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Bacteriological profile and antibiotic susceptibility pattern in neonatal sepsis in a tertiary care hospital

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Abstract: Neonatal sepsis is a significant cause of morbidity and mortality worldwide, yet there is no clear consensus definition, with positive blood cultures serving as the gold standard for diagnosis. Diagnosing sepsis in neonates is challenging due to varying definitions of "culture negative" or "suspected" sepsis.
Method: A total of 350 blood cultures were performed on neonates suspected of having septicaemia.The antibiotic susceptibility of the isolates was determined using the disc diffusion method, as per CLSI guidelines.
Results: During the study period, 350 blood samples were collected from clinically suspected cases of neonatal sepsis admitted to the Neonatal Intensive Care Unit (NICU). Blood culture reports were positive in 72 cases, indicating a positivity rate of 20.57%. Among these culture-positive cases, there were 52 males (72.2%) and 20 females (27.8%), resulting in a male-to-female ratio of 2.6:1.
Conclusion: Recent research has revealed a concerning trend: a significant increase in resistance is observed among various Gram-negative bacilli. This alarming development emphasizes the critical need for comprehensive antibiotic susceptibility testing to ensure the proper and effective use of antibiotics.

Introduction

Neonatal sepsis is a critical and serious condition that significantly contributes to morbidity and mortality rates among newborns. In the United States, the incidence of this syndrome is estimated to range from 1 to 4 cases per 1,000 live births, although this figure varies significantly across different regions worldwide. [1]

This clinical syndrome presents with a spectrum of symptoms and laboratory findings indicative of a systemic inflammatory response resulting from a complex, multifaceted infection. These infections can be caused by a diverse array of pathogens, including bacteria, viruses, and fungi. The severity of neonatal sepsis is underscored by its potential to trigger multiorgan dysfunction and failure, leading to devastating outcomes, including

death, within the critical first 28 days of a newborn's life. Early recognition and prompt intervention are crucial in managing this life-threatening condition. [1,2]

A patient is considered to have suspected sepsis when they exhibit clinical signs such as fever, increased heart rate, or altered mental status, yet laboratory tests, including blood cultures, return negative for infection. In such cases, it is important to initiate empirical treatment with broad-spectrum antibiotics within the first few hours of diagnosis. This treatment should be continued for at least five days to effectively manage the potential infection and prevent further complications. [3]

The World Health Organization (WHO) recommends two empiric antibiotic regimens for the treatment of suspected neonatal sepsis (NS). These regimens include a combination of ampicillin with either gentamicin or cefotaxime. Ampicillin plays a crucial role in combating infections caused by Listeria monocytogenes and other Gram-positive bacteria, such as Group B Streptococcus (GBS), which are common culprits in severe infections in newborns. On the other hand, gentamicin and cefotaxime are targeted against Gram-negative bacteria, known for their resistance and potential to cause serious health complications. These carefully selected antibiotic combinations aim to provide broad-spectrum coverage, ensuring effective treatment for vulnerable neonates suspected of having sepsis. [4,5] The purpose of this study was to evaluate the onset, bacteriological profile, and antibiotic sensitivity patterns in cases of neonatal sepsis.

MATERIAL & METHODS

The study took place at the Index Medical College, Hospital, and Research Centre in Indore, Madhya Pradesh. This observational study was conducted from May 2022 to May 2023, following approval from the ethics committee. A total of 300 blood cultures were performed on neonates suspected of having septicaemia.

During the study period, 10 mL of blood was collected for culture, and 5- 8 mL of the sample was inoculated into 30 mL BHI (Brain Heart Infusion) media (to get a dilution of 1:5). 2 mL of blood was allowed to clot before being centrifuged and separated into aliquots for serological investigations. A second blood sample was obtained in the same manner within 48 hours and inoculated into another BHI medium for culture.

During the study period, blood cultures were performed for all neonates suspected of having clinical sepsis. All the blood cultures were performed on an automated blood culture System.After the bottles were flagged positive, Gram stain was performed on the broth for preliminary identification of the type of organisms isolated. Subcultures were made in blood agar and MacConkey agar and incubated at 37°c for 18–24 hours. The grown bacteria were identified by colony morphology, Gram stain, and bio-chemical tests. Species identifications of all isolates were confirmed byVITEK 2 COMPACT (bioMérieux, U.S.A).

