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Bacteriological Profile and Antibiotic Susceptibility Pattern of Septicemia in Neonatal Intensive Care Unit in a Tertiary Care Hospital: A Prospective Observational Study

Dr Gopi Kishan Sharma[1], Dr Mohit Ajmera[2], Dr Harmandeep Kaur[3], Dr Jitendra Kumar Jain[4], Dr Dinesh Kumar Meena[5]

[1]Associate Professor, Dept of Pediatrics, GMC, Kota
[2]Associate Professor, Dept of Pediatrics, GMC, Kota
[3]PG Resident, Dept of Pediatrics, GMC, Kota
[4]Assistant Professor, Dept of Pediatrics, GMC, Kota
[5]Assistant Professor, Dept of Pediatrics, GMC, Kota

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Corresponding Author:

Dr Gopi Kishan Sharma
Associate Professor, Dept of Pediatrics, GMC kota
Email: dr. gopikishansharma6162@gmail.com

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ABSTRACT

Background: Neonatal sepsis remains a leading cause of morbidity and mortality among neonates, particularly in NICU settings of tertiary care hospitals. The emergence of multidrug-resistant organisms further complicates treatment outcomes. This study aimed to evaluate the microbiological profile and antimicrobial sensitivity patterns of culture-proven neonatal sepsis cases.
Methods: A prospective observational study was conducted over 12 months, in the NICU of a tertiary care hospital. A total of 243 neonates with clinical sepsis were enrolled. Specimens type was different for each patient depending upon the source or site of infection (blood or endotracheal tip and secretions or urine or cerebrospinal fluid (CSF) or umbilical tip). These samples were analyzed for bacteriological profile and antibiotic susceptibility pattern. Statistical analysis was conducted in accordance with standard biostatistical methods.
Results: Out of 243 neonates, 186 (76.5%) had bacteriological confirmed sepsis. Among 243, 147 were males and 96 females. The most commonly isolated organisms were Klebsiella pneumoniae (n=60), Acinetobacter spp. (n=39), and Escherichia coli (n=36). Klebsiella was resistant to cephalosporins but sensitive to colistin. Acinetobacter showed similar resistance patterns, remaining sensitive to colistin and ampicillin. Among the culture-positive cases, 138 (74.2%) neonates were discharged, while 48 (25.8%) expired. Culture-positive neonates had significantly higher mortality compared to culture-negative neonates (25.8% versus 15.8%, $p = 0.01$).
Conclusion: This study highlights the predominance of multidrug-resistant gram-negative organisms, especially Klebsiella and Acinetobacter. Continuous surveillance of antimicrobial susceptibility patterns is vital to guide empirical therapy and reduce neonatal mortality associated with sepsis.

Keywords: Acinetobacter, Antibiotic resistance, Colistin, Klebsiella, Neonatal sepsis.

INTRODUCTION

Neonatal sepsis is one of the leading causes of neonatal morbidity and mortality worldwide, particularly in developing countries, where access to timely diagnosis and optimal neonatal care remains limited [1]. Despite substantial improvements in perinatal care and advances in neonatal intensive care practices, sepsis continues to be a major contributor to neonatal deaths globally [2]. In India, neonatal sepsis represents a significant public health concern and accounts for a considerable proportion of neonatal mortality and long-term morbidity [3]. The burden of neonatal sepsis is particularly high in neonatal intensive care units (NICUs), where critically ill neonates are exposed to multiple invasive procedures,

prolonged hospital stays, and broad-spectrum antibiotic therapy [4]. Over recent years, the emergence and rapid spread of multidrug-resistant (MDR) organisms have further complicated the management of neonatal sepsis, resulting in limited therapeutic options and poorer clinical outcomes [5]. Multidrug-resistant gram-negative organisms, in particular, have been associated with increased mortality, prolonged hospitalization, and higher healthcare costs [6]. Early diagnosis and prompt initiation of appropriate antimicrobial therapy remain the cornerstones of effective management of neonatal sepsis [7]. However, the choice of empirical antibiotics is often challenging due to changing microbial patterns and increasing antimicrobial resistance. The bacteriological profile of neonatal sepsis is known to vary widely across different geographical regions [8], between healthcare institutions [9], and even within the same center over time [10]. Knowledge of the local microbial flora and their antibiotic susceptibility patterns is therefore essential for formulating effective empirical antibiotic policies [11,12]. Regular surveillance of pathogens through NICU-specific antibiograms plays a crucial role in optimizing antibiotic use, preventing inappropriate therapy, and curbing the emergence of antimicrobial resistance [13,14].

