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Original Article

Bacterial isolates from Neonatal Septicemia with antimicrobial susceptibility and correlation with CRP and Procalcitonin

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ABSTRACT

Introduction: Neonatal sepsis still remains a major contributor to illness and death in newborns globally around 25%, with the majority occurring in developing nations.

Materials And Methods: Blood samples from clinically diagnosed Neonatal sepsis were collected and Blood cultures done as per the standard protocol, Antibiotic susceptibility testing by Kirby-Bauer disc diffusion and interpretation as per CLSI guidelines. CRP estimation using the CRP-latex (slide agglutination) test kit from Beacon Diagnostics and PCT with Elecsys BRAHMS PCT assay from Roche Diagnostics by using electrochemiluminescence immunoassay (ECLIA)cobase immunoassay analyzers.

Results: Out of 100 blood samples, 42 were culture positives and male neonates were predominant (57.1%), preterm neonates (69%), early onset sepsis 76.2%. Klebsiella pneumoniae were predominant (31.1%) followed by Acinetobacter baumannii (21.4%), Escherichia coli (19%), Coagulase Negative Staphylococcus (16.66%) and Staphylococcus aureus (11.9%). Gram positive cocci were more sensitive to Tigecycline, Clindamycin, Linezolid and Teicoplanin. Among Gram negative bacilli, Klebsiella pneumoniae and Escherichia coli were sensitive to Ceftazidime avibactam and Acinetobacter baumannii to Amikacin and Piperacillin-Tazobactam. Among Klebsiella pneumoniae 64.7% Extended-Spectrum Beta-Lactamases and 55.6% carbapenem resistant and in Escherichia coli 35.29% and 44.4% respectively. 35 were culture positive out of 56 CRP-positive and 7 of CRP negatives. CRP showed 83.3% sensitivity, 63.7% specificity, 62.5% PPV, and 84.09% NPV. 65 samples were PCT-positive, of which 38 were culture positive.

Conclusion: While Blood culture is considered the gold standard as it takes longer time, biomarkers such as CRP and PCT are crucial in initiating early treatment.

Keywords: *Neonatal Septicemia, ESBL, CRE, CRP, Procalcitonin.*

INTRODUCTION

Neonatal sepsis still remains a major contributor to illness and death in newborns globally.
It accounts for nearly 25% of neonatal deaths globally, with the majority occurring in developing nations. According to the Sample Registration System (SRS) Statistical Report of 2020, India witnessed notable progress in reducing the Neonatal Mortality Rate (NMR), decreasing from 22 deaths per 1,000 live births in 2019 to 20 in 2020. (1,2)

It is generally categorized into early-onset septicemia (EOS), which develops during the initial 72 hours after birth, and late-onset septicemia (LOS), which develops beyond the first 72 hours of life. Early-onset sepsis (EOS) mainly stems from infections acquired from the mother's genital tract and often shows signs like breathing difficulties or pneumonia in

newborns. Late-onset sepsis (LOS) can originate either from hospital environments (nosocomial) or from the community and it typically presents as bloodstream infections, pneumonia, or inflammation of the meninges (meningitis). (3,4)

The definitive method for diagnosing neonatal sepsis is the isolation of microorganisms from blood samples. Recent trends indicate a rise in infections caused by Coagulase-Negative Staphylococcus (CONS). Increased prevalence of Extended-Spectrum Beta-Lactamases (ESBLs), Methicillin-Resistant Staphylococcus aureus (MRSA) and multidrug-resistant (MDR) strains is a cause of concern in Neonatal Intensive Care units (NICU) globally. (1)

As blood culture results take a longer time from 3 to 7 days, with the increased risk of morbidity and mortality in neonatal sepsis, there is a need for faster and more accurate ways to diagnose this condition. Recently, screening of serological markers such as C-Reactive Protein (CRP), Procalcitonin (PCT), and various cytokines has been suggested for early diagnosis of sepsis in neonates.

