DOI: https://doi.org/10.54393/pjhs.v7i7.2663

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 7, Issue 07 (July 2026)

Original Article

OPEN ACCESS

Microbial Profiles and Antibiotic Resistance Patterns in Neonatal Sepsis: A Frequency Analysis

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ARTICLE INFO

Keywords:
Neonatal Sepsis, *Escherichia Coli*, Antibiotic Susceptibility, Antimicrobial Resistance, Neonates

How to Cite:
Qaisar, I., Ahmed, I., Hussain, A., Ullah, I., Rafique, A., & Sheikh, S. A. (2026). Microbial Profiles and Antibiotic Resistance Patterns in Neonatal Sepsis: A Frequency Analysis: Microbial Profiles and Antibiotic Resistance Patterns in Neonatal Sepsis. Pakistan Journal of Health Sciences, 7(7), 98-102. https://doi.org/10.54393/pjhs.v7i7.2663

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Received Date: 18th December, 2024
1st Revision Received: 11th March, 2026
Acceptance Date: 25th March, 2026
Published Date: 31st July, 2026

ABSTRACT

Neonatal sepsis remains an important cause of neonatal morbidity, mortality, and prolonged hospitalization, and the spectrum of causative organisms and their antibiotic susceptibility varies across settings. Updated local microbiological data are therefore essential for appropriate empirical therapy and antimicrobial stewardship. **Objective:** To determine the frequency of different microorganisms and their antibiotic susceptibility pattern in neonatal sepsis. **Methods:** This descriptive cross-sectional study was conducted in the Pediatric Ward of Bahawal Victoria Hospital, Bahawalpur, from 09-02-2024 to 08-08-2024. A total of 119 term neonates with clinical features of sepsis were included. Blood cultures were processed by standard microbiological methods, and antibiotic susceptibility was assessed against commonly used antibiotics. Data were analyzed using SPSS version 25.0. Post-stratification Chi-square test was applied, and $p \leq 0.05$ was considered statistically significant. **Results:** *Escherichia coli* was the most frequent isolate, identified in 41 (34.45%) cases, followed by *Staphylococcus aureus* in 24 (20.17%) and *Klebsiella* species in 19 (15.97%). Overall, gram-negative organisms predominated. Amikacin showed the highest sensitivity, 111 (93.28%), followed by gentamicin, 107 (89.92%), whereas relatively lower sensitivity was observed for ceftazidime, 85 (71.43%), cefotaxime, 86 (72.27%), and tobramycin, 91 (76.47%). Stratification by age and gender showed no statistically significant differences in microorganism distribution or antibiotic susceptibility (all $p > 0.05$). **Conclusions:** *Escherichia coli* was the leading pathogen in neonatal sepsis in this cohort. The comparatively higher sensitivity to amikacin and gentamicin, together with lower sensitivity to some cephalosporins and tobramycin, supports the need for hospital-specific empirical antibiotic policies, regular antibiogram updates, and antimicrobial stewardship.

INTRODUCTION

Neonatal sepsis remains a major global cause of neonatal morbidity and mortality, especially in low- and middle-income countries where the burden of severe neonatal infection continues to be disproportionately high [1]. Recent evidence indicates that neonatal sepsis contributes substantially to preventable neonatal deaths, prolonged hospitalization, increased healthcare costs, and greater antibiotic exposure during the neonatal period [2]. Because the clinical presentation may be subtle or nonspecific, delayed recognition can rapidly worsen outcomes in affected newborns [1, 3]. Another important challenge in neonatal sepsis is the evolving microbiological pattern across different hospitals, countries, and clinical settings. Contemporary studies have shown that the spectrum of pathogens is not uniform, with some units reporting a predominance of gram-positive organisms, while others continue to report a high burden of gram-negative pathogens such as *Escherichia coli* and *Klebsiella* species [3, 4]. Recent reports have also suggested an increasing importance of *Escherichia coli* in early-onset neonatal sepsis, while other centers continue to observe significant roles for coagulase-negative *staphylococci*,

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Staphylococcus aureus, and other opportunistic organisms [3, 4]. These geographic and temporal variations make it unsafe to depend on older epidemiological data when selecting empirical antibiotics. Antimicrobial resistance has further complicated the management of neonatal sepsis. Recent studies have highlighted the growing resistance of neonatal pathogens to commonly used first-line antibiotics, creating difficulty in choosing effective empirical regimens and increasing the risk of treatment failure [4-6]. At the same time, overuse of broad-spectrum antibiotics in neonatal units may itself accelerate resistance, emphasizing the need for careful stewardship and unit-specific treatment protocols [5-7]. Systematic reviews have shown that surveillance of antimicrobial susceptibility and implementation of stewardship programs can reduce unnecessary antibiotic exposure without worsening neonatal outcomes [6-8]. Recent hospital-based studies from different regions continue to report marked variation in both pathogen frequency and susceptibility patterns [8-10].

