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Microbiological Profile, Antimicrobial Susceptibility Patterns, and Clinicopathological Characteristics of Neonatal Sepsis in Western Indian Population

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ABSTRACT

Background: Neonatal sepsis remains a leading cause of neonatal morbidity and mortality, particularly in low- and middle-income countries. Regional data on pathogen distribution and antimicrobial susceptibility are essential for guiding empirical antibiotic therapy and improving clinical outcomes.

Methods: This retrospective observational study included 150 neonates with clinically suspected sepsis admitted to the neonatal intensive care unit of a tertiary care teaching hospital in Western India. Clinical characteristics, laboratory findings, blood culture results, antimicrobial susceptibility patterns, treatment details, and outcomes were retrieved from hospital records. Bacterial identification and antimicrobial susceptibility testing were performed using standard microbiological methods in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Of the 150 neonates, 96 (64%) had early-onset sepsis and 54 (36%) had late-onset sepsis. Blood cultures were positive in 62 (41.3%) cases. Gram-negative bacteria accounted for 74.2% of isolates, with *Klebsiella pneumoniae* (32.3%) being the predominant pathogen, followed by *Escherichia coli* (19.4%) and *Acinetobacter baumannii* (12.9%). Respiratory distress (72%), poor feeding (68.7%), and lethargy (60%) were the most common clinical manifestations. High resistance to ampicillin and third-generation cephalosporins was observed, whereas meropenem, amikacin, piperacillin–tazobactam, vancomycin, and linezolid demonstrated good antimicrobial activity. The overall mortality rate was 16%.

Conclusion: Gram-negative organisms predominated in neonatal sepsis and exhibited substantial resistance to commonly used empirical antibiotics. Continuous microbiological surveillance and institution-specific antibiograms are essential to optimize empirical therapy, strengthen antimicrobial stewardship, and improve neonatal outcomes.

Key-words: Neonatal sepsis; Antimicrobial susceptibility; Microbiological profile; Clinicopathological characteristics; Antibiotic resistance; Western India

INTRODUCTION

Neonatal sepsis is a life-threatening systemic infection that occurs within the first 28 days of life. It remains a major cause of neonatal morbidity and mortality world-wide, particularly in low- and middle-income countries (LMICs). [1,2] Despite advances in neonatal intensive care, delayed diagnosis, limited microbiological facilities, and inappropriate antimicrobial use continue to contribute to poor neonatal outcomes. According to the World Health Organization (WHO), neonatal infections account for a substantial proportion of neonatal deaths, highlighting the need for timely diagnosis and appropriate treatment. [1,2]

Neonatal sepsis is classified as early-onset sepsis (EOS), occurring within the first 72 hours of life, and late-onset sepsis (LOS), occurring after 72 hours. EOS is primarily

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associated with vertical transmission of pathogens from the mother, whereas LOS is commonly acquired from hospital or community sources. [3,4] Prematurity, low birth weight, prolonged rupture of membranes, maternal infections, birth asphyxia, invasive procedures, and prolonged hospitalization are well-recognized risk factors for neonatal sepsis. [3,4]

The diagnosis of neonatal sepsis remains challenging because its clinical manifestations are often nonspecific, including respiratory distress, poor feeding, lethargy, temperature instability, and apnea. Although laboratory markers such as complete blood count, C-reactive protein, and procalcitonin aid in diagnosis, blood culture remains the gold standard for pathogen identification and antimicrobial susceptibility testing. [5,6]

The microbiological profile of neonatal sepsis varies across geographical regions and healthcare settings. Gram-negative organisms, including *K. pneumoniae*, *E. coli*, *A. baumannii*, and *Pseudomonas aeruginosa*, predominate in many developing countries, whereas Gram-positive organisms such as coagulase-negative staphylococci, *Staphylococcus aureus*, and Group B *Streptococcus* are more frequently reported in developed nations. [7,8] Increasing antimicrobial resistance, particularly to ampicillin and third-generation cephalosporins, has become a major challenge, emphasizing the need for continuous surveillance and institution-specific antibiotic policies. [9–11]

India continues to bear a substantial burden of neonatal sepsis, with considerable regional variation in pathogen distribution and antimicrobial susceptibility patterns. [12–14] Therefore, regular microbiological surveillance is essential to optimize empirical antibiotic therapy and strengthen antimicrobial stewardship. Moreover, clinicopathological correlation of clinical manifestations with hematological and microbiological findings may facilitate early diagnosis and improve patient outcomes.

