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Molecular Characterization of Antimicrobial Resistance Genes and Their Association with Clinical Outcomes Among Neonates with Sepsis: A Prospective Clinical and Molecular Study

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Abstract

Background
Neonatal sepsis is a significant contributor to neonatal morbidity and mortality and AMR is a challenge to effective treatment and clinical outcomes.
Objective
To molecularly characterize antimicrobial resistance genes among neonates with sepsis and determine their association with antimicrobial susceptibility patterns and clinical outcomes
Methodology
A prospective clinical and molecular research was carried out from January 2024 to December 2025. A consecutive series of 392 neonates with sepsis were included. Culture was used to identify bacterial pathogens, antimicrobial susceptibility testing was done and selected AMR genes such as *blaCTX-M*, *blaTEM*, *blaSHV*, *blaNDM*, *blaOXA-48-like* and *blaVIM* were detected by polymerase chain reaction. Of the 231 culture-positive isolates, 117 (50.65%) were identified as AMR gene-positive and 114 (49.35%) were identified as AMR gene-negative based on the detection of at least one of the AMR genes tested. Association of AMR gene status, phenotypic resistance and clinical outcomes was evaluated.

Results
Among the 392 neonates, 211 (53.83%) were male and 181 (46.17%) female, while 231 (58.93%) had culture-positive isolates. *Klebsiella pneumoniae* was the most common pathogen (71, 30.74%), followed by *Escherichia coli* (54, 23.38%). Ampicillin showed the highest resistance (177, 76.62%). Among the 231 culture-positive isolates, *blaCTX-M* was most prevalent (96, 41.56%), followed by *blaTEM* (82, 35.50%), *blaSHV* (61, 26.41%), and *blaNDM* (39, 16.88%). Compared with the AMR gene-negative group, AMR gene-positive cases had higher treatment failure (55, 47.01% vs. 31, 27.19%; p=0.002), prolonged hospitalization (48, 41.03% vs. 32, 28.07%; p=0.039), sepsis-related complications (42, 35.90% vs. 26, 22.81%; p=0.029), septic shock (27, 23.08% vs. 13, 11.40%; p=0.019), organ dysfunction (22, 18.80% vs. 10, 8.77%; p=0.027), and mortality (31, 26.50% vs. 14, 12.28%; p=0.006).
Conclusion
Neonatal sepsis isolates were found to contain molecular AMR determinants, which were significantly linked to antimicrobial resistance and clinical outcomes, thereby justifying integrated molecular surveillance and prompt effective antimicrobial therapy.

Introduction

Neonatal sepsis is still one of the most severe causes of morbidity and mortality in the first weeks of life, especially in low and middle income countries [1]. The immaturity of neonatal immune responses, underdeveloped skin and mucosal barriers, and exposure to healthcare-associated pathogens makes neonates vulnerable to invasive infections [2]. The clinical features of neonatal sepsis are often non-specific, such as respiratory distress, temperature instability, poor feeding, lethargy, apnea, and circulatory abnormalities, and early diagnosis and adequate antimicrobial treatment is often difficult [3]. Failure to provide timely effective treatment can quickly result in septic shock, organ dysfunction, longer hospital stays, and death [4].

Additionally, the rise in antimicrobial resistance (AMR) has made the treatment of neonatal sepsis more challenging [5]. Empirical antibiotic therapy is frequently started before microbiological confirmation due to the rapid onset of infection, but may be inappropriate or inadequate if the organism has resistance mechanisms [6]. While conventional antimicrobial susceptibility testing offers valuable phenotypic information, it does not necessarily reveal the genetic determinants of antimicrobial resistance [7]. Molecular characterization of antimicrobial resistance genes can help to identify the resistance mechanisms, such as genes related to resistance to β-lactam, aminoglycoside, fluoroquinolone, macrolide and other commonly used antimicrobial agents [8]. The presence of these genes could also help to clarify the differences between the susceptibility pattern and treatment response in the clinical setting [9].

The importance of antimicrobial resistance in neonates is highlighted because resistant infections can be linked to treatment failure, complications, length of hospital stay, and death, as well as delayed effective therapy [10,11]. But the presence of a resistance gene does not necessarily equate to the same clinical result; pathogen type, infection severity, birth characteristics, timing of treatment, antibiotic exposure and underlying neonatal conditions all play a role in the outcome [12]. Therefore, combining molecular data with clinical and microbiological data may help to gain a better understanding of neonatal sepsis and its outcomes.

Research Objective

To molecularly characterize antimicrobial resistance genes among neonates with sepsis and determine their association with antimicrobial susceptibility patterns and clinical outcomes, including treatment response, duration of hospitalization, complications, and mortality.

Methodology

Study Design
A prospective clinical and molecular study was done to describe the antimicrobial resistance (AMR) genes of neonates with sepsis and its correlation with antimicrobial susceptibility pattern and clinical outcome. The study combined clinical evaluation, microbiological investigation, antimicrobial susceptibility testing and molecular identification of selected antimicrobial resistance genes. Neonates were recruited from the eligible group and followed up during their hospital stay to record treatment response, complications, hospital stay and final clinical outcomes.

Study Setting
The study was carried out at Pakistan Institute of Medical Sciences (PIMS) Islamabad. PIMS is a tertiary care health care centre offering specialised neonatal and paediatric care and a large volume of neonatal sepsis cases. All neonates who presented to the neonatal and pediatric units during the study period were screened for eligibility. Clinical, microbiological and molecular investigations carried out in the institutional laboratory following institutional laboratory procedures and established diagnostic protocols.

