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RESEARCH ARTICLE

Detection of ESBL and Carbapenemase-Producing Gram-Negative Bacteria in Neonatal Sepsis and their Correlation with Semi-Quantitative CRP

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Abstract

Background: Neonatal sepsis continues to be a major contributor to morbidity and mortality globally, especially when induced by multidrug-resistant Gram-negative bacteria. This study sought to identify Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-producing Gram-negative bacteria isolated from neonates with sepsis and to connect these findings with semi-quantitative C-Reactive Protein (CRP) levels, and correlated with the presence of resistant organisms. **Aim and Objectives:** This study seeks to isolate Gram-negative bacteria from neonates with clinically suspected sepsis admitted to the Neonatal Intensive Care Unit (NICU), identify the presence of ESBL and carbapenemase production among these isolates, and evaluate their correlation with semi-quantitative CRP levels. **Materials and Methods:** A cross-sectional descriptive study was performed over three months. Blood samples were collected for culture and CRP testing. Isolates were identified using standard microbiological methods. ESBL production was confirmed by the combined disk diffusion method, and carbapenemase production by the E-test and Carbapenem Inactivation Method. CRP levels were measured using a latex agglutination assay. **Results:** Out of 75 Gram-negative isolates from neonatal sepsis cases, *Klebsiella pneumoniae* was predominant (50.7%), followed by *Acinetobacter baumannii* (24%) and *Escherichia coli* (13.3%). ESBL production was observed in 18 isolates (24%), predominantly in *K. pneumoniae* (39.5%). Carbapenemase production was noted in 8 isolates (10.7), predominantly in *A. baumannii* (11.1). No significant statistical correlation was found between CRP levels and resistance ($p > 0.05$). However, elevated CRP levels (>24 mg/L) were more commonly associated with resistant isolates, suggesting potential as an early indicator. **Conclusion:** This study highlights the increasing burden of antibiotic resistance in neonatal sepsis, emphasising the need for routine detection of ESBL and carbapenemase producers. Semi-quantitative CRP can be a valuable, rapid, and cost-effective tool to aid in early diagnosis and guide empirical treatment strategies.

Keywords: Antimicrobial Resistance, C-Reactive Protein, Carbapenemase, ESBL, Gram-Negative Bacteria, Neonatal Sepsis

1. Introduction

Neonatal sepsis remains a significant global health issue, contributing to increased morbidity and mortality rates, especially in developing countries like India. It is marked by a systemic inflammatory response to infection and can swiftly advance to life-threatening consequences if not recognised and treated expeditiously[1]. In India, newborn septicemia is a primary cause of mortality among neonates in NICUs[2].

This issue is further more severe by the rising incidence of Multidrug-Resistant (MDR) Gram-negative bacteria, especially those that produce ESBLs and carbapenemases. These organisms have emerged as significant pathogens in neonatal septicemia[2,3]. ESBL-producing organisms such as *E. coli* and *K.pneumoniae* exhibit resistance to third-generation cephalosporins, while carbapenemase producers are resistant even to carbapenems- considered the last resort antibiotics in many clinical scenarios[4,5].

Among multidrug-resistant pathogens, extended-spectrum beta-lactamase and carbapenemase

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producers present a significant clinical issue owing to their swift global dissemination and restricted therapeutic alternatives. This highlights the necessity of ongoing monitoring, the development of local antibiograms and the careful selection of empirical antibiotics for newborn sepsis. The selection of suitable first-line antibiotics is especially intricate in the context of these drug-resistant bacteria[3,6].

CRP is a widely used acute-phase reactant that elevates the response to inflammation. Elevated CRP levels can assist in the early detection of sepsis and provide clinicians with supportive diagnostic evidence[7]. Although CRP is a non-specific marker, semi-quantitative CRP testing offers a rapid, cost-effective estimate of infection severity and the inflammatory response in neonates[8]. Correlating CRP levels with the presence of ESBL and carbapenemase-producing organisms may help in early risk stratification, thereby guiding effective empirical therapy and improving clinical outcomes.

This study is designed to detect ESBL and carbapenemase-producing Gram-negative bacteria in neonates with sepsis and to evaluate their correlation with semi-quantitative CRP levels. The findings may aid in optimising antimicrobial strategies and strengthening infection control measures in neonatal care settings.

2. Aim and Objectives

2.1 Aim

This study aims to isolate Gram-negative bacteria from neonates with clinically suspected sepsis and to evaluate their potential to produce ESBLs and carbapenemases, while assessing the correlation between these resistant strains and semi-quantitative CRP levels.

2.2 Objectives

- To isolate Gram-negative bacteria from neonates having clinically suspected sepsis admitted to the NICU.
- To perform antibiotic susceptibility testing of these isolates.
- To detect ESBL and Carbapenemase production in the Gram-negative bacteria.
- To correlate the presence of ESBL/ carbapenemase-producing organisms with CRP levels in neonatal sepsis.

