

## Spectrum And Antimicrobial Susceptibility Pattern Of Isolates In Neonatal Sepsis

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### ABSTRACT

**Introduction:** Neonatal sepsis is a significant cause of morbidity and mortality, particularly in developing countries. Identifying causative pathogens and their antimicrobial resistance patterns is critical for effective management. This study aimed to assess microbial diversity, antimicrobial resistance, and recommend effective treatment strategies for neonatal sepsis at a tertiary care hospital.

**Material & Methods:** This study was carried out at the Department of Microbiology, National Institute of Medical Sciences and Research. A total of 120 neonates with suspected sepsis were included. Blood cultures were performed using the Bact/ALERT system, and antimicrobial susceptibility testing followed CLSI guidelines.

**Results:** Of 120 neonates, 59 (49.2%) had EOS and 61 (50.8%) had LOS. Blood culture positivity was 44/120 (36.7%). The most common isolates were *Klebsiella pneumoniae* (31.8%), *Pseudomonas aeruginosa* (11.4%), and *Staphylococcus aureus* (4.5%). Gram-negative isolates showed 100% resistance to ceftriaxone and cefotaxime, 82.1% to imipenem, and 75.0% to meropenem. Colistin (100% sensitivity,  $p < 0.001$ ) and tigecycline (95.7%,  $p = 0.002$ ) were the most effective agents.

**Conclusion:** The study highlights a high prevalence of Gram-negative bacterial infections and rising antimicrobial resistance in neonatal sepsis. Based on resistance patterns, **colistin, tigecycline, and minocycline** should be considered for multidrug-resistant infections, while **glycopeptides (vancomycin, teicoplanin)** remain effective for Gram-positive infections. The findings emphasize the need for routine surveillance, antimicrobial stewardship, and judicious antibiotic use to optimize neonatal sepsis treatment

**Keywords:** Blood culture, Neonatal sepsis, Gram negative, antimicrobial stewardship

### 1. INTRODUCTION

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Neonatal sepsis encompasses systemic infections of the newborn including septicemia, meningitis and pneumonia. [1] Of the One hundred and thirty million babies born every year, about four million die in the first four weeks of life- the neonatal period. The World Health Organization (WHO) estimates that, worldwide, approximately five million neonates die each year and that 98% of these deaths occur in developing countries. [2] Neonatal sepsis can be divided into two main classes depending on the onset of symptoms related to sepsis. Early onset sepsis (EOS) usually presents within the first 72 hours of life. Late onset sepsis (LOS) usually presents after 72 hours of life. [1]

#### Scientific Case Definition for Sepsis (Early vs. Late-Onset):

Neonatal sepsis was defined using **Sepsis-3 criteria**, which includes **systemic inflammatory response syndrome (SIRS) with a proven or suspected bloodstream infection**. Cases were classified as:

- **Early-onset sepsis (EOS):** Occurring within the **first 72 hours of life** often associated with maternal transmission.
- **Late-onset sepsis (LOS):** Occurring **after 72 hours**, usually due to hospital-acquired or environmental infections (Haque, 2005).

Newborn Sepsis gives the main problem which occurs commonly in developing countries. Neonatal Sepsis is being caused by an infection which activates the inflammatory cascade may lead to shock and multi-organ system failure [3]. The etiological newborn sepsis is commonly gram-negative bacilli in developing countries. The commonly detected organism in the sample included *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus spp*, *Citrobacter spp*, and Coagulase negative *Staphylococcus*. [4]

This study aims to **identify the microbial diversity and antimicrobial resistance patterns in neonatal sepsis cases** at a tertiary care hospital. It seeks to **determine the prevalence of early- and late-onset sepsis, evaluate resistance trends, and recommend effective treatment strategies**, with a goal to enhance **clinical management and antimicrobial stewardship within the study duration of January 2024 to September 2024**

## 2. MATERIAL & METHODS

This cross-sectional study was conducted in the Department of Microbiology and Neonatology Unit, Department of Pediatrics, NIMS Hospital, from January 2024 to September 2024. A total of 120 suspected septicemia cases. **Scientific Case Definition for Sepsis (Early vs. Late-Onset):**

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- **Late-onset sepsis (LOS):** Occurring **after 72 hours**, usually due to hospital-acquired or environmental infections (Haque, 2005).
- **Inclusion Criteria for Neonatal Sepsis Cases:** Newborns suspected of septicemia, presenting with symptoms such as fever, respiratory distress, lethargy, poor feeding, and abnormal laboratory findings.
- **Exclusion Criteria:** Neonates with congenital malformations, neonatal infections diagnosed prior, or those who had received antibiotics before admission were excluded from the study.

