti-mycological resistance. Because of their increasing prevalence, NAC species identification is important for prompt management, appropriate anti-mycological medication selection, and preventing the development of antifungal resistances.

Keywords: NAC species, Premature birth, Biofilm, Hemolysin, Neonatal *candidemia.*

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1. Introduction

Neonatal septicemia, a blood culture-confirmed systemic bacterial or fungal infection within the first 28 days of life, continues to be a major cause of neonatal mortality and morbidity in India. It is a major cause of disease and mortality in neonates worldwide, particularly in neonatal intensive care units. In the intensive care unit (ICU), Candida sp. has been identified as the fourth most common organism responsible for bloodstream disorders.[1] Bloodstream disorders are often caused by Candida species.[2-4] About 9–13% of neonatal septicemia is thought to be caused by late-onset sepsis, of which *candidemia* the third is most common cause.[5] 20-34% fatality associated with neonatal *candidemia*.[6] Although *C. albicans* is commonly found to be the cause of neonatal *candidemia*, recent research has shown a mycological shift toward non-*albicans Candida* (NAC) species.[7,8] Prematurity, low birth weight, the use of stronger antibiotics, central catheters, and ventilator support are risk factors for the development of neonatal *candidemia*. Although the urinary tract, meninges, or blood are the primary sites of infection, illness frequently spreads to multiple systems.[9,10] The hands of healthcare professionals and genital tract of the mother can also spread Candida.[11]

The main issue is that NAC species are becoming more resistant to anti-mycological medications, which is increasing disease and mortality.[2,3] Thus, the goal of the

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current study was to investigate risk factors associated with shifting trends in NICU *candidemia*.

2. Materials and Methods

The retrospective study was conducted in a rural tertiary referral teaching hospital between January and December of 2022. When a newborn exhibited clinical symptoms and a positive blood culture demonstrating the growth of pure Candida species, *candidemia* developed. IBM's SPSS 22 was used to calculate the sample size and perform statistical analysis.

2.1. Inclusion criteria

1. Newborn less than one month old admitted to the NICU.
2. Neonates with risk factors like prematurity, low birth weight, intravascular catheter & prolonged antimicrobial therapy
3. Neonates with *Candida* species detected in their blood cultures

2.2. Exclusion criteria

1. Neonates on empirical antibiotic therapy.
2. Neonates with bacterial growth detected in their blood culture.
3. Neonates with mixed growth detected in their blood culture

The Institutional Ethical Committee granted ethical clearance through letter Ref. No. 79/IEC/ACPMMC/Dhule. Dated March 28, 2023.

2.3. Blood culture

2 ml of aseptically obtained blood via venipuncture is inoculated in Brain Heart Infusion Broth and incubated at 37^0C for overnight incubation. Subculture done on blood agar and Sabouraud's dextrose agar slant with 0.05% chloramphenicol which kept at 37°C for 2 to 15 days. The research team identified *Candida* Sp. through fungal culture criterions. The primary screening evaluated *Candida* Sp. growth on SDA slants and CHROME agar plates and through Raynold's Braude phenomenon and Corn meal agar growth and sugar fermentation and assimilation tests. The NAC species were distinguished by the color they produced on CHROME agar.[12]

2.4. Phospholipase detection

10µl of suspension in sterile saline solution was placed on the surface of agar containing egg yolk (pH = 4.3). These agar dishes were kept at 37°C for 4 days. Formation of colony surrounded by opaque zone detects phospholipase. Opaque zone of enzyme activity was calculated by dividing the colony diameter by the sum of colony diameter & the opaque zone. An opaque zone of 1.0 was assessed as negative (–), 0.99-0.9 as weak (+), 0.89-0.8 as mild (++), 0.79- 0.7 as relatively strong (+++) and <0.69 (++++) as very strong positive.[13] The positive control used was *Candida albicans* ATCC 90028 strain.