Such differences underline the importance of local data when framing empirical therapy and infection-control strategies. In our setting, limited recent local literature is available on the current microbial profile and antibiotic sensitivity pattern in neonatal sepsis. Therefore, the study aimed to determine the frequency of different microorganisms isolated in neonatal sepsis and to evaluate their antibiotic susceptibility pattern so that local treatment decisions may be better aligned with current microbiological evidence.

METHODS

This descriptive cross-sectional study was carried out in the Pediatric Ward of Bahawal Victoria Hospital, Bahawalpur, from February 2024 to August 2024 (letter No. 2359/DME/QAMC, Bahawalpur; dated 9th February 2024). The sample size was calculated by the formula $n = Z^2P(1–P)/d^2$ using a 95% confidence level, anticipated Escherichia coli frequency of 9.1% from a recent neonatal sepsis study [11], and an absolute precision of 5.2%, yielding a required sample of approximately 119 neonates. Non-probability consecutive sampling was used. Eligible participants were term neonates aged 0–28 days of either gender presenting with clinical features suggestive of sepsis for ≤24 hours. Neonates born preterm, those who had already received antibiotics before presentation, those with previous surgery, and those whose parents/guardians did not provide consent were excluded. After ethical approval and informed consent, 3–5 mL blood samples were collected aseptically using sterile syringes and inoculated into blood culture bottles. Blood cultures were processed by the BACTEC method and monitored for bacterial growth over 24–48 hours. Positive cultures were identified by standard microbiological techniques, including Gram staining and routine organism identification procedures. Antibiotic susceptibility testing was performed for ampicillin, amoxicillin-clavulanate, gentamicin, amikacin, tobramycin, cefotaxime, and ceftazidime using routine laboratory protocols, with interpretation based on standard quality-controlled laboratory practice.

Data were entered and analyzed using IBM SPSS Statistics version 25.0. Age was expressed as mean ± standard deviation. Gender, isolated microorganisms, and antibiotic susceptibility results were presented as frequencies and percentages. Stratification was performed with respect to age and gender. Post-stratification Chi-square test was applied, and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 119 neonates were included in the study, with a mean age of 7.82 ± 5.41 days. Overall, *Escherichia coli* was the most frequent microorganism isolated, followed by *Staphylococcus aureus* and *Klebsiella* species. In antibiotic susceptibility testing, amikacin and gentamicin showed the highest sensitivity, while ceftazidime, cefotaxime, and tobramycin showed comparatively lower sensitivity (Table 1).

Table 1: Frequency of Different Microorganisms and Antibiotic Susceptibility in Neonatal Sepsis

Microorganisms	Frequencies	Sensitive, n (%)	Resistant, n (%)
Microorganism Susceptibility			
Escherichia coli	41 (34.45%)	–	–
Klebsiella species	19 (15.97%)	–	–
Staphylococcus aureus	24 (20.17%)	–	–
Acinetobacter species	12 (10.08%)	–	–
Staphylococcus epidermidis	10 (8.40%)	–	–
Enterobacter cloacae	7 (5.88%)	–	–
Moraxella species	6 (5.04%)	–	–
Antibiotic Susceptibility			
Ampicillin (10 µg)	–	99 (83.19%)	20 (16.81%)
Amoxicillin-clavulanate (20/10 µg)	–	101 (84.87%)	18 (15.13%)
Gentamicin (10 µg)	–	107 (89.92%)	12 (10.08%)
Amikacin (30 µg)	–	111 (93.28%)	8 (6.72%)
Tobramycin	–	91 (76.47%)	28 (23.53%)
Cefotaxime	–	86 (72.27%)	33 (27.73%)
Ceftazidime	–	85 (71.43%)	34 (28.57%)

Across age groups, *Escherichia coli* and *Klebsiella* species were numerically more frequent in the 0–14-day group than in the 15–28-day group, but none of the age-based differences reached statistical significance (Table 2).

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Table 2: Stratification of Different Microorganisms with Respect to Age

Microorganisms	Status	0–14 days (n=100)	15–28 days (n=19)	p-value
Escherichia coli	Yes	38 (38.0%)	3 (15.79%)	0.062
	No	62 (62.0%)	16 (84.21%)	
Klebsiella species	Yes	18 (18.0%)	1 (5.26%)	0.165
	No	82 (82.0%)	18 (94.74%)	
Staphylococcus aureus	Yes	21 (21.0%)	3 (15.79%)	0.604
	No	79 (79.0%)	16 (84.21%)	
Acinetobacter species	Yes	9 (9.0%)	3 (15.79%)	0.368
	No	91 (91.0%)	16 (84.21%)	
Staphylococcus epidermidis	Yes	9 (9.0%)	1 (5.26%)	0.590
	No	91 (91.0%)	18 (94.74%)	
Enterobacter cloacae	Yes	6 (6.0%)	1 (5.26%)	0.900
	No	94 (94.0%)	18 (94.74%)	
Moraxella species	Yes	5 (5.0%)	1 (5.26%)	0.962
	No	95 (95.0%)	18 (94.74%)	

With respect to gender, *Escherichia coli* was numerically more common in female neonates, whereas *Klebsiella* species and *Moraxella* species were more frequent in male neonates. However, these differences were not statistically significant (Table 3).

