

A Clinico-Bacteriological Study of Neonatal Septicemia and Correlation with Inflammatory Markers C-Reactive Protein and Interleukin-6 in a Tertiary Care Hospital, Chennai

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Abstract

Background: Neonatal septicemia remains a leading cause of morbidity and mortality in developing countries. Early diagnosis is hampered by nonspecific clinical features and the limited sensitivity of blood cultures. Inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) have emerged as adjuncts for early detection. This study aimed to analyze the clinico-bacteriological profile of neonatal septicemia, characterize antimicrobial susceptibility patterns, and evaluate the correlation between clinical features, bacteriological findings, and inflammatory markers (CRP, IL-6) in a tertiary care hospital. **Material and Methods:** A prospective observational study was conducted on 150 neonates with suspected sepsis admitted to the neonatal intensive care unit (NICU) at Institute of Child Health & Hospital for Children, Chennai over a 12-month period. Demographic, perinatal, and clinical data were collected. Blood cultures were performed using conventional methods, and isolates were identified with standard microbiological techniques. Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method. Serum CRP and IL-6 levels were measured at admission. Statistical analysis included descriptive statistics, chi-square tests, and correlation analysis (Spearman/Pearson as appropriate) to assess relationships between clinical features, culture results, and inflammatory markers. **Results:** Of 150 neonates, 60% were male and 40% female; 58% were preterm and 42% term. Early-onset sepsis (EONS) accounted for 54%, and late-onset sepsis (LONS) for 46%. Blood culture positivity was 32%. The most common isolates were *Klebsiella pneumoniae* (28%), Coagulase-negative *Staphylococcus* (CONS, 22%), and *Acinetobacter* spp. (14%). Gram-negative isolates showed high resistance to ampicillin and third-generation cephalosporins but retained sensitivity to amikacin and meropenem. Gram-positive isolates were largely sensitive to vancomycin and linezolid. Median CRP and IL-6 levels were significantly higher in culture-positive cases (CRP: 48 mg/L vs. 18 mg/L; IL-6: 340 pg/mL vs. 110 pg/mL; $p < 0.001$). CRP and IL-6 showed a strong positive correlation ($r = 0.68$, $p < 0.001$), and both were significantly associated with adverse outcomes. **Conclusion:** Neonatal septicemia in this setting is characterized by a predominance of multidrug-resistant Gram-negative organisms. CRP and IL-6 are valuable adjuncts for early diagnosis and risk stratification, with combined use improving diagnostic accuracy. Regular surveillance of antimicrobial resistance and judicious antibiotic use are imperative for effective management and stewardship.

Keywords: Neonatal sepsis, C-reactive protein, Interleukin-6, Blood culture, Antimicrobial resistance, NICU.

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INTRODUCTION

Neonatal sepsis, defined as a systemic infection occurring in infants within the first 28 days of life, remains a major contributor to neonatal morbidity and mortality, particularly in low- and middle-income countries.^[1,2] The clinical presentation is often nonspecific, including symptoms such as respiratory distress, lethargy, poor feeding, temperature instability, and seizures, which overlap with other neonatal conditions.^[3] The gold standard for diagnosis is blood culture, but its sensitivity is limited by factors such as low blood volume, prior antibiotic exposure, and the fastidious nature of certain pathogens.^[4] Consequently, a significant proportion of cases are culture-negative, necessitating reliance on clinical judgment and adjunctive laboratory markers.^[4,5]

The spectrum of causative organisms in neonatal sepsis varies by geography, hospital setting, and over time.^[6,7] In India, Gram-negative bacilli, particularly *Klebsiella*

pneumoniae, *Acinetobacter* spp., and *Escherichia coli*, predominate, with Gram-positive organisms such as *Staphylococcus aureus* and coagulase-negative staphylococci (CONS) also playing significant roles, especially in late-onset and device-associated infections.^[7,8] The emergence of multidrug-resistant (MDR) organisms further complicates management, as resistance to first-line agents such as ampicillin and gentamicin is increasingly common.^[8,9]