3. Result

Out of 383 suspected neonates, 269 (70.23%) had positive blood cultures, and 87 (32.34%) had positive Candida species results. There were 68 (78.16%) NAC species and 19 (21.84%) *C. albicans*. The most common NAC species was *C. tropicalis* (45.58%), which was followed by *C. glabrata* (33.82%) and *C. krusei* (13.23%) (**Table 1**). The most common risk factors were low birth weight and premature birth, followed by intravascular catheterization and prolonged antibiotic treatment (**Table 2**). Among all Candida isolates, hemolysin production (44.82%) was the most common virulence factor, followed by the genesis of biofilm (39.08%) and proteases (36.78%). (**Table 3**)

Table 1: Classification of NAC species detected from cases

Species	No. of isolates (%)
C. tropicalis	31/68 (45.58)
C. glabrata	23/68 (33.82)
C. krusei	9/68 (13.23)
C. parapsilosis	5/68 (7.35)

Table 2: Predisposing factors in no. of cases

Predisposing factors	No. of cases (%)
Prematurity	54/68 (79.41)
Low Birth Weight	50/68 (73.52)
Antibiotic use	39/68 (57.35)
Intravascular Catheter	35/68 (51.47)
Ventilator	29/68 (42.64)

Table 3: Virulence expressed by total no. of isolates (%)

	Total isolates	*Candida albicans*	*Candida tropicalis*	*Candida glabrata*	*Candida krusei*	*Candida parapsilosis*
	87	19	31	23	9	5
Adherence assay	87 (100)	19 (100)	31 (100)	23 (100)	9 (100)	5 (100)
Phospholipase	17 (19.54)	3 (15.78)	7 (22.58)	4 (17.39)	3 (33.33)	0
Lipase	6 (6.89)	1 (5.26)	5 (16.12)	0	0	0
Protease	32 (36.78)	8 (42.1)	10 (32.25)	13 (56.52)	4 (44.44)	0
Hemolysin	39 (44.82)	8 (42.1)	16 (51.61)	7 (30.43)	3 (33.33)	5 (100)
Biofilm	34 (39.08)	9 (47.36)	16 (51.61)	6 (26.08)	3 (33.33)	0
Coagulase	16 (18.39)	5 (26.31)	11 (35.48)	0	0	0

4. Discussion

Bloodstream infections in neonates admitted to the neonatal intensive care unit are frequently caused by Candida infections. Recent research shows a shift toward NAC species, even though Candida albicans is still the most commonly found fungal species in neonatal *candidemia*.[8,14] These NAC species are linked to increased mortality and are becoming more resistant to the most common azole groups of antifungal treatments. One of the possible causes of the increasing isolation rate of NAC species is the widespread use of azole antifungal medications, especially fluconazole.

32.34% of newborns in the current study had bloodstream disorders caused by Candida species, indicating that this is a common cause of nosocomial bloodstream disease. Candida sp. was found in blood samples in 30.1% and 34.7% of the studies by Sardana *et al*. and Rani *et al*., respectively, which is consistent with our findings.[15,16]

According to the current study, *C. albicans* was detected in roughly 21.84% and 78.16% of neonatal *candidemia* cases caused by NAC species. This supports the findings of other international researchers.[17,18]

Currently, *C. tropicalis* is the main cause of nosocomial *candidemia* in India. According to epidemiological studies, *C. tropicalis* is implicated in 67-90% of *candidemia* cases.[19] The increasing prevalence of non-albicans Candida, especially *C. tropicalis*, over *C. albicans* has been attributed primarily to the growing use of fluconazole. NAC species of Candida, mainly *C. tropicalis*, have been found to be increasing in frequency throughout the country.[20] According to the current study, Candida tropicalis was the most common species of Candida found in *candidemia* cases (45.58%), followed by *Candida glabrata* (33.82%), *Candida albicans* (21.84%), *Candida krusei* (13.23%), and *Candida parapsilosis* (6.9%). *C. tropicalis* was the most common species in neonatal *candidemia* (43%), our findings were consistent with those of a study by Goel *et al*.,[21] and 44 % Gunjan S. *et al*.[22] respectively.