The sample collection was carried out randomly from the Neonatology Unit of the Pediatrics Department, ensuring randomization to avoid bias in selection. Neonatal blood samples were collected using aseptic techniques for microbiological testing and to rule out infection.

### Sample Size Calculation:

The sample size of **120 neonates with suspected sepsis** was determined based on an estimated **sepsis prevalence of 35%** (from prior studies), with a **95% confidence interval** and a **margin of error of 5%**. Using standard power calculations, this sample size ensures sufficient statistical power to detect significant differences in microbial prevalence and resistance patterns.

- **Sample Type:** Blood samples were collected from the septicemia cases for blood culture and IL-6 detection.
- **Ethical Considerations:** The study was approved by the Institutional Ethical Committee (IEC), and informed consent was obtained from the parents or guardians of the newborns.

**Laboratory procedure:** Blood cultures were performed by BacT/ALERT system method using BD Blood culture bottles which contains soybean-casein digest broth with anticoagulant (sodium polyanetholsulfonate) with 2-3 ml of blood from each patient and incubate in BacT/ALERT system. When any microbial growth occurs within culture bottle indicates by a beep. Subcultures were done on Blood agar and MacConkey's agar on 24 hours, 48 hours and once before discarding the culture bottle i.e after 5 days. The organisms were identified by their colony morphology, staining character, pigment production, motility and relevant biochemical tests as per standard methods [5].

Data were analyzed using **SPSS version 25.0**. Results were summarized as **percentages for categorical data**. The **Chi-Square test ( $\chi^2$ )** was used to compare **blood culture positivity and antibiotic resistance rates**. A **p-value < 0.05** was considered statistically significant.

### 3. RESULT

Table 1: Age & Gender of neonates studied		
Neonates characteristics	No. of neonates	Percentage
Age in days		
0-3 Days (Early Onset)	59	49.16
4-28 Days (Late Onset)	61	50.84
Gender		
Male	84	70
Female	36	30

Out of 120 neonates with clinical features of septicemia 59(49.16%) belonged to early onset septicemia and 61(50.84%) belonged to late onset septicemia (4-28 days) respectively. Among neonates 70% were males and 30% were females respectively.