3. Review of Literature

Neonatal sepsis is a severe illness and a major contributor to neonatal morbidity and mortality, particularly in low- and middle-income countries. Neonatal sepsis is a systemic illness that manifests in babies during the initial 28 days of life and continues to be a major contributor to neonatal morbidity and mortality globally. The WHO indicates that it accounts for about one-third of all newborn fatalities, especially in low- and middle-income nations[9]. In India, the burden is quite high, with the National Infant Perinatal Database (NNPD) indicating that sepsis constitutes approximately 30–50 % of infant fatalities in NICUs[10]. Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, *A. baumannii*, and Pseudomonas aeruginosa, are frequently linked with both types of sepsis and are often characterised by multidrug resistance[11,12].

3.1 Extended-Spectrum Beta-Lactamases (ESBLS)

ESBLs are microbes capable of hydrolysing third-generation cephalosporins and monobactams. They are typically vulnerable to carbapenems and cephamycins. All were mediated by plasmids, thereby facilitating easy transfer between organisms. ESBLs are enzymes that provide resistance to penicillins, cephalosporins, and aztreonam, yet are susceptible to inhibition by beta-lactamase inhibitors such as clavulanic acid. The principal ESBLs are Temoneira (TEM), Sulfhydryl Variable (SHV), and Cefotaximase (CTX-M) type[13]. They can be inhibited by beta-lactam inhibitors such as tazobactam, clavulanate, and sulbactam. ESBLs are more common within the Enterobacteriaceae family. The CTX-M type is currently the most widespread ESBL type worldwide[3]. ESBL-producing organisms have been increasingly documented in newborn facilities, frequently resulting in outbreaks, extended hospitalisations, and restricted treatment alternatives[14].

3.2 Risk Determinants for ESBL Production

- Infection or colonisation with ESBL-producing organisms
- Prolonged length of hospital stay
- Low birth weight
- The presence of catheters - vascular or urinary

- Gut colonisation
- Prior treatment with antibiotics.

3.3 Carbapenemase-Producing Gram-Negative Bacteria

Carbapenems are frequently regarded as the ultimate line of defence against severe Gram-negative infections. The rise of Carbapenemase-Producing Organisms (CPOs) has emerged as a significant public health issue.

Mechanism of resistance:

The resistance mechanism is mediated by

1) Enzymatic degradation from beta-lactamases (carbapenemases).
2) Reduced drug permeability due to porin mutations.
3) Increased drug efflux.
4) Alteration in target penicillin-binding proteins.

There are various types of Carbapenemases: Class A Serine Carbapenemases (KPC), Class D Serine Carbapenemases (OXA-type Carbapenemases), Class B Metallo-β-lactamases (NDM, IMP, and VIM). The Prevalent carbapenemases comprise KPC (*K. pneumoniae* carbapenemase), OXA-48, and Verona integron-encoded metallo-beta-lactamase (VIM). And New Delhi Metallo-beta-lactamase (NDM)[8]. The NDM gene is notably prevalent in India. The proliferation of CPOs in NICUs correlates with outbreaks that are challenging to manage, and their existence frequently requires the administration of very toxic antibiotics such as polymyxins and tigecycline[15].

3.4 Procalcitonin in Bacterial Infections

Procalcitonin (PCTcpo), a 116-amino-acid prohormone of calcitonin, is normally produced in low levels by thyroid C-cells. During systemic bacterial infections, it is released in large amounts from various organs in response to endotoxins and pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), while its production is suppressed in viral infections by interferon-γ. PCT levels rise within 3–6 hours, peak at 6–24 hours, and decline with effective treatment, making it a valuable biomarker for distinguishing bacterial from viral infections in conditions like sepsis, pneumonia, and meningitis. Elevated levels correlate with infection severity, bacteremia, and organ dysfunction. Clinically, PCT supports early diagnosis and guides antibiotic therapy, helping reduce unnecessary use[16].

3.5 CRP in Neonatal Sepsis

CRP is an acute-phase reactant produced by the liver in response to inflammation. It is widely employed as a biomarker for the early detection and management of neonatal sepsis[17]. Although CRP levels cannot identify a specific cause, higher levels are frequently observed in bacterial infections, particularly those triggered by resistant strains[6]. Semi-quantitative CRP assays are straightforward, expeditious, and economical, rendering them advantageous in resource-constrained environments[18].

3.6 Correlation Between CRP and MDR Pathogens

Recent investigations indicate a potential link between elevated CRP levels and infections caused by multidrug-resistant organisms, especially ESBL and carbapenemase producers[19]. Elevated CRP levels in newborns may indicate infection severity and resistance, leading physicians to contemplate resistant Gram-negative bacteria before the availability of culture data[20]. Further establishment of this link may strengthen early risk classification and facilitate empirical antibiotic decision-making, perhaps improving newborn outcomes.

4. Materials and Methods

4.1 Sample Collection and Processing

This cross-sectional descriptive study was performed in the Department of Microbiology at Government Kilpauk Medical College from May to July 2025, following previous approval from the Institutional Ethics Committee.