The work conducted by Sardana *et al.*[15] detects *C. glabrata* (39%) as a frequent neonatal *candidemia* reason & *C. tropicalis* (26.4%) and *C. parapsilosis* (14.5%). The work conducted by Sirinivas Rao, MS, *et al.*[23] from Hyderabad detects *C. tropicalis* (36.53%) as a frequent source of neonatal *candidemia* & *C. albicans* (26.92%) and *C. glabrata* (19.23%). The same results of neonatal *candidemia* were recorded by other international researchers also.[4]

Our results are comparable to the current pattern, where elevated rates of NAC have been reported by numerous researchers from various locations in India and abroad, despite the fact that Candida albicans was the most common species causing bloodstream diseases during the last few decades. The selection of less susceptible antifungal agents, like fluconazole, as well as the increased use of invasive devices, antibiotics, extensive surgical procedures, and advanced life support on transplant recipients are generally thought to be the causes of the occurrence of NAC species.

Infection rates may surge in specific situations (IV catheters, elevated IV glucose levels). These fungi are almost impossible to destroy, even though *C. parapsilosis* is less contagious.[24] Since it is difficult to find effective treatments for these diseases, there are significant implications for the development of new medications. Babies are seriously at risk when *C. parapsilosis* colonizes healthcare workers' hands, catheters, and IV fluids.[25] According to recent research, *C. parapsilosis* (57.35%) poses a serious risk of fungal disease due to its ability to form biofilms and its tendency to colonize on foreign objects like catheters.[26] There have previously been reports of *C. parapsilosis* BSI increases in NICUs.[27]

Prematurity and LBW were frequently identified as predisposing factors associated with *candidemia* in the current study, which was followed by prolonged antimicrobial treatment and supportive care. Juyal D *et al.*[8] reported that the most common predisposing threats were LBW (67.42%) and prematurity (73.49%), which is consistent with our findings. Romeo *et al*.[29] and Narang *et al*.[28] reported that, prematurity was found in 85% and 94% of cases of neonatal *candidemia*, respectively.

5. Conclusion

This study shows that NAC species is now a major cause of septicemia in newborns. The prevalence of NAC species caused *candidemia* has resulted in increased rates of disease, mortality, and complications. In neonatal *candidemia*, a mycological shift toward the identification of NAC species indicates a changed tendency and the escalating issue of anti-mycological resistance. Because of its increasing prevalence, NAC species identification is important for prompt management, appropriate anti-mycological medication selection, and preventing the development of antifungal resistances.

6. Ethical Clearance

The letter Ref. No. 79/IEC/ACPMMC/Dhule from the Institutional Ethical Committee dated March 28, 2023.

7. Source of Funding

None.

8. Conflict of Interest

None.

9. Acknowledgement

None.

