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Epidemiology and antimicrobial resistance patterns of neonatal sepsis in a tertiary NICU in Western Ukraine: a 2023–2025 retrospective surveillance study

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Abstract

Background

Neonatal sepsis remains a major cause of morbidity and mortality in neonatal intensive care units, particularly in critically ill and mechanically ventilated neonates. Increasing antimicrobial resistance among hospital-associated pathogens substantially complicates empirical treatment and infection control strategies. This study aimed to evaluate the microbiological profile of neonatal sepsis and antimicrobial resistance patterns in a tertiary neonatal intensive care unit in Western Ukraine during 2023–2025.

Methods

A retrospective observational study was conducted in the neonatal intensive care unit of Chernivtsi Regional Clinical Hospital. Sixty-one neonates with clinically suspected or microbiologically confirmed sepsis admitted between 2023 and 2025 were included. Microbiological investigations included blood cultures, cerebrospinal fluid cultures, and endotracheal aspirates obtained during mechanical ventilation. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing recommendations. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages.

Results

Positive blood cultures were identified in 44/61 cases (72.1%). Gram-positive organisms predominated, mainly coagulase-negative staphylococci, including *Staphylococcus haemolyticus* (43.2%) and *Staphylococcus epidermidis* (13.4%). Gram-negative pathogens accounted for 31.3% of bloodstream isolates, with increasing detection of *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* during the study period. Cerebrospinal fluid cultures were positive in 55.7% of cases, while 90.4% of endotracheal aspirates from mechanically ventilated neonates yielded positive cultures. Eight fatal neonatal sepsis cases were recorded, predominantly associated with multidrug-resistant Gram-negative pathogens. High resistance rates were observed against cephalosporins, aminoglycosides, and carbapenems.

Conclusions

Neonatal sepsis in this tertiary neonatal intensive care unit was characterized by a high prevalence of multidrug-resistant pathogens and increasing representation of Gram-negative organisms during 2023–2025. The findings emphasize the importance of continuous microbiological surveillance, institution-

specific antimicrobial stewardship strategies, and strengthened infection prevention measures in neonatal intensive care settings.

Trial registration

Not applicable.

BACKGROUND

Neonatal sepsis remains one of the leading causes of neonatal morbidity and mortality worldwide [1], particularly among preterm and critically ill neonates requiring intensive care support [2]. Despite advances in neonatal intensive care, sepsis continues to contribute substantially to prolonged hospitalization, neurodevelopmental complications, and neonatal death, especially in low- and middle-income healthcare settings [3].

The epidemiology of neonatal sepsis has evolved considerably over recent decades [4]. Although Gram-positive organisms, particularly coagulase-negative staphylococci, remain predominant causes of late-onset sepsis in neonatal intensive care units (NICUs), increasing prevalence of Gram-negative multidrug-resistant (MDR) pathogens has become a major global concern. Organisms such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are associated with severe nosocomial infections, limited therapeutic options, and increased mortality in neonatal populations [5].

Antimicrobial resistance poses a substantial challenge in NICU practice due to frequent empirical use of broad-spectrum antibiotics, prolonged hospitalization, invasive procedures, and mechanical ventilation [6]. Continuous microbiological surveillance is therefore essential for optimization of empirical antimicrobial therapy and implementation of effective infection prevention strategies.

The ongoing Russian-Ukrainian war has placed additional strain on healthcare systems across the country [7–9]. Although Western Ukraine remains geographically distant from active combat zones, regional healthcare institutions have experienced increased patient flow, internal displacement, and elevated demand for intensive care services [10]. These conditions may contribute to changes in hospital microbial ecology and antimicrobial resistance patterns [11–12].

However, data regarding neonatal sepsis epidemiology and antimicrobial resistance trends in Ukrainian NICUs during the ongoing war remain limited [13–14]. The present study aimed to evaluate the microbiological profile of neonatal sepsis, characterize antimicrobial resistance patterns, and assess temporal changes in pathogen distribution in a tertiary NICU in Western Ukraine during 2023–2025. The aim of this study was to evaluate the etiological structure of neonatal sepsis and antimicrobial resistance patterns among pathogens isolated from neonates treated in a tertiary neonatal intensive care unit in Western Ukraine during 2023–2025.

METHODS

Aim, study design and setting

This study aimed to describe the etiological spectrum of neonatal sepsis and antimicrobial resistance patterns among pathogens isolated from neonates treated in a tertiary neonatal intensive care unit (NICU) in Western Ukraine between 2023 and 2025.