A total of 75 blood samples were obtained from neonates in the NICU under stringent aseptic settings. Venous blood was inserted into blood culture bottles containing Brain Heart Infusion (BHI) broth and incubated aerobically at 37°C.

The bottles underwent ocular inspection for signs of development (hemolysis, turbidity) between 6 to 18 hours. Blind subcultures from BHI broth must be conducted on Nutrient, Blood, and MacConkey agar plates on the first day, irrespective of the lack of apparent growth.

Isolates were identified using colony morphology, Gram staining, and conventional biochemical assays, emphasising the identification of Gram-negative organisms in accordance with accepted microbiological protocols.

4.2 The Antimicrobial Susceptibility Test

The Kirby-Bauer disk diffusion method was executed on Mueller-Hinton Agar (MHA). The inhibition zones for several antibiotics include Ampicillin (10µg), Amoxicillin clavulanate (20/10µg), Piperacillin-tazobactam (100/10µg), Ceftazidime, Meropenem (10µg), Imipenem (10µg), Cefoxitin (30µg), and Cefazolin (30µg). Cefepime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Gentamicin (10µg), Amikacin (30µg), Tetracycline (30µg), and Ciprofloxacin (5µg) were documented and assessed in accordance with Clinical and Laboratory Standards Institute (CLSI) 2024 criteria.

4.3 Detection of ESBL By Screening Method

For ESBL (Extended Spectrum β-Lactamase) producers, the screening method involves a disc diffusion test using Ceftazidime (30µg) or Cefotaxime (30µg) discs. It is considered an ESBL if the organism is resistant to at least one of the third-generation cephalosporins. According to CLSI 2024 guidelines, the zone of inhibition cut-offs were Ceftazidime ≤ 22 mm, Cefotaxime ≤ 27mm, and Ceftriaxone ≤ 24mm.

Confirmatory Tests for ESBL Detection:

4.3.1 Double Disc Diffusion Method

A lawn culture of the screened-resistant isolate was prepared on Mueller-Hinton Agar, and discs of either Ceftazidime (30µg) with Ceftazidime-clavulanate (30/10 µg) or Cefotaxime (30µg) with Cefotaxime-clavulanate (30/10µg) were placed 24mm apart. Plates were incubated at 37°C for 18 to 24 hours. An increase of ≥5mm in zone diameter with the clavulanate combination relative to cephalosporin alone signifies ESBL generation, according to CLSI recommendations.

4.3.2 E – Test (Epsilometer Test)

This test is based on the principle of gradient diffusion. A lawn culture of the test organism is made on Mueller-Hinton Agar, and an E-strip containing Ceftazidime on one end and Ceftazidime with Clavulanate on the other is placed on the surface. The plate is incubated overnight at 35–37°C. The Minimum Inhibitory Concentration (MIC) is read where the elliptical zone of inhibition intersects the strip. A reduction of ≥3 twofold concentration decrease (i.e., ≥8-fold) in an MIC of Ceftazidime in the presence of Clavulanate compared to Ceftazidime alone indicates positive ESBL production.

4.3.3 Detection of Carbapenemase Producers

The screening for carbapenem resistance is performed using the disc diffusion method with Imipenem (10µg) or Meropenem (10µg) discs. The confirmatory tests include the Modified Carbapenem Inactivation Method (mCIM), often used in conjunction with the Enhanced Carbapenem Inactivation Method (eCIM). Another confirmatory approach is the E-test based on gradient diffusion, which specifically detects Metallo-β-Lactamase (MBL) production in Gram-negative bacteria. This method compares the minimum inhibitory concentrations of Imipenem/Meropenem alone and Imipenem/Meropenem combined with Ethylenediaminetetraacetic Acid (EDTA). An≥8-fold reduction in the MIC value of Imipenem in the presence of EDTA is interpreted as MBL positive, indicating the presence of metallo-β-lactamase enzymes.

4.3.4 Phenotypic Detection of Carbapenemase-Producing Enterobacteriaceae

Carbapenemase was identified in all CRE isolates using the mCIM and eCIM methodologies. A 1µL aliquot of the test isolate was suspended in 2mL of Trypticase Soy Broth (TSB) in two tubes, one containing EDTA (eCIM) and the other devoid of it (mCIM). A meropenem (10µg) disc was introduced into each tube and incubated at 35°C for 4 hours ± 15 minutes. The disks were thereafter removed from the E. tubes and placed on MHA agar plates, where a lawn culture of the carbapenem-susceptible strain of *E. coli* ATCC 25922 was created. The plates were incubated at 35 °C for 16 to 20 hours, after which the diameters of the zones were recorded.

