RESEARCH PAPER

Bacteriological Profile and Antibiotic Sensitivity Pattern of Isolates from Neonatal Septicaemia in a Tertiary Care Hospital

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ABSTRACT

Background and Objectives

Neonatal septicaemia remains one of the foremost contributors to neonatal morbidity and mortality in developing nations, including India. Neonates represent a uniquely susceptible population due to the immaturity of their immunological defenses, and any delay in initiating appropriate antimicrobial therapy can lead to fatal outcomes. Comprehensive knowledge of the prevailing microbial flora in neonatal intensive care units (NICUs) and their corresponding antibiotic susceptibility profiles is imperative for the rational formulation of empirical treatment strategies. The escalating prevalence of multidrug-resistant (MDR) pathogens in hospital settings has further complicated the clinical management of neonatal sepsis. The present investigation was undertaken with the dual objectives of identifying the predominant bacterial etiological agents responsible for neonatal septicaemia and delineating their antibiotic susceptibility patterns at a tertiary care institution.

Methods

A hospital-based prospective observational study was conducted over a duration of twelve months in the Department of Microbiology at a tertiary care teaching hospital following institutional ethical committee approval. A total of 250 neonates presenting with clinical features suggestive of septicaemia were enrolled. Blood specimens were aseptically collected and processed using the BacT/ALERT automated microbial detection system. Bacterial identification was performed employing standard colony morphology assessment and a battery of biochemical tests. Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion methodology in strict compliance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

Of the 250 clinically suspected neonatal septicaemia cases evaluated, bacteriological confirmation was achieved in 23 cases, representing a culture positivity rate of 9.0%. Gram-negative organisms were significantly predominant, accounting for 78% (n=18) of all isolates, whereas Gram-positive organisms constituted 22% (n=5). Klebsiella pneumoniae emerged as the leading pathogen (26%), followed by Escherichia coli (21.7%), Acinetobacter baumannii (17.3%), Pseudomonas aeruginosa (8.6%), Staphylococcus aureus (8.6%), coagulase-negative staphylococci (CoNS) (8.6%), Enterobacter cloacae (4.3%), and Enterococcus faecium (4.3%). Gram-positive isolates demonstrated universal sensitivity to Levofloxacin and Linezolid. One Vancomycin-Resistant Enterococcus (VRE) strain was detected. Gram-negative organisms were uniformly susceptible to Polymyxin-B and Colistin, with 83% sensitivity to Tigecycline. Resistance to carbapenems, third-generation cephalosporins, and fluoroquinolones was notably high. The overall mortality rate among suspected neonatal septicaemia cases was 6%.

Conclusion

Gram-negative bacilli constitute the predominant etiological agents in neonatal septicaemia, with a substantial proportion exhibiting multidrug resistance. The findings highlight the indispensability of routine blood culture-guided therapy, stringent antimicrobial stewardship, and rigorous infection prevention practices within NICUs to curb the emergence and dissemination of resistant pathogens.

Keywords: Neonatal septicaemia; Bacteriological profile; Antibiotic susceptibility; Multidrug resistance; Neonatal ICU; Empirical therapy; Antimicrobial stewardship.

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1. INTRODUCTION

Neonatal septicaemia is defined as a systemic clinical syndrome arising from infection during the first 28 days of life, characterised by physiological derangements and bacteraemia [1]. It represents a leading cause of neonatal morbidity and mortality in both developed and developing nations, with the burden disproportionately borne by low- and middle-income countries (LMICs) such as India [2]. Globally, neonatal infections account for an estimated 26% of all neonatal deaths, with septicaemia being the third most common cause of

neonatal fatality, following preterm birth complications and intrapartum events [3].

Neonatal sepsis is stratified into two principal categories based on the timing of disease manifestation. Early-onset sepsis (EOS) typically presents within the initial 72 hours of life and is predominantly attributable to vertical transmission of pathogens from the maternal genitourinary tract, most notably Group B Streptococcus (GBS) and Gram-negative enteric organisms [4]. Late-onset sepsis (LOS), occurring after 72 hours, is frequently nosocomial or community-acquired in origin, with multidrug-resistant hospital-adapted organisms playing a disproportionately significant role, particularly in infants requiring prolonged hospitalisation and invasive device support [5].

The epidemiological landscape of neonatal sepsis varies markedly between geographic regions and institutional settings. In the Indian context, data from the National Neonatal Perinatal Database (NNPD) indicates that *Klebsiella pneumoniae, Escherichia coli*, and *Staphylococcus aureus* are the predominant causative organisms in EOS, while *Staphylococcus aureus* and *Streptococcus pyogenes* are frequently implicated in LOS [6]. This pattern diverges substantially from the GBS-dominated etiology observed in high-income countries, necessitating region-specific empirical treatment protocols rather than uncritical adoption of international guidelines [7].

