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Original Research Article

A Study on the Bacteriological Spectrum and Diagnostic Utility of Acute Phase Markers in Neonatal Septicaemia

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Abstract

Background: Neonatal sepsis remains a major cause of morbidity and mortality in developing countries and requires timely diagnosis and appropriate antimicrobial therapy.

Objectives: To determine the bacteriological spectrum and antimicrobial-resistance pattern of neonatal sepsis and to assess the diagnostic utility of C-reactive protein (CRP) and Procalcitonin (PCT).

Methods: A hospital-based cross-sectional observational study was conducted among 110 neonates with suspected sepsis admitted to the NICU and SNCU of Bankura Sammilani Medical College & Hospital (March 2024–August 2025). Blood cultures were processed using the BACT/ALERT 3D system, isolates were identified by the Vitek 2 Compact System, and antimicrobial susceptibility testing was performed as per CLSI 2023 guidelines. Serum C-reactive protein (CRP) and procalcitonin (PCT) levels were measured by immunoturbidimetric and ELFA methods, respectively. Diagnostic performance was assessed using ROC curve analysis.

Results: Of 110 neonates evaluated, 33 (30.0%) were culture-positive. Late-onset sepsis accounted for the majority of cases (69.7%). Gram-positive bacteria constituted 51.5% of isolates, predominantly Staphylococcus aureus and coagulase-negative staphylococci, while Gram-negative bacilli accounted for 21.2%. Fungal isolates (Candida species) represented 27.3% of cases. Gram-positive isolates showed complete sensitivity to vancomycin and linezolid, whereas Gram-negative organisms demonstrated multidrug resistance. ROC analysis showed good diagnostic accuracy for CRP (AUC = 0.87) and excellent accuracy for PCT (AUC = 0.98). At optimal cut-offs (CRP ≥14.27 mg/L; PCT ≥1.77 ng/mL), PCT demonstrated higher sensitivity and specificity than CRP.

Conclusion: Gram-positive cocci were the predominant bacterial pathogens, with a notable proportion of fungal sepsis among neonates. Procalcitonin proved to be a more reliable biomarker than CRP for early diagnosis of neonatal septicaemia. Integration of biomarker assessment with microbiological surveillance may facilitate early detection, guide rational antimicrobial therapy, and improve management of neonatal sepsis.

Keywords: Neonatal septicaemia; acute phase markers; CRP; Procalcitonin; Blood culture; Bacteriological profile.

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Introduction

Neonatal sepsis is a bloodstream infection occurring in infants up to 28 days of age and remains a major contributor to neonatal morbidity and mortality worldwide. [1] Bacteraemia refers to the transient or persistent presence of bacteria in the bloodstream, whereas septicaemia denotes systemic dissemination of microorganisms accompanied by clinical manifestations. [2]

In developing countries, neonatal sepsis accounts for approximately 30–50% of total neonatal deaths, reflecting persistent gaps in early diagnosis and effective management. [3] Based on the time of onset, neonatal sepsis is classified into early-onset sepsis (EOS) and late-onset sepsis (LOS). [4] EOS typically presents within the first 72 hours of life and is primarily caused by organisms acquired from the maternal genital tract. [5] Major risk factors include prolonged rupture of membranes (>12 hours), prolonged labour (>24 hours), maternal febrile illness within two weeks prior to delivery, prematurity (<36 weeks), low birth weight (<2.5 kg), impaired immunity, and the need for resuscitation procedures. [6]

In contrast, LOS develops after 72 hours of life and is usually associated with nosocomial or community-acquired infections. Predisposing

factors for LOS include prematurity, low birth weight, invasive procedures, prolonged hospitalization, and extended neonatal intensive care unit stay. [7]

The incidence of neonatal sepsis varies considerably across geographical regions. In industrialized nations, the reported incidence ranges from 0.3 to 0.8 per 1,000 live births for EOS and approximately 6 per 1,000 live births for LOS, whereas substantially higher rates are observed in developing countries. [8] Clinical manifestations are often subtle and non-specific, including respiratory distress, temperature instability, bradycardia, lethargy, poor feeding, and cyanosis, which frequently delay recognition and initiation of appropriate therapy. [9]

