

## Prevalence of Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Bacteria in Neonatal Septicemia: An Observational Study from a Tertiary Care Center in Kanpur, India

Kayyum Khan<sup>1</sup>, Prajakta Radke<sup>2</sup>, Zaki Shaikh<sup>3</sup>, Waqas Alauddin<sup>4</sup>

<sup>1</sup>Assistant Professor, Department of Microbiology, Naraina Medical College and Research Centre, Kanpur, IND

<sup>2</sup>Associate Professor, Department of Physiology, MGM Medical College, Nerul, Navi Mumbai, India

<sup>3</sup>Associate Professor, Department of Physiology, MGM Medical College, Nerul, Navi Mumbai, India

<sup>4</sup>Assistant Professor, Department of Physiology, Naraina Medical College and Research Centre, Kanpur, India

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Corresponding author: Dr. Waqas Alauddin

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### Abstract

**Background:** Neonatal septicemia remains a leading cause of morbidity and mortality in low- and middle-income nations. In India, the rising incidence of multidrug-resistant Gram-negative organisms, particularly extended-spectrum beta-lactamase (ESBL) producers, has undermined the effectiveness of standard empirical therapy. This study aimed to determine the prevalence and clinical impact of ESBL-producing Gram-negative organisms among neonates with sepsis in a tertiary hospital in Kanpur, India.

**Methods:** A prospective observational study was carried out between January and December 2024. Blood cultures from neonates with suspected sepsis were processed using automated systems. Gram-negative isolates were identified, and ESBL production was confirmed according to Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines. Clinical variables, including demographic data, onset of sepsis, and patient outcomes, were analyzed.

**Results:** Out of 520 blood cultures, 312 (60%) showed growth, including 190 Gram-negative isolates. The predominant pathogens were *Klebsiella* spp. (50.5%), *Escherichia coli* (29.5%), *Acinetobacter* spp. (10.5%), and *Pseudomonas* spp. (9.5%). ESBL production was confirmed in 35 isolates (18.4%), with *Klebsiella* spp. representing the largest proportion (45.7%). Male neonates constituted 57.9% of cases, and early-onset sepsis occurred in 65%. Mortality among neonates with ESBL-positive infections was 20%, compared with 12% in ESBL-negative cases ( $p < 0.05$ ).

**Conclusion:** ESBL-producing Gram-negative bacilli are an important contributor to neonatal sepsis in Kanpur. The predominance of *Klebsiella* spp. and higher mortality in ESBL-associated infections highlight the urgent need for ongoing resistance surveillance, rational antimicrobial use, and stringent infection control strategies.

**Keywords:** Extended-Spectrum Beta-Lactamase (ESBL), Neonatal Sepsis, Gram-Negative Bacilli.

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### Introduction

Neonatal sepsis is a major global health problem, disproportionately affecting low- and middle-income countries. India bears a significant share of this burden due to suboptimal infection control, premature births, and the widespread misuse of antimicrobials [1,2].

The emergence of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacilli further complicates treatment, as these enzymes hydrolyze third-generation cephalosporins and related beta-lactams [3,4]. In neonatal intensive care units (NICUs), ESBL-producing organisms are associated with prolonged hospitalization,

increased healthcare costs, and higher mortality rates [5]. The frequent use of cephalosporins has accelerated this trend [6].

While several multicenter studies have documented ESBL prevalence in Indian metropolitan hospitals [7,8], data from semi-urban centers such as Kanpur are scarce.

Local surveillance data are crucial to refine empirical treatment protocols and inform antimicrobial stewardship [9].

This study aimed to determine the prevalence of ESBL-producing Gram-negative organisms in

neonatal sepsis in Kanpur and to evaluate their clinical implications.

### Materials and Methods

**Study Design and Population-** This prospective observational study was conducted in the Department of Microbiology, a tertiary care hospital in Kanpur, from January to December 2024. Informed consent was provided by parents or guardians.

Neonates aged 0–28 days admitted with clinical suspicion of sepsis (fever, lethargy, poor feeding, or respiratory distress) were included. Exclusion criteria were prior antibiotic therapy for >48 hours and incomplete clinical data.

**Sample Collection and Processing-** Two milliliters of venous blood were inoculated into pediatric blood culture bottles and incubated in an automated system (BACTEC, Becton Dickinson). Positive cultures were sub-cultured on MacConkey and blood agar. Organisms were identified using biochemical methods and confirmed by VITEK-2 (bioMérieux).

**Antimicrobial Susceptibility and ESBL Detection-** Antimicrobial susceptibility testing was performed

by the Kirby–Bauer disk diffusion method, interpreted according to CLSI 2018. Screening for ESBL was done using ceftazidime (30 µg) and cefotaxime (30 µg). Confirmatory testing used ceftazidime-clavulanate and cefotaxime-clavulanate disks. An increase in inhibition zone diameter  $\geq 5$  mm in the presence of clavulanate was considered ESBL-positive.

**Clinical Data and Analysis:** Clinical variables (sex, age of onset, NICU stay, and outcomes) were recorded. Statistical analysis was performed using SPSS version 22.0. Chi-square tests were applied for categorical variables;  $p < 0.05$  was considered significant.

### Results

**Distribution of Isolates-** of 520 blood cultures, 312 (60%) were positive. Gram-negative bacilli accounted for 190 isolates (60.9%), while 122 (39.1%) were Gram-positive.

