RESEARCH
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PREVALENCE OF NON-ALBICANS *CANDIDA* IN CASES OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL

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Abstract
Background: Neonatal candidiasis is becoming more common, mostly as a result of the improved survival of low-birth-weight neonates, preterm deliveries, medical advancements, life support systems, a state of relative immunodeficiency, and the widespread use of broad-spectrum antibiotics. The dominance of *Candida albicans* has gradually given way to non-albicans *Candida* species during the past few decades. We in this study tried to analyse the prevalence of non-albicans *Candida* in cases of neonatal sepsis cases.
Methods: Blood samples of 1 – 2ml were done by arterial and venous samples, finger, or heel prick-capillary sampling, as well as newly inserted umbilical catheters. The ready-to-use BacT/ALERT PF Plus culture vials (yellow colour coded for paediatric usage) were injected with around 1 ml of blood and well-shaken. After scanning the bottle's barcode, the culture bottles were inserted into the apparatus and placed in the incubator.
Results: Out of n=23 culture-proven sepsis cases 30.44% (n=07) were bacterial isolates and 69.44%(n=16) were fungal isolates. there was a twofold rise in fungal isolates compared to bacterial isolates in the present study. Among the fungal isolates, *C. glabrata* 34.78% (n=8) constituted the majority of isolates followed by *C. tropicalis* 30.43%(n=7) and *C. cruzi* 4.35%(n=01). 50% of *C. glabrata* cases demonstrated resistance to commonly used antifungal drugs like fluconazole, *C. tropicalis* isolates, 42.8% showed resistance to fluconazole, 28.6% exhibited resistance to flucytosine, and 14.2% demonstrated resistance to amphotericin B.
Conclusion: There is a predominance of *candida* species in neonatal septicemia cases and a shift of strains to non-albicans *candida* is noted. Therefore, the importance to routinely identifying *Candida* isolates to the species level and conducting antifungal susceptibility tests to detect resistant strains. Continuous monitoring of *candidemia* through surveillance is essential to track changes in epidemiological trends, antifungal susceptibility patterns, and to guide the development and evaluation of preventive strategies.
Keyword: Non-albicans candida, Neonatal sepsis, neonatal candidemia.

INTRODUCTION

The occurrence of candidemia is on the rise in various countries worldwide. An analysis of NNIS data from the 1990s showed that Candida fungal infections represented 8% of all hospital-acquired bloodstream infections, making them the fourth most prevalent cause. This condition poses a substantial risk to patient well-being and contributes to serious health issues and increased risk of death [1-3] *Candida* species tend to colonize patients in NICU shortly after birth. This colonization primarily occurs in the Gastrointestinal and respiratory tracts within the first fourteen days of life, often through vertical transmission during childbirth. However, after the initial two-week period, the skin becomes the most common site of colonization. This shift may be attributed to the handling of patients by healthcare personnel. [4, 5]In NICUs, 9%–13% of all blood isolates are of the *Candida* species.[6] The majority of isolated species, *C. albicans*, causes invasive candidiasis in 50% to 70% of cases.[7, 8] The usage of fluconazole and itraconazole, however, appears to have increased the occurrence of nonalbicans *candidial* septicemia, according to recent investigations.[9] Neonatal septicemia patients are increasingly isolating *Candida tropicalis, Candida glabrata*, and *Candida parapsilosis*.[10] Broad-spectrum antibiotic usage, low birth weight, preterm, and intravenous catheter use are risk factors for candidemia.[11] An increase in the isolation rate of non-albicans Candida species from newborn septicemia cases served as the impetus for this investigation. Since most centres currently do not routinely test for further *Candida sp*. speciation and susceptibility, no accurate information about the estimated usage of antifungals in hospitals is available from our region. A major issue in the healthcare system, especially in tertiary care facilities, is the frequency of Non-albicans *Candida* species in instances of neonatal sepsis. Healthcare professionals have a great deal of difficulty while treating neonatal sepsis, a potentially fatal illness typically

brought on by bacterial or fungal infections in babies. Neonatal sepsis's etiological profile has changed noticeably in recent years, with non-albicans *Candida* species emerging as major culprits. This change is linked to a number of things, including longer hospital stays, a rise in invasive medical operations performed in neonatal intensive care units, and an increase in the use of broad-spectrum antibiotics. Moreover, considering the substantial geographical variations in Candida epidemiology, local expertise is crucial for preventing and managing invasive Candida infections. This expertise can guide the initiation of empirical antibiotic therapy, which is essential for managing neonatal sepsis.