The emergence and spread of antimicrobial resistance (AMR) among neonatal pathogens have become a critical global health concern. Multidrug-resistant Gram-negative bacilli, particularly members of the Enterobacteriaceae family and non-fermenting Gram-negative organisms, are increasingly identified as causative agents of neonatal sepsis in NICUs [8]. These organisms harbour sophisticated resistance mechanisms including extended-spectrum beta-lactamase (ESBL) production, carbapenemase production, and efflux pump upregulation, rendering many conventional antibiotic regimens ineffective [9]. The consequences of inappropriate empirical therapy in neonates are particularly severe given their limited physiological reserves and the rapidity with which infection can progress to septic shock and multi-organ failure [10].

Blood culture remains the gold standard for the confirmatory diagnosis of neonatal septicaemia, and its results are indispensable for directing definitive antimicrobial therapy [11]. Ancillary markers of sepsis, collectively termed 'sepsis screen' parameters—including C-reactive protein (CRP), the immature-to-total neutrophil (I/T) ratio, micro-erythrocyte sedimentation rate (ESR), absolute neutrophil count (ANC), and total leucocyte count (TLC)—provide supportive diagnostic information but cannot supplant microbiological confirmation [12]. Given the dynamic nature of pathogen profiles and resistance patterns across institutions and over time, periodic surveillance of the local bacteriological landscape is essential for informing empirical treatment guidelines.

The present study was designed to provide a contemporary analysis of the aerobic bacteriological profile and antibiotic susceptibility patterns of organisms causing neonatal septicaemia at a tertiary care hospital, thereby contributing to evidence-based empirical treatment selection and antimicrobial stewardship in the NICU setting.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

A hospital-based prospective observational study was carried out over a period of twelve months in the Department of Microbiology of a tertiary care teaching hospital following approval from the Institutional Ethics Committee (IEC). The study adhered to the principles of the Declaration of Helsinki and applicable local regulatory requirements.

2.2 Study Population and Sample Size

A total of 250 neonates admitted to the NICU with clinical features suggestive of septicaemia were enrolled consecutively during the study period. Inclusion criteria encompassed neonates of either gender presenting with signs and symptoms of systemic infection, including but not limited to temperature instability, respiratory distress, feeding intolerance, altered sensorium, hypotonia, seizures, skin changes, and haemodynamic instability. Neonates with gross congenital anomalies and those who had undergone surgical procedures were systematically excluded to minimise confounding.

2.3 Blood Culture Collection and Processing

Blood specimens ranging from 0.5 to 4 mL were aseptically collected from each enrolled neonate by trained nursing personnel using strict sterile precautions. The samples were inoculated immediately into BacT/ALERT PF Plus paediatric culture bottles (colour-coded yellow, bioMerieux) and transported without delay to the Microbiology laboratory. Upon receipt, the bottles were loaded into the BacT/ALERT automated microbial detection system (bioMerieux, France) and incubated continuously at 37°C for a maximum period of five days with automated monitoring for growth signals.

When an alert signal was triggered, Gram stains were prepared from the culture broth and subcultured onto Blood Agar and MacConkey Agar plates, which were incubated aerobically at 37°C for 18–24 hours. Growth was assessed, and pure cultures were processed for identification and susceptibility testing.

2.4 Organism Identification

Bacterial identification was performed through a systematic approach encompassing colony morphological characteristics, Gram staining, and a comprehensive panel of standard biochemical tests as described in the textbook by Sastry and Bhat [13]. These included catalase and coagulase tests for Gram-positive

cocci, and oxidase, triple sugar iron (TSI) agar reactions, urease, indole, methyl red, Voges-Proskauer (MR-VP), citrate utilisation, and sugar fermentation profiles for Gram-negative organisms.

2.5 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar, strictly following the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Standard commercial antibiotic disks of established potency were employed. Inhibition zone diameters were measured and interpreted as sensitive (S), intermediate (I), or resistant (R) in accordance with current CLSI breakpoints. Quality control was maintained using reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

2.6 Statistical Analysis

Categorical data were expressed as frequencies and percentages. Descriptive statistics were computed for all relevant variables. Culture positivity rates, species distribution, and sensitivity percentages were tabulated and compared with published literature. Data entry and analysis were performed using Microsoft Excel 2016 (Microsoft Corporation, USA).

