

## Microbial Spectrum and Antibiotic Sensitivity Patterns in Neonatal Blood Cultures: A NICU Based Study from Quetta, Pakistan

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### ABSTRACT

**Objective:** To document the microbial spectrum and antibiotic sensitivity patterns in blood cultures of suspected neonatal sepsis.

**Study Design:** Cross-sectional study.

**Place and duration:** Neonatal Intensive Care Unit (NICU) of Combined Military Hospital, Quetta, Pakistan from May 2023 to Oct 2023.

**Methodology:** The study analyzed neonates diagnosed with sepsis based on specific risk factors and clinical signs of bacterial infections. A thorough clinical assessment was conducted to confirm the presence of suspected sepsis. Specimens of neonates were processed using standard microbiological techniques for culture and sensitivity analysis.

**Results:** In a total of 119 neonates, 68(57.1%) were boys. The median age was 2.00 days (1.00-4.00). Culture proven sepsis was found in 33(27.7%) specimens. Among 33 neonates with culture proven sepsis, *Serratia marcescens*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were found to be the most frequent isolates, noted in 16(48.5%), 4(12.1%) and 4(12.1%) cases, respectively. *Serratia marcescens* were found to be highly resistant (100.0%) to ampicillin, cefipime, cefotaxime, and doxycycline whereas these were 100.0% sensitive to Tazocin. *Acinetobacter baumannii* were found resistant 3rd generation cephalosporins but found 100.0% sensitive to Polymyxin B. *Klebsiella pneumoniae* were 100% sensitive to Polymyxin B. *Enterococcus species* were 100.0% sensitive to Linezolid.

**Conclusion:** Gender, birth weight, gestational age, and mode of delivery had significant association with culture-proven neonatal sepsis. The identification of *Serratia marcescens*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* as most commonly found isolates and high resistance patterns to most commonly antibiotics highlight the imperative for precise antibiotic selection guided by local resistance profiles.

**Keywords:** *Acinetobacter baumannii*, Blood culture, *Klebsiella pneumoniae*, Neonate, Sepsis, *Serratia marcescens*.

**How to Cite This Article:** Ahmed SM, Shoaib M, Ahmed W, Yasmeen S, Ali HM, Najeebulah. Microbial Spectrum and Antibiotic Sensitivity Patterns in Neonatal Blood Cultures: A NICU Based Study from Quetta, Pakistan. *Pak Armed Forces Med J* 2026; 76(SUPPL-1): S254-S259.

DOI: <https://doi.org/10.51253/pafmj.v76iSUPPL-1.11430>

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### INTRODUCTION

Neonatal mortality accounts for 70% of the infant mortality rate while the primary factors contributing to neonatal mortality are prematurity (38%), neonatal infections (31%), and congenital malformations (16%).<sup>1</sup> Neonatal sepsis remains a significant global health concern. Sepsis in newborns can be life-threatening and is often caused by bacteria or other pathogens transmitted during childbirth, through the mother, or from the environment.<sup>2</sup> The incidence of neonatal sepsis varies between 7 to 38 per 1000 live births in Asia, 7-23 per 1000 live births in Africa, 4-9 per 1000 live births in South America and 9 per 1000 live births in America.<sup>3,4</sup> In low- and middle-income countries, neonatal sepsis is one of the most common causes behind neonatal mortality.<sup>5</sup>

Diagnosing neonatal sepsis presents numerous

challenges due to nonspecific clinical manifestations, especially in the early stages. Symptoms of neonatal sepsis can include temperature instability (fever or hypothermia), respiratory distress, poor feeding, lethargy, and abnormal vital signs.<sup>6,7</sup> These symptoms can overlap with those of other neonatal conditions, making it challenging to differentiate sepsis from non-infectious conditions. Early recognition and diagnosis are crucial, but this remains difficult due to the absence of definitive diagnostic tests and reliance on clinical judgment.<sup>8</sup> Untreated or inadequately managed neonatal sepsis can lead to severe consequences. Survivors might face long-term health issues, including developmental delays, neurological impairments, hearing loss, and other disabilities.<sup>9</sup> Sepsis-associated complications can significantly impact the quality of life for affected infants and their families.<sup>10</sup>