The present retrospective observational study was undertaken to evaluate the microbiological profile, antimicrobial susceptibility patterns, and clinicopathological characteristics of neonatal sepsis in a Western Indian population. The findings are expected to provide contemporary regional evidence to guide empirical antimicrobial therapy and improve neonatal care.

MATERIALS AND METHODS

Study Design and Setting- This retrospective observational study was conducted in the Department of Pediatrics and Neonatal Intensive Care Unit (NICU) in collaboration with the Department of Microbiology and the Department of Pathology at a tertiary care teaching hospital in Western India. The study included neonates admitted with clinically suspected neonatal sepsis during the study period January 2024 to December 2025. Hospital medical records, microbiology laboratory registers, and pathology reports were reviewed to obtain relevant clinical, laboratory, and microbiological data.

Study Population- All neonates (aged ≤28 days) admitted to the NICU with a clinical diagnosis of suspected neonatal sepsis and who underwent blood culture examination were eligible for inclusion.

Inclusion Criteria

- Neonates aged ≤28 days admitted with suspected neonatal sepsis.
- Neonates with blood cultures performed before initiation of antimicrobial therapy whenever feasible.
- Availability of complete clinical, laboratory, microbiological, and treatment records.

Exclusion Criteria

- Neonates with major congenital anomalies incompatible with life.
- Neonates with incomplete medical records or missing microbiological data.
- Duplicate admissions during the same episode of illness (only the first admission was included).

Data Collection- Demographic, maternal, clinical, laboratory, microbiological, treatment, and outcome data were retrospectively extracted from the hospital medical records using a standardized data collection form. Neonatal variables included age at presentation, sex, gestational age, birth weight, mode of delivery, Apgar score, and age at onset of symptoms. Maternal risk factors, including prolonged rupture of membranes (>18 hours), maternal fever, chorioamnionitis, urinary tract infection, meconium-stained liquor, intrapartum antibiotic use, and mode of delivery, were also recorded. Clinical characteristics such as poor feeding, lethargy, respiratory distress, apnea, fever, hypothermia,

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irritability, seizures, jaundice, abdominal distension, vomiting, and shock were documented.

Laboratory parameters comprised complete blood count, total leukocyte count, absolute neutrophil count, immature-to-total neutrophil ratio, platelet count, hemoglobin concentration, C-reactive protein levels, and other relevant biochemical investigations, where available. Microbiological data included blood culture results, bacterial isolates, and antimicrobial susceptibility profiles. Clinical outcomes, including duration of neonatal intensive care unit stay, requirement for mechanical ventilation, discharge, referral, and in-hospital mortality, were also recorded for analysis.

Definitions- Neonatal sepsis was defined as the presence of clinical signs suggestive of systemic infection occurring within the first 28 days of life, supported by laboratory evidence and/or positive blood culture.

EOS was defined as sepsis occurring within the first 72 hours of life, whereas LOS was defined as sepsis occurring after 72 hours of birth. Multidrug-resistant (MDR) organisms were defined as bacterial isolates demonstrating resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Microbiological Evaluation- Blood samples were collected under strict aseptic precautions before initiation of empirical antimicrobial therapy whenever possible. Approximately 1–2 mL of venous blood was inoculated into pediatric blood culture bottles and processed according to the standard microbiological protocols followed by the institutional microbiology laboratory. Positive blood culture isolates were identified using conventional microbiological techniques and/or automated identification systems available at the study center. Organisms were identified to the species level using colony morphology, Gram staining, biochemical reactions, and automated methods where applicable.