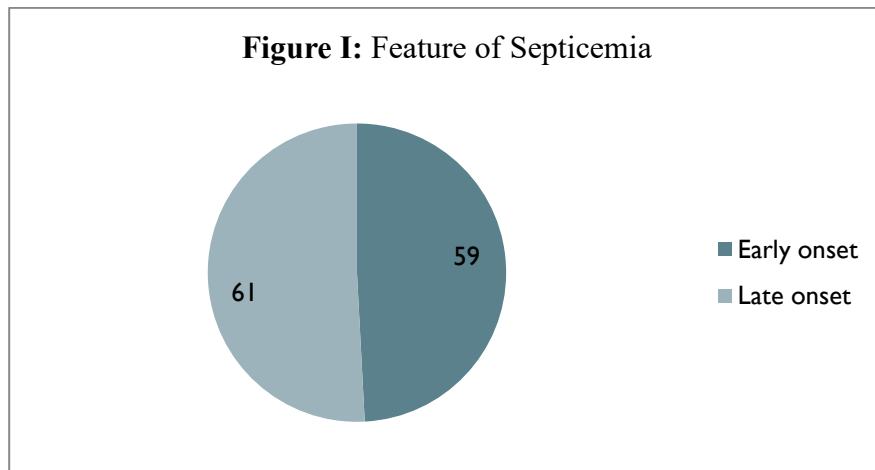


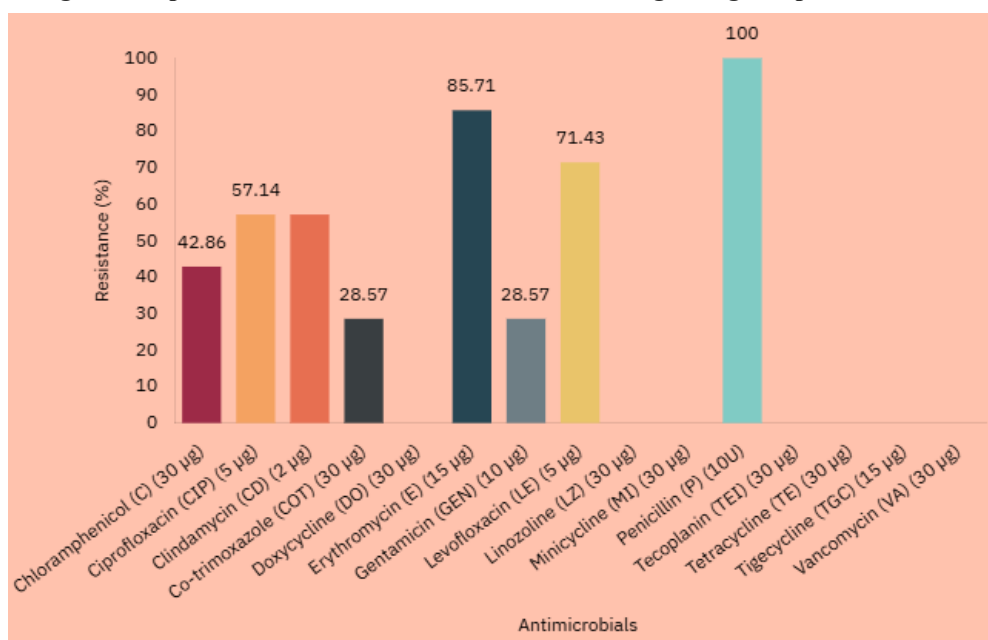
Table 2: Spectrum of microorganisms isolated from blood culture		
Culture findings	No. of Isolates	Percentage
Blood culture positive	44	36.67
Blood culture negative	76	63.34
<i>Staphylococcus aureus</i>	02	4.54
<i>Enterococcus faecalis</i>	01	2.27
Coagulase negative staphylococcus	04	9.09
<i>Micrococcus</i> spp.	02	4.54
<i>Bacillus</i> spp.	01	2.27
<i>Escherichia coli</i>	04	9.09
<i>Klebsiella pneumonia</i>	14	31.81
<i>Acinetobacter baumannii</i>	04	9.09

<i>Pseudomonas aeruginosa</i>	05	11.36
<i>Burkholderia cepacia</i>	01	2.27
<i>Candida</i> spp.	06	13.63
Total	44	100

Out of 44 isolates, *Staphylococcus aureus* was 2(4.54%), *CONS* 4(9.09%), *Enterococcus faecalis* 1(2.27%), *Micrococcus* spp. 2(9.09%), *Bacillus* spp. 1(2.27%), *Escherichia coli* 4(9.09%), *Klebsiella pneumonia* 14(31.81%), *Acinetobacter baumannii* 4(9.09%), *Pseudomonas aeruginosa* 5(11.36%), *Burkholderia cepacia* 1(2.27%) and *Candida* spp. 6(13.63%).

Table 3: Spectrum of antimicrobial agents used against gram positive isolates		
Antibiotics	Resistant	Sensitive
Gentamicin (GEN) (10 µg)	02 (28.57%)	05(71.43%)
Ciprofloxacin (CIP) (5 µg)	04 (57.14%)	03(42.86%)
Co-trimoxazole (COT) (30 µg)	02 (28.57%)	05(71.43%)
Doxycycline (DO) (30 µg)	-	07(100%)
Levofloxacin (LE) (5 µg)	05(71.43%)	02(28.57%)
Tigecycline (TGC) (15 µg)	-	07(100%)
Erythromycin (E) (15 µg)	06(85.71%)	01(14.29%)
Clindamycin (CD) (2 µg)	04 (57.14%)	03(42.86%)
Penicillin (P) (10U)	07 (100%)	-
Tecoplanin (TEI) (30 µg)	-	07(100%)
Linazoline (LZ) (30 µg)	-	07(100%)
Minicycline (MI) (30 µg)	-	07(100%)
Vancomycin (VA) (30 µg)	-	07(100%)
Tetracycline (TE) (30 µg)	-	07(100%)
Chloramphenicol (C) (30 µg)	03(42.86%)	04(57.14%)