Study Duration
This study was conducted for 2 years (January 2024 to December 2025). In this timeframe, eligible neonates were prospectively recruited, clinical information and specimens were collected, the bacterial pathogens were identified, antimicrobial susceptibility testing was conducted, and selected genes for antimicrobial resistance were characterized by molecular techniques. Follow-up was performed until discharge, transfer, or death and clinical outcomes were recorded.

Study Population
The neonates (0-28 days) admitted to relevant units of PIMS with suspected or laboratory confirmed sepsis were included in the study population. The clinical and microbiological evaluation of neonates was performed based on pre-established criteria. Prospective records were made of demographic characteristics, clinical presentation, laboratory findings, antimicrobial treatment, microbiological results, molecular resistance profile and clinical outcome.

Sample Size Determination
A total of 392 neonates with suspected or confirmed sepsis were enrolled in the study. Sample size was determined according to the prevalence of antimicrobial resistance in neonatal sepsis reported in similar studies at 95% confidence level and 5% margin of error. An additional 10% was assumed to be due to incomplete clinical data, specimens that could not be evaluated, or loss to follow-up. The total number of neonates in the final study sample of 392 was sufficient to identify bacterial pathogens, antimicrobial resistance patterns, selected antimicrobial resistance genes, and to evaluate their correlation to clinical outcomes.

Sampling Technique
Consecutive sampling was used for participant recruitment. Every neonate who came to the study site during the study period was evaluated based on the set criteria. Neonates were consecutively selected until the target sample size of 392 was reached, then the eligible neonates were included if their parents or legally authorized guardians had given informed consent.

Inclusion Criteria and Exclusion Criteria

Neonates (0–28 days) with clinical signs of sepsis and who were investigated for suspected bacterial infection were included. Eligible neonates included those who were admitted to PIMS during the study period where blood or other clinically indicated samples were collected. Parental/legally authorized guardian written informed consent was obtained prior to enrollment. Neonates who died without being clinically and microbiologically assessed, were transferred to another health care facility without sufficient clinical and microbiological data, and neonates who had conditions incompatible with survival were not included. Parents/legal guardians who refused to participate were also excluded. In addition, samples that were not collected or processed properly to allow for microbiological evaluation were not included in the relevant analysis.

Clinical Assessment and Data Collection

A systematic clinical evaluation was performed at the time of enrollment in the study for each neonate. Demographic and clinical data such as age, sex, gestational age, birth weight, mode of delivery, symptoms, vital signs, feeding status, respiratory status and relevant neonatal or maternal risk factors were documented. Temperature instability, difficulty breathing, apnea, lethargy, poor feeding, irregular heart rate, slow capillary refill, hypotension, and other indications of systemic infections were noted as clinical manifestations suggestive of sepsis. Other laboratory investigations performed as part of routine clinical management (such as complete blood count, C-reactive protein, blood glucose and other clinically indicated investigations) were also documented.

Specimen Collection and Processing

Blood samples were drawn in strict aseptic conditions, ideally prior to the start of antibiotic treatment, if possible. Samples were collected in the appropriate blood culture bottles and with the use of standard microbiological procedures. Samples were collected and immediately brought to the microbiology lab for processing. Contamination and loss of specimen quality was minimized by proper labeling, documentation, transportation, and processing procedures.

Microbiological Identification of Bacterial Pathogens

Blood culture was done in a standard microbiological manner. All positive cultures were stained and subcultured on proper culture media according to Gram staining. Conventional biochemical tests were used to identify the bacterial isolates and, if available, an automated bacterial identification system. Each clinically significant bacterial isolate's identity was recorded prior to the antimicrobial susceptibility testing. The isolation and identification process was carried out with appropriate quality control procedures.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out on clinically important bacterial isolates using standard laboratory techniques like the Kirby–Bauer disk diffusion method or using an appropriate automated susceptibility testing system. The choice of antimicrobial agents was based on the identified bacterial species, the range of antimicrobial agents used in neonatal infections and the institutional laboratory protocols. Susceptibility results were evaluated by the study laboratory using current applicable guidelines of the Clinical and Laboratory Standards Institute or the European Committee on Antimicrobial Susceptibility Testing. Results were classified as susceptible, intermediate or resistant. Multidrug resistance was defined using internationally accepted definitions based on resistance to multiple antimicrobial classes.

Molecular Characterization of Antimicrobial Resistance Genes

Clinical isolates with antimicrobial resistance patterns of clinical significance were

chosen for molecular characterization. DNA from bacteria was isolated using a validated commercial extraction kit or an appropriate standardized laboratory method. Selected antimicrobial resistance genes were detected by polymerase chain reaction (PCR) based on the observed resistance phenotype and the bacterial species. The main molecular targets were important beta-lactam resistance genes (*blaCTX-M*, *blaTEM*, *blaSHV*) and carbapenemase associated genes (*blaNDM*, *blaOXA-48-like*, *blaVIM*) as appropriate for the species of bacteria identified. Appropriate species-specific resistance determinants were investigated for Gram-positive resistant isolates based on the observed phenotypic resistance and available laboratory resources. For PCR analysis appropriate primers and positive and negative controls were used. Products were amplified and analyzed by agarose gel electrophoresis and visualized with an appropriate DNA detection system. A phenotypic antimicrobial susceptibility profile was correlated with molecular data for each bacterial isolate.

