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RESEARCH ARTICLE

A PROSPECTIVE STUDY ON THE PROFILE OF BACTERIAL PATHOGENS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN NEONATAL SEPSIS AT OUR TERTIARY CENTER

Juwairiah Abdur Raheem¹, Pavani Gandham², Grace Olive³ and Kumuda Arumugam⁴

1. Post-graduate, Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad.
2. Professor and HOD, Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad.
3. Assistant Professor, Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad.
4. Senior Resident, Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad.

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Abstract

Background: Neonatal sepsis in India has the highest incidence worldwide, ranging from 14.3% to 23% and is the second leading cause of mortality among newborns. This study was conducted to isolate the microorganisms causing neonatal sepsis and to determine their antibiotic sensitivity patterns.

Materials and Methods: A prospective study was conducted for a period of 3 months. 100 blood cultures samples were processed from newborns admitted to Neonatal Intensive Care Unit (NICU) with signs and symptoms suggestive of neonatal sepsis. Blood collected was loaded into BacT/ALERT 3D culture system. From bottles flagged positive, gram stain was performed and sub-cultures were made onto Blood agar and MacConkey agar. Bacterial colonies were identified by gram stain, biochemical tests and the organism confirmed by VITEK 2 Compact ID cards. Antimicrobial susceptibility testing of the isolates was performed using VITEK 2 Compact AST cards and the data was analyzed using descriptive statistics. Conclusions were made based on the observed distribution of bacterial isolates and their antimicrobial susceptibility patterns.

Results: Out of the 100 samples processed, 10 (10%) were culture positive, 80% (n=8) of them were Gram-negative bacteria and 20% (n=2) were Gram-positive bacteria. The most common organisms isolated were *Klebsiella pneumoniae* (n=2, 20%), *Escherichia coli* (n=2, 20%) and *Acinetobacter baumannii* (n=2, 20%). Gram-negative isolates were most susceptible to Amikacin, Meropenem and Piperacillin/Tazobactam combination, whereas Gram-positive pathogens were susceptible to Vancomycin, Teicoplanin and Linezolid.

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Conclusion: Blood culture positivity was 10%, with Gram-negative bacteria accounting for 80% of the isolates. *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii* and Coagulase-Negative Staphylococcus were

the most frequently isolated pathogens. The findings reaffirm these organisms as important causes of neonatal sepsis and support the continued effectiveness of broad-spectrum agents such as Piperacillin/Tazobactam, Meropenem and aminoglycosides against Gram-negative pathogens and Vancomycin, Teicoplanin and Linezolid against Gram-Positive organisms.

Introduction:-

Neonatal sepsis is defined as a life-threatening, dysregulated inflammatory response to bloodstream infection in infants under 28 days [1,2] and is a leading cause of morbidity and mortality. In India, it is the second major cause of mortality, with an incidence ranging from 14.3% to 23%.[3] Globally, of the estimated three million annual neonatal sepsis cases, India has the highest incidence of clinical sepsis (17,000/ 1,00,000 live births).[4]

Recent global evidence highlights neonatal sepsis as a major contributor of neonatal deaths, particularly in low- and middle-income countries.[5] The predominant organisms isolated across various studies include *Klebsiella pneumoniae*, *Acinetobacter* species, Coagulase-Negative Staphylococci and *Staphylococcus aureus*. [6,7]

The emergence of Multi-Drug Resistance (MDR) organisms has become a serious public health concern. Antimicrobial resistance in neonatal sepsis is on the rise due to the use of reserve antibiotics as first- and second-line drugs. Increased rates of multi-drug resistance are observed in *Acinetobacter* (82%), *Klebsiella* (54%) and *Escherichia coli* (38%) isolates.[8] The present study was undertaken to determine the bacterial profile and antimicrobial susceptibility pattern of neonatal sepsis in a tertiary care center.

Materials and Methods:-

A prospective study was conducted for a period of 3 months from 24th February 2025 to 25th May 2025. The study was approved by the Institutional Ethics Committee and a total of 100 blood culture samples were collected and processed aseptically in the Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad, India.

Inclusion criteria:-

Newborns admitted to the NICU with clinical signs and symptoms suggestive of neonatal sepsis were included in the study after obtaining informed consent from parents/guardians.

Exclusion criteria:-

Neonates who received antibiotic therapy for more than 48 hours prior to blood sample collection, those with insufficient blood volume for culture processing and samples found to be contaminated were excluded from the study.

Bacterial isolation:-

Under aseptic precautions, 4ml of blood was collected and injected into the PF Plus blood culture bottle and loaded into BacT/ALERT 3D culture system and incubated at 37°C for a maximum of 5 days. When the bottles were flagged positive, gram stain was performed and sub-cultures were made on Blood agar and MacConkey agar and incubated at 37°C for 18-24 hours. The sample was reported sterile if no growth was observed for 5 days.