4.3.5 CRP Measurement

CRP detection is based on latex agglutination. The test specimen is mixed with CRP- latex reagent. Visible agglutination indicates CRP > 0.6 mg/dL; absence of

agglutination indicates CRP < 0.6 mg/dL. For semi-quantitative analysis, the serum is serially diluted (1:2 to 1:64) with normal saline. One drop of each dilution is mixed with an equal drop of latex reagent on a slide. The slide is gently rocked, and agglutination is observed within 2 minutes. Results are interpreted as negative, weak, moderate, or strong positive based on the highest dilution showing agglutination. The CRP concentration is calculated by multiplying the highest dilution factor showing agglutination by the reagent sensitivity (0.6 mg/dL).CRP in mg/dl = D*S (D = Highest dilution showing positive reaction and S = Sensitivity of the test is 0.6mg/dl).

4.3.6 Statistical Analysis

The sample size was determined using a prevalence of 22.9%, a confidence level of 95%, and a precision of 10%. The final sample size was modified to 74, accounting for a 10% non-response rate. Data were collected and statistically analysed using SPSS software (version 28.0.1.1). Results were expressed as percentages or rates. The Correlation between CRP levels and the presence of ESBL/ carbapenemase-producing organisms was assessed. P values were calculated using the Chi-square test. The Chi-square test was employed for statistical analysis, with a p-value of less than 0.05 being statistically significant.

5. Results (Including Observations)

A total of 75 Gram-negative bacterial isolates[21] were obtained from blood cultures of neonates diagnosed with sepsis. Among these, the most commonly isolated organism was *K. pneumoniae*, accounting for 38 isolates (50.7%), making it the predominant pathogen in this study.

The second most frequently isolated organism was Acinetobacter baumannii, with 18 isolates (24%), followed by *E. coli*, which was detected in 10 cases (13.3%). *Citrobacter koseri* was isolated in 6 cases (8%), while *P. aeruginosa* was the least common isolate, found in 3 cases (4%).

Out of the 75 Gram-negative bacterial isolates analysed, 18 isolates (24%) were found to be ESBL producers.

The highest number of ESBL-producing organisms was seen in *K. pneumoniae*, with 15 out of 38 isolates (39.5%) testing positive. Escherichia coli showed ESBL production in 2 out of 10 isolates (20%), while *C. koseri* had 1 ESBL-positive isolate out of 6 (16.7%).

Out of the 75 Gram-negative isolates tested, 8 isolates (10.7%) were found to be carbapenemase producers, indicating resistance to carbapenem antibiotics.

The majority of carbapenem-resistant isolates were observed in *K. pneumoniae*, where 4 out of 38 isolates (10.5%) tested positive. *Acinetobacter baumannii* also showed notable resistance, with 2 out of 18 isolates (11.1%) being carbapenemase positive.

There was no statistically significant correlation between CRP levels and ESBL/ Carbapenemase production ($p > 0.05$).

Clinical trend observed: Higher CRP levels (>24 mg/L) are more frequently associated with resistant isolates.

This suggests that semi-quantitative CRP may be a useful early marker of likely antimicrobial resistance in neonates, though not conclusive in this sample size.

6. Discussion

This study highlights the distribution of Gram-negative bacteria causing neonatal sepsis, their antibiotic resistance patterns, and the correlation of these patterns with semi-quantitative CRP levels in a cohort of 75 neonates over 3 months.

The most commonly isolated organism was *K. pneumoniae*, accounting for 50.7% of all isolates (Table 1). This finding is consistent with multiple Indian and global studies, where *K. pneumoniae* remains the predominant cause of sepsis in NICU settings due to its strong colonisation potential and ability to survive in hospital environments (Roy *et al.*; Joseph *et al.*,)[12,22]. The second most frequent isolate was A. baumannii (24%),

Table 1. Distribution of isolated gram-negative bacteria in neonatal sepsis (n=75).

Distribution of Isolated Gram-Negative Bacteria in Neonatal Sepsis (n=75)	
Organism	**No. of Isolates**
Klebsiella pneumoniae	38(50.7%)
Acinetobacter baumannii	18(24%)
Escherichia coli	10(13.3%)
Citrobacter koseri	6(8%)
Pseudomonas aeruginosa	3(4%)
Total Isolates	75(100%)

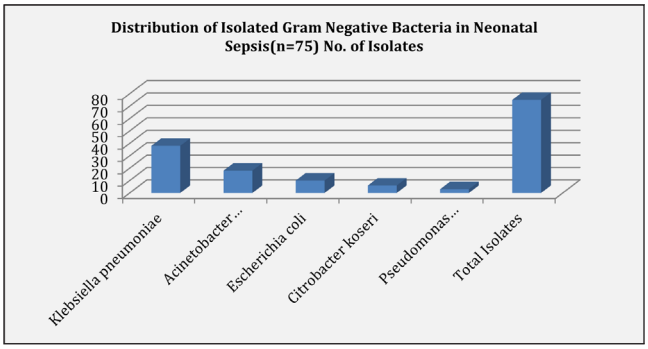

Chart 1. Chart Distribution of Isolated Gram-Negative Bacteria in Neonatal Sepsis (n=75).

an opportunistic pathogen, particularly in ventilated and low-birth-weight neonates. Its rising prevalence is concerning due to its multidrug resistance capabilities and environmental resilience. Other isolates, such as *C. koseri* (8%) and *P. aeruginosa* (4%), though less common, are notable

for their inherent resistance to multiple antibiotics and their role in neonatal sepsis in chart 1. Overall, this microbial profile highlights the dominance of *K. pneumoniae*, underlining the need for strict infection control measures, targeted antimicrobial therapy, and ongoing surveillance of resistance trends in neonatal care units.