Antimicrobial Susceptibility Testing- Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, and the results were interpreted according to the CLSI guidelines applicable during the study period. Appropriate quality control procedures were followed using standard American Type Culture Collection (ATCC) reference strains. The antimicrobial panel included commonly prescribed neonatal antibiotics such as ampicillin, amikacin, gentamicin, cefotaxime, ceftriaxone, ceftazidime, piperacillin-tazobactam, meropenem, imipenem, ciprofloxacin, vancomycin, linezolid, and colistin, depending on the bacterial isolate and institutional protocol.

Clinicopathological Correlation- Clinicopathological correlation was performed by comparing the clinical presentation with hematological and inflammatory markers, microbiological findings, and antimicrobial susceptibility profiles. Associations between isolated pathogens, laboratory abnormalities, and clinical outcomes were evaluated.

Outcome Measures- The primary outcome was to determine the microbiological profile and antimicrobial susceptibility patterns among neonates with sepsis. Secondary outcomes included distribution of bacterial pathogens causing neonatal sepsis along with frequency of multidrug-resistant organisms. Clinicopathological characteristics of neonatal sepsis and clinical outcomes, including recovery, duration of hospitalization, and mortality.

Statistical Analysis- Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version XX.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution. Categorical variables were summarized as frequencies and percentages. Comparisons between groups were performed using the Chi-square test or Fisher's exact test for categorical variables and the independent Student's *t*-test or Mann–Whitney *U* test for continuous variables, as appropriate. A *p*-value of <0.05 was considered statistically significant.

Ethical Approval- As this was a retrospective record-based study, the requirement for obtaining informed consent was waived by the Ethics Committee. Patient confidentiality and anonymity were maintained throughout the study in accordance with the principles of the Declaration of Helsinki.

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RESULTS

A total of 150 neonates with clinically suspected neonatal sepsis were included in the study. Of these, 96 (64%) had EOS, while 54 (36%) had LOS. The mean gestational age was 35.8 ± 2.6 weeks, and the mean birth weight was 2.34 ± 0.61 kg. Male neonates constituted 58% (87/150) of the study population. Preterm birth and low birth weight were observed in 54.7% and 63.3% of neonates, respectively in Table 1. Blood culture positivity was observed in 62 (41.3%) neonates. Culture positivity was significantly higher among preterm neonates than term neonates and among low birth weight neonates compared with those weighing ≥2.5 kg.

Table 1: Baseline demographic and clinicopathological characteristics of neonates with sepsis

Variable	Overall (N=150)	Culture Positive (n=62)	Culture Negative (n=88)
Male sex, n (%)	87 (58)	39 (62.9)	48 (54.5)
Preterm birth, n (%)	82 (54.7)	42 (67.7)	40 (45.5)
Low birth weight, n (%)	95 (63.3)	47 (75.8)	48 (54.5)
Early-onset sepsis	96 (64)	37 (59.7)	59 (67)
Respiratory distress	108 (72)	49 (79)	59 (67)
Poor feeding	103 (68.7)	46 (74.2)	57 (64.8)
Elevated CRP	105 (70)	55 (88.7)	50 (56.8)
Thrombocytopenia	58 (38.7)	34 (54.8)	24 (27.3)

Gram-negative bacteria accounted for 74.2% of all isolates, whereas Gram-positive organisms constituted 25.8%. *K. pneumoniae* was the predominant pathogen (32.3**%**), followed by *E. coli* (19.4**%**) and *A. baumannii* (12.9%). Among Gram-positive isolates, *S. aureus* represented 14.5%, followed by coagulase-negative *Staphylococcus* species (8.1%) in Table 2.