Figure II: Spectrum of antimicrobial resistance used against gram positive isolates



The antimicrobial susceptibility testing revealed significant resistance patterns among Gram-positive isolates. **Penicillin resistance was 100%**, underscoring its limited efficacy in empirical therapy. Similarly, **high resistance was noted for Erythromycin (85.71%) and Levofloxacin (71.43%)**, reflecting a concerning trend of macrolide and fluoroquinolone resistance. **Clindamycin resistance (57.14%)** suggests the possible presence of **MLSB resistance mechanisms**.

On the other hand, **Glycopeptides (Vancomycin, Teicoplanin) and Linezolid retained 100% susceptibility**, reinforcing their role as **first-line agents against resistant Gram-positive infections**. **Tetracycline-class antibiotics (Doxycycline, Minocycline, Tetracycline) also showed complete sensitivity (100%)**, suggesting their potential as alternative therapies. **Chloramphenicol resistance (42.86%) and Co-trimoxazole resistance (28.57%)** indicate moderate efficacy, while **Tigecycline (100% susceptibility)** emerges as a promising option for **MDR infections**.

These findings highlight the **urgent need for antimicrobial stewardship and continuous surveillance** to combat **emerging resistance trends**, ensuring the **rational use of last-resort antibiotics** in clinical settings.

**Table 4: Spectrum of antimicrobial agents used against gram negative isolates**

Antibiotics	Resistant	Sensitive
Ampicillin (AMP) (10µg)	09 (100%)	-
Amoxicillin clavulanic acid (AMC) (30 µg)	21 (95.45%)	01 (4.55%)
Tobramycin (TOB) (10 µg)	22 (78.57%)	06 (21.43%)
Meropenem(MRP) (10µg)	21(75.00%)	07 (25.00%)
Imipenem(IPM) (10 µg)	23 (82.14%)	05 (17.86%)
Gentamicin (GEN) (10 µg)	19 (67.86%)	09 (32.14%)
Amikacin (AK) (5 µg)	18 (64.29%)	10 (37.71%)
Ciprofloxacin (CIP) (5 µg)	14 (50.00%)	14 (50.00%)
Piperacillin-tazobactam (100/10 µg)	11(39.29%)	17 (60.71%)
Ceftriaxone (CTR) (30 µg)	23 (100.00%)	-
Cefotaxime(CTX) (30 µg)	23 (100.00%)	-
Cefepime(CPM) (30 µg)	21(75.00%)	07 (25.00%)
Ceftadizime (CAZ) (30 µg)	24(85.71%)	04 (14.29%)
Cefixime(CXM) (5 µg)	17(73.91%)	06 (26.09%)
Tetracycline (TE) (30 µg)	11(47.83%)	12 (52.17%)
Co-tromoxazole(COT) (25 µg)	14(60.87%)	09 (39.13%)
Ticarcillinclavulanic acid (TCC) (75/10 µg)	05(50.00%)	05 (50.00%)
Tigecycline (TGC) (15 µg)	01(4.35%)	22 (95.65%)
Doxycycline (DO) (30 µg)	03 (60.00%)	02 (40.00%)
Minocycline (MI) (30 µg)	04 (14.29%)	24 (85.71%)
Chloramphenicol (C)	09 (37.5%)	15 (62.5%)
Colistin (CL) (10 µg)	-	27 (100.00%)
*Intrinsic resistant drugs were not tested as per CLSI 2022		

The antimicrobial susceptibility testing of **Gram-negative isolates** revealed **widespread resistance to β-lactams**, with **100% resistance to Ampicillin, Ceftriaxone, and Cefotaxime**, indicating extensive **ESBL (Extended-Spectrum Beta-Lactamase) production**. **Amoxicillin-clavulanic acid (95.45% resistance)** further underscores **limited efficacy of β-lactam/β-lactamase inhibitors**.