Meropenem and Imipenem demonstrated excellent efficacy, with 89% (34/38) of isolates exhibiting susceptibility (Table 2). Cefepime and Tetracycline also showed considerable activity, with sensitivity rates of 76% and 74%, respectively. A notable degree of resistance was observed against β-lactam antibiotics, fluoroquinolones, and selected aminoglycosides, highlighting emerging antimicrobial challenges (table 3).

This study revealed that out of 75 Gram-negative bacterial isolates from neonatal sepsis cases, 18 isolates (24%) were ESBL producers (Table 4). The predominant ESBL-producing organism was *K. pneumoniae*, with 15 out of 38 isolates (39.5%) testing positive in chart 2. This aligns with previous studies that have identified

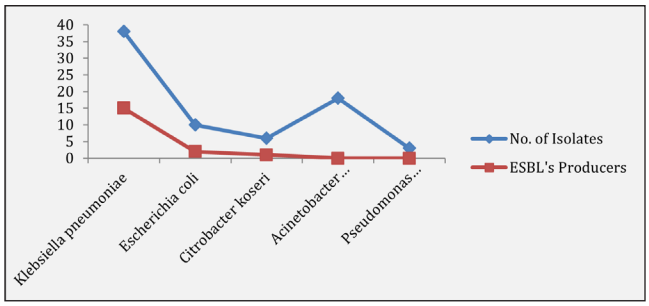

Chart 2. Chart ESBL's Producers among Gram negative Isolates (n=75).

Table 2. Antibiotic sensitivity pattern for total isolates in GNB (n = 75)

Antibiotic (µg)	***Klebsiella pneumoniae* (n = 38)**	***Escherichia coli* (n = 10)**	***Citrobacter koseri* (n =6)**	***Acinetobacter baumannii* (n = 18)**	***Pseudomonas aeruginosa* (n = 3)**
Ampicillin (10)		1	1		
Amox–Clav (20/10)	9	3	2		
Ciprofloxacin (5)	15	5	3	8	2
Cefazolin (30)	22	2			
Cefoxitin (30)	20	5	3		
Ceftriaxone (30)	21	9	6	16	
Cefotaxime (30)	23	9	5	16	
Ceftazidime (30)	18	4	3	1	1
Cefepime (30)	29	5	3	16	2
Gentamicin (10)	19	6	4	10	2
Amikacin (30)	25	8	5	14	3
Tetracycline (30)	28	6	4		
Pip–Taz (100/10)	22	7	4	6	2
Imipenem (10)	34	9	5	16	3
Meropenem (10)	34	10	5	16	3
		Indicates either intrinsically resistant or not effective *in vivo*			

Table 3. Antibiotic sensitivity pattern for *Klebsiella pneumonia* (n = 38)

Table: Antibiotic Sensitivity Pattern for Klebsiella pneumoniae(n = 38)		
Antibiotic (µg)	**Sensitive**	**Resistant**
Ampicillin (10)		
Amox–Clav (20/10)	9 (24%)	29 (76%)
Ciprofloxacin (5)	15 (39%)	23 (61%)
Cefazolin (30)	22 (58%)	16 (42%)
Cefoxitin (30)	20 (53%)	18 (47%)
Ceftriaxone (30)	21 (55%)	17(45%)
Cefotaxime (30)	23 (61%)	15 (39%)
Ceftazidime (30)	18 (47%)	20 (53%)
Cefepime (30)	29 (76%)	9 (24%)
Gentamicin (10)	19 (50%)	19 (50%)
Amikacin (30)	25 (66%)	13 (34%)
Tetracycline (30)	28 (74%)	10 (26%)
Pip–Taz (100/10)	22 (58%)	16 (42%)
Imipenem (10)	34(89%)	4 (11%)
Meropenem (10)	34 (89%)	4 (11%)
	Indicates either intrinsically resistant or not effective *in vivo*	

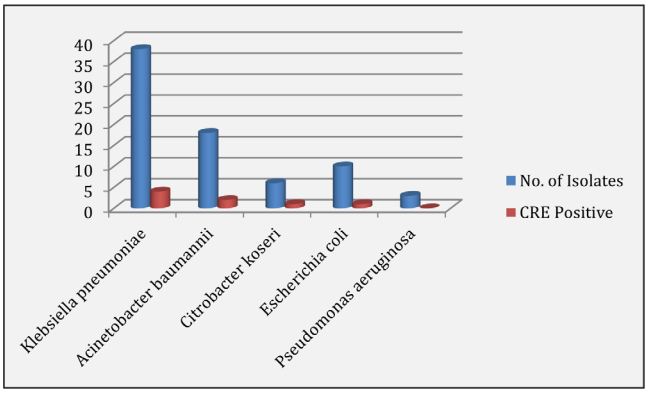

Chart 3. Chart Carbapenemase detection among Gram negative Isolates (n=75).