The knowledge about the bacteriological profile and its antibiotic sensitivity pattern is extremely beneficial in saving the lives of newborns with septicemia. In suspected cases of clinical septicemia, it is crucial to begin appropriate empirical therapy and once the culture and sensitivity results are obtained, antibiotic therapy should be re-evaluated. (1,5)

The present study was done to know the common bacterial agents causing neonatal sepsis, evaluate their antibiotic susceptibility patterns and assess the role of serological markers such as CRP and PCT in cases of neonatal septicemia.

MATERIALS AND METHODOLOGY

Blood samples from clinically diagnosed Neonatal sepsis cases were collected for culture, CRP and procalcitonin analysis. Isolation and identification of organism was done as per the standard protocol in the laboratory. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and interpretation as per CLSI guidelines. CRP estimation was done using the CRP-latex (slide agglutination) test kit from Beacon Diagnostics.PCT estimation was done using Elecsys BRAHMS PCT assay from Roche Diagnostics. The electrochemiluminescence immunoassay (ECLIA) was intended for use on cobas e immunoassay analyzers.

RESULTS

All the blood samples were processed for culture sensitivity and CRP and PCT tests were performed from November 2023 to April 2025 at the Department of Microbiology, Government Medical College, Srikakulam.

Out of 100 blood samples, 42 were culture positives and among the culture positives, male neonates were predominant (57.1%), preterm neonates (69%), early onset sepsis accounts for 76.2% and Positive blood cultures were more among neonates weighing less than 1 kg (30.95%), followed by those weighing 1.5-2.5kg (28.57%).

Out of 42 positive samples, Klebsiella pneumoniae were the predominant isolates (31.1%) followed by Acinetobacter baumannii (21.4%), Escherichia coli (19%), Coagulase Negative Staphylococcus (16.66%) and Staphylococcus aureus (11.9%), respectively.

Staphylococcus aureus was 100% sensitive to Clindamycin and Tigecycline, followed by Linezolid (80%) and Teicoplanin (80%). Staphylococcus aureus was 100% resistant to Penicillin and showed 80% resistance to both Cefoxitin and Erythromycin. Coagulase-negative Staphylococcus showed 100% sensitivity to Tigecycline and Teicoplanin, followed by 85.71% to Clindamycin, Vancomycin, Linezolid and 71.42% to Ciprofloxacin. Coagulase-negative Staphylococcus was 100% resistant to Penicillin, followed by 71.42% to Erythromycin and 42.85% to resistant to Gentamicin and Cefoxitin. 80% of Staphylococcus aureus were methicillin-resistant.

Klebsiella pneumoniae were 92.3% sensitive to Ceftazidime avibactam followed by 76.9% sensitive to Levofloxacin, and 69.23% sensitive to Gentamicin, Imipenem and Colistin. Acinetobacter baumannii showed 88.8% sensitivity to Amikacin and Piperacillin Tazobactam, followed by 87.5% to Gentamicin, Levofloxacin and Imipenem. Escherichia coli showed 87.5% sensitivity to Ceftazidime avibactam followed by 75% to Amikacin and Colistin and 62.5% to Levofloxacin, Gentamicin and Ceftazidime.

Out of 21 isolates of Enterobacteriaceae, 64.7% of Klebsiella pneumoniae and 35.29% of Escherichia coli have produced Extended-Spectrum Beta-Lactamases (ESBLs) and 55.6% of Klebsiella pneumoniae and 44.4% of Escherichia coli were carbapenem resistant.

Out of 100 samples, 56 were CRP-positive, out of which 35 were also positive for blood culture. Among the 44 CRP-negative samples, 7 were positive for blood culture. CRP showed 83.3% sensitivity, 63.7% specificity, 62.5% positive predictive value, and 84.09% negative predictive value.

Out of 100 samples, 65 were PCT-positive, out of which 38 were also positive for blood culture. Among the 35 PCT-negative samples, 4 were positive for blood culture. Procalcitonin showed 90.4% sensitivity, 53.4% specificity, 58.4% positive predictive value, and 88.57% negative predictive value.