MATERIAL AND METHODS

Neonatal intensive care unit of our tertiary care hospital served as setting for this cross sectional study. Institutional Ethical approval was obtained for the study of Human Subject's Research and Written informed consent was taken from the mother.

Inclusion criteria

Neonates who are going to be started on antibiotics with:

1. Neonatal factors such as low birth weight, preterm, prematurity, or twins presenting with signs and symptoms of severe sepsis
2. Maternal risk factors
   - Urinary tract infection
   - Cesarean section
   - Twin pregnancy
   - One unclean or more than 3 sterile vaginal examinations
   - In vitro conception
   - Pre-eclampsia
   - Gestational diabetes mellitus
   - Cervical stitch in situ.

Exclusion criteria

1. Neonates who had birth asphyxia, aspiration syndrome
2. Laboratory findings which are suggestive of inborn errors of metabolism
3. Neonates with congenital anomalies
4. Referred cases already treated with antibiotics.

The sample size calculation was performed using the formula: Sample size = $(Z\alpha)^2$ x sensitivity x (100 - sensitivity) / (Relative error) ^{2} x prevalence. Given the following values: Zα (constant) = 1.96 Sensitivity (S) = 81 Relative error (d) = 5% Prevalence (p) = 7%. Adding in the values, we have Sample size = $(1.96)^2$ x 81 x (100 - 81) / $(0.05)^2$ x 7 Sample size ≈ 35.17 ≈ 36. Therefore, the calculated sample size is approximately 36. The collection of blood samples of 1 – 2ml was done by arterial and venous samples, finger, or heel prick-capillary sampling, as well as newly inserted umbilical catheters. The ready-to-use BacT/ALERT PF Plus culture vials (yellow color coded for pediatric usage) were injected with around 1 ml of blood and well-shaken. After scanning the bottle's barcode, the culture bottles were inserted into the apparatus and placed in the incubator. The BacT/ALERT Microbial Detection System identified whether culture bottles were positive or negative. Only seven days of incubation were required before blood cultures were deemed negative.[12]

RESULTS

The current study observed a positive blood culture in 63.89% (n=23) of suspected sepsis cases, while it was negative in 36.1% (n=1) of cases. Therefore, the blood culture positivity rate in this study was 63.89%. Among the total of n=36 cases examined, n=23 cases were confirmed for sepsis through culture. No cases of late-onset sepsis were observed. Of the sepsis cases, n=15 were males and n=8 were females, resulting in an approximate male-to-female ratio of 2:1. Regarding the n=36 suspected sepsis cases, 2.78% (n=1) were term neonates, while 97.22% (n=35) were preterm neonates. Among the n=23 culture-proven sepsis cases, 4.35% (n=1) were term neonates, and 95.65% (n=22) were preterm neonates (see Table 1).

Table 1: Gestational age and separation of different sepsis based on this factor

Gestational age	*Suspected sepsis cases*		*Culture-proven sepsis cases*	
	Frequency	*Percentage*	*Frequency*	*Percentage*
Preterm (<37weeks)	35	97.22	22	95.65
Term (>37 weeks)	1	2.78	1	4.35
Total	36	100.0	23	100.0

Among the n=36 neonates suspected of sepsis, the majority, 91.65% (n=33), had low birth weight (<2500 gm), while a smaller proportion, 8.35% (n=03), had normal birth weight. Among the n=23 neonates with culture-proven sepsis, 91.3% (n=21) had low birth weight, and 8.70% (n=02) had normal birth weight. Notably, among the culture-proven sepsis cases, the highest rate of culture positivity was observed in low-birth-weight neonates, with 91.3% (n=21) showing positive results. Only 8.70% (n=02) of neonates with normal birth weight exhibited culture positivity (refer to Figure 1).

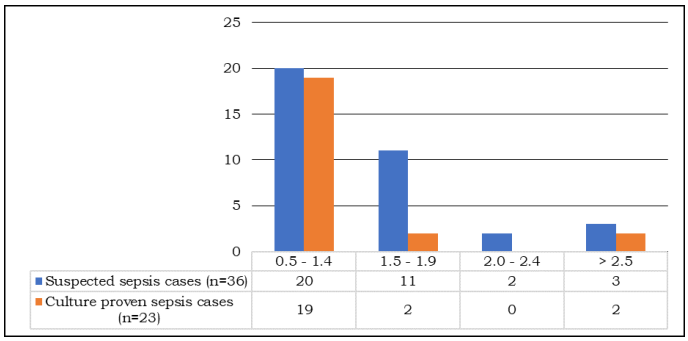

Figure 1: Birth Weight and separation of different cases of sepsis based on this factor

Among the n=23 culture-proven sepsis cases, the highest proportion of cases, 21.74%, were associated with

premature/prolonged rupture of membranes as the maternal risk factor. This was followed by cases related to pre-eclampsia, accounting for 17.40% of the cases. Prolonged labor and maternal pyrexia were each observed in 4.35% of the cases. The remaining 52.16% of the cases were attributed to various other risk factors, such as twin pregnancy, in vitro conception, gestational diabetes mellitus, cervical stitch in situ, home delivery, cervical cerclage, and so on (refer to Figure 2).