Association Between AMR Genes and Antimicrobial Susceptibility

Molecular detection of each individual antimicrobial resistance gene was compared to the corresponding phenotypic antimicrobial susceptibility results. Specific resistance genes was assessed in relation to resistance to the corresponding antimicrobial agent(s) or antimicrobial classes. This analysis identified how much of the observed antimicrobial resistance patterns of the bacterial isolates from neonates with sepsis corresponded to the identified antimicrobial resistance genes.

Assessment of Clinical Outcomes

Clinical outcomes were assessed in a prospective fashion during the entire hospitalization. The clinical response to antimicrobial therapy, the need for antimicrobial modification or escalation, the length of time in hospital, complications, intensive neonatal care, septic shock, organ dysfunction and outcome on discharge were documented. Survival/mortality was also recorded. The link between bacterial pathogens, antimicrobial resistance profile, presence of antimicrobial resistance genes and clinical outcome was then assessed.

Treatment Outcome Assessment

The clinical improvement, resolution or significant reduction of manifestations of sepsis and need for antimicrobial modification or escalation were used to evaluate treatment responses. Failure of treatment was defined as failure to improve or deteriorate clinically, need for escalation or modification of antimicrobial treatment due to inadequate response, development of serious complications or death as a result of sepsis. The results were contrasted based on the presence or absence of certain antimicrobial resistance genes.

Quality Control and Assurance

The study included quality-control procedures for both the clinical and microbiological and molecular aspects. Specimen collection, bacterial culture, identification, DNA extraction, PCR amplification and interpretation of molecular results were conducted according to standard operating procedures. Where applicable, suitable reference strains, positive and negative controls were added to laboratory procedures. Laboratory equipment was kept and calibrated as per institutional requirement and data collection forms were checked periodically for completeness and consistency.

Statistical Analysis

The data were fed to a computer-based data base and analyzed by the Statistical Package for the Social Sciences (SPSS) version 27. Data were presented as a mean ±

SD or median (IQR) depending on the distribution of the data, and frequencies and percentages were used for categorical data. Associations between categorical variables were analysed by the Chi-square test and for continuous variables the independent-samples t-test was used. Association between antimicrobial resistance genes, phenotypic resistance and clinical outcomes were assessed using appropriate regression analysis. The independent predictors of adverse clinical outcomes (treatment failure and mortality) were determined following adjustment for relevant clinical and demographic factors through binary logistic regression. Odds ratios with 95% confidence intervals were presented and a p-value < 0.05 was defined as being statistically significant.

Data Confidentiality

The study identification code for each enrolled neonate was unique and information that could directly identify the neonate was not included in the analytical dataset. All clinical records, lab results and molecular findings were kept safely and only shared with authorized personnel on the research team. The data collected were only used for the purposes of the study and the results of the study were presented in aggregate form to ensure the confidentiality and privacy of all the neonates studied.

Ethical Considerations

Ethical approval was obtained from the relevant Institutional Ethical Review Committee of Pakistan Institute of Medical Sciences (PIMS), Islamabad, before commencement of the study. Written informed consent was obtained from the parents or legally authorized guardians of all participating neonates. Participation was voluntary, and refusal or withdrawal did not affect the medical care provided. Confidentiality was maintained by anonymizing participant information, and all collected data were securely stored and used solely for research purposes.

Results

Among the 392 neonates with sepsis, 211 (53.83%) were male and 181 (46.17%) were female (table 1). The largest age group was ≤7 days (176, 44.90%), while 244 (62.24%) were term neonates and 235 (59.95%) had birth weight ≥2.5 kg. Late-onset sepsis was more common (223, 56.89%) than early-onset sepsis (169, 43.11%). Poor feeding (60.71%), respiratory distress (54.59%), and elevated CRP (58.42%) were frequent clinical findings. Significant associations were observed for age, gestational age, birth weight, poor feeding, respiratory distress, temperature instability, apnea, elevated CRP, and thrombocytopenia ($p<0.05$).

Table 1. Demographic, Clinical, and Laboratory Characteristics of Neonates with Sepsis (N = 392)

Variable	Category	Number of Patients (n)	Frequency (%)	χ^2	p-value
Gender	Male	211	53.83	2.30	0.129
	Female	181	46.17		
Age	≤7 days	176	44.90	8.42	0.015
	8–14 days	119	30.36		
	15–28 days	97	24.74		
Gestational age	Preterm	148	37.76	4.91	0.027
	Term	244	62.24		
Birth weight	<2.5 kg	157	40.05	6.73	0.009
	≥2.5 kg	235	59.95		
Sepsis onset	Early-onset	169	43.11	3.64	0.056

	Late-onset	223	56.89		
Poor feeding	Present	238	60.71	9.18	0.002
	Absent	154	39.29		
Respiratory distress	Present	214	54.59	7.64	0.006
	Absent	178	45.41		
Temperature instability	Present	167	42.60	5.31	0.021
	Absent	225	57.40		
Apnea	Present	96	24.49	4.26	0.039
	Absent	296	75.51		
Elevated CRP	Present	229	58.42	11.27	<0.001
	Absent	163	41.58		
Thrombocytopenia	Present	143	36.48	6.82	0.009
	Absent	249	63.52		

Among the 231 culture-positive isolates, *Klebsiella pneumoniae* was the most frequently isolated pathogen, accounting for 71 (30.74%) cases, followed by *Escherichia coli* 54 (23.38%), *Staphylococcus aureus* 38 (16.45%), *Acinetobacter baumannii* 32 (13.85%), and *Enterobacter* spp. 21 (9.09%), shown in figure 1. Other Gram-negative and Gram-positive organisms accounted for 10 (4.33%) and 5 (2.16%) isolates, respectively, indicating a predominance of Gram-negative bacterial pathogens.