3. RESULTS

3.1 Culture Positivity and Sepsis Classification

Among the 250 clinically suspected neonatal septicaemia cases enrolled during the study period, bacteriological confirmation by blood culture was achieved in 23 cases (9.0%). Of the 23 culture-positive cases, early-onset septicaemia (EOS), defined as onset within the first 72 hours of life, accounted for 16 cases (69.6%), while late-onset septicaemia (LOS) was identified in 7 cases (30.4%). The overall culture positivity rate, the distribution of EOS versus LOS, and the relative proportions of isolates are summarised in the tables below.

Table 1: Comparison of culture positivity and time of onset of septicaemia (n=23)

Time of sepsis	Culture positive isolates
Early onset sepsis(EOS)	16(70%)
Late onset sepsis (LOS)	7(30%)
Total	23(100%)

Table 2: Distribution of Gram-Positive and Gram-Negative Isolates from Neonatal Septicaemia Cases (n=23)

Organism Category	Number of Isolates	Percentage (%)
Gram-Positive Organisms	5	22.0%
Gram-Negative Organisms	18	78.0%
Total	23	100.0%

3.2 Distribution of Bacterial Isolates

Among the Gram-negative organisms (n=18), *Klebsiella pneumoniae* was the most frequently isolated pathogen, identified in 6 cases (26.0%). This was followed by *Escherichia coli* in 5 cases (21.7%), *Acinetobacter baumannii* in 4 cases (17.3%), *Pseudomonas aeruginosa* in 2 cases (8.6%), and *Enterobacter cloacae* in 1 case (4.3%). Among the Gram-positive organisms (n=5), *Staphylococcus aureus* was identified in 2 cases (8.6%), coagulase-negative staphylococci (CoNS) in 2 cases (8.6%), and *Enterococcus faecium* in 1 case (4.3%). The complete bacteriological profile is presented in Table 3.

Table 3: Bacteriological Profile of Neonatal Septicaemia – Organism-Wise Distribution (n=23)

Bacterial Isolate	Number of Isolates	Percentage (%)
Klebsiella pneumoniae	6	26.0%
Escherichia coli	5	21.7%
Acinetobacter baumannii	4	17.3%
Pseudomonas aeruginosa	2	8.6%
Staphylococcus aureus	2	8.6%
Coagulase-Negative Staphylococci (CoNS)	2	8.6%
Enterobacter cloacae	1	4.3%
Enterococcus faecium	1	4.3%
Total	23	100.0%

3.3 Antibiotic Sensitivity Pattern of Gram-Positive Isolates

Among Gram-positive organisms, all five isolates (100%) demonstrated complete sensitivity to Levofloxacin and Linezolid. Vancomycin sensitivity was noted in 80% of isolates (4 out of 5); notably, one *Enterococcus faecium* strain exhibited resistance to Vancomycin, establishing the presence of a Vancomycin-Resistant Enterococcus (VRE) in this study cohort. Both *Staphylococcus aureus* isolates were fully susceptible to Vancomycin. Lesser degrees of sensitivity were recorded for Penicillin, Ampicillin, Cefoxitin, and Amikacin, while Gentamicin, Clindamycin, and Erythromycin elicited minimal sensitivity responses. The detailed antibiotic sensitivity profiles for Gram-positive organisms are presented in Table 4.

Table 4: Antibiotic Sensitivity Pattern of Gram-Positive Isolates from Neonatal Septicaemia

Antibiotic	S. aureus (n=2)	CoNS (n=2)	E. faecium (n=1)	Total (n=5)
Penicillin	1 (50%)	1 (50%)	0 (0%)	2 (40%)
Ampicillin	1 (50%)	0 (0%)	1 (100%)	2 (40%)

Cefoxitin	1 (50%)	1 (50%)	0 (0%)	2 (40%)
Erythromycin	1 (50%)	0 (0%)	0 (0%)	1 (20%)
Clindamycin	1 (50%)	1 (50%)	0 (0%)	2 (40%)
Vancomycin	2 (100%)	2 (100%)	0 (0%)*	4 (80%)
Linezolid	2 (100%)	2 (100%)	1 (100%)	5 (100%)
Gentamicin	0 (0%)	1 (50%)	0 (0%)	1 (20%)
Amikacin	1 (50%)	1 (50%)	0 (0%)	2 (40%)
Levofloxacin	2 (100%)	2 (100%)	1 (100%)	5 (100%)

**One Vancomycin-Resistant Enterococcus (VRE) detected*

3.4 Antibiotic Sensitivity Pattern of Gram-Negative Isolates

All 18 Gram-negative isolates (100%) were uniformly susceptible to Polymyxin-B and Colistin. Tigecycline retained activity in 15 of 18 isolates (83%). Third-generation cephalosporins (Ceftazidime and Cefotaxime) demonstrated sensitivity in 61% of isolates, with Cefepime active in 55.5%. Meropenem retained activity in 44.4% and Imipenem in 33.3% of isolates, reflecting a concerning level of carbapenem resistance. Resistance to Ciprofloxacin was profound, with only 16.6% sensitivity overall. High rates of resistance were also noted for Piperacillin (22.2%), Amoxicillin-clavulanate (22.2%), and Amikacin (22.2%). Table 5 provides the complete sensitivity data for all Gram-negative isolates.