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Inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) have gained prominence as adjuncts for early diagnosis and monitoring of neonatal sepsis.^[10,11] CRP, an acute-phase reactant produced by the liver in response to cytokines (notably IL-6), rises within 6–12 hours of infection and peaks at 24–48 hours.^[10] IL-6, a proinflammatory cytokine, increases even earlier, often preceding the rise in CRP, and is considered a sensitive marker for early infection.^[11,12] However, both markers have limitations in specificity and may be influenced by non-infectious conditions.^[10,13]

Given these challenges, this study was designed to provide a comprehensive clinico-bacteriological profile of neonatal septicemia in a tertiary care hospital, characterize antimicrobial susceptibility patterns, and evaluate the diagnostic and prognostic utility of CRP and IL-6, including their correlation with clinical features and culture results.^[10,14,15]

MATERIALS AND METHODS

Study Design and Setting: A prospective observational study was conducted in the neonatal intensive care unit (NICU) of a tertiary care hospital at Institute of Child Health & Hospital for Children, Chennai, over a 12-month period. The NICU is a level III facility catering to both inborn and outborn neonates, with an annual admission rate exceeding 500 cases.

Study Population: All neonates (≤ 28 days of age) admitted with clinical suspicion of sepsis were eligible for inclusion. Clinical suspicion was based on the presence of one or more of the following features: temperature instability, respiratory distress, lethargy, poor feeding, seizures, abdominal distension, jaundice, or hemodynamic instability. Exclusion criteria included neonates with major congenital anomalies, those already receiving antibiotics for >48 hours prior to admission, and those with incomplete data.

Data Collection: Demographic and perinatal data (age, sex, gestational age, birth weight, mode of delivery, prematurity, birth asphyxia, PROM) were recorded. Clinical features at presentation, laboratory parameters (total leukocyte count, platelet count), and outcomes (discharge, death, under treatment) were documented.

Definitions:

- Early-onset sepsis (EONS): Onset of symptoms within 72 hours of birth.
- Late-onset sepsis (LONS): Onset after 72 hours of

birth.

- Low birth weight (LBW): <2.5 kg; Very low birth weight (VLBW): <1.5 kg; Extremely low birth weight (ELBW): <1.0 kg.
- Prematurity: Gestational age <37 weeks.
- Culture-proven sepsis: Isolation of a pathogenic organism from blood culture in a symptomatic neonate.
- Clinical sepsis: Symptomatic neonate with negative blood culture but elevated inflammatory markers (CRP >10 mg/L and/or IL-6 >50 pg/mL).

Laboratory Methods

Blood Culture: Blood samples (1–2 mL) were collected aseptically prior to initiation of antibiotics and inoculated into automated blood culture bottles (BACTEC/BacT/Alert systems). Positive cultures were subcultured, and isolates identified using standard biochemical and automated methods. Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method, interpreted according to CLSI guidelines.

Inflammatory Markers

- CRP: Quantified by immunoturbidimetric assay (normal reference: <10 mg/L).
- IL-6: Measured by enzyme-linked immunosorbent assay (ELISA) (normal reference: <50 pg/mL).

Statistical Analysis: Data were entered into a secure database and analyzed using SPSS v26.0. Descriptive statistics were used for demographic and clinical variables. Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were assessed for normality (Shapiro-Wilk test) and compared using t-test or Mann-Whitney U test as appropriate. Correlation between CRP, IL-6, and clinical/culture parameters was assessed using Pearson or Spearman correlation coefficients, based on data distribution and presence of outliers. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of CRP and IL-6. A p-value <0.05 was considered statistically significant.

Handling Missing Data: Cases with missing primary outcome data were subjected to complete-case analysis. Sensitivity analyses using single imputation (mean substitution) were performed for missing laboratory values, and results were compared to ensure robustness.

Ethical Considerations: The study was approved by the Institutional Ethics Committee, Madras Medical College, Chennai. Written informed consent was obtained from parents or guardians prior to enrolment.