Antibiotic susceptibility testing was performed using the disc diffusion method with isolated organisms, following the guidelines of the Clinical and Laboratory Standards Institute.

The data were analysed using the Statistical Package for the Social Sciences (SPSS) software.

RESULTS

During the study period, 350 blood samples were collected from clinically suspected cases of neonatal sepsis admitted to the Neonatal Intensive Care Unit (NICU). Blood

culture reports were positive in 72 cases, indicating a positivity rate of 20.57%. Among these culture-positive cases, there were 52 males (72.2%) and 20 females (27.8%), resulting in a male-to-female ratio of 2.6:1.

The incidence of early-onset sepsis was found to be higher than that of late-onset sepsis. Of the 131 cases evaluated, 56 (77.8%) had early-onset sepsis, while 16 (22.2%) had late-onset sepsis. (Table 1)

Table 1: Distribution of culture-proven septic neonates according to onset of sepsis.

Category	Total number (N=72)	Percentage
Early onset	56	77.8%
Late onset	16	22.2%

Out of 72 isolates, 42 (58.3%) were gram-negative bacilli and 30 (41.7%) were Gram-positive cocci. In the case of Gram-negative bacilli, Klebsiella sp. was most common, and in the case of Gram-positive cocci, CoNS was most common. (Table 2)

Table 2: Distribution of isolated organisms

Organism	Number (N=72)	Early onset	Late onset
Klebsiella Spp	23(31.9%)	17	6
Pseudomonas Spp	9(12.5.%)	7	2
E. coli	5(6.9%)	4	1
Acinetobacter Spp	5(6.9%)	2	3
CoNS	18(25%)	13	3
Staphylococcus aureus	9(12.5%)	8	1
Enterococcus Spp	3(4.2%)	3	0

Table 3: Antibiotic sensitivity of Gram-negative organisms.

Antibiotics	Klebsiella Spp (N=23)	Pseudomonas Spp(N=9)	Acinetobacter Spp(N=5)	E. coli (N=5)
Amikacin	9(39.1%)	Not tested	0	4(80%)
Tobramycin	8(34.7%)	Not tested	0	4(80%)
Gentamycin	9(39.1%)	Not tested	0	4(80%)
Ciprofloxacin	3(13%)	2(22.2%)	0	3(60%)
Ceftazidime	6(26.1%)	4(44.4%)	0	3(60%)
Ceftriaxone	6(26.1%)	4(44,4%)	0	3(60%)
Ampicillin/Sulbactam	10(43.5%)	Not tested	2(40%)	4(80%)
Pipracillin/tazobactam	10(43.5%)	2(22.2%)	2(40%)	4(80%)
Imipenem	23(78%)	8(88.9%)	3(60%)	4(80%)
Meropenem	23(78%)	8(88.9%)	3(60%)	4(80%)
Colistin	23(100%)	9(100%)	6(100%)	5(100%)
Aztreonam	NT	6(66.7%)	Not tested	Not tested

Table 4: Antibiotic sensitivity of Gram-positive organisms

Antibiotics	*Staphylococcus aureus (N=9)*	*S.epidermidis (N=18)*	*Enterococcus Spp (N=3)*
Penicillin	3(33.3%	9(50%)	29(66.7%)
Erythromycin	4(44.4%)	7(38.9%)	Not tested
Clindamycin	4(44.4%)	8(44.4%)	Not tested
Tetracycline	5(55.6%)	9(50%)	2(66.7%)
Doxycycline	5(55.6%)	9(50%)	2(66.7%)
Minocycline	5(55.6%)	9(50%)	2(66.7%)
Ciprofloxacin	3(33.3%)	10(55.6%)	1((33.3%)
Gentamicin	5(55.6%)	9(50%)	Not tested
Rifampin	4(44.4%)	9(50%)	Not tested
Vancomycin	9(100%)	18(100%)	3(100%)
Linezolid	9(100%)	18(100%)	3(100%)
Cefoxitin	5(55.6%)	Not tested	Not tested
Gentamycin high level	Not tested	Not tested	2(66.7%)