*Klebsiella* spp. (50.5%) was the predominant Gram-negative organism, followed by *E. coli* (29.5%), *Acinetobacter* spp. (10.5%), and *Pseudomonas* spp. (9.5%).

**Table 1: Distribution of Gram-negative isolates and ESBL production**

Organism	Total Isolates (n=190)	% of GN Isolates	ESBL-Positive (n=35)	% of ESBL Producers
<i>Klebsiella</i> spp.	96	50.5%	16	45.7%
<i>Escherichia coli</i>	56	29.5%	12	34.2%
<i>Acinetobacter</i> spp.	20	10.5%	5	14.3%
<i>Pseudomonas</i> spp.	18	9.5%	2	5.8%
Total	190	100%	35	18.4%

**Clinical Characteristics and Outcomes:** Male neonates represented 57.9% of infections. Early-onset sepsis ( $\leq 72$  hours) accounted for 65% of cases, while 35% were late-onset. Mortality was significantly higher in ESBL-positive infections (20%) compared with ESBL-negative infections (12%,  $p < 0.05$ ).

**Table 2: Clinical characteristics and outcomes of neonates with Gram-negative septicemia**

Clinical Parameter	ESBL-Positive (n=35)	ESBL-Negative (n=155)	p-value
Male sex	21 (60%)	89 (57.4%)	0.81
Early-onset sepsis	22 (62.9%)	102 (65.8%)	0.74
Mortality	7 (20%)	19 (12.3%)	<0.05*

\* p-value <0.05 is significant

### Discussion

This study demonstrates that ESBL-producing Gram-negative organisms are a significant cause of neonatal sepsis in Kanpur, with a prevalence of 18.4%. These findings are consistent with national data reporting ESBL rates of 15–30% in NICUs [9,10]. *Klebsiella* spp. emerged as the most frequent ESBL producer, followed by *E. coli*, consistent with earlier studies [11,12]. *Klebsiella*'s high propensity for plasmid-mediated resistance transmission underscores its epidemiological importance [13]. Male predominance in neonatal sepsis was again confirmed, in line with global and

regional data attributing this to immunological differences [14]. Early-onset cases predominated, suggesting significant perinatal or vertical transmission, particularly in resource-limited maternity environments [15].

Mortality was significantly higher among ESBL-positive cases, likely reflecting delays in effective therapy due to initial empirical use of cephalosporins. Similar findings have been reported internationally [5,15]. This study underscores the urgent need to revise empirical therapy protocols in NICUs, moving away from cephalosporins in high-resistance settings,

alongside stringent infection control and antimicrobial stewardship.

### Conclusion

ESBL-producing Gram-negative organisms constitute a notable threat in neonatal septicemia in Kanpur, with *Klebsiella* spp. as the leading pathogen. ESBL-positive infections were linked to significantly higher mortality.

Continuous surveillance, strict antibiotic stewardship, and improved infection prevention are essential to reduce the burden of neonatal sepsis.

### References

1. Vergnano S, et al. Neonatal sepsis: an international perspective. *Arch Dis Child Fetal Neonatal Ed.* 2005;90(3): F220–F224.
2. Sankar MJ, et al. Neonatal sepsis in South Asia: progress and challenges. *Semin Perinatol.* 2016;40(6):433–443.
3. Karanika S, et al. Clinical outcomes of infections caused by ESBL-producing Enterobacteriaceae. *Clin Infect Dis.* 2016; 63(6): 759–766.
4. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev.* 2005;18(4):657–686.
5. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae. *J Infect Dis.* 2017;215(suppl\_1): S28–S36.
6. Kaur P, et al. Prevalence of ESBL-producing Enterobacteriaceae in neonatal sepsis: a tertiary care experience. *Indian J Pathol Microbiol.* 2017;60(4):541–546.
7. Bhat YR, Lewis LE. Bacterial pathogens and resistance patterns in neonatal sepsis in India. *J Pediatr Infect Dis.* 2011;6(4):227–234.
8. Sundaram V, et al. Blood culture confirmed bacterial sepsis in neonates in India. *Pediatr Infect Dis J.* 2009;28(12):1079–1082.
9. Bhattacharya S. ESBL-producing Enterobacteriaceae in neonatal sepsis: Indian scenario. *Indian Pediatr.* 2013;50(7):583–587.
10. Sharma CM, et al. Neonatal septicemia in Nepal: early-onset vs late-onset. *Int J Pediatr.* 2018; 2018:3797025.
11. Roy S, et al. Prevalence of multidrug-resistant Gram-negative bacteria in NICU settings. *Indian J Med Microbiol.* 2019;37(1):46–52.
12. Dutta S, et al. Antimicrobial resistance in neonatal sepsis: Indian NICU study. *J Trop Pediatr.* 2014;60(5):377–382.
13. Yadav S, et al. Gender differences in neonatal sepsis outcomes. *Indian Pediatr.* 2017; 54(4): 345–349.
14. Stoll BJ, et al. Early-onset neonatal sepsis: maternal and neonatal risk factors. *J Pediatr.* 2011;159(1):72–78.
15. Tziella C, et al. New insights into neonatal sepsis: impact of multidrug resistance. *Front Pediatr.* 2018; 6:285.