Blood culture remains the gold standard for the identification of causative organisms and for guiding antimicrobial therapy. However, it is time-consuming, requires technical expertise, and may yield false-negative results due to prior antibiotic exposure or low-level bacteraemia. [10-11] consequently, there is increasing reliance on rapid and reliable biomarkers for early diagnosis. Acute phase reactants such as C-reactive protein (CRP) and procalcitonin (PCT) have emerged as useful adjuncts in the early detection and monitoring of neonatal sepsis. [12-13]

In view of regional variations in bacteriological profiles and antimicrobial resistance patterns, as well as the need for timely diagnosis, continuous evaluation of diagnostic strategies is essential. The present study was undertaken to determine the bacteriological spectrum and to assess antibiotic susceptibility pattern, and diagnostic utility of CRP and PCT in neonatal septicaemia at a tertiary-care hospital in West Bengal.

Materials and Methods

A hospital-based cross-sectional observational study was conducted in the Departments of Microbiology and Paediatrics, Bankura Sammilani Medical College & Hospital, from March 2024 to August 2025. Neonates with clinical suspicion of sepsis admitted to the Neonatal Intensive Care Unit (NICU) and Sick Newborn Care Unit (SNCU) were included after obtaining written informed consent from parents or guardians. The sample size was calculated using the standard single-proportion formula and finalized at 110 after adjusting for possible non-response and sample loss.

Under strict aseptic precautions, 1–2 mL of venous blood was collected and inoculated into BACT/ALERT 3D/60 culture bottles and incubated for up to seven days. Bottles showing positivity were subcultured on blood agar, MacConkey agar, and nutrient agar, followed by incubation at 37 °C for 18–24 hours. Bacterial isolates were identified by colony morphology, Gram staining, standard biochemical tests, and confirmed using the Vitek 2 Compact System (bioMérieux). Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method and automated Vitek 2 system in accordance with Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.

Serum C-reactive protein level was estimated by immunoturbidimetric assay, and procalcitonin levels were measured by enzyme-linked fluorescent assay. Demographic, clinical, and laboratory data were recorded in a structured proforma. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 24.0. Normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were performed using Mann–Whitney U test for continuous variables. Receiver operating characteristic curve analysis was used to evaluate diagnostic performance, and optimal cut-off values were determined using the Youden index. Sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy were calculated. A p-value <0.05 was considered statistically significant. Ethical approval was obtained from the Institutional Ethics Committee of Bankura Sammilani Medical College prior to study initiation.

Results

Out of 110 neonates evaluated for suspected septicaemia, 33 (30.0%) were confirmed as culture-positive. Early-onset sepsis constituted 30% of the proven cases, while late-onset sepsis accounted for 70%. Male neonates were predominant; however, culture positivity did not differ significantly between sexes. Low-birth-weight infants (<2.5 kg) represented 27% of the confirmed cases. The distribution of neonatal septicaemia cases and bacterial isolates is summarized in Table 1.

Microbiological analysis showed that Gram-positive cocci constituted the majority of isolates (51.5%), with Staphylococcus aureus and coagulase-negative staphylococci being the predominant organisms. Gram-negative bacilli accounted for 21.2% of isolates and included Burkholderia cepacia complex, Acinetobacter baumannii, and Klebsiella pneumoniae. Rest 27.3% were fungal isolates (Table 1).

Haematological and biomarker analysis revealed abnormal total leukocyte counts in 66% of culture-positive neonates. Elevated C-reactive protein levels were observed in 97% of proven cases, while raised procalcitonin levels were detected in 96%. Culture-positive neonates had significantly higher CRP, PCT, and WBC counts compared to culture-negative neonates ($p < 0.001$). No significant difference was observed with respect to birth weight and gestational age ($p > 0.05$) (Table 2).

Antimicrobial susceptibility testing revealed that all Gram-positive isolates were uniformly sensitive to vancomycin and linezolid. In contrast, high levels of resistance were observed against amoxiclav, gentamicin, and macrolides. Gram-negative isolates demonstrated multidrug resistance, particularly to beta-lactam antibiotics and carbapenems. The antimicrobial susceptibility patterns of the major isolates are detailed in Table 3.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of CRP and PCT in predicting culture-proven neonatal sepsis. Procalcitonin demonstrated excellent diagnostic performance with an area under the curve (AUC) of 0.98 [Figure 1(a)], while CRP showed good diagnostic accuracy with an AUC of 0.87 [Figure 1(b)]. At an optimal cut-off value of ≥1.77 ng/mL, PCT showed a sensitivity of 90.9%, specificity of 98.7%, positive predictive value of 96.8%, negative predictive value of 96.2%, and overall diagnostic accuracy of 96.4%.