A retrospective observational study was conducted in accordance with the STROBE guidelines. The study is descriptive in nature and is intended to be hypothesis-generating. Clinical and laboratory data were obtained from the Department of Pediatrics and Pediatric Infectious Diseases, Bukovinian State Medical University (Chernivtsi, Ukraine), and the NICU of the Chernivtsi Regional Clinical Hospital (Chernivtsi, Ukraine), a tertiary referral center.

National statistical mortality data were used only for contextual epidemiological background and were not directly linked to the NICU cohort.

Study population

A total of 61 neonates with a clinical diagnosis of neonatal sepsis treated in the NICU between 2023 and 2025 were included. Inclusion criteria were: (1) clinical diagnosis of neonatal sepsis, (2) availability of microbiological investigations, and (3) hospitalization in the NICU.

Exclusion criteria included incomplete clinical or laboratory data and transfer to another healthcare facility before completion of diagnostic evaluation.

Due to the retrospective design and incomplete availability of structured severity indicators in medical records, stratification by gestational age, birth weight, and standardized severity scores was not performed.

Microbiological investigations

Microbiological evaluation was performed to describe the etiological profile of neonatal sepsis. The following clinical specimens were analyzed:

- 61 blood cultures,
- 61 cerebrospinal fluid (CSF) samples,
- 98 endotracheal aspirates obtained from mechanically ventilated neonates.

Endotracheal aspirate sampling was routinely available only for ventilated patients, and systematic collection became standardized from 2024 onward; therefore, this subgroup represents a selected ventilated population.

All samples were processed in the certified bacteriological laboratory of the Chernivtsi Regional Clinical Hospital using standard culture-based methods.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Bacterial suspensions were standardized to a 0.5 McFarland turbidity standard and inoculated onto Mueller–Hinton agar. Plates were incubated at 35–37°C for 18–24 hours. Zone diameters were interpreted according to EUCAST clinical breakpoints.

Intermediate susceptibility was interpreted according to EUCAST recommendations and included in the non-susceptible category for MDR classification. Quality control was ensured using reference strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Definitions

Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial classes. ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) were analyzed as markers of healthcare-associated antimicrobial resistance.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee of Bukovinian State Medical University (protocol #4 18.12.2025). Given the retrospective design and anonymized dataset, informed consent was waived. The study complied with national infection prevention and antimicrobial stewardship regulations issued by the Ministry of Health of Ukraine [15–16].

Statistical analysis

Statistical analysis was performed using TIBCO Statistica 13.5.0.17 (TIBCO Software Inc., USA) and Microsoft Excel 16.

The study was primarily descriptive and hypothesis-generating. Therefore, no formal sample size calculation or statistical power analysis was performed.

Continuous variables were expressed as mean ± standard deviation (M ± SD). Depending on distribution, parametric or non-parametric methods were applied as appropriate. A p-value < 0.05 was considered

statistically significant for exploratory comparisons. Missing data accounted for < 10% of variables and were handled using complete-case analysis without imputation.

Graphical visualization was performed using R software (R Foundation for Statistical Computing, Vienna, Austria) in RStudio (Posit Software, Boston, MA, USA). Figures illustrate temporal and descriptive trends in pathogen distribution, antimicrobial resistance, and outcomes.

Data availability

The datasets generated and analyzed during the current study are not publicly available due to institutional data protection policies but are available from the corresponding author upon reasonable request.

RESULTS

Contextual mortality trends

National statistical data were analyzed to provide epidemiological context for neonatal sepsis during the study period (2023–2025). These data were not directly linked to the NICU cohort.

The proportion of infant deaths attributed to neonatal sepsis increased from 10.0% (0.66‰) in 2023 to 15.6% (0.88‰) in 2024 and 27.3% (1.73‰) in 2025, corresponding to a change from fourth to second leading cause of infant mortality.

Neonatal sepsis-associated mortality showed an overall increase over the study period. This increase occurred concurrently with a higher proportion of Gram-negative pathogens and MDR isolates observed in the NICU cohort (Fig. 1), without implying causal inference.

Blood culture results and etiological profile

Positive blood cultures were obtained in 44 cases (72.1%). Gram-positive organisms predominated (71.5%), mainly coagulase-negative staphylococci (58.1%), including *Staphylococcus haemolyticus* (43.2%) and *Staphylococcus epidermidis* (13.4%). Enterococci accounted for 13.4%.