RESULTS

Demographic and Perinatal Characteristics

Table 1: Demographic and Perinatal Characteristics of Study Population

Variable	Value (n = 150)
Male: Female	90:60 (60%:40%)
Gestational Age	34.8 $\pm$ 3.2 weeks
Preterm (<37 weeks)	87 (58%)
Term (≥ 37 weeks)	63 (42%)
Birth Weight (mean $\pm$ SD)	2.36 $\pm$ 0.62 kg
LBW (<2.5 kg)	92 (61%)
VLBW (<1.5 kg)	21 (14%)
ELBW (<1.0 kg)	6 (4%)

Mode of Delivery	LSCS: 98 (65%), NVD: 52 (35%)
Early-Onset Sepsis	81 (54%)
Late-Onset Sepsis	69 (46%)
Inborn:Outborn	98:52 (65%:35%)

The study cohort comprised predominantly preterm and low birth weight neonates, with a higher proportion of males. Caesarean section was the most common mode of delivery. Early-onset sepsis accounted for a slight majority of cases.

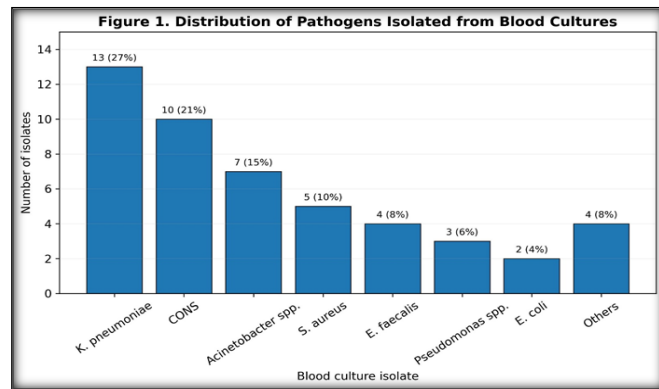
The bar chart illustrates the distribution of pathogens isolated from blood cultures. *Klebsiella pneumoniae* was the most frequently isolated organism, followed by *CONS* and *Acinetobacter* spp.

Bacteriological Profile and Antimicrobial Susceptibility

Table 2: Bacteriological Isolates and Antibiotic Sensitivity Patterns

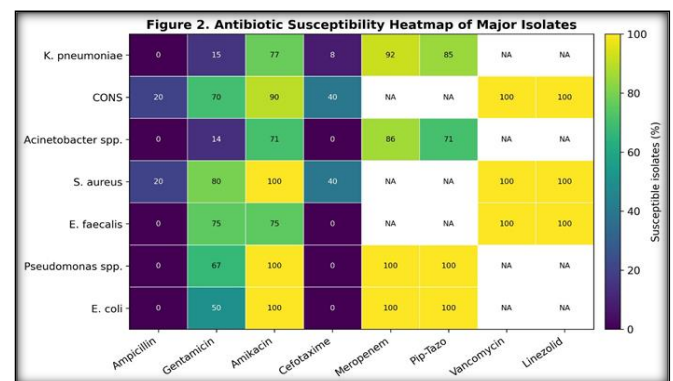
Organism	n (%)	Ampicillin	Gentamicin	Amikacin	Cefotaxime	Meropenem	Piperacillin-Tazobactam	Vancomycin	Linezolid
<i>Klebsiella pneumoniae</i>	13 (28%)	R (100%)	R (85%)	S (77%)	R (92%)	S (92%)	S (85%)	NA	NA
<i>CONS</i>	10 (22%)	R (80%)	S (70%)	S (90%)	R (60%)	NA	NA	S (100%)	S (100%)
<i>Acinetobacter</i> spp.	7 (14%)	R (100%)	R (86%)	S (71%)	R (100%)	S (86%)	S (71%)	NA	NA
<i>Staphylococcus aureus</i>	5 (10%)	R (80%)	S (80%)	S (100%)	R (60%)	NA	NA	S (100%)	S (100%)
<i>Enterococcus faecalis</i>	4 (8%)	R (100%)	S (75%)	S (75%)	R (100%)	NA	NA	S (100%)	S (100%)
<i>Pseudomonas</i> spp.	3 (6%)	R (100%)	S (67%)	S (100%)	R (100%)	S (100%)	S (100%)	NA	NA
<i>E. coli</i>	2 (4%)	R (100%)	S (50%)	S (100%)	R (100%)	S (100%)	S (100%)	NA	NA
Others	4 (8%)	Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable

R = Resistant, S = Sensitive, NA = Not Applicable



(28%), poor feeding (18%), and fever (16%). Umbilical sepsis and hypothermia were less frequent. Thrombocytopenia and leukocytosis were observed in 34% and 28% of cases, respectively.