The bacteriological profile and antibiotic susceptibility patterns of neonatal sepsis are known to differ across geographical regions and healthcare settings and continue to evolve over time. However, data from several regions of India, especially from certain parts of Rajasthan, remain limited or underreported. Generating region-specific data is essential, as empirical antibiotic policies based on data from other regions may not accurately reflect the local resistance patterns and may lead to inappropriate antibiotic use. In this context, the present study was conducted to determine the bacteriological profile and antibiotic susceptibility pattern among culture-proven neonatal sepsis cases admitted to the NICU of a tertiary care hospital.

MATERIALS AND METHODS

This prospective observational study was conducted in the Neonatal Intensive Care Unit (NICU) of a tertiary care hospital attached with Govt Medical College, Kota, Rajasthan, over a period of 12 months from July 2024 to June 2025. All neonates admitted to the NICU during the study period who exhibited clinical features suggestive of sepsis were assessed for eligibility. A total of 243 neonates with clinical suspicion of sepsis were enrolled in the study by consecutive sampling method. Neonates were included in the study if they had clinical signs and symptoms suggestive of sepsis and if appropriate culture samples were obtained prior to the initiation of antibiotic therapy. Neonates were excluded if informed consent was not obtained, antibiotics had been started before sample collection, had inborn errors of metabolism, discharged or expired before the availability of culture reports, or had major congenital malformations.

Approval from the Institutional Ethics Committee was obtained before the commencement of the study. Prior written informed consent was taken from the parents or legal guardians. Detailed demographic and clinical characteristics of each enrolled neonate, including gestational age, birth weight, sex and mode of delivery were recorded at the time of admission using a predesigned and structured proforma. Specimen collection was performed based on the clinical presentation and suspected focus of infection. Samples included blood cultures and, where clinically indicated, endotracheal tip or secretion samples, urine, cerebrospinal fluid (CSF), and umbilical tip specimens. Samples were processed using standard microbiological techniques (blood samples were inoculated into pediatric blood culture bottles and incubated in the BACTEC automated blood culture system and other samples were cultured on BLOOD AGAR and MACCONKEY AGAR. Organisms were identified based on standard microbiological techniques including colony morphology, Gram staining, and relevant biochemical tests and antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines [13].

Clinical details, microbiological findings, and outcomes variables were entered into the predesigned proforma and subsequently compiled for analysis. Outcome variables were analyzed in terms of Discharged successfully, mortality and need of mechanical ventilation. Data were analyzed using appropriate statistical software (SPSS 24). Categorical variables such as demographic characteristics, bacteriological profile, antibiotic susceptibility patterns, and clinical outcomes were described in number and percentages. Associations of outcome measures were analyzed using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Total 243 suspected cases of sepsis were enrolled out of 8245 admitted neonates during the study. 186 cases (76.5%) had found culture positive sepsis. We found male predominance (141, 58%). 173 (71.1%) neonates had less than 2.5 kg weight while 157 cases (64.6 %) were preterm (Gestational age < 37 week). Demographic profile of the enrolled neonates is demonstrated in Table 1.

Table 1: Demographic profile of the enrolled neonates

Parameter	Culture-Positive Neonates (N = 186)	Culture-Negative Neonates (N = 57)	Total (N=243)

Sex	Male	111	30	141(58%)
	Female	75	27	102 (42%)
Weight	Low birth weight (1.5 kg – 2.49 kg)	72	25	97 (39.9%)
	Very low birth weight (1 kg to 1.49 kg)	41	16	57(23.4%)
	Extremely low birth weight (less than 1 kg)	13	6	19 (7.8%)
Gestatinal age	Term (> 37 week)	74	12	86 (35.4%)
	Preterm (< 37 week)	112	45	157 (64.6%)
Mode of delivery	Cesarean section	40	17	57 (23.4%)
	Vaginal	146	40	186 (76.5%)

Among the 186 culture-positive neonatal sepsis cases, *Klebsiella pneumoniae* was the most commonly isolated organism (60, 32.3%). This was followed by *Acinetobacter* species (39, 21.0%), and *Escherichia coli* (36, 19.4%). The detail of isolated organisms is illustrated in Table 2 and Figure 1.