K. pneumoniae as a major reservoir of ESBL genes in NICU environments due to horizontal gene transfer and nosocomial spread (Ghosh *et al.*; Ramesh *et al.*, 2021)[23]. Escherichia coli exhibited ESBL production in 20% of its isolates, consistent with its role in early neonatal sepsis. *Citrobacter koseri* showed ESBL positivity in 16.7%, though based on smaller isolate numbers. Importantly, no ESBL production was observed in *A. baumannii* or *P. aeruginosa* in this study.

Table 4. ESBL producers among gram-negative isolates (n=75)

Organism	No. of Isolates	ESBL's Producers
Klebsiella pneumoniae	38	15 (39.5%)
Escherichia coli	10	2 (20.0%)
Citrobacter koseri	6	1 (16.7%)
Acinetobacter baumannii	18	0 (0%)
Pseudomonas aeruginosa	3	0 (0%)
Total	75	18 (24.0%)

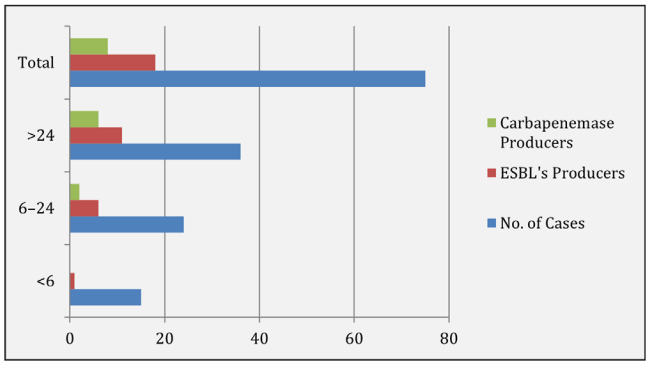

Chart 4. Chart Correlation Between semi quantitative CRP Levels and ESBL/ Carbapenemase Positivity (n=75).

While these organisms are commonly multidrug-resistant, their resistance typically stems from other mechanisms (e.g., efflux pumps, carbapenemases) rather than ESBL's. The overall ESBL prevalence of 24% is a significant finding, suggesting that nearly 1 in 4 Gram-negative infections in neonates may not respond to third-generation cephalosporins, the mainstay of empirical therapy in many NICUs. This highlights the urgent need for local antibiograms and early detection methods for ESBL.

This study identified carbapenemase-producing Gram-negative bacteria in 8 out of 75 neonatal sepsis isolates (Table 5), yielding a carbapenem resistance rate of 10.7%. CPO, also referred to as CRE, is of major clinical concern, especially in vulnerable populations such as neonates, due to limited treatment options and high mortality rates. Among the isolates, *K. pneumoniae* contributed the highest number of CRE cases (4/38, 10.5%). This organism is widely recognised for its capacity to acquire and disseminate carbapenemase genes (e.g., KPC, NDM), especially in NICU outbreaks. A CRE rate of 11.1% (2/18) was

Table 5. Carbapenemase detection among gram-negative isolates (n=75)

Table. Carbapenemase detection among Isolates (n=75)		
Organism	**No. of Isolates**	**CRE Positive**
Klebsiella pneumoniae	38	4 (10.5%)
Acinetobacter baumannii	18	2(11.1%)
Citrobacter koseri	6	1 (16.7%)
Escherichia coli	10	1 (10.0%)
Pseudomonas aeruginosa	3	0 (0.0%)
Total	75	8

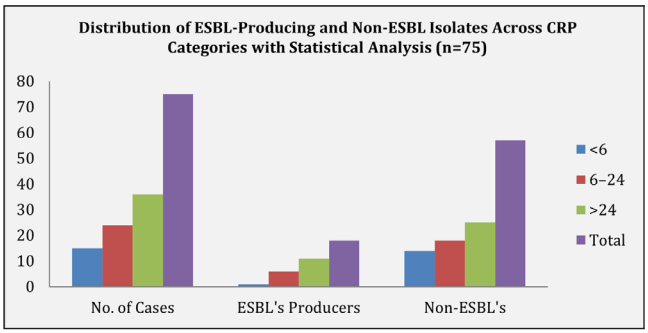

Chart 5. Chart Distribution of ESBL-Producing and Non-ESBL Isolates Across CRP Categories with Statistical Analysis (n=75).

observed in *A. baumannii*, adding to the complexity of treatment in chart 3.