DISCUSSION

Neonatal septicemia is often challenging to diagnose clinically due to its nonspecific signs and symptoms. Early detection is crucial for the initiation of timely and suitable therapy. The prompt identification of the causative microorganisms, along with their antibiotic susceptibility profiles, is essential for guiding clinicians in selecting both empirical and definitive antimicrobial therapies. (1)

The bacteriological profile keeps changing from region to region and hospital to hospital in the same city or country and with the passage of time. To support the management of neonatal septicemia as resistant bacteria are emerging, we need to do surveillance of NICU and formulate few periodic guidelines in antibiotic policy for empirical treatment.

Globally, the bacteriological pattern of neonatal septicemia has shifted from a predominance of Gram-negative to Gram-positive bacterial isolates. Several recent studies have also highlighted the emergence of new pathogens, including coagulase negative staphylococci (CONS), non-fermenting Gram-negative organisms (NFGO), and Candida species, as significant causative agents of neonatal septicemia. (1)

Various leukocyte indices and acute-phase proteins have been investigated as diagnostic markers for sepsis. Among these, C-reactive protein (CRP) is widely utilized as an acute-phase reactant in the diagnosis of sepsis. However, elevated CRP levels can also be observed in conditions such as autoimmune disorders, surgical interventions, meconium aspiration, and following recent vaccinations. Additionally, CRP levels typically do not rise markedly until 14 to 48 hours after the onset of infection. (4)

Serum Procalcitonin has been reported as a measurable laboratory marker of inflammatory response to infection. Macrophages and monocyte cells of liver increase procalcitonin secretion during the sepsis process. It is a promising early new marker as it increases rapidly 6-8 hours, reaching a plateau between 12-48 hours. It increases in bacterial and fungal infections, but no change in viral infections and other inflammatory diseases.

In the present study, culture positivity was observed in 42% of the cases, which correlates with the findings of Thakur et al (1), Zakariya et al (3) and Aditi Rawat et al (6). In contrast, lower culture positivity rates were reported by Vrishali et al (7), Nirmal K. Mohakud et al (8), Mamatha P. Samaga et al (9), and P. Jyothi et al (10), whereas a higher culture positivity rate was reported by Emad A. Morad et al (11).

Among the culture positives, male neonates accounted for 57.1%, which correlates with the findings of Santosh Kotgire et al (12) and Sathyamurthi et al (13) . Whereas Nirmal K. Mohakud et al (8) and Arati Bhurle et al (14) (51%) reported a lower incidence. Higher incidences were noted by S. Thakur et al (1) and P. Jyothi et al (10) .

Among the positive cases, 31% were term neonates and 69% were preterm ,which aligns with the findings reported by Sathyamurthi et al (13) and Arati Bhurle et al (14), Nirmal K. Mohakud et al (8) , Veereswara et al (5) and Santosh Kotgire et al (12) . Whereas, Thakur et al showed a predominance of term neonates)61% and 39%), in contrast to the present study.

In the present study, early-onset sepsis (EOS) accounted for 76.2%, which correlates with the findings of P. Jyothi et al (10) and Vrishali et al (7). The highest incidence of EOS was observed in the study by Arati Bhurle et al (14) . The lower incidence was reported by Saurabh et al (15) and Nirmal K. Mohakud et al (8) . For LOS, the highest incidence was observed in the study by Nirmal K. Mohakud et al (8) and Vrishali et al (7) , while the lowest incidence was reported by Arati Bhurle et al (14) in contrast to the present study (23.8%).

In the current study out of total positive cases, 80.95% of neonates were of low birth weight, similar to findings by Arati Bhurle et al (14) (84%) and Veereswara et al (5) (73.2%) and Nirmal K. Mohakud et al (8) . However, Thakur et al (1). reported a predominance of normal birth weight neonates, differing from the findings of the current study.

In this study out of 42 isolates, Gram-negative bacilli (71.5%) were more prevalent, which is consistent with the findings of Zakariya et al (3) , Vrishali et al (7) , Satyamurthi et al (13), Tanya et al (16) and Aarti Bhurle et al (14). In contrast, studies by S. Thakur et al (1), Veereswara et al (5) , and Ashish Vijay et al (17) reported a higher prevalence of Gram-positive cocci.