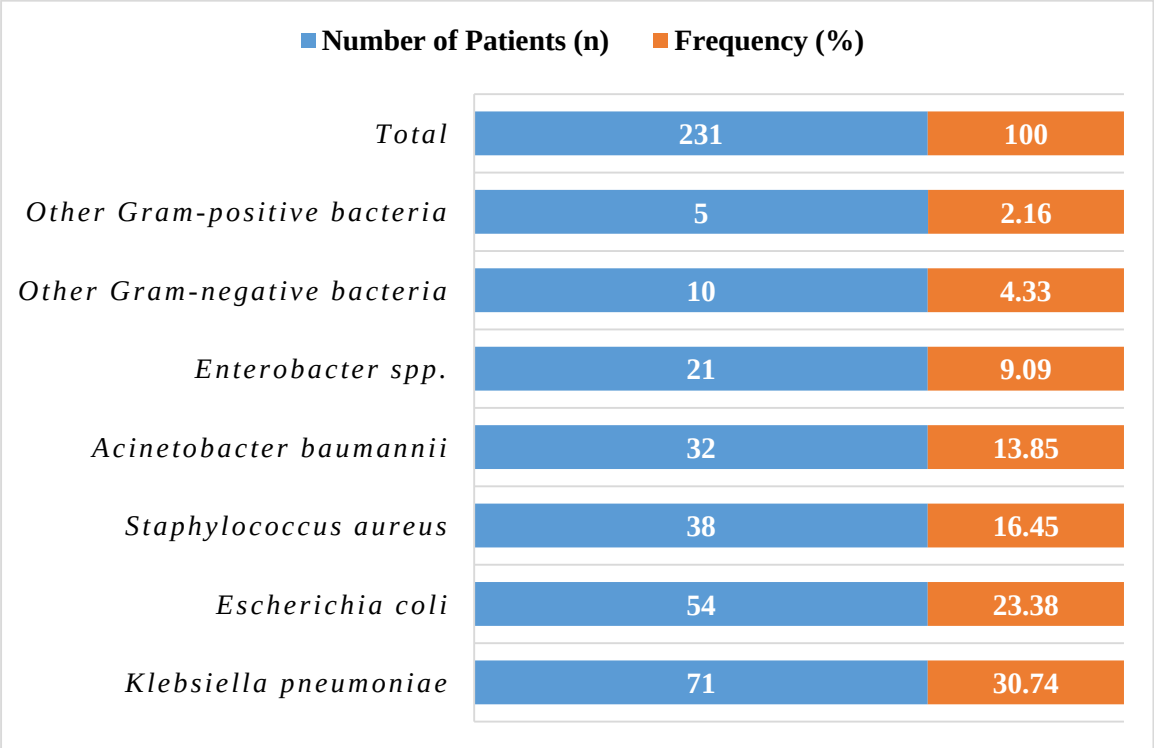

Figure 1. Distribution of Bacterial Pathogens Isolated from Blood Cultures

The antimicrobial susceptibility results showed substantial resistance among the bacterial isolates (table 2). Ampicillin had the highest resistance rate, with 177 (76.62%) isolates resistant, followed by ceftriaxone 162 (70.13%) and cefotaxime 158 (68.40%). Resistance to gentamicin and amikacin was observed in 119 (51.52%) and 83 (35.93%) isolates, respectively. Meropenem demonstrated comparatively better activity, with 179 (77.49%) isolates susceptible and 47 (20.35%) resistant. Significant differences were observed for most antimicrobial agents, whereas amikacin was not statistically significant (p=0.057).

Table 2. Antimicrobial Susceptibility Pattern of Bacterial Isolates

Antimicrobial agent	Resistant n (%)	Intermediate n (%)	Susceptible n (%)	χ^2	p-value
Ampicillin	177 (76.62)	12 (5.19)	42 (18.18)	28.64	<0.001
Cefotaxime	158 (68.40)	14 (6.06)	59 (25.54)	19.73	<0.001
Ceftriaxone	162 (70.13)	11 (4.76)	58 (25.11)	21.46	<0.001
Gentamicin	119 (51.52)	15 (6.49)	97 (41.99)	8.92	0.012
Amikacin	83 (35.93)	12 (5.19)	136 (58.87)	5.73	0.057
Meropenem	47 (20.35)	5 (2.16)	179 (77.49)	32.18	<0.001

Molecular analysis demonstrated a substantial presence of antimicrobial resistance genes among the 231 bacterial isolates. *blaCTX-M* was the most prevalent gene, detected in 96 (41.56%) isolates, followed by *blaTEM* in 82 (35.50%), *blaSHV* in 61 (26.41%), and *blaNDM* in 39 (16.88%), shown in table 3. The *blaOXA-48-like* gene was detected in 28 (12.12%) isolates, whereas *blaVIM* was least frequent, occurring in 11 (4.76%) isolates. These findings demonstrate the presence of both β-lactamase- and carbapenemase-associated resistance determinants.