Antibiotic resistance poses a growing threat in treating neonatal sepsis. Prolonged or inappropriate

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**Received:** 13 Jan 2024; **revision received:** 25 Dec 2025; **accepted:** 21 Jan 2026

use of antibiotics in neonates can contribute to the emergence of multidrug-resistant organisms.<sup>11</sup> This resistance complicates treatment options, leading to increased morbidity, prolonged hospital stays, and higher healthcare costs. Limited antibiotic choices due to resistance can result in the use of broad-spectrum antibiotics, which may not always be the most effective or safe option for newborns.

Understanding the spectrum of pathogens causing neonatal sepsis and their antibiotic sensitivity patterns is crucial for tailoring effective and targeted antibiotic treatments. Identifying the most common pathogens and their sensitivity profiles can guide clinicians in selecting the most appropriate antibiotics promptly, thereby potentially reducing morbidity and mortality rates in neonatal populations. Data from this study can contribute to formulating or refining antibiotic guidelines specific to NICU settings, aiding in the judicious use of antibiotics and combating antibiotic resistance. The objectives of study were to document the microbial spectrum and antibiotic sensitivity patterns in blood cultures of suspected neonatal sepsis.

## METHODOLOGY

This cross-sectional study was conducted in Neonatal Intensive Care Unit (NICU) of Combined Military Hospital, Quetta, Pakistan during May 2023 to October 2023. Approval from Institutional Ethical Committee was acquired (Letter number 391, CMH QTA-IERB/18/2023, dated April 2023). Considering the anticipated prevalence of neonatal sepsis as 12.8%,<sup>11</sup> with 95% confidence level, and 6% margin of error, the sample size was calculated to be 119. The sample size was calculated using the formula:  $n=z^2*p*(1-p)/e^2$ . The study enrolled neonates diagnosed with sepsis based on specific risk factors and clinical signs of bacterial infections. The risk factors considered were low birthweight (<2500 grams) or prematurity (<37 weeks gestation), maternal fever within 2 weeks before delivery, meconium-stained amniotic fluid, suspected chorioamnionitis, prolonged rupture of membranes (>18 hours), prolonged labor (>24 hours), and perinatal asphyxia (Apgar score <4 at 1 minute). Clinical signs indicating sepsis included poor reflexes, lethargy, respiratory distress, bradycardia, apnea, fever, convulsions, abdominal distension, and bleeding.

**Exclusions criteria:** Were neonates whose mothers were unavailable for interviews to supplement the data, even if the neonates met inclusion criteria. This

exclusion aimed to avoid potential data inconsistencies or incompleteness. Additionally, critically ill newborns unable to undergo necessary laboratory evaluations were also excluded.

**Inclusion Criteria:** Neonates meeting the inclusion criteria were identified from the NICU of Combined Military Hospital, Quetta, and informed as well as written consents were obtained from the parents or guardians of the neonates involved in the study. Interviews with mothers or guardians were conducted to gather additional information complementing the clinical data. A thorough clinical assessment of enrolled neonates was conducted to confirm the presence of suspected sepsis. This assessment involved observing specific risk factors and clinical signs as outlined in the inclusion criteria. Data regarding the gender, age at the time of admission (days), and birth weight (as per medical record) were noted. Onset of sepsis either early-onset sepsis (EOS, within the first 72 hours of life) or late-onset (LOS, after 72 hours), was documented. Gestational age and mode of delivery were also noted. Neonates specimens were processed using standard microbiological techniques for culture and sensitivity analysis. Antibiotic sensitivity testing was performed on isolated pathogens to determine their response to various antibiotics. Collected data were meticulously organized, ensuring accuracy and consistency. Anonymization procedures were employed to maintain confidentiality and privacy.