Table 2: Bacteriological profile of isolated organisms

Organism	**Number (N = 186)**	**Percentage (%)**
Klebsiella pneumoniae	60	32.3
Acinetobacter spp.	39	21.0
Escherichia coli	36	19.4
Enterococcus	32	17.2
Staphylococcus aureus	10	5.4
Enterobacter	3	1.6
Pseudomonas	3	1.6
Citrobacter	2	1.1
CONS	1	0.5

The antibiotic susceptibility pattern of the common isolates revealed a high level of resistance to frequently used antibiotics. *Klebsiella pneumoniae* showed resistance to ampicillin and cephalosporins, with variable sensitivity to aminoglycosides and reduced sensitivity to carbapenems. *Acinetobacter* species demonstrated sensitivity to ampicillin but were resistant to cephalosporins, exhibited variable susceptibility to aminoglycosides, and showed reduced sensitivity to carbapenems. *Escherichia coli* isolates were resistant to ampicillin and cephalosporins, showed variable sensitivity to aminoglycosides, and exhibited moderate sensitivity to carbapenems. Notably, all three major organisms—*Klebsiella pneumoniae*, *Acinetobacter* species, and *Escherichia coli*—remained uniformly sensitive to colistin, highlighting its role as an effective last-resort antibiotic against multidrug-resistant neonatal sepsis pathogens.

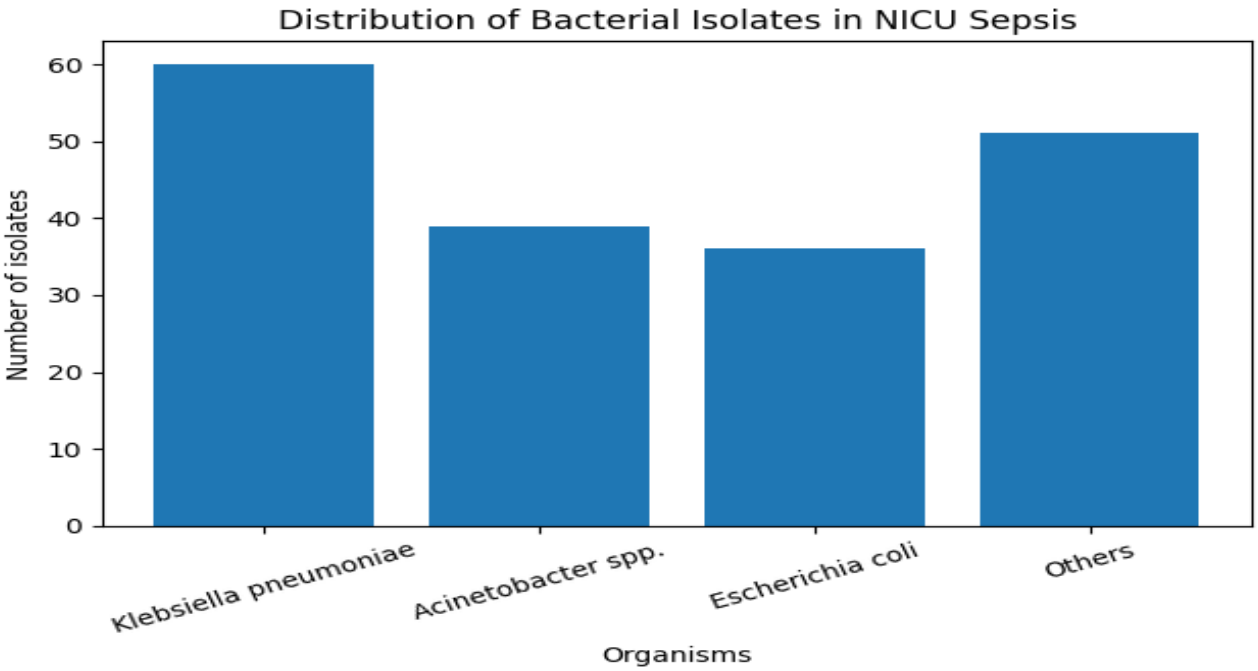

Figure 1: Distribution of bacterial isolates causing neonatal sepsis in NICU

Among the 186 culture-positive neonates, 138 (74.2%) were successfully discharged following treatment, while 48 (25.8%) succumbed to the illness. Culture-positive neonates had significantly higher mortality compared to culture-negative neonates (25.8% versus 15.8%, p = 0.01). The requirement for mechanical ventilation was higher among culture-positive cases than culture-negative cases ((55.9% versus 31.6%, p = 0.06). The data of outcome variables are shown in table 3.