Table 3. Molecular Detection of Antimicrobial Resistance Genes

AMR gene	Positive (n)	Positive (%)	Negative (n)	Negative (%)	χ^2	p-value
blaCTX-M	96	41.56	135	58.44	14.82	<0.001
blaTEM	82	35.50	149	64.50	9.67	0.002
blaSHV	61	26.41	170	73.59	5.24	0.022
blaNDM	39	16.88	192	83.12	8.31	0.004
blaOXA-48-like	28	12.12	203	87.88	6.17	0.013
blaVIM	11	4.76	220	95.24	2.41	0.121

A significant association was observed between AMR gene positivity and phenotypic antimicrobial resistance. Among *blaCTX-M*-positive isolates, 89 (92.71%) demonstrated phenotypic resistance compared with 69 (51.11%) of gene-negative isolates (χ^2= 44.91, p<0.001), shown in table 4. Similarly, resistance was observed in 73 (89.02%) *blaTEM*-positive and 55 (90.16%) *blaSHV*-positive isolates, compared with 57.05% and 60.59% of the respective negative groups. The highest phenotypic resistance among gene-positive isolates was observed for *blaNDM* (37, 94.87%), compared with 63.02% among gene-negative isolates (χ^2=19.62, p<0.001).

Table 4. Association Between AMR Genes and Phenotypic Antimicrobial Resistance

AMR gene	Phenotypic resistance n (%)	No phenotypic resistance n (%)	χ^2	p-value
blaCTX-M positive	89 (92.71)	7 (7.29)	44.91	<0.001
blaCTX-M negative	69 (51.11)	66 (48.89)		
blaTEM positive	73 (89.02)	9 (10.98)	26.83	<0.001
blaTEM negative	85 (57.05)	64 (42.95)		
blaSHV positive	55 (90.16)	6 (9.84)	22.74	<0.001
blaSHV negative	103 (60.59)	67 (39.41)		

blaNDM positive	37 (94.87)	2 (5.13)	19.62	<0.001
blaNDM negative	121 (63.02)	71 (36.98)		

Among the 231 culture-positive isolates, 117 (50.65%) were AMR gene-positive and 114 (49.35%) were AMR gene-negative (table 5). AMR gene-positive neonates had significantly higher treatment failure (47.01% vs. 27.19%, p=0.002), prolonged hospitalization (41.03% vs. 28.07%, p=0.039), sepsis-related complications (35.90% vs. 22.81%, p=0.029), septic shock (23.08% vs. 11.40%, p=0.019), organ dysfunction (18.80% vs. 8.77%, p=0.027), and mortality (26.50% vs. 12.28%, p=0.006) than AMR gene-negative neonates.

Table 5. Clinical Outcomes According to AMR Gene Status

Clinical outcome	AMR gene positive (n=117)	AMR gene negative (n=114)	χ^2	p-value
Treatment failure	55 (47.01%)	31 (27.19%)	9.70	0.002
Prolonged hospitalization	48 (41.03%)	32 (28.07%)	4.28	0.039
Sepsis-related complications	42 (35.90%)	26 (22.81%)	4.76	0.029
Septic shock	27 (23.08%)	13 (11.40%)	5.50	0.019
Organ dysfunction	22 (18.80%)	10 (8.77%)	4.87	0.027
Mortality	31 (26.50%)	14 (12.28%)	7.44	0.006

Comparison of continuous variables showed that AMR gene-positive neonates had a higher mean age at admission than AMR gene-negative neonates (10.42 ± 6.18 vs. 8.76 ± 5.91 days; p=0.030), shown in table 6. Mean birth weight was significantly lower in the AMR-positive group (2.31 ± 0.64 vs. 2.52 ± 0.59 kg; p=0.005). AMR gene-positive neonates also had a significantly longer hospitalization duration (15.84 ± 8.26 vs. 11.37 ± 6.41 days; p<0.001) and higher mean CRP levels (48.63 ± 21.74 vs. 36.29 ± 18.52 mg/L; p<0.001).

Table 6. Comparison of Continuous Clinical Variables by AMR Gene Status

Variable	AMR Gene Positive Mean ± SD	AMR Gene Negative Mean ± SD	t-value	p-value
Age at admission (days)	10.42 ± 6.18	8.76 ± 5.91	2.18	0.030
Birth weight (kg)	2.31 ± 0.64	2.52 ± 0.59	-2.87	0.005
Duration of hospitalization (days)	15.84 ± 8.26	11.37 ± 6.41	4.82	<0.001
CRP (mg/L)	48.63 ± 21.74	36.29 ± 18.52	5.03	<0.001

Multivariable logistic regression showed that AMR gene positivity independently increased the odds of treatment failure (adjusted OR=2.84, 95% CI: 1.76–4.58, p<0.001) and mortality (adjusted OR=2.96, 95% CI: 1.62–5.41, p<0.001), shown in table 7. Carbapenemase gene positivity was associated with approximately threefold higher odds of treatment failure (OR=3.17, 95% CI: 1.71–5.89) and mortality (OR=3.48, 95% CI: 1.72–7.04; both p<0.001). Multidrug-resistant isolates, preterm birth, and low birth weight were also significant predictors of adverse outcomes. Delayed effective antimicrobial therapy independently predicted treatment failure (OR=2.76, 95% CI: 1.67–4.56, p<0.001), while septic shock was strongly associated with mortality

(OR=4.16, 95% CI: 2.24–7.72, p<0.001).