Identification and AST of bacterial isolates:-

The bacteria grown was identified by colony morphology, gram stain and bio-chemical tests and confirmed by VITEK 2 Compact ID cards. Antibiotic sensitivity of the organism was analyzed by VITEK 2 Compact AST cards. AST cards N405 and N406 were used to determine the antibiotic susceptibility of Gram-negative fermenter bacteria and Gram-negative non-fermenter bacteria respectively, while P628 AST cards were utilized for Gram-positive bacteria. The antimicrobial susceptibility test results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Data was entered into Microsoft Excel and analyzed using descriptive statistics. The prevalence of culture positivity, distribution of bacterial pathogens and antimicrobial susceptibility patterns were expressed as frequencies and percentages. The identified organism and its antibiotic sensitivity pattern were immediately shared with the primary team and all cases were followed till discharge.

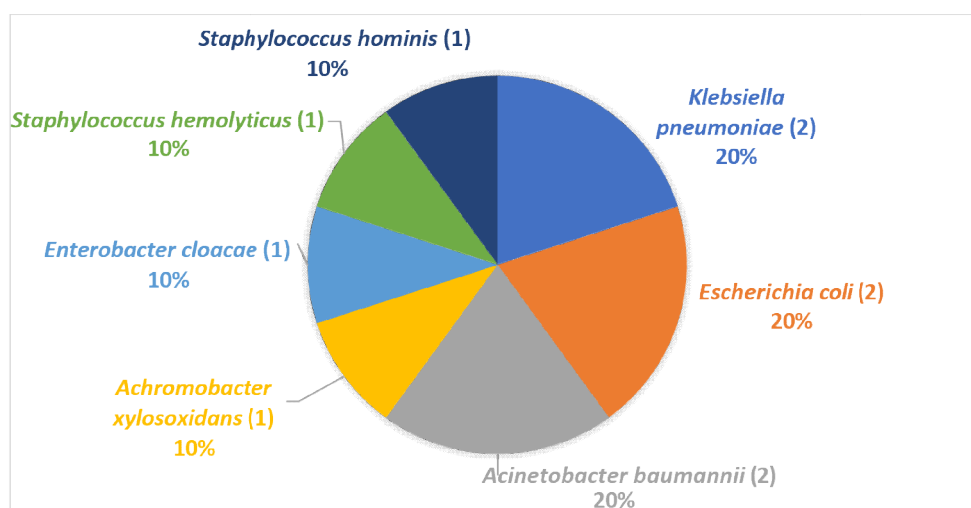
Results/Discussion:-

In the present study, 100 samples were received with the clinical suspicion of neonatal sepsis, of which 10 (10%) were culture positive. Other studies had a higher percentage of positive blood cultures, 19.2% culture positivity by Jyothi P et al.[6] and 15.3% culture positivity by Siddiqui T et al.[7] This could be due to the differences in geographical location and hospital setting. Both studies were done in North India and included a larger sample size over a longer study period.

In our study, 80% (n=8) of organisms isolated were Gram-negative bacteria and 20% (n=2) were Gram-positive bacteria. The most common organisms isolated were *Klebsiella pneumoniae* (n=2, 20%), *Escherichia coli* (n=2, 20%) and *Acinetobacter baumannii* (n=2, 20%). Other Gram-negative bacteria isolated were *Achromobacter xylosoxidans* (n=1, 10%) and *Enterobacter cloacae* (n=1, 10%).

Gram-positive organisms isolated were Coagulase-Negative Staphylococcus (n=2, 20%) (*Staphylococcus hemolyticus* and *Staphylococcus hominis*) (Figure 1).

Figure 1: Type and percentage of bacterial isolates



Several other studies have also reported Gram-negative bacteria as the most frequently isolated pathogen in neonatal sepsis.[6,7,9,10] In a study by Zakariya BP et al., *Klebsiella pneumoniae* (66%) was the most common organism isolated followed by Coagulase-Negative Staphylococcus (12%).[9] Another study by Jyothi P et al, concluded that *Klebsiella*, *Acinetobacter*, Coagulase-Negative Staphylococcus and *Staphylococcus aureus* as the leading cause of neonatal sepsis,[6] whereas, Lamba M et al.'s study revealed that Coagulase-Negative Staphylococcus (17.43%) was the predominant isolate followed by *Klebsiella* species (16.11%).[11]

Therefore, *Klebsiella pneumoniae* is the most common Gram-negative bacteria, while Coagulase-Negative Staphylococci is the most common Gram-positive organism isolated in neonatal septicemia.