Table 3: Comparison of outcome variables between culture-positive and culture-negative neonates

Variable	Culture positive (n = 186)	Culture negative (n = 57)	P value*
Discharged successfully	138 (74.2%)	48 (84.2)	0.01
Mortality	48 (25.8%)	9 (15.8%)	0.01
Need for mechanical ventilation	104 (55.9%)	18 (31.6%)	0.06

*** Chi-square test**

DISCUSSION

In the present study, the culture positivity rate among clinically suspected neonatal sepsis cases was 76.5%. This relatively high culture positivity rate is comparable to reports from other tertiary care NICUs in India and reflects the high burden of neonatal infections in referral centers that cater to critically ill and high-risk neonates [9].

A male predominance was observed among culture-positive neonates in this study. Similar findings have been consistently reported in earlier studies. This gender difference may be attributed to genetic and immunological factors, including X-linked immune regulatory mechanisms, which may predispose male neonates to a higher risk of infections [1]. This study demonstrated a clear predominance of gram-negative organisms as the causative agents of neonatal sepsis. *Klebsiella pneumoniae* was the most frequently isolated organism, followed by *Acinetobacter* species and *Escherichia coli*. This bacteriological pattern is consistent with several NICU-based studies from India and reflects a shift toward gram-negative predominance in neonatal sepsis in developing countries [15].

A major concern identified in this study was the high level of resistance to commonly used antibiotics, particularly third-generation cephalosporins, among *Klebsiella* and *Acinetobacter* isolates. The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing gram-negative organisms causing neonatal sepsis has been widely reported and poses a significant therapeutic challenge [16]. In the present study, Colistin emerged as the most effective antibiotic against multidrug-resistant gram-negative organisms. This finding is in agreement with earlier reports that identify Colistin as a last-resort drug for the treatment of MDR neonatal infections, particularly in settings with high resistance to first-line and second-line antibiotics [17]. However, reliance on colistin raises concerns regarding toxicity and the potential emergence of colistin resistance. The rising resistance to first-line antibiotics underscores the urgent need for rational antibiotic use in

NICUs. Regular monitoring of antimicrobial susceptibility patterns, strict adherence to antibiotic stewardship programs, and periodic revision of empirical antibiotic policies are essential strategies to limit the spread of antimicrobial resistance [18].

The mortality rate among culture-positive neonates in this study was 25.8%. High mortality associated with neonatal sepsis has been reported previously, particularly among preterm and low birth weight neonates. Infections caused by multidrug-resistant organisms further worsen outcomes by delaying effective therapy and limiting treatment options [3].

The present study has certain limitations that should be acknowledged. Being a single-center study conducted in a tertiary care NICU, the findings may not be fully generalizable to other healthcare settings or regions with different patient populations and microbial profiles. Although a reasonable number of neonates were included, the sample size was relatively limited, which may restrict the statistical power to detect significant associations between certain variables. In addition, the study focused only on aerobic bacterial pathogens; anaerobic and fungal cultures were not performed, which may have led to an underestimation of the complete spectrum of pathogens responsible for neonatal sepsis.

The present study highlights the predominance of multidrug-resistant gram-negative organisms as the major causative agents of neonatal sepsis in a tertiary care NICU. *Klebsiella pneumoniae* and *Acinetobacter* species were the most frequently isolated pathogens among culture-positive neonates A high level of resistance to commonly used antibiotics, particularly cephalosporins, was observed, while Colistin remained effective against most multidrug-resistant isolates. These findings emphasize the shrinking arsenal of effective antibiotics for neonatal sepsis and the growing dependence on last-line agents. Continuous surveillance of antimicrobial susceptibility patterns is essential to guide empirical antibiotic therapy in NICUs. Regular updating of unit-specific antibiograms, strict implementation of antibiotic stewardship principles, and rational antibiotic prescribing practices are crucial to reduce antimicrobial resistance, improve treatment outcomes, and ultimately decrease neonatal mortality.

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