Table 3: Stratification of Different Microorganisms with Respect to Gender

Microorganisms	Status	Male (n=79)	Female (n=40)	p-value
Escherichia coli	Yes	24 (30.38%)	17 (42.50%)	0.189
	No	55 (69.62%)	23 (57.50%)	
Klebsiella species	Yes	16 (20.25%)	3 (7.50%)	0.073
	No	63 (79.75%)	37 (92.50%)	
Staphylococcus aureus	Yes	15 (18.99%)	9 (22.50%)	0.652
	No	64 (81.01%)	31 (77.50%)	
Acinetobacter species	Yes	8 (10.13%)	4 (10.0%)	0.983
	No	71 (89.87%)	36 (90.0%)	
Staphylococcus epidermidis	Yes	5 (6.33%)	5 (12.50%)	0.252
	No	74 (93.67%)	35 (87.50%)	
Enterobacter cloacae	Yes	5 (6.33%)	2 (5.0%)	0.771
	No	74 (93.67%)	38 (95.0%)	
Moraxella species	Yes	6 (7.59%)	0 (0.0%)	0.074
	No	73 (92.41%)	40 (100.0%)	

Antibiotic susceptibility by age group showed some numerical variation, but none of the differences between the 0–14-day and 15–28-day groups were statistically significant (Table 4).

Table 4: Stratification of Antibiotic Susceptibility with Respect to Age

Antibiotics	Status	0–14 days (n=100)	15–28 days (n=19)	p-value
Ampicillin (10 µg)	Sensitive	84 (84.0%)	15 (78.95%)	0.589
	Resistant	16 (16.0%)	4 (21.05%)	
Amoxicillin-clavulanate (20/10 µg)	Sensitive	85 (85.0%)	16 (84.21%)	0.929
	Resistant	15 (15.0%)	3 (15.79%)	
Gentamicin (10 µg)	Sensitive	90 (90.0%)	17 (89.47%)	0.944
	Resistant	10 (10.0%)	2 (10.53%)	
Amikacin (30 µg)	Sensitive	95 (95.0%)	16 (84.21%)	0.085
	Resistant	5 (5.0%)	3 (15.79%)	
Tobramycin	Sensitive	78 (78.0%)	13 (68.42%)	0.367
	Resistant	22 (22.0%)	6 (31.58%)	
Cefotaxime	Sensitive	72 (72.0%)	14 (73.68%)	0.881
	Resistant	28 (28.0%)	5 (26.32%)	
Ceftazidime	Sensitive	70 (70.0%)	15 (78.95%)	0.429
	Resistant	30 (30.0%)	4 (21.05%)	

Likewise, antibiotic susceptibility by gender did not show statistically significant differences (Table 5).

Table 5: Stratification of Antibiotic Susceptibility with Respect to Gender

Antibiotics	Status	Male (n=79)	Female (n=40)	p-value
Ampicillin (10 µg)	Sensitive	65 (82.28%)	34 (85.0%)	0.708
	Resistant	14 (17.72%)	6 (15.0%)	
Amoxicillin-clavulanate (20/10 µg)	Sensitive	68 (86.08%)	33 (82.50%)	0.607
	Resistant	11 (13.92%)	7 (17.50%)	
Gentamicin (10 µg)	Sensitive	71 (89.87%)	36 (90.0%)	0.983
	Resistant	8 (10.13%)	4 (10.0%)	
Amikacin (30 µg)	Sensitive	74 (93.67%)	37 (92.50%)	0.809
	Resistant	5 (6.33%)	3 (7.50%)	
Tobramycin	Sensitive	59 (74.68%)	32 (80.0%)	0.518
	Resistant	20 (25.32%)	8 (20.0%)	
Cefotaxime	Sensitive	57 (72.15%)	29 (72.50%)	0.968
	Resistant	22 (27.85%)	11 (27.50%)	
Ceftazidime	Sensitive	58 (73.42%)	27 (67.50%)	0.499
	Resistant	21 (26.58%)	13 (32.50%)	