Among 36 neonates with CRP >24 mg/L, 11 (30.5%) had ESBL-producing infections and 5 (16.6%) had carbapenemase-producing organisms (Table 6 and chart 4). A positive correlation was observed between higher CRP levels and antibiotic resistance, especially with CRP >24 mg/L, suggesting that the inflammatory response correlates with infection severity and resistance pattern. A notable observation was the significant correlation between elevated CRP levels and antimicrobial resistance. Of the 36 neonates with CRP>24 mg/L, 47.2% harboured ESBL or carbapenemase-producing organisms in chart 5. This aligns with findings by Ghosh *et al.* and Joshi *et al.* (2021), who reported that CRP >24 mg/L may be a predictor of severe, resistant infections[23].

A Chi-square test was used to assess the correlation between semi-quantitative CRP levels and ESBL positivity. Although a higher number of ESBL and CRE cases were observed in patients with

Table 6. Correlation between semi-quantitative CRP levels and ESBL carbapenemase positivity (n=75)

Table: correlation between semi-quantitative CRP levels and ESBL/ carbapenemase positivity (n=75)			
CRP Levels	**no. of cases**	**ESBL Producers**	**Carbapenemase Producers**
<6	15	1	0
6–24	24	6	2
>24	36	11	6
Total	75	18	8

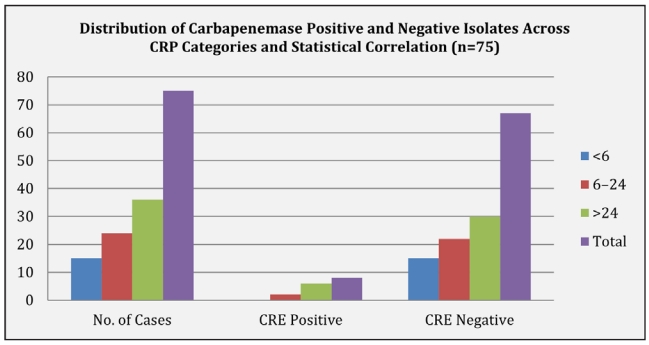

Chart 6. Chart Distribution of Carbapenemase Positive and Negative Isolates Across CRP Categories and Statistical Correlation (n=75).

Table 7. Distribution of ESBL-producing and non-ESBL isolates across crp categories with statistical analysis (n=75)

Table. Distribution of ESBL-Producing and Non-ESBL Isolates Across CRP Categories with Statistical Analysis (n=75)					
CRP Range (mg/L)	**No. of Cases**	**ESBL's Producers**	**Non-ESBL's**	**P value**	**Chi-square**
<6	15	1	14	~0.157	~3.7
6–24	24	6	18		
>24	36	11	25		
Total	75	18	57		

CRP >24 mg/L, the association was not statistically significant (χ^2 = 3.7, p = 0.157 (Table 7) and χ^2 = 5.0, p = 0.082 (Table 8 and chart 6)). These data indicate that although increased CRP may signify the inflammatory burden and heighten clinical suspicion of drug-resistant infections in newborn sepsis, it cannot independently forecast ESBL or CRE positivity in this study population (Ray P, Manchanda V, Bajaj J, *et al*)[24]. High CRP values reflect intense inflammatory responses often triggered by virulent