At a cut-off value of ≥14.27 mg/L, CRP demonstrated a sensitivity of 81.8%, specificity of 100%, positive predictive value of 100%, negative predictive value of 92.8%, and diagnostic accuracy of 94.5%. These findings indicate superior diagnostic utility of PCT over CRP for early detection of neonatal septicaemia.

Table 1: Distribution of neonatal septicaemia cases and bacterial isolates (n = 33)

Parameter	Category	Number (%)
Type of Sepsis	Early-Onset (≤ 72 h)	10 (30.3)
	Late-Onset (> 72 h)	23 (69.7)
Sex Distribution	Male	18 (54.5)
	Female	15 (45.5)
Organisms Isolated	Staphylococcus aureus	7 (21.2)
	CoNS	7 (21.2)
	Enterococcus spp.	3 (9.1)
	Burkholderia cepacia	3 (9.1)
	Acinetobacter baumannii	3 (9.1)
	Klebsiella pneumoniae	1 (3.0)
	Candida species	9 (27.3)

Table 2: Comparison of variables between culture positive vs culture negative cases (n = 110)

Parameter	Median		Mann-Whitney U, p-value
	Culture negative	Culture positive	
Birth weight (kg)	2.40	2.44	1200.5, 0.650
Gestational age (weeks)	35.50	36.00	1192, 0.611
CRP (mg/L)	7.58	22.39	319.5, <0.001
PCT (ng/mL)	0.98	3.27	62.5, <0.001
WBC count (cells/µL)	11035	18685	79.0, <0.001

Table 3: Antimicrobial susceptibility pattern of major isolates

Organism	Most Sensitive Antibiotics (%)	Major Resistant Antibiotics (%)
S. aureus	Vancomycin (100), Linezolid (100), Gentamicin (82)	Penicillin (100), Erythromycin (100)
CoNS	Vancomycin (100), Linezolid (100)	Penicillin (100), Levofloxacin (100)
Enterococcus spp.	Linezolid (100), Vancomycin (100) Gentamycin (100)	No resistance found
Burkholderia cepacia	Meropenem (100), Levofloxacin (100)	Imipenem (70), Amikacin (80), Ceftazidime, Cefepime
Acinetobacter baumannii	Meropenem, Imipenem, Tigecycline	Carbapenems (60), Gentamicin (70)
K. pneumoniae	Amikacin (80), Meropenem (70), Tigecycline	Cefotaxime, Ampicillin (100)