In this study, among the Gram-negative isolates, Klebsiella pneumoniae was the predominant organism (31.1%), followed by Acinetobacter baumannii (21.4%) and Escherichia coli, which correlates with Tanya et al (16), P. Jyothi et al (10) ,Vrishali et al (7), Sathyamurthi et al (13), Veereshwara et al (5), and Ashish Vijay et al (17). S. Thakur et al (1) reported

Acinetobacter baumannii as the predominant isolate (40%), while Nirmal K. Mohakud et al (8) isolated Escherichia coli as the most prevalent isolate (9.1%).

Among the Gram-positive isolates, Coagulase Negative Staphylococcus (16.66%) and Staphylococcus aureus (11.9%) were isolated, which correlates with the findings of Tanya et al (16) (22.23%,18.89%) and P. Jyothi et al (10) (27.5%,10.6%). However, studies by Vrishali et al (7) (6.3%,22.9%), Veereshwara et al (5) (9.83%,21.8%), Sathyamurthi et al (13) (13.98%,18.18%), Ashish Vijay et al (17) (14.75%,32.79%) and S. Thakur et al (1) (31%,66%) reported Staphylococcus aureus as the predominant Gram-positive isolate in their studies. (Table1)

In this study, the sensitivity pattern of Gram-positive cocci was similar to the studies reported by Saurabh et al (15), Thakur et al (1) , Nirmal K. Mohakud et al (8) and Santhosh et al (12) . However, Aditi Rawat et al (6) demonstrated a lower sensitivity towards Clindamycin and Linezolid , while the sensitivity pattern to Teicoplanin was comparable to the present studies.(Table 2)

In the present study, the sensitivity pattern of Gram-negative bacilli exhibited correlate with the studies conducted by Nirmal K. Mohakud et al (8) , Saurabh et al (15), and Santhosh et al (12) . In contrast, Aditi Rawat et al (6) reported a decreased sensitivity pattern to Levofloxacin, Gentamicin , Piperacillin-Tazobactam , Amikacin and Imipenem , although the sensitivity to Colistin (66.6%) remained consistent with the present study.(Table 3)

In the present study, 80% of the isolates were identified as MRSA, which correlates with the findings of Arati Bhurle et al (14) (75%) and Aditi Rawat et al (6) (58.3%). In contrast, a lower prevalence of MRSA was reported by Vrishali et al (7) (18.1%) and Nirmal K. Mohakud et al (8) (18%).

In the current study, the prevalence of ESBL producers was found to be 80.95%, which correlates with the findings of Veereswara et al (5) (80.32%) and Coralith Garcia et al (18) (73.3%). A higher prevalence was reported by Arati Bhurle et al (14) (97.05%), Zakariya et al (3) (97.05%) and S. Thakur et al (1) (90%). Lower prevalence was reported by Mamatha P. Samaga et al (9) (47.36%) and Vrishali et al (7) (28%).

In the current study, 42.85% of the isolates were identified as CRE producers, which correlates with the findings by Mubashir Hassan Shah et al (19) (46.42%) and Chandra Madhur Sharma et al (20) (54.68%). A higher prevalence of CRE was reported by Sathyamurthi et al (13) (70.9%) and Ashish Vijay et al (17) (95.2%). Lower prevalence rates were reported by Aditi Rawat et al (6) (14.28%) and Veereswara et al (5) (9.8%)

In the current study, C-reactive protein (CRP) demonstrated a sensitivity of 83.3%, specificity of 63.7%, positive predictive value (PPV) of 62.5%, and negative predictive value (NPV) of 84.09%, which correlates with the findings of Thejaswini et al (4), who reported a sensitivity of 83.3%, specificity of 59.43%, PPV of 53.76%, and NPV of 86.3%. The highest CRP sensitivity was reported in the studies by Nuntnarumit P et al (21) (100%) and Anuradha et al (22) (100%). Whereas, the lowest sensitivity was observed in the studies by Aditi Rawat et al (6) (45%) and Shalini Tripathi et al (23) (41%). Highest specificity was observed by Naher B. S. et al (24) , while the lowest was observed in the study conducted by Effat Hisamuddin et al (25) (53.49%).