Table 5: Antibiotic Sensitivity Pattern of Gram-Negative Isolates from Neonatal Septicaemia (n=18)

Antibiotic	*K. pneumoniae* (n=6)	*E. coli* (n=5)	*A. baumannii* (n=4)	*P. aeruginosa* (n=2)	*E. cloacae* (n=1)	Total (n=18)
Amoxyclav	2 (33%)	1 (20%)	0 (0%)	1 (50%)	0 (0%)	4 (22.2%)
Piperacillin	1 (17%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	4 (22.2%)
Ceftazidime	3 (50%)	2 (40%)	4 (100%)	1 (50%)	1 (100%)	11 (61.1%)
Cefotaxime	3 (50%)	2 (40%)	4 (100%)	1 (50%)	1 (100%)	11 (61.6%)
Cefepime	3 (50%)	2 (40%)	4 (100%)	0 (0%)	1 (100%)	10 (55.5%)
Pip+Tazobactam	3 (50%)	2 (40%)	0 (0%)	1 (50%)	0 (0%)	4 (22.2%)
Imipenem	2 (33%)	1 (20%)	1 (25%)	1 (50%)	1 (100%)	6 (33.3%)
Meropenem	2 (33%)	2 (40%)	2 (50%)	1 (50%)	1 (100%)	8 (44.4%)
Gentamicin	2 (33%)	3 (60%)	1 (25%)	0 (0%)	1 (100%)	7 (38.3%)
Amikacin	1 (17%)	1 (20%)	0 (0%)	1 (50%)	1 (100%)	4 (22.2%)
Ciprofloxacin	1 (17%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	3 (16.6%)
Tigecycline	6 (100%)	4 (80%)	3 (75%)	1 (50%)	1 (100%)	15 (83%)
Polymyxin-B	6 (100%)	5 (100%)	4 (100%)	2 (100%)	1 (100%)	18 (100%)
Colistin	6 (100%)	5 (100%)	4 (100%)	2 (100%)	1 (100%)	18 (100%)
Ampicillin	1 (17%)	1 (20%)	1 (25%)	0 (0%)	1 (100%)	4 (22.2%)

4. DISCUSSION

Neonatal septicaemia continues to impose a substantial burden on healthcare systems in developing nations. The present study documents the contemporary bacteriological profile and antibiotic susceptibility patterns in a tertiary care NICU setting, with findings that are broadly concordant with existing Indian literature while raising important concerns regarding the trajectory of antimicrobial resistance.

The blood culture positivity rate of 9.0% observed in this study is consistent with data from comparable Indian studies. Verma et al. (2015) reported a positivity rate of approximately 7.6–8.0%, Tagare et al. (2010) documented 10%, and Ghelbi et al. noted an 11% positivity rate, while Dhanawade et al. (2015) reported 12.4% [15,16,17,18]. The relatively modest culture positivity rates across these studies reflect the inherent limitations of blood culture in neonates, including the small volumes of blood obtainable, intermittent or low-grade bacteraemia, prior administration of antibiotics to mothers during intrapartum care, and early empirical antibiotic treatment commenced before blood sampling in some instances [19,20].

The predominance of early-onset septicaemia (69.6%) in our cohort aligns with observations by Goyal et al., who reported an EOS frequency of 69.03% [21]. Risk factors for EOS in the Indian setting include low birth weight, preterm delivery, prolonged rupture of membranes, and deliveries occurring outside institutional settings without adequate obstetric monitoring [22]. The relatively lower proportion of late-onset septicaemia likely reflects the shorter average NICU stays in resource-limited settings and potentially better initial empirical antibiotic coverage.

The predominance of Gram-negative organisms (78%) in the present study is a characteristic feature of neonatal septicaemia in Indian healthcare settings and aligns with reports from Tagare et al. (75%) and Goyal et al. (71%) [23,24]. In contrast, GBS, the leading pathogen in high-income countries, was conspicuously absent from our isolates. This is a recurring observation in Indian neonatal sepsis studies and may be explained by the high transplacental transfer of maternal antibodies against endemic GBS serotypes, the widespread intrapartum antibiotic prophylaxis practices, and potentially differing serotype virulence profiles [25].