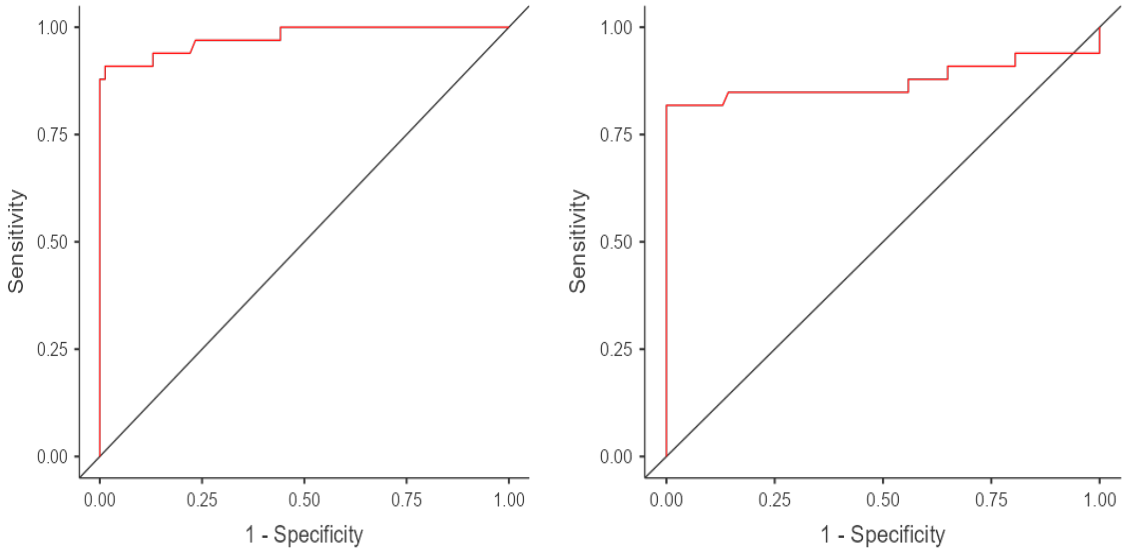

Figure 1(a,b): ROC curve for (a) Procalcitonin and (b) C - reactive protein

Discussion

Neonatal sepsis continues to be a major contributor to neonatal morbidity and mortality, particularly in developing countries where early diagnosis remains challenging due to nonspecific clinical manifestations. In the present study, late-onset sepsis constituted the majority of culture-positive cases (69.7%). Similar finding had been reported by Ali et al. where healthcare-associated factors such as invasive procedures, prolonged hospitalization, and prematurity increase the risk of late-onset infections. [14] Male predominance and low-birth-weight association corresponded with previous observations by Herz et al. and Belachew & Tewabe. [15–16]

The bacteriological spectrum in the present study showed predominance of Gram-positive organisms (51.5%), mainly Staphylococcus aureus and coagulase-negative staphylococci. These organisms are frequently associated with hospital-acquired infections and the use of indwelling devices in neonatal intensive care units. Comparable pattern had been reported by Kariniotaki et al., indicating a shift toward Gram-positive pathogens in neonatal sepsis. [17] However, geographic variability exists, and several investigations have reported Gram-negative organisms such as Klebsiella and Acinetobacter as predominant pathogens in neonatal units.

Gram-negative bacilli accounted for 21.2% of isolates in our study and included Burkholderia cepacia, Acinetobacter baumannii, and Klebsiella pneumoniae. These organisms are well recognized causes of nosocomial infections and are frequently associated with multidrug resistance. The resistance patterns observed in the present study are consistent with recent surveillance reports highlighting increasing antimicrobial resistance among Gram-negative pathogens in neonatal intensive care settings.

An important observation in this study was the notable proportion of fungal isolates, with Candida species accounting for 27.3% of culture-positive cases. Fungal sepsis has been reported in neonates, particularly among preterm infants and those receiving prolonged broad-spectrum antibiotics or intensive care support as seen in study by Samad et al. [18] This finding underscores the importance of considering fungal pathogens in suspected cases of neonatal sepsis.

Evaluation of biomarkers demonstrated significantly higher CRP, PCT, and WBC counts among culture-positive neonates. ROC curve analysis showed that procalcitonin had excellent diagnostic performance (AUC = 0.98), whereas CRP demonstrated good accuracy (AUC = 0.87). Similar findings have been reported in recent systematic review by Sundara et al. showing that procalcitonin has higher sensitivity and specificity than CRP for early detection of neonatal sepsis. [19] Although CRP remains widely used because of its accessibility and cost-effectiveness, combining CRP with procalcitonin and hematological parameters may improve diagnostic accuracy and facilitate early targeted therapy.

Overall, the present study highlights the importance of continuous microbiological surveillance, biomarker-guided diagnosis, and rational antibiotic stewardship in neonatal intensive care units to improve early detection and management of neonatal sepsis.

Conclusion

The present study demonstrates that late-onset neonatal sepsis predominates in the studied population, with Gram-positive cocci—particularly

Staphylococcus aureus and coagulase-negative staphylococci—being the leading bacterial pathogens. A notable proportion of cases were also attributable to fungal organisms, highlighting the emerging significance of fungal sepsis in neonatal intensive care settings. Gram-positive isolates retained complete susceptibility to vancomycin and linezolid, whereas Gram-negative bacilli exhibited considerable multidrug resistance, underscoring the need for continuous antimicrobial surveillance and rational antibiotic policies.

Procalcitonin showed superior diagnostic performance compared with C-reactive protein, demonstrating higher sensitivity and specificity for early detection of neonatal septicaemia. Incorporation of biomarker-guided diagnostic strategies alongside conventional microbiological methods may facilitate earlier diagnosis, optimize targeted therapy, and reduce unnecessary antibiotic exposure. Strengthening infection control measures, maintaining updated local antibiograms, and conducting multicentric studies will be essential for improving neonatal sepsis management and reducing associated morbidity and mortality.

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