Table 2: Microbiological profile of culture-positive neonatal sepsis (n = 62)

Organism	Number (%)
Klebsiella pneumoniae	20 (32.3)
Escherichia coli	12 (19.4)
Acinetobacter baumannii	8 (12.9)
Pseudomonas aeruginosa	6 (9.7)
Staphylococcus aureus	9 (14.5)
Coagulase-negative *Staphylococcus*	5 (8.1)
Enterococcus spp.	2 (3.2)

Respiratory distress (72%), poor feeding (68.7%), lethargy (60%), and temperature instability (46.7%) were the most frequent presenting clinical manifestations. Elevated C-reactive protein was observed in 70% of neonates, thrombocytopenia in 38.7%, leukocytosis in 34%, and leukopenia in 12%. Elevated CRP and thrombocytopenia were significantly associated with culture-positive sepsis.

Antimicrobial susceptibility testing demonstrated marked resistance to ampicillin (18% susceptible) and cefotaxime (36% susceptible) among Gram-negative isolates. The highest susceptibility was observed with meropenem (92%), followed by amikacin (82%) and piperacillin–tazobactam (78%). All Gram-positive isolates remained susceptible to vancomycin and linezolid (100%) in Table-3. The prevalence of multidrug-resistant

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organisms was, with Gram-negative bacteria contributing the majority of resistant isolates. Overall mortality was 16% (24/150). Mortality was significantly higher among neonates with culture-positive sepsis than culture-negative sepsis. Likewise, infection with multidrug-resistant organisms was associated with a significantly increased risk of mortality.

Table 3: Antimicrobial susceptibility profile of bacterial isolates

Antibiotic	Gram-negative Susceptibility (%)	Gram-positive Susceptibility (%)
Ampicillin	18	35
Gentamicin	58	72
Amikacin	82	80
Cefotaxime	36	-
Piperacillin–Tazobactam	78	-
Meropenem	92	-
Vancomycin	-	100
Linezolid	-	100

DISCUSSION

Neonatal sepsis remains one of the leading causes of neonatal morbidity and mortality worldwide, particularly in low- and middle-income countries, despite significant advances in neonatal intensive care and antimicrobial therapy. [15,16] The present study evaluated the microbiological profile, antimicrobial susceptibility patterns, and clinicopathological characteristics of neonatal sepsis in a Western Indian population. The findings provide important regional epidemiological data that may facilitate evidence-based empirical antibiotic selection and strengthen antimicrobial stewardship practices. . [15,16]

In the present study, early-onset sepsis constituted the majority of cases. Similar observations have been reported in several studies from developing countries, where maternal and perinatal risk factors, including prolonged rupture of membranes, maternal infections, prematurity, and low birth weight, continue to contribute substantially to neonatal sepsis. [16,17] The predominance of early-onset sepsis emphasizes the importance of improving antenatal screening, intrapartum infection control, and timely administration of appropriate maternal antibiotic prophylaxis whenever indicated.

The microbiological profile observed in our study demonstrated the predominance of Gram-negative organisms, with *K. pneumoniae* being the most frequently isolated pathogen, followed by *E. coli* and *A. baumannii*. This pattern is consistent with reports from several Indian neonatal intensive care units and the Delhi Neonatal Infection Study (DeNIS), which demonstrated that Gram-negative bacilli account for the majority of culture-confirmed neonatal sepsis and are associated with increased mortality and antimicrobial resistance. [17, 18] In contrast, studies from high-income countries have frequently reported Group B *Streptococcus* and coagulase-negative staphylococci as the predominant pathogens, reflecting differences in maternal colonization, infection prevention practices, and healthcare infrastructure.[15,16]

The clinical manifestations identified in the present study, including respiratory distress, poor feeding, lethargy, temperature instability, and apnea, are in agreement with previous studies demonstrating that neonatal sepsis often presents with nonspecific clinical features. [19] Because these manifestations frequently overlap with other neonatal disorders, laboratory investigations remain indispensable for early diagnosis. Elevated C-reactive protein, abnormal leukocyte counts, and thrombocytopenia observed in our study have similarly been reported as useful adjunctive markers, although blood culture continues to remain the gold standard for definitive microbiological diagnosis. [20]

Blood culture positivity in the present study was comparable with previously published Indian studies.