The data analysis was performed employing "IBM-Statistical Package for Social Sciences" version 26.0. Categorical variables were depicted using frequency and percentages. Continuous variables like age were shown as median and interquartile range (IQR) as it was non-normally distributed (checked by Shapiro-Wilk test). Effect modifiers like gender, age groups, birth weight, onset of sepsis, gestational age, delivery mode, maternal age, and specimen type were stratified to see their effect on outcome (culture proven sepsis). Post-stratification, chi-square test was applied taking  $p<0.05$  as significant.

## RESULTS

In a total of 119 neonates, 68(57.1%) were boys. The median age was 2.00 days (1.00-4.00). Birth weight was 2.5 kg or more in 77(64.7%) neonates. Early onset sepsis was noted in 87(73.1%) cases. Gestational age distribution showed 70(58.8%) neonates were term. Maternal age distribution indicated that 66(55.5%) mothers were below 30 years. Blood specimens

constituted the majority, 111(93.3%). Table-I is showing characteristics of neonates studied.

Table-I: Characteristics of Neonates (n=119)

Characteristics		Frequency (%)
Gender	Boys	68(57.1%)
	Girls	51(42.9%)
Age (days)	1	57(47.9%)
	2-7	37(31.1%)
	8-28	25(21.0%)
Birth weight (kg)	<2.5	42(35.3%)
	≥2.5	77(64.7%)
Onset of sepsis	Early onset sepsis	87(73.1%)
	Late onset sepsis	32(26.9%)
Gestational age	Pre-term	49(41.2%)
	Term	70(58.8%)
Delivery mode	Vaginal delivery	55 (46.2%)
	Cesarean section	64(53.8%)
Maternal age (years)	<30	66(55.5%)
	≥30	53(44.5%)
Specimen type	Blood	111(93.3%)
	Cerebral spinal fluid	6(5.0%)
	Urine	2(1.7%)

Culture proven sepsis was found in 33(27.7%) specimens. It was found that girls were having significant association with culture proven sepsis (57.6% vs. 37.2%,  $p=0.044$ ). Low birth weight ( $<2.5$  kg) was having significant association with culture proven sepsis (54.5% vs. 27.9%,  $p=0.006$ ). Pre-term neonates were found to have significant association with culture proven sepsis (63.6% vs. 32.6%,  $p=0.002$ ). Mode of delivery as cesarean section was found to have significant association with culture proven sepsis (72.7% vs. 46.5%,  $p=0.010$ ). Table-II is showing comparison of baseline characteristics of neonates with respect to culture findings.

Table-II: Comparison of Baseline Characteristics of Neonates with Respect to Culture Findings (n=119)

Characteristics	Culture Proven Sepsis		$p$ -value
	Yes (n=33)	No (n=86)	
Gender	Boys	14(42.4%)	0.044
	Girls	19(57.6%)	
Age (days)	1	13(39.4%)	0.088
	2-7	8(24.2%)	
	8-28	12(36.4%)	
Birth weight (kg)	<2.5	18(54.5%)	0.006
	≥2.5	15(45.5%)	
Onset of sepsis	Early onset sepsis	21(63.6%)	0.149
	Late onset sepsis	12(36.4%)	
Gestational age	Pre-term	21(63.6%)	0.002
	Term	12(36.4%)	
Delivery mode	Vaginal delivery	9(27.3%)	0.010
	Cesarean section	24(72.7%)	
Maternal age (years)	<30	15(45.5%)	0.174
	≥30	18(54.5%)	
Specimen type	Blood	30(90.9%)	0.320
	Cerebral spinal fluid	3(9.1%)	
	Urine	-	
		2 (2.3%)	

Among 33 neonates with culture proven sepsis, *Serratia marcescens*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were found to be the most frequent isolates, noted in 16(48.5%), 4(12.1%) and 4(12.1%) cases, respectively. Complete frequency distribution of various types of isolates found in positive cultures among neonates is shown in Figure-I.