Table 7. Binary Logistic Regression Analysis of Predictors of Treatment Failure and Mortality Among Neonates with Sepsis

Predictor	Treatment Failure Adjusted OR (95% CI)	p-value	Mortality Adjusted OR (95% CI)	p-value
AMR gene positivity	2.84 (1.76–4.58)	<0.001	2.96 (1.62–5.41)	<0.001
Multidrug-resistant isolate	2.31 (1.42–3.76)	<0.001	2.43 (1.36–4.34)	0.003
Carbapenemase gene positivity	3.17 (1.71–5.89)	<0.001	3.48 (1.72–7.04)	<0.001
Preterm birth	1.68 (1.05–2.69)	0.030	1.74 (1.01–3.00)	0.046
Low birth weight	1.91 (1.20–3.04)	0.006	2.21 (1.27–3.85)	0.005
Delayed effective antimicrobial therapy	2.76 (1.67–4.56)	<0.001	—	—
Septic shock	—	—	4.16 (2.24–7.72)	<0.001

Note: OR = Odds Ratio; CI = Confidence Interval; AMR = Antimicrobial Resistance. Adjusted ORs were obtained from multivariable binary logistic regression models. The treatment-failure model included predictors relevant to treatment response, whereas the mortality model included predictors relevant to mortality. An adjusted OR >1 indicates increased odds of the respective adverse outcome.

Discussion

A total of 392 neonates were evaluated for sepsis in the present study with 53.83% male and 46.17% female. The largest age group was ≤7 days (44.90%), while 37.76% were preterm and 40.05% had birth weight <2.5 kg. Clinically, poor feeding (60.71%), respiratory distress (54.59%), elevated CRP (58.42%), temperature instability (42.60%), and thrombocytopenia (36.48%) were common. The results highlight the significant clinical impact of neonatal sepsis and are largely consistent with earlier studies that indicate that prematurity, low birth weight and non-specific systemic signs are significant neonatal sepsis characteristics. Another systematic review and meta-analysis by Fleischmann et al., (2021) also highlighted the substantial neonatal sepsis-related mortality and morbidity around the world, especially in low-resource areas [13]. The microbiological results showed that *Klebsiella pneumoniae* (30.74%), *Escherichia coli* (23.38%), *Staphylococcus aureus* (16.45%), and *Acinetobacter baumannii* (13.85%) were the most common pathogens. This dominance of *Klebsiella* is also similar to that of Bandyopadhyay et al. (2018) who found *Klebsiella* to be the most frequent individual pathogen (31.1%) in culture positive neonatal sepsis cases. Their study also found significant multidrug resistance in *Klebsiella*, *E. coli* and *Acinetobacter* isolates. Similarly, a recent systematic review conducted in the Gulf region showed that *Klebsiella* was one of the major causes of neonatal sepsis and high resistance to commonly used β-lactam antibiotics [14]. The similarities indicate that Gram-negative organisms continue to be important causes of neonatal sepsis and antimicrobial resistance.

The antimicrobial susceptibility results were especially high for ampicillin (76.62%), ceftriaxone (70.13%), and cefotaxime (68.40%) resistance and 51.52% for gentamicin. Meropenem, on the other hand, had comparatively lower resistance (20.35%) and 77.49% of the isolates were susceptible. The results show high resistance to the most frequently used β-lactam and aminoglycoside drugs. In a 2025 systematic review, third generation cephalosporin resistance rates were reported to be about 15–35% and aminoglycoside resistance to be 20–45% in neonatal sepsis studies, which are higher than the resistance rates in the present study [15]. Similarly, Moftian et al., (2023) found that there was a

high prevalence of antimicrobial resistance among Gram-negative pathogens associated with neonatal sepsis, raising concerns about the ability of empirical antibiotics to effectively treat infections in the future [16].

Molecular analysis identified *blaCTX-M* as the most frequently detected resistance gene (41.56%), followed by *blaTEM* (35.50%), *blaSHV* (26.41%), *blaNDM* (16.88%), *blaOXA-48-like* (12.12%), and *blaVIM* (4.76%). Importantly, the presence of *blaCTX-M*, *blaTEM*, *blaSHV* and *blaNDM* was significantly associated with phenotypic resistance, as 92.71%, 89.02%, 90.16% and 94.87% of positive isolates were respectively phenotypically resistant (all $p<0.001$). The results are in line with molecular research that has shown that ESBL-associated genes are widespread in neonatal pathogens. In another study, the distribution of *blaCTX-M*-15, *blaTEM*, and *blaSHV* was studied in bloodstream isolates of *Klebsiella* and *E. coli* from neonates; all ESBL-positive isolates were found to carry *blaCTX-M*-15, whereas *blaTEM* and *blaSHV* were also detected [17]. Recently, molecular characterization of ESBL-producing *K. pneumoniae* in preterm babies revealed the presence of *blaCTX-M*, *blaTEM*, *blaSHV*, *blaNDM*, blaOXA-48, and *blaVIM*, corroborating the wide range of genetic diversity seen in the present study [18].

The clinical findings also revealed an important relationship between AMR gene positivity and adverse outcome. Of the 231 culture-positive isolates, 117 (50.65%) were identified as AMR gene-positive and 114 (49.35%) as AMR gene-negative, due to the presence or absence of at least one of the resistance genes being tested. Treatment failure was observed in 55 (47.01%) AMR gene-positive patients and 31 (27.19%) AMR gene-negative patients ($p=0.002$). Prolonged hospitalization occurred in 48 (41.03%) versus 32 (28.07%) cases ($p=0.039$), while sepsis-related complications occurred in 42 (35.90%) versus 26 (22.81%) ($p=0.029$). Septic shock occurred in 27 (23.08%) versus 13 (11.40%) ($p=0.019$), and organ dysfunction occurred in 22 (18.80%) versus 10 (8.77%) cases ($p=0.027$), respectively. In addition, neonates with AMR gene-positive isolates had a significantly higher mortality rate (31 (26.50%) versus 14 (12.28%) for AMR gene-negative isolates; $p=0.006$). The results suggest that clinically relevant AMR determinants may be linked to poor treatment outcomes and complications in neonates with culture proven sepsis. In previous neonatal studies, resistant infections were also reported to have worse outcomes, such as increased hospital stay and mortality, in ESBL-associated neonatal infections; and higher infectious complications and sepsis-attributable mortality in ESBL cases in Taiwan [19].