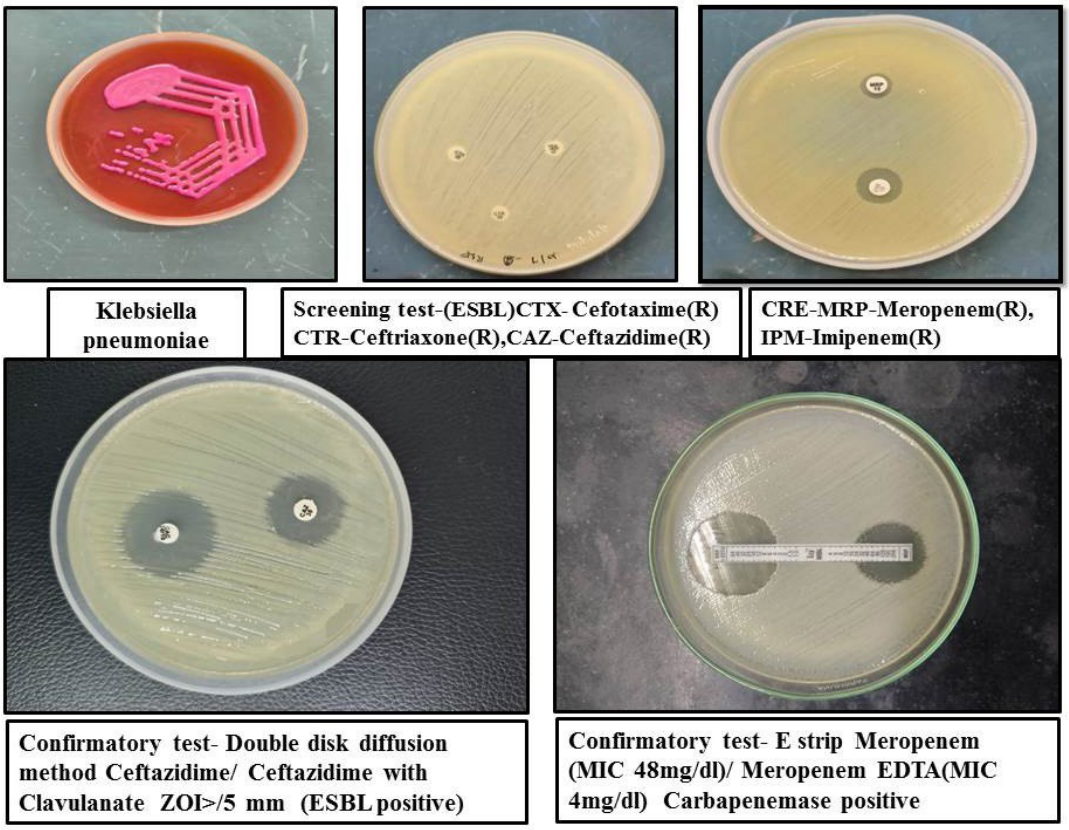

Figure 1. *Klebsiella pneumoniae.* 2. Screening test (ESBL): CTX–Cefotaxime (R), CTR–Ceftriaxone (R), CAZ–Ceftazidime (R). 3. CRE: MRP–Meropenem (R), IPM–Imipenem (R). 4. Confirmatory test – Double disk diffusion: CAZ/CAZ with clavulanate, ZOI ≥ 5 mm (ESBL). 5. E-strip: MRP (MIC 48 mg/dL) / MRP+EDTA (MIC 4 mg/dL) – CRE.

Table 8. Distribution of carbapenemase-positive and negative isolates across CRP categories and statistical correlation (n=75)

Table: Distribution of Carbapenemase Positive and Negative Isolates Across CRP Categories and Statistical Correlation (n=75)					
CRP Range (mg/L)	**No. of Cases**	**CRE Positive**	**CRE Negative**	**P value**	**Chi-square**
<6	15	0	15	~0.082	~5.0
6–24	24	2	22		
>24	36	6	30		
Total	75	8	67		

or resistant strains. Therefore, CRP can aid in early suspicion of resistance, guiding prompt escalation to appropriate antimicrobials before lab confirmation. ESBL or carbapenemase infections, justifying the need for empirical coverage with higher-end antibiotics while awaiting culture results[20]. Overall, this study underlines the need for routine surveillance of resistance patterns in NICUs, Incorporation of CRP monitoring into sepsis management protocols, and judicious use of antibiotics guided by antibiograms and inflammatory markers.

These findings highlight the importance of early detection of resistant organisms and reinforce the role of combining microbiological testing with inflammatory markers in guiding empirical therapy. These results provide valuable guidance for empirical therapy protocols and the development of antibiotic stewardship strategies.

7. Summary and Conclusion

7.1 Summary

- *Klebsiella pneumoniae* was the most frequently isolated organism (50.7%) in this study.
- High Sensitivity was observed for Meropenem and Imipenem: 89% (34/38 isolates) were sensitive, Cefepime: 76% sensitivity (29/38), Tetracycline: 74% sensitivity (28/38).

- Significant resistance to β-lactams, fluoroquinolones, and some aminoglycosides highlights the importance of regular antimicrobial stewardship and local antibiogram updates.
- Among the 75 isolates, 18 (24%) were identified as ESBL producers.
- *Klebsiella pneumoniae* was the predominant ESBL producer at 39.5%, accompanied by *E. coli* at 20% and *C. koseri* at 16.7%.
- 8 out of 75 isolates (10.7%) were CRE positive.
- *Klebsiella pneumoniae* (10.5%) and *A. baumannii*. (11.1%) were the leading contributors to CRE infections.
- A Chi-square test revealed no statistically significant association between semi-quantitative CRP levels and ESBL or CRE positivity, despite a higher number of resistant cases in the >24 mg/L CRP group. While elevated CRP may indicate increased inflammatory burden and raise suspicion of drug resistance in neonatal sepsis, it cannot independently predict ESBL or CRE positivity.
- These findings highlight the emergence of multidrug-resistant pathogens in NICU sepsis, demanding early identification, infection control vigilance, and antibiotic stewardship.

7.2 Conclusion

This study highlights the concerning prevalence of ESBL- and carbapenemase- producing Gram-negative bacteria in neonatal sepsis, with Klebsiella pneumoniae emerging as the predominant pathogen. Although higher CRP levels were frequently associated with drug-resistant organisms (ESBL and CRE), the correlation did not reach statistical significance in the current study population (n = 75).

Nevertheless, the trend suggests that CRP may serve as a supportive marker for early clinical suspicion of antimicrobial resistance. The integration of CRP testing with prompt microbiological investigations can facilitate early therapeutic decisions and promote rational antibiotic use.

To combat the growing threat of resistance, regular surveillance, rapid diagnostics, and robust antimicrobial stewardship programs remain essential for improving outcomes in neonatal care.

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