References

1. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *Lancet Infect. Dis*. 2003;3(11):685–702.
2. Chander J. Texbook of Medical Mycology, 4th edition: Jaypee Brothers Medical Publisher; 2018.
3. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P. et al, editors. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Philadelphia: Lippincott Williams & Wilkins; 2006.
4. Caggiano G, Lovero G, De Giglio O, Barbuti G, Montagna O, Laforgia N, Montagna MT. *Candidemia* in the Neonatal Intensive Care Unit: A Retrospective, Observational Survey and Analysis of Literature Data. *BioMed Res Int*. 2017;2017:7901763.
5. Benjamin DK, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, et al. National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis among extremely low birth weight infants: Risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. *Pediatrics*. 2006;117(1):84–92.
6. Shetty SS, Harrison LH, Hajjeh RA, Taylor T, Mirza SA, Schmidt AB, et al. Determining risk factors for *candidemia* among newborn infants from population-based surveillance: Baltimore, Maryland, 1998-2000. *Pediatr Infect Dis J*. 2005;24(7):601–4.
7. Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K. Non – *albicans candida* species in blood stream infections in a tertiary care hospital at New Delhi, India. *Indian J Med Res*. 2012;136(6):997–1003.
8. Juyal D, Adekhandi S, Negi V, Sharma N. An Outbreak Of Neonatal *Candidemia* Due To Non-*Albicans Candida* Species In A Resource Constrained Setting Of Uttarakhand State. *Ind J Clin Neonatal*. 2013;2(4):183–6.
9. Chapman RL. Candida infections in the neonate. *Curr Opin Pediatr*. 2003;15(1):97–102.
10. Agarwal J, Bansal S, Malik GK, Jain A. Trends in neonatal septicemia: emergence of non-albicans Candida. *Indian Pediatr*. 2004;41(7):712-5
11. Adib SM, Bared EE, Fanous R, Kyriacos S. Practices of Lebanese gynecologists regarding treatment of recurrent vulvovaginal candidiasis. *N Am J Med Sci*. 2011;3(9):406–10.
12. McGinnis MR. Laboratory Handbook of Medical Mycology. 1st Edition. New York: Academic Press. 1980:337–73.
13. Price MF, Cawson RA. Phospholipase activity in Candida albicans. *Sabouraudia*. 1977;15(2):179–85.
14. Nazir A. Non-*albicans Candida* in Neonatal Septicemia: An emerging clinical entity. *Int J Biomed Res*. 2016;7(2):47–50.
15. Sardana V, Pandey A, Madan M, Goel SP, Asthana AK. Neonatal *candidemia*: A changing trend. *Indian J Pathol Microbiol*. 2012;55(1):132-3.
16. Rani R, Mohapatra NP, Mehta G, Randhawa VS. Changing trends of Candida species in neonatal septicemia in a tertiary North Indian hospital. *Indian J Med Microbiol*. 2002;20(1):42–4.
17. Baradkar VP, Mathur M, Kumar S, Rathi M. Candida glabrata emerging pathogen in neonatal sepsis. *Ann Trop Med Public Health*. 2008;1:5–8.
18. Kothari A, Sagar V. Epidemiology of Candida bloodstream infections in a tertiary care institute in India. *Indian J Med Microbiol*. 2009;27(2):171–2.
19. Kothavade RJ, Kura MM, Valand AG, Panthaki MH. Candida tropicalis: Its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol*. 2010;59(pt.8):873–80.
20. Giri S, Kindo AJ. A review of Candida species causing blood stream infection. *Indian J Med Microbiol*. 2012;30(3):270–8.
21. Goel N, Rajan PK, Aggarwal R, Chaudhary U, Sanjeev N. Emergence of NAC species in neonatal septicemia and antifungal susceptibility: Experience from a tertiary care center. *J Lab physicians*. 2009;1(2):53–5.
22. Shrivastava G, Bajpai T, Bhatambare GS, Chitnis V, Deshmukh AB. Neonatal *candidemia*: Clinical importance of species identification. *Sifa Med J*. 2015;2(2):37–40.
23. Srinivas Rao MS, Surendranath M, Sandeepthi M. Prevalence of neonatal *candidemia* in a tertiary care institution in Hyderabad, South India. *Int J Res Med Sci*. 2014;2(3):1016–9.
24. Hartung de Capriles C, Mata-Essayag S, Azpiróz A, Ponente A, Magaldi S, Pérez C, et al. Neonatal candidiasis in Venezuela: Clinical and epidemiological aspects. *Rev Latinoam Microbiol*. 2005;47(1-2):11–20.
25. Trofa D, Gácser A, Nosanchuk JD. Candida parapsilosis, an emerging fungal pathogen. *Clin Microbiol Rev*. 2008;21(4):606–25.
26. Bonassoli LA, Bertoli M, Svidzinski TI. High frequency of Candida parapsilosis on the hands of healthy hosts. *J Hosp Infect*. 2005;59(2):159–62.
27. Almirante B, Rodríguez D, Cuenca-Estrella M, Almela M, Sanchez F, Ayats J, et al. Epidemiology, risk factors, and prognosis of Candida parapsilosis bloodstream infections: Case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. *J Clin Microbiol*. 2006;44(5):1681–5.
28. Narang A, Agarwal PB. Chakrabarti A, Kumar P. Epidemiology of systemic candidiasis in a tertiary care neonatal unit. *J Trop Pediatr*. 1998;44(2):104–8.
29. Romeo Reyes MC, Fernandez Gutierrez F, Poyato Dominguez JL, Párraga Quiles MJ, Huertas Muñoz MD, Cabañas JG, et al. Neonatal systemic candidiasis in the nineties. *An Esp Pediatr.* 1996;44(3):257–61.

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