Lastly, multivariable logistic regression showed that AMR was independently associated with poor clinical outcomes after adjustment for other risk factors. AMR gene positivity increased the odds of treatment failure by 2.84-fold (95% CI: 1.76–4.58; $p<0.001$) and mortality by 2.96-fold (95% CI: 1.62–5.41; $p<0.001$). Carbapenemase gene positivity showed an even stronger association with treatment failure (OR=3.17, 95% CI: 1.71–5.89; $p<0.001$) and mortality (OR=3.48, 95% CI: 1.72–7.04; $p<0.001$). Other important predictors were preterm birth and low birth weight, with septic shock being a strong predictor of mortality (OR=4.16, 95% CI: 2.24–7.72; $p<0.001$). These results are in line with previous studies, which have shown antimicrobial resistance, severity of illness and neonatal vulnerability to be significant factors in adverse outcomes. Bandyopadhyay et al. (2018) also found independent clinical predictors of neonatal death in culture positive sepsis [20]. In conclusion, the results of this study provide support to the implementation of molecular AMR surveillance in addition to standard susceptibility testing, to help detect resistant infections earlier and to guide antimicrobial therapy more appropriately.

Study Strengths and Limitations

One of the strengths of this study is the prospective clinical and molecular design, which allowed the incorporation of clinical characteristics, bacterial culture, antimicrobial susceptibility testing, molecular detection of genes conferring antimicrobial resistance,

and patient outcome in neonates with sepsis. The large number of neonates (392) and the number of culture-positive isolates (231) allowed for a significant amount of data to be used to assess the significance of important resistance determinants, such as *blaCTX-M*, *blaTEM*, *blaSHV*, *blaNDM*, *blaOXA-48-like*, and *blaVIM*. The study also reinforced the clinical implications of the molecular results by comparing the association between the molecular results and treatment failure, hospital stay, complications, septic shock, organ dysfunction and mortality, and then by multivariable logistic regression to control for relevant neonatal and clinical risk factors. But there are a few drawbacks to keep in mind. The study was performed in a single tertiary care hospital and may not be generalizable to other hospitals and areas. The results are based on molecular testing of selected bacterial isolates, and may not reflect the results of all neonates that had clinically suspected sepsis, especially those that were culture negative. The study did not include all resistance genes and may not have included other genetic mechanisms that play a role in antimicrobial resistance. Furthermore, the observational study design makes it difficult to draw a direct causal inference between carriage of the AMR gene and adverse clinical outcomes. Multivariable adjustment may not fully control for confounding due to the severity of the disease, antimicrobial exposure, comorbidities, and timing of effective therapy. Lastly, the study took place over a specific 2-year period, and any changes in the prescribing of antimicrobials and the emergence of antimicrobial resistance may affect the generalizability of the results to future populations.

Conclusion

This prospective clinical and molecular study revealed significant prevalence of antimicrobial resistance in the neonates with sepsis. Important resistance genes, such as *blaCTX-M*, *blaTEM* and *blaSHV* were more prevalent, and *blaNDM* and *blaOXA-48-like* were detected as important carbapenemase-associated determinants. Major AMR genes were significantly correlated with phenotypic AMR, suggesting that there is a significant link between the molecular resistance determinants and observed susceptibility patterns. Neonates with AMR gene-positive isolates had worse clinical outcomes, such as higher treatment failure rate, longer hospital stay, higher rates of complications related to sepsis, septic shock, organ dysfunction, and death than those with AMR gene-negative isolates. Multivariable analysis also confirmed that both AMR gene positivity and CAR were independently associated with poor clinical outcomes. The results of this study underscore the clinical value of using both molecular AMR detection and conventional culture and AMR testing in neonatal sepsis. To achieve better clinical outcomes and decrease the burden of antimicrobial resistance neonatal sepsis, the four elements of early recognition of resistant pathogens, prompt initiation of appropriate antimicrobial therapy, antimicrobial stewardship and enhanced infection control must be implemented.

References

Sands K, Spiller OB, Thomson K, Portal EA, Iregbu KC, Walsh TR. Early-onset neonatal sepsis in low-and middle-income countries: current challenges and future opportunities. Infection and drug resistance. 2022 Jan 1:933-46. https://doi.org/10.2147/IDR.S294156

Popescu CR, Cavanagh MM, Tembo B, Chiume M, Lufesi N, Goldfarb DM, Kissoon N, Lavoie PM. Neonatal sepsis in low-income countries: epidemiology, diagnosis and prevention. Expert review of anti-infective therapy. 2020 May 3;18(5):443-52. https://doi.org/10.1080/14787210.2020.1732818

Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. The lancet. 2017 Oct 14;390(10104):1770-80. https://doi.org/10.1016/S0140-6736(17)31002-4

Kariniotaki C, Thomou C, Gkentzi D, Panteris E, Dimitriou G, Hatzidaki E. Neonatal sepsis: a comprehensive review. Antibiotics. 2024 Dec 25;14(1):6.