DISCUSSION

In the present study, *Escherichia coli* was the most frequent isolate, followed by *Staphylococcus aureus* and *Klebsiella* species, with an overall predominance of gram-negative organisms. This pattern is partly comparable to recent studies showing that pathogen distribution in neonatal sepsis is highly variable across centers. Majigo *et al.* reported that gram-positive bacteria predominated in their Tanzanian cohort, but *Escherichia coli* remained the leading gram-negative isolate, and amikacin showed comparatively lower resistance among gram-negative pathogens [11]. The difference from our findings may reflect variation in case mix, neonatal unit practices, and local epidemiology. Our findings also differ from those of Karimi *et al.* reported *Klebsiella* pneumoniae and coagulase-negative staphylococci as the most frequent isolates in culture-positive neonatal sepsis, highlighting substantial regional diversity in pathogen prevalence [12]. Similarly, Siwakoti *et al.* reported a different bacteriological profile in Nepal, again emphasizing that empirical therapy for neonatal sepsis should be guided by local microbiological data rather than borrowed directly from

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other institutions [13]. These differences support the reviewer's concern that the discussion should be contextualized using recent regional and global literature. The numerical predominance of Escherichia coli in the 0–14-day age group in our study may carry practical implications even though the difference did not reach statistical significance. Early neonatal predominance of gram-negative organisms may justify maintaining adequate gram-negative empirical coverage in clinically suspected cases, while also reinforcing maternal screening, aseptic delivery practices, and early neonatal infection-prevention measures. This interpretation is consistent with recent reports describing evolving early-onset and late-onset pathogen trends in neonatal sepsis [14, 15]. We also observed some gender-based numerical variation in microorganism distribution, with *Escherichia coli* more common among female neonates and *Klebsiella* species more common among male neonates, but these differences were not statistically significant. Such variation may simply reflect limited subgroup sizes, random variation, or unmeasured exposure-related factors rather than a true biological sex-based difference. Therefore, our data do not support gender-specific empirical antibiotic protocols. Recent hospital-based studies likewise emphasize the importance of unit-level epidemiology over subgroup assumptions [15, 16]. Regarding antibiotic susceptibility, amikacin and gentamicin showed the highest activity in our study, whereas relatively lower sensitivity was observed for ceftazidime, cefotaxime, and tobramycin. This has important therapeutic implications. Lower susceptibility to ceftazidime and tobramycin suggests that these agents may be less reliable as stand-alone empirical choices in our setting, particularly when gram-negative organisms predominate. Recent studies have similarly highlighted changing susceptibility patterns, rising multidrug resistance, and the need for regular surveillance-driven empirical policy revision [14-17]. Yang *et al.* further demonstrated that epidemiological shifts may occur over time even within the same institution, which reinforces the need for ongoing monitoring rather than one-time antibiogram review [17]. Our findings are also consistent with broader recent evidence showing the continuing burden of gram-negative neonatal sepsis and the importance of choosing effective empirical therapy early. Utomo *et al.* reported a predominance of gram-negative organisms in culture-proven neonatal sepsis, while Solomon *et al.* documented very high resistance to commonly used first-line agents among gram-negative bloodstream infections, with amikacin retaining comparatively lower resistance [18, 19]. These observations align with our results, where amikacin remained the most active antibiotic. In addition, Lee *et al.* reported that contemporary neonatal sepsis management remains challenging due to evolving microbial patterns and the need to optimize antimicrobial use in neonatal units [20].

The fact that no statistically significant differences were found in antibiotic susceptibility across age and gender strata in our study has practical value. It suggests that, at present, a unit-level empirical antibiotic policy may be more appropriate than subgroup-specific protocols in this setting. However, these nonsignificant findings should not be interpreted as proof of the absence of clinically meaningful variation; rather, they point to the need for larger multicenter studies with sufficient power to detect subgroup differences. Meanwhile, hospital infection-control policies should focus on hand hygiene, aseptic blood culture sampling, rational antibiotic prescribing, and periodic antibiogram review. This study has some limitations. It was conducted at a single center, included a modest sample size, and was restricted to term neonates, which may limit generalizability. In addition, advanced molecular resistance characterization was not performed. Nevertheless, the study provides locally relevant microbiological and susceptibility data that may help guide empirical therapy and support antimicrobial stewardship in our setting.

CONCLUSIONS

Escherichia coli was the most frequent pathogen isolated in neonatal sepsis in this study, followed by Staphylococcus aureus and *Klebsiella* species, with overall predominance of gram-negative organisms. Amikacin and gentamicin showed the highest sensitivity, whereas relatively lower sensitivity was observed for ceftazidime, cefotaxime, and tobramycin. These findings support the use of periodic local microbiological surveillance, regular antibiogram-based revision of empirical antibiotic policies, and strengthened antimicrobial stewardship and infection-control practices in neonatal care.

Authors' Contribution

Conceptualization: IQ
Methodology: IQ
Formal analysis: IA, AR, SAS
Writing and Drafting: IQ, IA
Review and Editing: IQ, IA, IU

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflict of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

DOI: https://doi.org/10.54393/pjhs.v7i7.2663

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