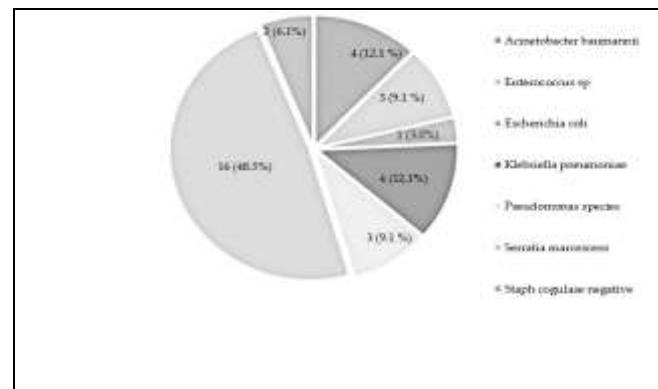


Figure-I: Frequency Distribution of Isolates Organisms (n=33)

*Serratia marcescens* were found to be highly resistant (100%) to ampicillin, cefipime, cefotaxime, doxycycline, and polymyxin B whereas these were 100% sensitive to Tazocin. *Acinetobacter baumannii* were found resistant 3<sup>rd</sup> generation cephalosporins but found 100% sensitive to Polymyxin B. *Klebsiella pneumoniae* were 100% sensitive to Polymyxin B. *Enterococcus* species were 100% sensitive to Linezolid. Details about the antibiotic susceptibility and resistance profiles of isolated organisms is shown in table-III.

## DISCUSSION

The findings of this study shed light on the prevalence and microbial spectrum of neonatal sepsis. We found the overall culture-proven sepsis rate of 27.7% highlighting the substantial burden of neonatal sepsis. In hospital-based reports from South Asia, the collective incidence of culture-positive neonatal sepsis stands at 15.8 per 1000 live births (95% CI 12.7 to 18.8).<sup>14</sup> This rate notably surpasses the incidence reported in higher-income countries like England and the United States, marking a two to fourfold higher occurrence.<sup>15,16</sup> Recent local and regional data has reported culture proven neonatal sepsis rates between 8.3% to 43%.<sup>17-19</sup> Despite advancements in healthcare, the incidence of neonatal sepsis in this region has shown little evidence of decline over the past decade, reflecting persistent challenges in combating this critical health issue.<sup>20</sup> Alarmingly, approximately one-

Table-III: Antibiotic Susceptibility and Resistance Profile of Isolated Organisms

Antibiotics	Acinetobacter baumannii (n=4)		Enterococcus species (n=3)		Klebsiella pneumoniae (n=4)		Pseudomonas species (n=3)		Serratia marcescens (n=16)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amikacin	-	100	33	67	-	100	100	-	19	81
Ampicillin	-	100	-	100	-	100	-	-	-	100
Co-amoxiclav	-	100	67	33	-	100	-	-	13	87
Cefipime	-	50	-	-	-	100	-	-	-	100
Cefotaxime	-	-	-	-	-	-	-	-	-	100
Ceftazidime	-	-	-	-	-	-	67	33	-	-
Ceftriaxone	-	100	-	-	-	100	33	67	81	19
Ciprofloxacin	-	100	-	-	-	100	100	-	69	31
Cotrimoxazole	-	100	-	-	-	100	-	-	81	19
Doxycycline	-	100	-	-	-	100	-	-	-	100
Gentamycin	-	-	67	33	-	-	-	-	-	-
Linezolid	-	-	100	-	-	-	-	-	-	-
Meropenem	-	100	-	-	50	50	100	-	69	31
Polymyxin B	100	-	-	-	100	-	-	-	-	100
Tazocin	-	100	-	-	50	50	100	-	100	-
Vancomycin	-	-	67	33	-	100	-	-	-	-

third of neonates diagnosed with culture-positive sepsis succumb to the condition, demonstrating a median case fatality rate of 34.4%, indicating the severe toll this condition takes on newborns in these settings.<sup>14</sup>