https://doi.org/10.3390/antibiotics14010006
Li G, Bielicki JA, Ahmed AN, Islam MS, Berezin EN, Gallacci CB, Guinsburg R, da Silva Figueiredo CE, Santarone Vieira R, Silva AR, Teixeira C. Towards understanding global patterns of antimicrobial use and resistance in neonatal sepsis: insights from the NeoAMR network. Archives of disease in childhood. 2020 Jan;105(1):26-31. https://doi.org/10.1136/archdischild-2019-316816
Boscarino G, Romano R, Iotti C, Tegoni F, Perrone S, Esposito S. An overview of antibiotic therapy for early-and late-onset neonatal sepsis: current strategies and future prospects. Antibiotics. 2024 Mar 10;13(3):250. https://doi.org/10.3390/antibiotics13030250
Geleta D, Abebe G, Tilahun T, Abdissa A, Mihret A, Cataldo RJ, Workneh N, Negash AA, Beyene G. Molecular and clinical insights into extended-spectrum β-lactamase genes of Klebsiella pneumoniae isolated from neonatal sepsis in Ethiopia. BMC Infectious Diseases. 2024 Dec 18;24(1):1442. https://doi.org/10.1186/s12879-024-10344-w
Chatterjee S, Datta S, Roy S, Ramanan L, Saha A, Viswanathan R, Som T, Basu S. Carbapenem resistance in Acinetobacter baumannii and other Acinetobacter spp. causing neonatal sepsis: focus on NDM-1 and its linkage to IS Aba125. Frontiers in microbiology. 2016 Aug 8;7:1126. https://doi.org/10.3389/fmicb.2016.01126
Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, Blair JM. Molecular mechanisms of antibiotic resistance revisited. Nature Reviews Microbiology. 2023 May;21(5):280-95. https://doi.org/10.1038/s41579-022-00820-y
Folgori L, Bielicki J. Future challenges in pediatric and neonatal sepsis: emerging pathogens and antimicrobial resistance. Journal of pediatric intensive care. 2019 Mar;8(01):017-24. DOI: 10.1055/s-0038-1677535
Rallis D, Giapros V, Serbis A, Kosmeri C, Baltogianni M. Fighting antimicrobial resistance in neonatal intensive care units: rational use of antibiotics in neonatal sepsis. Antibiotics. 2023 Mar 3;12(3):508. https://doi.org/10.3390/antibiotics12030508
Coșovanu ET, Balan TA, Coșovanu EO, Ionescu S, Damian C, Petroaie AD, Coman EA, Grigore M, Socolov D, Balan RA, Iancu LS. Neonatal Infections Caused by Multidrug-Resistant Bacteria: An Analysis of Prevalence, Risk Factors, and Therapeutic Implications—A Narrative Review. Pathogens. 2026 Apr 26;15(5):469. https://doi.org/10.3390/pathogens15050469
Fleischmann, C., Reichert, F., Cassini, A., Horner, R., Harder, T., Markwart, R., Tröndle, M., Savova, Y., Kissoon, N., Schlattmann, P., Reinhart, K., Allegranzi, B., & Eckmanns, T. (2021). Global incidence and mortality of neonatal sepsis: A systematic review and meta-analysis. *Archives of Disease in Childhood, 106*(8), 745–752. https://doi.org/10.1136/archdischild-2020-320217 Journal article/DOI
Bandyopadhyay, T., Kumar, A., Saili, A., & Randhawa, V. S. (2018). Distribution, antimicrobial resistance and predictors of mortality in neonatal sepsis. *Journal of Neonatal-Perinatal Medicine, 11*(2), 145–153. https://doi.org/10.3233/NPM-1765 Journal article/DOI
Soni, P., Matoria, R., & Nagalli, M. M. (2025). Antibiotic strategies for neonatal sepsis: Navigating efficacy and emerging resistance patterns. *European Journal of Pediatrics, 184*, 439. https://doi.org/10.1007/s00431-025-06271-w Journal article/DOI
Moftian, N., Rezaei-Hachesu, P., Arab-Zozani, M., Samad-Soltani, T., Esfandiari, A., Tabib, M. S., & Mirnia, K. (2023). Prevalence of gram-negative bacteria and their antibiotic resistance in neonatal sepsis in Iran: A systematic review and meta-analysis. *BMC Infectious Diseases, 23*, 534. https://doi.org/10.1186/s12879-023-08508-1 Journal article/DOI

Roy S, Gaind R, Chellani H, Mohanty S, Datta S, Singh AK, Basu S. Neonatal septicaemia caused by diverse clones of Klebsiella pneumoniae & Escherichia coli harbouring blaCTX-M-15. Indian J Med Res. 2013 Apr;137(4):791-9.

Benboubker M, Oumokhtar B, Oukachou D, Elfakir S, Belchkar S, Rossi M, Massik A, Yahyaoui G, Moutaouakkil K, Hmami F. ESBL-producing Klebsiella pneumoniae gut colonisation and subsequent health-care associated bacteraemia in preterm newborns: a descriptive cohort with nested case-control study. Epidemiol Infect. 2025 Oct 6;153:e121. doi: 10.1017/S0950268825100630

Sehgal R, Gaind R, Chellani H, Agarwal P. Extended-spectrum beta lactamase-producing gram-negative bacteria: clinical profile and outcome in a neonatal intensive care unit. Ann Trop Paediatr. 2007 Mar;27(1):45-54. doi: 10.1179/146532807X170501.

Bandyopadhyay T, Kumar A, Saili A, Randhawa VS. Distribution, antimicrobial resistance and predictors of mortality in neonatal sepsis. J Neonatal Perinatal Med. 2018;11(2):145-153. doi: 10.3233/NPM-1765. PMID: 29991144.