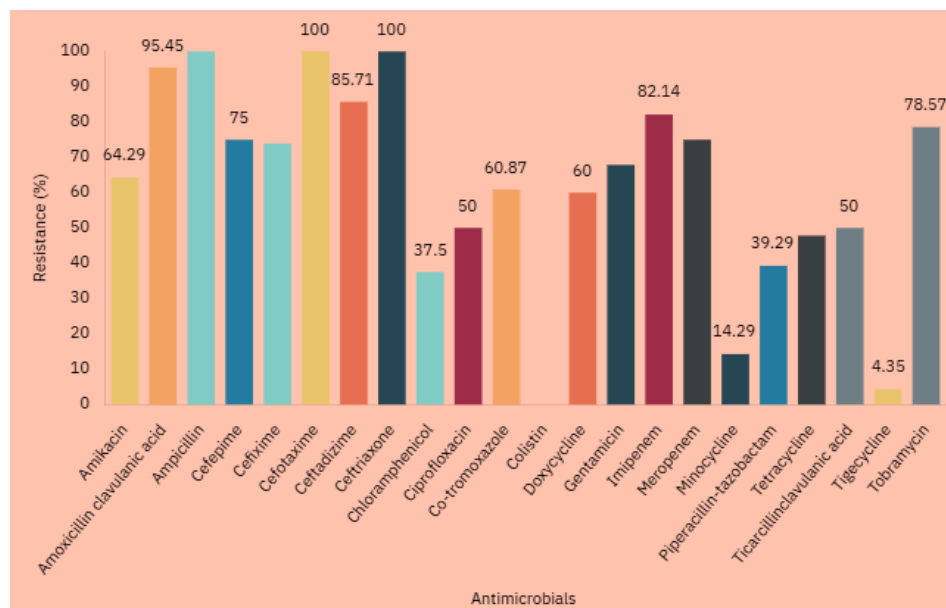
Carbapenem resistance was **notably high**, with **Imipenem (82.14%)** and **Meropenem (75.00%)** showing reduced effectiveness, suggesting the possible presence of **carbapenemase-producing isolates** (e.g., NDM, KPC, OXA-48). **Third and fourth-generation cephalosporins** (Ceftazidime, Cefepime) showed resistance rates of **85.71% and 75.00%, respectively**, reinforcing concerns over **multi-drug resistance (MDR)** in Gram-negative pathogens.

Among aminoglycosides, **Tobramycin (78.57%)** and **Gentamicin (67.86%)** showed high resistance, while **Amikacin** retained relatively better efficacy (64.29% resistance, 37.71% sensitivity). Fluoroquinolone resistance was moderate (**Ciprofloxacin 50.00%**), indicating potential **efflux pump-mediated resistance** or **topoisomerase mutations**.

Notably, **Tigecycline (95.65% sensitivity)** and **Minocycline (85.71% sensitivity)** remained highly effective, highlighting their role as **viable treatment options** against MDR isolates. **Colistin** exhibited **100% susceptibility**, reinforcing its critical role as a **last-resort antibiotic** in treating carbapenem-resistant Gram-negative infections.

The observed resistance patterns emphasize the **urgent need for routine surveillance, antimicrobial stewardship, and molecular characterization of resistance genes** to curb the spread of MDR and extensively drug-resistant (XDR) Gram-negative pathogens.

**Figure III: Spectrum of antimicrobial resistance used against gram negative isolates**



**Table 5: Microbiological and Antimicrobial Susceptibility Data in Neonatal Sepsis**

Variable	Category	n (%)	Statistical Test	p-value
Neonatal Sepsis Type	Early-Onset Sepsis (EOS)	59 (49.2%)	Chi-Square Test ( $\chi^2$ )	p = 0.42 (NS)
	Late-Onset Sepsis (LOS)	61 (50.8%)		
Blood Culture Positivity	Positive Cases	44 (36.7%)	Chi-Square Test ( $\chi^2$ )	p = 0.03*
	Negative Cases	76 (63.3%)		
Most Common Isolates	<i>Klebsiella pneumoniae</i>	14 (31.8%)	Chi-Square Test ( $\chi^2$ )	p = 0.02*
	<i>Pseudomonas aeruginosa</i>	5 (11.4%)		
	<i>Staphylococcus aureus</i>	2 (4.5%)		
Antibiotic Resistance	Ceftriaxone	100%	Chi-Square Test ( $\chi^2$ )	p < 0.001**
	Cefotaxime	100%		
	Imipenem	82.1%		