Treatment with antibiotics is the mainstay treatment for neonatal sepsis. Maximum sensitivity of *Klebsiella pneumoniae* isolates to Amikacin, Meropenem and Piperacillin/Tazobactam combination was observed in our study. Susceptibility to Ceftriaxone, Ciprofloxacin, Cefoperazone/Sulbactam, Imipenem, Aztreonam was 50%. Zakariya BP et al. also reported susceptibility of *Klebsiella pneumoniae* isolates to Amikacin and Meropenem, while showing resistance to other antibiotics.[9]

Both isolates of *Escherichia coli* were sensitive to Amikacin and only one of them was sensitive to Ceftazidime, Ceftriaxone, Ciprofloxacin, Cefoperazone/Sulbactam, Meropenem, Imipenem, Aztreonam and Piperacillin/Tazobactam. *Acinetobacter baumannii* showed resistance to all drugs, as also observed in another study. [8] Antibiotic susceptibility of other isolates is mentioned in Table 1.

Table 1: Antibiotic sensitivity pattern of the Gram-negative bacterial isolates

Antibiotic	Sensitivity pattern, n (%)				
	<i>Klebsiella pneumoniae</i> (n=2)	<i>Escherichia coli</i> (n=2)	<i>Acinetobacter baumannii</i> (n=2)	<i>Achromobacter xylosoxidans</i> (n=1)	<i>Enterobacter cloacae</i> (n=1)
Amikacin	2 (100%)	2 (100%)	0 (0%)	0 (0%)	1 (100%)
Ceftazidime	1 (50%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)
Ceftriaxone	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)
Ciprofloxacin	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)
Cefoperazone/Sulbactam	1 (50%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)
Meropenem	2 (100%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)
Imipenem	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)
Aztreonam	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)
Piperacillin/Tazobactam	2 (100%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)
Colistin	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate

Overall, Gram-negative isolates were most susceptible to Amikacin (n=5, 62.5%), Meropenem (n=5, 62.5%) and Piperacillin/Tazobactam combination (n=5, 62.5%). Jyothi P et al. also reported a similar pattern of susceptibility among Gram-negative bacteria, with highest sensitivity to Imipenem (93%), Amikacin (52%) and Netilmicin (41%).[6] Saima Inam et al., noted 80% susceptibility of isolates to Carbapenems like Meropenem and Imipenem.[12] This suggests that Amikacin and Meropenem can be considered for empirical therapy in neonatal sepsis due to Gram-negative bacteria.

All Gram-positive organisms isolated were susceptible to Vancomycin, Teicoplanin and Linezolid (Table 2). A study conducted by Li J et al. reported similar findings of Gram-positive bacteria exhibiting higher susceptibility to Vancomycin, Tigecycline and Linezolid,[13] suggesting that Vancomycin, Teicoplanin and Linezolid can be considered for Gram-positive bacteria. *Staphylococcus hominis* further showed susceptibility to Clindamycin and Gentamicin. Panigrahi P et al., also observed extremely low resistance to Gentamicin and Amikacin.[10] Hence, Aminoglycosides can be considered for the treatment of *Staphylococcus hominis* in neonatal septicemia.

Table 2: Antibiotic sensitivity pattern of the Gram-positive bacterial isolates

Antibiotic	Sensitivity pattern, n (%)	
	<i>Staphylococcus hemolyticus</i> (n=1)	<i>Staphylococcus hominis</i> (n=1)
Amikacin	0 (0%)	0 (0%)
Clindamycin	0 (0%)	1 (100%)
Erythromycin	0 (0%)	0 (0%)
Ampicillin	0 (0%)	0 (0%)
Doxycycline	0 (0%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)
Vancomycin	1 (100%)	1 (100%)
Teicoplanin	1 (100%)	1 (100%)
Gentamicin	0 (0%)	1 (100%)
Linezolid	1 (100%)	1 (100%)
Minocycline	0 (0%)	0 (0%)

Conclusion:-

Klebsiella pneumoniae, *Escherichia coli* and *Acinetobacter baumannii* were the predominant Gram-negative pathogens and Coagulase-Negative Staphylococci was the predominant Gram-positive pathogen isolated in the study. Increased susceptibility to Piperacillin/Tazobactam, Meropenem and Aminoglycosides against Gram-negative bacteria and Vancomycin, Teicoplanin and Linezolid against Gram-positive bacteria was observed. Therefore, these agents may be considered as effective first-line drugs for the treatment of neonatal sepsis. The results of the study underscore the importance of ongoing surveillance of local antimicrobial resistance patterns to guide in optimal empirical therapy in neonatal sepsis.

Limitations:-

This study was conducted in a single tertiary care center with a limited sample size over a short duration, which may not fully represent broader regional or seasonal variations in pathogen distribution and antimicrobial resistance patterns.

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