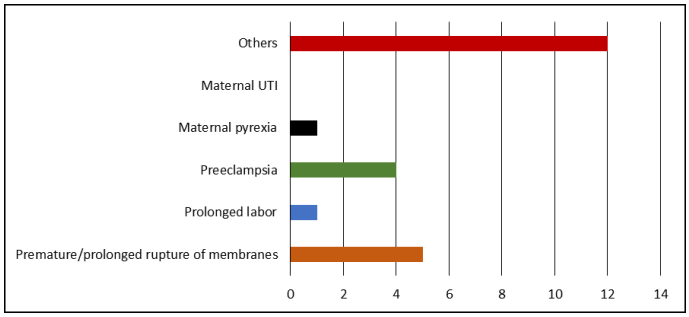

Figure 2: Neonatal sepsis and the risk factors during maternity associated to it.

Out of n=23 culture-proven sepsis cases, 30.44% (n=07) were bacterial isolates and 69.44%(n=16) were fungal isolates. there was a twofold rise in fungal isolates compared to bacterial isolates in the present study. Among the fungal isolates, *Candida glabrata* 34.78% (n=8) constituted the majority of isolates followed by *Candida tropicalis* 30.43%(n=7) and *Candida cruzi* 4.35%(n=01). Among the bacterial isolates *Klebsiella pneumoniae* constituted majority of isolates n=2 (8.09%) followed by *Pseudomonas aeruginosa* n=1(4.35%), *Escherichia coli* n=1(4.35%), *Enterobacter cloacae* n=1(4.35%), *Staphylococcus epidermidis* n=1(4.35%), *Staphylococcus hemolyticus* n=1(4.35%) given in table 2.

Table 2: Distribution of the Culture Isolates

Organism	*No. of isolates (n=23)*	*Percentage*
Candida glabrata	8	34.78
Candida tropicalis	7	30.43
Candida cruzi	1	4.35
Klebsiella pneumonia	2	8.09
Pseudomonas aeruginosa	1	4.35
Escherichia coli	1	4.35
Enterobacter species	1	4.35
Staphylococcus epidermidis	1	4.35
Staphylococcus haemolyticus	1	4.35
Total	23	100.0

Table 3: Clinical manifestations associated with neonatal candidemia

Sign / Symptom	*Frequency (n=16)*	*Percentage*
Respiratory Distress	12	75.00
Failure to thrive	11	68.75
Lethargy	8	50.00
Feed intolerance	9	56.25
Convulsions	6	37.5
Bleeding Tendency	4	25.00

In this study, it was found that 50% of *C. glabrata* cases demonstrated resistance to commonly used antifungal drugs like fluconazole, and flucytosine resistance was observed in 25% of cases, while amphotericin B resistance was seen in 12.5% of cases. Among *C. tropicalis* isolates, 42.8% showed resistance to fluconazole, 28.6% exhibited resistance to flucytosine, and 14.2% demonstrated resistance to amphotericin B. *C. cruzi* isolates displayed a higher resistance to azoles, particularly fluconazole. Despite the observed resistance patterns, all isolates in the study demonstrated consistent sensitivity to micafungin, voriconazole, and caspofungin.