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However, blood culture sensitivity in neonatal sepsis remains limited because of prior antibiotic exposure, inadequate blood sample volume, intermittent bacteremia, and delayed sample collection.[20] Recent advances in molecular diagnostic techniques may improve pathogen detection in culture-negative neonatal sepsis, although their routine availability remains limited in many resource-constrained settings. [20]

One of the most important findings of our study was the high level of antimicrobial resistance among Gram-negative isolates. Resistance to ampicillin and third-generation cephalosporins was substantial, whereas relatively higher susceptibility was observed with carbapenems, aminoglycosides, and piperacillin-tazobactam. Similar resistance patterns have been reported from neonatal intensive care units across India and other low- and middle-income countries, reflecting increasing selective pressure resulting from widespread empirical antibiotic use. [20,21] The emergence of multidrug-resistant organisms poses a major therapeutic challenge and has been associated with prolonged hospitalization, increased healthcare expenditure, treatment failure, and higher mortality. [20,21]

The preserved susceptibility of Gram-positive isolates to vancomycin and linezolid observed in the present study is encouraging and is comparable with previous reports. [5] Nevertheless, these reserve antibiotics should be used judiciously within antimicrobial stewardship programs to minimize the emergence of further resistance. Periodic revision of institutional antibiotic policies based on local antibiograms is therefore essential to optimize empirical therapy while preserving the efficacy of last-resort antimicrobial agents. [20,21]

The findings of this study have important clinical implications. Continuous surveillance of local pathogen distribution and antimicrobial susceptibility patterns should form an integral component of neonatal intensive care practice. Development of institution-specific empirical antibiotic guidelines, implementation of infection prevention bundles, strict hand hygiene, environmental decontamination, and antimicrobial stewardship programs are likely to reduce healthcare-associated infections and improve neonatal survival. [15,16]

STRENGTHS

The present study has several noteworthy strengths. It provides comprehensive data on the microbiological profile, antimicrobial susceptibility patterns, and clinicopathological characteristics of neonatal sepsis from a tertiary care teaching hospital in Western India, thereby contributing valuable region-specific epidemiological evidence. The integration of clinical, pathological, and microbiological findings enabled a holistic assessment of neonatal sepsis and facilitated meaningful clinicopathological correlation. Furthermore, the study generated a local antibiogram that may assist clinicians in selecting appropriate empirical antimicrobial therapy and support the development of institution-specific antibiotic stewardship policies. The inclusion of consecutive eligible neonates during the study period also minimized selection bias and enhanced the representativeness of the study population.

LIMITATIONS

The present study has certain limitations that should be acknowledged. First, its retrospective observational design relied on hospital records, making it susceptible to incomplete documentation and missing data. Second, the study was conducted at a single tertiary care center, which may limit the generalizability of the findings to other healthcare settings or geographic regions. Third, only bacterial pathogens identified by conventional blood culture techniques were evaluated; molecular diagnostic methods and routine fungal investigations were not available, potentially resulting in underestimation of culture-negative or non-bacterial neonatal sepsis. In addition, antimicrobial susceptibility patterns are dynamic and may change over time due to evolving resistance mechanisms and antibiotic prescribing practices. Therefore, periodic multicenter surveillance studies with larger sample sizes are warranted to validate the present findings and to provide more representative national epidemiological data.

CONCLUSIONS

In conclusion, neonatal sepsis continues to represent a major cause of neonatal morbidity and mortality in Western India. Gram-negative bacteria, particularly K. pneumoniae, were the predominant causative organisms, and a high prevalence of resistance to commonly prescribed empirical antibiotics was observed.

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The findings underscore the importance of continuous microbiological surveillance, regular monitoring of antimicrobial susceptibility patterns, and the implementation of institution-specific empirical antibiotic guidelines. Early clinical recognition, prompt microbiological diagnosis, rational antimicrobial use, and effective infection prevention and control measures remain essential to improving neonatal outcomes. Future prospective multicenter studies incorporating advanced molecular diagnostic techniques are recommended to further elucidate the evolving epidemiology of neonatal sepsis and to strengthen antimicrobial stewardship initiatives.

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