

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, neonatal sepsis is the second major cause of mortality, with an incidence ranging from 14.3% to 23%. [3] Globally, of the three million annual neonatal sepsis cases, India has the highest incidence of clinical sepsis (17,000/ 1,00,000 live births). [4]

The predominant organisms isolated across various studies include, *Klebsiella pneumoniae*, *Acinetobacter* species, Coagulase-Negative Staphylococci and *Staphylococcus aureus*. [5,7]

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. High rates of multi-drug resistance are observed in *Acinetobacter* (82%), *Klebsiella* (54%) and *Escherichia coli* (38%) isolates. [6]

Materials and Methods

A prospective study was conducted for a period of 3 months from 24th February 2025 to 25th May 2025. Newborns admitted to NICU with clinical signs and symptoms suggestive of neonatal sepsis were included in the study. 100 blood culture samples were collected and processed aseptically in the Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad, India.

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 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 Pet al. [5] and 15.3% culture positivity by Siddiqui Tetal. [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 baumani* (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).

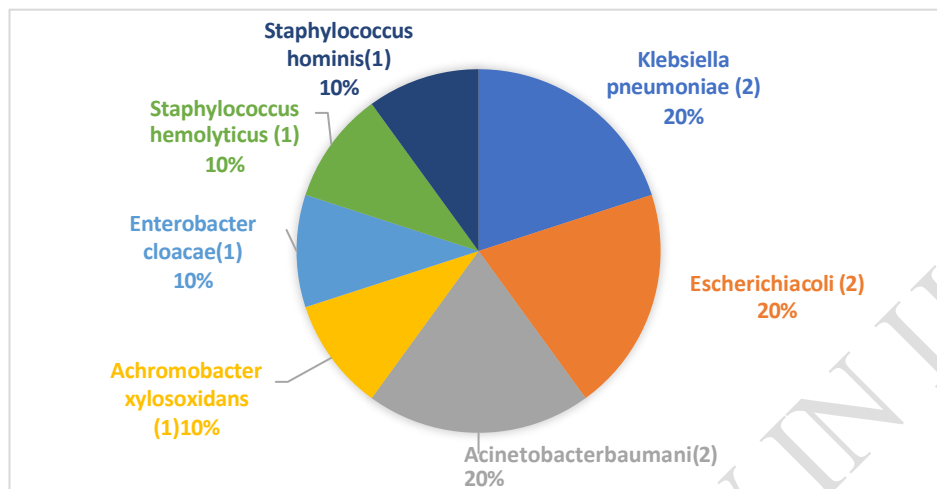


Figure 1: Type and percentage of bacterial isolates

Several other studies have also reported Gram-negative bacteria as the most frequently isolated pathogen in neonatal septicemia. [5,7,8,9] In a study of Zakariya BP et al., *Klebsiella pneumoniae* (66%) was the most common organism isolated followed by Coagulase Negative Staphylococcus (12%). [8] Another study by Jyothi P et al, concluded that *Klebsiella*, *Acinetobacter*, Coagulase Negative Staphylococcus and *Staphylococcus aureus* as the leading cause of neonatal sepsis [5], whereas, Lamba Metal.'s study revealed that Coagulase Negative Staphylococcus (17.43%) was the predominant isolate followed by *Klebsiella species* (16.11%). [10]

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* isolate to Amikacin and Meropenem, while showing resistance to other antibiotics. [8]

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 baumanii showed resistance to all drugs, as also observed in another study. [6] Antibiotic susceptibility of other isolates is mentioned in Table 1.

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

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

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%). [5] Saima Inam et al., noted 80% susceptibility of isolates to Carbapenems like Meropenem and Imipenem.[11] 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, [12] 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.[9] Hence, Aminoglycosides can be considered for the treatment of *Staphylococcus hominis* in neonatal septicemia.

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

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

Conclusion

The present study highlights the profile of bacterial pathogens responsible for neonatal sepsis and their antibiotic sensitivity patterns. *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, we recommend the use of these agents as 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 cases of neonatal sepsis.

References

1. Singh M, Alsaleem M, Gray CP. Neonatal sepsis.
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016 Feb 23;315(8):801-10.
3. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015 Jan 10;385(9963):117-71.
4. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. *Lancet Respir Med*. 2018 Mar;6(3):223-230.
5. Jyothi P, Basavaraj MC, Basavaraj PV. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. *Journal of natural science, biology, and medicine*. 2013 Jul;4(2):306.
6. Chaurasia S, Sankar MJ, Agarwal R, Yadav CP, Arya S. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. *Lancet Glob Health*. 2016;4(10):e752-60.
7. Siddiqui T, Dubey A, Kar M, Patel SS, Sahu C, Ghoshal U. Bacteriological profiles and antibiotic susceptibility of neonatal sepsis in a university hospital of Northern India. *Journal of Family Medicine and Primary Care*. 2023 Mar 1;12(3):493-8.
8. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary care hospital in South India: bacteriological profile and antibiotic sensitivity pattern. *The Indian Journal of Pediatrics*. 2011 Apr;78:413-7.
9. Panigrahi P, Chandel DS, Hansen NI, Sharma N, Kandefer S, Parida S, et al. Neonatal sepsis in rural India: timing, microbiology and antibiotic resistance in a population-based prospective study in the community setting. *Journal of Perinatology*. 2017 Aug;37(8):911-21.
10. Lamba M, Sharma R, Sharma D, Choudhary M, Maheshwari RK. Bacteriological spectrum and antimicrobial susceptibility pattern of neonatal septicaemia in a tertiary care hospital of North India. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2016 Dec 16;29(24):3993-8.
11. Inam S, Ikram S, Saeed MT, Munir S, Raza A, Inam A, et al. Spectrum and antibiotic susceptibility of Gram negative organisms associated with neonatal sepsis. *Journal of Population Therapeutics and Clinical Pharmacology*. 2024 Jan 20;31(1):1152-9.
12. Li J, Xia S, Liu Y, Zhang S, Jin Z. Bacteriological profile and antibiotic susceptibility pattern of neonatal septicemia and associated factors of ICU hospitalization days. *Infection and drug resistance*. 2022 Jan 1:427-38.