Klebsiella pneumoniae emerged as the single most prevalent organism (26%), corroborating its well-established role as a nosocomial pathogen in NICUs across India and South Asia [26]. Klebsiella spp. are uniquely adapted to healthcare environments, capable of colonising the hands of healthcare workers, surviving on abiotic surfaces, and acquiring resistance determinants through horizontal gene transfer. *E. coli* (21.7%) ranked second, consistent with findings by Tamboli and Nilekar (2011) [27]. The isolation of *Acinetobacter baumannii* in 17.3% of culture-positive cases is particularly concerning, as this opportunistic pathogen demonstrates a remarkable propensity for acquiring and expressing diverse resistance mechanisms, often resulting in pan-drug-resistant (PDR) strains that limit therapeutic options [28,29].

Among Gram-positive organisms, the equal distribution of *S. aureus* and CoNS (8.6% each) warrants attention, as CoNS—once considered mere contaminants—are now recognised as clinically significant pathogens in neonates, particularly in association with central venous catheter use, prolonged hospitalisation, and parenteral nutrition [30]. The presence of VRE (Enterococcus faecium) in this study, albeit as a single isolate, signals the penetration of this high-level resistant pathogen into the NICU ecosystem and necessitates heightened surveillance.

The universal sensitivity of all Gram-positive isolates to Levofloxacin and Linezolid is noteworthy; however, the use of fluoroquinolones in neonates is constrained by concerns regarding cartilage toxicity, limiting their clinical applicability to salvage situations [31]. Linezolid, on the other hand, has demonstrated safety and efficacy in neonates and represents a valuable option for methicillin-resistant S. aureus (MRSA) and VRE infections [32]. The retention of Vancomycin activity against *S. aureus* and CoNS supports its continued role as a cornerstone of empirical Gram-positive coverage in NICUs, though the identification of a VRE strain underscores the importance of periodic susceptibility reassessment.

The absolute sensitivity of all Gram-negative isolates to Polymyxin-B and Colistin, with 83% susceptibility to Tigecycline, reflects the preserved activity of last-resort agents. These antibiotics are now increasingly relied upon for carbapenem-resistant Enterobacteriaceae (CRE) and MDR Acinetobacter infections [33]. The observed carbapenem resistance—44.4% to Meropenem and 66.7% to Imipenem—represents a particularly alarming finding, given that carbapenems have traditionally constituted the definitive therapeutic option for serious Gram-negative infections. The high resistance to Ciprofloxacin (83.4%) parallels findings reported by Zakariya et al. [34] and likely reflects decades of widespread fluoroquinolone usage in the Indian community and hospital setting. These data collectively reinforce the critical importance of antimicrobial stewardship and rational prescribing.

The overall mortality rate of 6% among all suspected neonatal septicaemia cases is within the range reported from comparable tertiary care institutions in India. Prompt institution of blood culture-guided therapy, supportive intensive care, and prevention of secondary infections are the principal modifiable determinants of outcome. Structured antimicrobial stewardship programmes (ASPs), incorporating systematic auditing of antibiotic prescriptions, dose optimisation, de-escalation protocols, and regular reporting of institutional antibiograms, have been demonstrated to reduce inappropriate antibiotic use, limit resistance emergence, and improve clinical outcomes in NICUs [35].

5. CONCLUSION

The present study demonstrates that Gram-negative bacilli, principally *Klebsiella pneumoniae* and *Escherichia coli*, constitute the dominant etiological agents of neonatal septicaemia in tertiary care settings, with a substantial burden of multidrug resistance. High-

level resistance to carbapenems, fluoroquinolones, and aminoglycosides, alongside preserved activity of Polymyxin-B, Colistin, and Linezolid, delineates the current therapeutic landscape. The detection of a VRE strain and the prevalence of MDR Gram-negative organisms underscore the urgency of microbiological surveillance and evidence-based empirical treatment formulation.

Blood culture prior to antibiotic initiation remains the cornerstone of sepsis management. A coordinated and multidisciplinary commitment to rational antibiotic use, implementation of robust antimicrobial stewardship programmes, rigorous infection prevention practices—including strict hand hygiene compliance—and continuous surveillance of pathogen profiles and resistance trends are imperative to contain the spread of resistant organisms in NICUs. These measures collectively form the foundation for reducing infection-related morbidity, mortality, and the emergence of untreatable pathogens in the neonatal care environment.

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Ethical Approval:
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