DISCUSSION

In NICUs, sepsis continues to be the leading cause of morbidity and mortality,[13] but during the past ten years, candidemia has increased in prevalence among patients admitted to ICUs.[14] Several perinatal factors increase the susceptibility of newborns to sepsis, but timely intervention can prevent it. Additionally, it is crucial to identify the microorganisms present in a patient's blood to understand their characteristics and determine antibiotic susceptibility to provide prompt treatment. Therefore, this study aims to expedite the diagnosis of sepsis and evaluate the diagnostic efficacy of limited rapid diagnostic tests against the established gold standard of blood culture for sepsis diagnosis. The present investigation, the prevalence of *Candida* isolation from patients of newborn septicemia was 27.12%, which is slightly higher than numerous previous findings indicating isolation rates ranging from 13.6% to 19.6%.[11] It could be due to the lesser number of sepsis cases included in the study. Our study's findings of non-albicans *Candida* as a significant contributor to newborn candidemia are noteworthy. Other similar research from other parts of India that have shown that non-albicans *Candida* predominates over *C. albicans* in newborn septicemia confirms our findings. [6,15] Mane et al., [16] in their study at Nagpur, reported all their candida isolates to be *candida* albicans. However, on the contrary, non-albicans *candida* predominated in the studies conducted by Roy et al.,[17] Gandhi et al., [18]and Sharma et al., [19] Thus, the recent reports suggest that *candida* albicans, which was responsible for nearly 80% of candidemia in the 1990s are being replaced by non-albicans *candida* species in the present years. Fungal sepsis poses a significant challenge in critically ill neonates, with mortality rates ranging from 21% to 76%, and definitive diagnosis is hindered by the time required for pathogen isolation. Fungal sepsis is difficult to diagnose definitively, and pathogen isolation may take some time. A thorough investigation into the frequency of Non-albicans Candida in instances of newborn sepsis was carried out at a tertiary care hospital. The results highlighted these fungal species' increasing importance in newborn illnesses. The higher prevalence of Non-albicans Candida sepsis in newborns has been linked to several risk factors, including low birth weight, preterm, and the use of indwelling catheters. Comprehending and mitigating these risk factors is crucial in devising efficacious preventative measures and treatment regimens customised to the distinct vulnerabilities of neonates. To improve the overall management and outcomes of neonatal care in tertiary settings, healthcare practitioners must adopt a nuanced strategy that takes into account the unique characteristics of Non-albicans Candida infections as they negotiate the difficulties of neonatal sepsis. Therefore, it would be wise to administer antifungal medication to infants suspected of having fungal sepsis while waiting for culture results. Understanding the prevalent fungal isolates from a certain unit

might inform empirical antifungal therapy. The pathogens responsible for neonatal sepsis exhibit not only geographical variability but also temporal variation within the same region, likely due to changing living conditions according to Jyothi P et al., [20] Newborns with low birth weight, premature birth, intravenous hyperalimentation, central catheters, mechanical ventilation, and endotracheal intubation are at an increased risk of developing fungal sepsis.[18]

In this investigation, 34.78% of the patients contained *C. glabrata,* 30.43% had *C. tropicalis,* and 4.35% contained *C. cruzi*. Nosocomial candidemia is most frequently caused by *C. tropicalalis,* according to another epidemiological research from India. [22, 23] The presence of *C. tropicalis* in the neonatal ICU has been associated with fungemia, and it is believed that the fungus can be transmitted through the hands of hospital personnel. One of the main factors contributing to its virulence is its ability to form clusters. When introduced to an immunocompromised host, *C. tropicalis* may exhibit greater virulence compared to *C. albicans*, leading to a rapid transition from colonization to invasion. The recent rise of newborn candidemia is caused by a variety of risk factors. Broad-spectrum antibiotic usage on a large scale, mucosal immunity loss, colonization, Low birth weight, and duration of hospital stay are a few of these.[24] In our study, neonates with candidemia most frequently had preterm delivery (78.5%) and low birth weight (73.5%). This agrees with the findings of other investigations. [25, 26] In non-albicans Candida species, our study found an increase in antifungal medication resistance, especially in the azole group. Tests for antifungal susceptibility showed that the non-albicans *C. glabrata, C. tropicalis, and C. cruzi* had increased azole resistance. Several studies have reported a growing prevalence of fluconazole resistance. Gupta et al., [24] found a resistance rate of 37.5%, Kothari et al., [27] reported 36%, and Xess et al. reported 11.7%. It's crucial to remember that C. krusei has an innate resistance to fluconazole. Flucytosine resistance varied in other trials; it ranged from 37% in Bhatt et al. [28] to 0% in Pahwa et al. [29]. Against each isolate, caspofungin, voriconazole, and micafungin showed consistent sensitivity.

CONCLUSION

Within the constraints of this research, it is reasonable to infer that there is a predominance of *candida* species in neonatal septicemia cases and a shift of strains to non albicans *candida* is noted. Therefore, it the importance to routinely identify Candida isolates to the species level and conduct antifungal susceptibility tests to detect resistant strains. It is crucial to continuously monitor candidemia through surveillance to observe changes in epidemiological characteristics and antifungal susceptibility, as well as to develop and assess prevention strategies. Additionally, implementing measures to minimize risk factors can significantly reduce morbidity, mortality, and the need for antifungal drugs in neonates.

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