In the present study, procalcitonin (PCT) showed a sensitivity of 90.4%, specificity of 53.4%, positive predictive value (PPV) of 58.4%, and negative predictive value (NPV) of 88.57%, which correlates with the findings of Thejaswini et al (4), who reported a sensitivity of 90%, specificity of 50.94%, PPV of 49.05%, and NPV of 90%. The highest sensitivity of PCT was reported by Emad A. Morad et al (11) (97.6%), while the lowest sensitivity was noted in studies by Naher B. S. et al (24) (65%) and Shalini Tripathi et al (23) (48%). The highest specificity was observed by Shalini Tripathi et al (23) (97%), whereas the lowest specificity was recorded in the study by Thejaswini et al (4) (50.94%).

TABLES AND FIGURES

TABLE 1: Corelation of CRP and Blood cultures(n=100) & Correlation of Procalcitonin and blood cultures (n=100)

CRP	Blood culture		Total
	Positive	Negative	
Positive	35	21	56
Negative	7	37	44
Total	42	58	100
Procalcitonin	Blood culture		Total
	POSITIVE	Negative	
Positive	38	27	65
Negative	4	31	35
Total	42	58	100

TABLE 2: ANTIBIOTIC SENSITIVITY PATTERN OF GRAM-POSITIVE ISOLATES FROM VARIOUS STUDIES

Studies	P	CX	CD	CIP	GEN	TEI	TIG	E	VA	LZ
P Jyothi et al (15)	10	-	-	52	40	-	-	35	-	91
Santhosh et al(1)	18.18	-	63.63	72.72	-	-	-	27.27	100	100
Aditi Rawat et al (24)	-	8.3	41.6	-	58.3	91.6	75	8.3	83.3	58.3
Thakur et al (3)	13	60	80	-	-	-	-	-	100	-
Saurabh et al (28)	13	-	-	52.1	50	-	-	-	100	100
Nirmal K Mohakud et al(29)	8	-	-	76	66	95	-	-	100	88
Present study	0	41.6	91.6	50	50	91.6	100	25	66.66	83.33

TABLE 3: ANTIBIOTIC SENSITIVITY PATTERN OF GRAM-NEGATIVE ISOLATES FROM VARIOUS STUDIES

Studies	AK	GEN	CTX	LE	PIT	IMP	CL	CAZ	CZA
P Jyothi et al(15)	52	51	43	37	-	93	-	-	-
Santhosh et al(1)	11.1	-	-	100	100	94.4	-	77.7	-
Aditi Rawat et al (24)	25	33.3	16.6	41.6	25	8.3	66.6	-	-
Thakur et al (3)	50	37	27	48	-	96	-	21	-
Saurabh et al (28)	55	45	38	76	79	86	-	-	-
Nirmal K Mohakud et al(29)	83	83	32	73	61	76	92	32	-
Present study	76.6	66.6	30	73.3	66.6	63.3	70	57.14	90.47

CONCLUSION

The present study highlights the bacteriological profile and antibiogram among the clinically suspected cases of neonatal septicemia and also the role of CRP &PCT in the early diagnosis. Organisms causing neonatal sepsis and their resistance trends vary geographically. With the variation in antibiotic spectrum, the empirical regimen should be modified based on the antibiogram of the isolates. While blood culture is considered the gold standard for diagnosis, biomarkers such as CRP and PCT are crucial in initiating early treatment. An antibiotic policy should be developed and regularly updated to avoid inappropriate use of antibiotics and the emergence of multidrugresistant organisms. Implementing an Antimicrobial Stewardship Programme can optimise antibiotic use and reduce the emergence of multidrug resistant bacteria. Early diagnosis and timely initiation of antibiotic therapy can decrease the neonatal mortality and morbidity caused by sepsis.

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