DISCUSSION

Neonatal sepsis causes significant morbidity and mortality among neonates worldwide and is a major cause of mortality in developing countries such as India [6]. World Health Organization has estimated that 1.6 million deaths occur globally every year because of neonatal infections and 40% of all neonatal deaths occur in developing countries. [6,7]

In India, the incidence of blood culture-proven sepsis was reported as 8.5 per 1,000 live births for the year 2002–2003 by the National Neonatal Perinatal Database.[13] In the present study, the blood culture positivity rate was 15.3%, which is in accordance with previous studies performed in India where the culture positivity ranged from 16% to 54%[6,8,9].

In our study out of 350 blood cultures specimens, 72 blood culture were positive, indicating a positivity rate of 20.57%. This is This finding is comparable with other reports [10,13]

Among these culture-positive cases, there were 52 males (72.2%) and 20 females (27.8%), resulting in a male-to-female ratio of 2.6:1. The incidence of early-onset sepsis was found to be higher than that of late-onset sepsis. Of the 131 cases evaluated, 56 (77.8%) had early-onset sepsis, while 16 (22.2%) had late-onset sepsis Different study shows that the culture-positivity for aerobic organisms in neonates vary from 25% to 60% [10-11], where a high blood culture-positivity rate in septicaemic children (56%) had been reported by Sharma et al.[18] and Jain et al.[19]

A low blood culture isolation rate could be due to the administration of antibiotics before blood collection from the primary centres or the possibility of infection with anaerobes.

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common and are mainly represented by Klebsiella, Escherichia coli, Pseudomonas, and Salmonella. Among the Gram-positive organisms, Staphylococcus aureus, CONS, Streptococcus pneumoniae, and S. pyogenes are the most isolated. [10,16]

A study done by Jyothi et al. [10] reported that Gram-negative and Gram-positive septicemia were encountered in 56% and 44% of the culture-positive cases,and another study done by Agnihotri et al [17] reported that Gram-negative and Gram-positive organisms were responsible for 59% and 41% of the septicemia casesrespectively. In our research,out of 72 isolates, 42 (58.3%) were gram-negative bacilli and 30 (41.7%) were Gram-positive cocci. Which are almost like that our study.

The report of the National Neonatal-Perinatal database showed Klebsiella as the predominant (29%) pathogen.[11]. In our study 23(31.9%) Klebsiella Spp predominant Gram-negative organisms which slightly higher than that previous study. Another by Jyothi et al [10] reported that Klebsiella spp. (31%) was the predominant Gram-negative species,which is almost like our study.

Antibiotic resistance is a global problem today. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are on the rise. The widespread availability of over-the-counter antibiotics and the improper use of broad-spectrum antibiotics in the community may contribute to this situation. It is challenging to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is highly variable [10]

Antibiotic susceptibility pattern was studied for all isolates causing neonatal sepsis. The analysis of antibiotic resistance patterns showed that, among gram-negative isolates, the maximum number, 97%, were resistant to ciprofloxacin and the lowest, 22%, were resistant to carbapenems.

The greater prevalence of resistance to commonly used antibiotics has also been reported by other studies. [10,18,19] Among aminoglycosides, amikacin was found to have an edge over tobramycin and gentamicin in Gram-negative septicemia, with sensitivity of 39%, 35%, and 39%, respectively. Previous research groups have made similar observations. [10,20]

In this study, maximum sensitivity 100% was observed in colistin, vancomycin and linezolid, but these two drugs should not be used indiscriminately and should be kept as a reserve drug. Otherwise, resistance to these drugs may develop, thereby threatening the treatment.

CONCLUSION

The current research highlights a concerning trend: a notable rise in resistance is observed among various Gram-negative bacilli. This alarming development underscores the critical importance of conducting thorough antibiotic susceptibility testing to ensure the appropriate and effective administration of antibiotics.

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