Notably, significant associations were observed between certain demographic and clinical factors with culture-proven sepsis. Girls exhibited a higher association with culture-proven sepsis compared to boys, aligning contrary to previous studies that have suggested male gender to be significantly more susceptible to neonatal sepsis.<sup>21,22</sup> The substantial association of low birth weight (<2.5 kg) and pre-term birth with culture-proven sepsis is consistent with the previous findings.<sup>23</sup> These observations further underscore the vulnerability of underweight and premature neonates to infections, likely due to their immature immune systems and underdeveloped barriers to infection. Significant association of cesarean section delivery with culture-proven sepsis implicates potential factors linked to the mode of delivery that may influence microbial colonization or susceptibility to infections in neonates, warranting further investigation.

The identification of specific pathogens in culture-positive cases, *Serratia marcescens*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, to be the most frequent ones, underscore the diversity and significance of these organisms in causing neonatal sepsis. Shaikh *et al.*, from Karachi noted *Staphylococcus aureus*, *pseudomonas*, and *klebsiella* species to be the most frequent micro-organisms involved in neonatal sepsis which is different to what we noted.<sup>18</sup> Regional data from hospital settings analyzing 24,273 isolates revealed that Gram-negative

organisms constituted the majority, accounting for 63% of the isolates studied and our findings are consistent with what has been described previously in the literature.<sup>24,25</sup> This detailed microbial profile highlights the predominance of specific bacterial species commonly encountered in hospital-acquired infections, underscoring the significance of these organisms in clinical settings.

This study highlighted high resistance of *Serratia marcescens* to multiple commonly used antibiotics calls for careful consideration when selecting empirical treatment regimens in settings where these pathogens are prevalent. The susceptibility profiles observed in this study, notably the resistance of *Acinetobacter baumannii* to third-generation cephalosporins echo the challenges in combating multidrug-resistant organisms commonly implicated in neonatal infections. Variations in the resistance profiles and predominant isolates underline the regional differences in microbial epidemiology and resistance patterns, emphasizing the necessity for local surveillance and tailored antibiotic stewardship protocols.

The findings of this study underscore the multifactorial nature of neonatal sepsis, emphasizing the potential roles of gender, birth weight, gestational age, and mode of delivery in influencing susceptibility. This study's insights into microbial profiles and antibiotic resistance provide valuable information for guiding empirical treatment strategies and emphasize the need for continual vigilance and antimicrobial stewardship programs to combat the rising challenge of neonatal sepsis caused by multidrug-resistant organisms. Understanding these facets of neonatal sepsis, details about the incidence,

diagnostic challenges, potential consequences, and the emergence of antibiotic resistance are vital for developing effective prevention strategies, improving diagnostic tools, and devising targeted treatment plans to combat this critical condition in newborns.

In this study, a limitation arose due to the unavailability of the medical record about the Apgar scores of the newborns upon delivery. This absence of Apgar scores limited our ability to comprehensively analyze the immediate health condition of the newborns at birth and potentially affected the depth of our assessment of early health indicators. Single center study setting with cross-sectional study design have some in-built limitations which must be addressed in the future trials.

## CONCLUSION

The study underscores the significant association between culture-proven neonatal sepsis and demographic factors like gender, birth weight, gestational age, and mode of delivery. The identification of *Serratia marcescens*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* as most commonly found isolates and high resistance patterns to most commonly antibiotics highlight the imperative for precise antibiotic selection guided by local resistance profiles. Tailored therapeutic approaches are crucial in managing neonatal sepsis effectively and curbing the escalating challenge of antimicrobial resistance.

**Conflict of Interest:** None.

**Funding Source:** None.

## Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SMA & MS: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

WA & SY: Data acquisition, data analysis, approval of the final version to be published.

HMA & N: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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