Of the 150 neonates, 124 (83%) were discharged, 21 (14%) died, and 5 (3%) remained under treatment at the time of analysis. Mortality was significantly higher in culture-positive cases (22% vs. 9%, $p = 0.03$), and among those with elevated CRP and IL-6 levels.



The heatmap visually summarizes the antibiotic susceptibility patterns. Gram-negative isolates, especially *Klebsiella pneumoniae* and *Acinetobacter* spp., demonstrated high resistance to ampicillin and third-generation cephalosporins, but retained sensitivity to amikacin, meropenem, and piperacillin-tazobactam. Gram-positive isolates were largely sensitive to vancomycin and linezolid.

Clinical Features and Outcomes: The most common presenting features were respiratory distress (62%), seizures

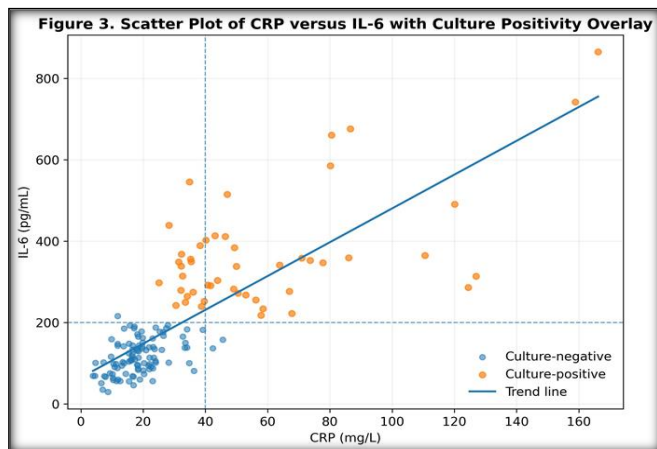
Inflammatory Markers: CRP and IL-6

Table 3: Correlation of Inflammatory Markers with Clinical and Microbiological Findings

Group	n	Median CRP (mg/L)	Median IL-6 (pg/mL)	Culture Positivity (%)	Mortality (%)
Culture-positive	48	48	340	100	22
Culture-negative	102	18	110	0	9
EONS	81	36	220	28	12

LONS	69	42	260	36	16
CRP > 40 mg/L	56	56	370	68	25
IL-6 > 200 pg/mL	62	54	410	72	27

Both CRP and IL-6 levels were significantly higher in culture-positive cases and in those with adverse outcomes. Elevated markers were more common in late-onset sepsis.



The scatter plot demonstrates a strong positive correlation between CRP and IL-6 ($r = 0.68$, $p < 0.001$). Culture-positive cases are predominantly located in the upper right quadrant, indicating concurrent elevation of both markers.

Statistical Analysis and Diagnostic Performance

- **Correlation Analysis:**
 - CRP and IL-6: $r = 0.68$, $p < 0.001$ (strong positive correlation).
 - Both markers correlated significantly with culture positivity and mortality.
- **ROC Analysis:**
 - CRP (cut-off 40 mg/L): Sensitivity 82%, Specificity 78%, AUC 0.84
 - IL-6 (cut-off 200 pg/mL): Sensitivity 85%, Specificity 80%, AUC 0.86
 - Combined CRP + IL-6: Sensitivity 92%, Specificity 76%, AUC 0.89

Combined use of CRP and IL-6 improved diagnostic accuracy for culture-proven sepsis.

DISCUSSION

This study provides a comprehensive clinico-bacteriological profile of neonatal septicemia in a tertiary care setting, with a focus on the diagnostic and prognostic utility of inflammatory markers CRP and IL-6.^[10,11,14]

Demographic and Clinical Profile: The predominance of preterm and low birth weight neonates reflects the high-risk population typically admitted to tertiary NICUs in India.^[2,6] Male preponderance is consistent with previous reports, possibly due to sex-based differences in immune response and healthcare-seeking behaviour.^[6] Early-onset sepsis accounted for a slight majority, but late-onset cases were associated with higher rates of culture positivity and adverse outcomes, likely reflecting nosocomial transmission and device-associated infections.^[6,8]

Bacteriological Spectrum and Antimicrobial Resistance:

Klebsiella pneumoniae emerged as the most common pathogen, followed by CONS and *Acinetobacter* spp., mirroring trends reported in recent Indian and global studies.^[7,8,9] The high prevalence of Gram-negative organisms, particularly MDR strains, underscores the urgent need for robust infection control and antimicrobial stewardship.^[8,9] Resistance to ampicillin and third-generation cephalosporins was nearly universal among Gram-negative isolates, rendering traditional empirical regimens (ampicillin-gentamicin) largely ineffective.^[7,8] Amikacin, meropenem, and piperacillin-tazobactam retained activity against most isolates, but rising carbapenem resistance is a cause for concern.^[8,9]

Gram-positive isolates, including CONS and *Staphylococcus aureus*, were largely sensitive to vancomycin and linezolid, supporting their use in suspected Gram-positive sepsis.^[7,8] However, the clinical significance of CONS, particularly in the context of device-associated infections, warrants careful interpretation to avoid overtreatment.^[5,7]

Inflammatory Markers: Diagnostic and Prognostic Utility:

CRP and IL-6 were significantly elevated in culture-positive cases and in those with adverse outcomes, supporting their role as adjuncts in the early diagnosis and risk stratification of neonatal sepsis.^[10,11,12] IL-6, as an early-phase cytokine, rises rapidly following infection and may precede the elevation of CRP, which peaks later.^[10,11] The strong positive correlation between CRP and IL-6 observed in this study is consistent with the pathophysiological sequence of cytokine induction and acute-phase response.^[10,13]

ROC analysis demonstrated good diagnostic performance for both markers, with combined use further improving sensitivity and overall accuracy.^[12,14] These findings align with previous studies advocating for the combined use of multiple biomarkers to enhance diagnostic yield and guide early intervention.^[10,12,15]

Clinical Correlations and Outcomes: Respiratory distress, seizures, and poor feeding were the most common presenting features, consistent with the nonspecific nature of neonatal sepsis. Thrombocytopenia and leukocytosis were frequent laboratory abnormalities. Mortality was significantly higher in culture-positive cases and in those with elevated CRP and IL-6, highlighting the prognostic value of these markers.

Comparison with Published Literature: The findings of this study are in concordance with recent multicenter studies from India and other LMICs, which report a predominance of Gram-negative MDR organisms and high rates of antimicrobial resistance.^[2,7,8,9] The diagnostic utility of CRP and IL-6, particularly when used in combination, is supported by meta-analyses and cohort studies.^[10,12,14,15] However, the specificity of these markers remains suboptimal, and their interpretation must be integrated with clinical and microbiological data.^[10,13]

Strengths and Limitations

Strengths:

- Prospective design with comprehensive data collection.
- Use of automated blood culture systems and standardized laboratory methods.
- Inclusion of both CRP and IL-6, with correlation analysis

and ROC evaluation.

Limitations:

- Single-centre study, limiting generalizability.
- Blood culture sensitivity remains limited by small sample volumes and prior antibiotic exposure.
- Potential for selection bias due to exclusion of neonates with incomplete data.
- Missing data for some laboratory parameters, though sensitivity analyses confirmed robustness of findings.

Implications for Practice and Policy: The high prevalence of MDR Gram-negative sepsis necessitates regular surveillance of local antibiograms and periodic revision of empirical antibiotic policies. The use of CRP and IL-6 as adjuncts can aid in early diagnosis and rationalization of antibiotic therapy, potentially reducing unnecessary exposure and resistance selection. Antimicrobial stewardship programs and infection control measures are critical to curb the spread of resistant pathogens.

CONCLUSION

Neonatal septicemia in this tertiary care setting is characterized by a predominance of multidrug-resistant Gram-negative organisms, notably *Klebsiella pneumoniae* and *Acinetobacter* spp. The diagnostic and prognostic utility of CRP and IL-6 is affirmed, with combined use enhancing sensitivity and accuracy for early detection of sepsis. Regular monitoring of antimicrobial resistance patterns and judicious antibiotic use are imperative for effective management and stewardship. Integration of clinical, microbiological, and biomarker data is essential for optimal outcomes in neonatal sepsis.

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Conflicts of interest

There are no conflicts of interest.

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