	Meropenem	75.0%		
Effective Antibiotics	Colistin	100% sensitive	Chi-Square Test ( $\chi^2$ )	p < 0.001**
	Tigecycline	95.7% sensitive	Chi-Square Test ( $\chi^2$ )	p = 0.002**

P value <0.05 Significant

#### 4. DISCUSSION

Diagnosis of neonatal sepsis is based on bacteraemia demonstrated by a positive blood culture, a method with well-known limitations in turnaround time, sensitivity, and specificity. There is no widely accepted definition for neonatal sepsis, but most definitions demand bacteraemia together with clinical signs of sepsis or increased inflammatory parameters [6]. Since blood culture has a poor sensitivity, sepsis treatment is often administered to patients with a clinical picture of sepsis but negative blood cultures; this condition is normally called clinical or suspected sepsis. The standard treatment for neonatal sepsis is intravenous broad spectrum antibiotics together with supportive intensive care. Neonatal sepsis causes increased mortality and morbidity [7,8,9], with consequences such as poor neurological outcome, bronchopulmonary dysplasia and necrotizing enterocolitis, leading to prolonged hospital stays and increased costs [10,11]. If neonatal sepsis was easier to diagnose, fewer infants would receive antibiotic treatment and the overall antibiotic consumption in neonatal intensive care could diminish.

Among total 120 suspected sepsis cases, 84 (70%) were males & 36 (30%) were females. A study by Gupta L.K *et al.* [12] also reported the male predominance among suspected cases. They observed out of 58 suspected sepsis cases, 36 (62.07%) were males & 22 (37.93%) were females.

Present study set out to determine the current prevalence, common bacterial pathogens and the antibiotic susceptibility pattern of neonatal sepsis in our facility. We observed a prevalence of 36.67% for positive blood cultures among neonates with clinical features of neonatal sepsis. The prevalence of confirmed neonatal sepsis reported in this study was similar to other study where they reported the prevalence from 34% to 35%. [13, 14]

The etiological agents of neonatal sepsis vary between developed and developing countries. *Klebsiella pneumoniae* and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from India [15, 16]. In contrast to Western nations where GBS is the predominant pathogen, Southeast Asian nations have distinct organism spectrums [17]. Consistent with this pattern, our investigation failed to identify a single group B streptococcus.

Among the gram positive isolates CONS and *Staphylococcus aureus* was the predominant. The finding of *Staphylococcus aureus* and CoNS as the predominant causative organisms of neonatal sepsis has also been reported by other researchers [18–20].

In the present study most of the antibiotics were resistant against gram negative isolates. We observed 100% cephalosporin resistance. In a similar study, 50–100% of the isolates were observed to be resistant to commonly used antibiotics especially gentamicin and the second and third generation cephalosporins [21]. In another study from North India, 30–80% of the Gram negative isolates were resistant to third-generation cephalosporins [15]. This suggests that the third-generation cephalosporins cannot be used alone for empirical treatment of neonatal sepsis.

In present study Minocycline, Tigecycline and Colistin were the most sensitive antibiotics against isolates from neonatal sepsis cases. A study from North India also reported, colistin was highly sensitive to treat neonatal sepsis [22].

In the resent study Teicoplanin, Minocycline, Tigecycline, Vancolylin and Linozoline were the higher sensitive. Similar findings was reported by Jatsho J *et al* [23]

#### 5. CONCLUSION

The prevalence of bacteraemia with positive blood cultures in children presenting with neonatal sepsis is still very high. *Staphylococcus aureus*, CoNS and *Klebsiella pneumoniae* are the prevalent pathogen. Regular surveillance of local antimicrobial resistance and review of antibiotic guidelines in the neonatal unit should be maintained.

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