Gram-negative organisms were identified in 31.3% of cases. A gradual increase in Enterobacterales was observed over time, particularly *Klebsiella aerogenes* (8.9%). *Escherichia coli* and *Klebsiella pneumoniae* were each identified in 1.5% of cases. Non-fermenting Gram-negative bacilli accounted for 6.0%, including *Acinetobacter baumannii* (4.5%) and *Pseudomonas aeruginosa* (1.5%). *Moraxella* spp. were detected in 10.4% of isolates.

Polymicrobial bloodstream infections were observed in 9.8% of cases, most frequently involving *Staphylococcus haemolyticus* in combination with Gram-negative bacteria or enterococci. Methicillin-

resistant staphylococcal isolates were identified in 2025.

Cerebrospinal fluid findings

CSF cultures were positive in 55.7% of cases. *Staphylococcus haemolyticus* was the most frequently isolated pathogen (44.1%). Methicillin-resistant staphylococcal strains (*S. haemolyticus* and *S. aureus*) were detected in 2025.

Klebsiella spp. represented the main Gram-negative pathogens in CSF, with *Klebsiella aerogenes* accounting for 23.5% of isolates and showing an increasing proportion over time. Mixed infections were identified in five cases.

Endotracheal aspirates (ventilated subgroup)

Among 52 mechanically ventilated neonates (2024–2025), 90.4% of endotracheal aspirates were culture-positive. Gram-negative organisms predominated (64.1%), mainly *Pseudomonas aeruginosa* (27.5%) and *Klebsiella* spp. (29.5%), followed by *Escherichia coli* (5.1%) and *Acinetobacter baumannii* (2.0%).

Gram-positive organisms included *Enterococcus faecalis* (17.3%) and coagulase-negative staphylococci (15.2%). MRSA was detected in 20.0% of staphylococcal isolates. *Candida albicans* was identified in 2.0% of samples. Polymicrobial infections occurred in four cases and were predominantly associated with resistant organisms.

Mortality-associated isolates

Eight fatal cases of neonatal sepsis were recorded. All fatal outcomes involved Gram-negative organisms, most frequently ESBL-producing *Klebsiella aerogenes* and *Klebsiella pneumoniae*. *Staphylococcus haemolyticus* was frequently present in polymicrobial infections. MDR ESKAPE pathogens were identified in 50% of fatal cases.

Antimicrobial resistance patterns

High antimicrobial resistance levels were observed across major pathogen groups (Table 1A-B). Very high resistance rates were detected in several pathogen-antibiotic combinations, particularly among Gram-negative bacteria, reflecting a highly selected population of critically ill neonates in a tertiary referral setting.

Table 1. Antimicrobial resistance patterns among major neonatal sepsis pathogens isolated in the NICU cohort

Resistance rates are presented as percentages (%).

Table 2

A. Antimicrobial resistance among Gram-negative pathogens

Antibiotic	***Klebsiella aerogenes***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Acinetobacter baumannii***
Cefotaxime	84.0	70.0	83.3	37.5	66.6
Cefepime	83.5	39.3	54.5	18.5	100
Ciprofloxacin	66.6	40.0	18.7	0	25.0
Meropenem	60.0	44.4	28.5	0	62.5
Amikacin	72.7	45.4	26.6	12.5	50.0
Gentamicin	68.0	30.0	37.5	12.5	28.5
Ampicillin	86.0	85.7	100	50.0	50.0
Piperacillin	70.8	54.5	21.4	12.5	0
Clindamycin	87.5	100	100	0	0
Linezolid	93.3	100	100	0	0
Tobramycin	100	0	66.6	0	0
Colistin	0	50.0	0	0	0

Table 2
B. Antimicrobial resistance among Gram-positive pathogens

Antibiotic	*Staphylococcus haemolyticus*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Enterococcus faecalis*
Cefotaxime	80.0	40.0	0	50.0
Cefepime	37.5	28.5	100	50.0
Ciprofloxacin	26.6	8.3	0	14.2
Meropenem	28.5	14.2	0	91.6
Amikacin	16.6	9.0	0	50.0
Gentamicin	53.8	9.0	0	44.4
Vancomycin	50.0	50.0	0	16.6
Ampicillin	42.8	50.0	0	11.1
Piperacillin	50.0	100	0	14.2
Clindamycin	16.1	22.2	0	50.0
Linezolid	20.0	20.0	0	20.0
Tobramycin	0	50.0	0	100

High antimicrobial resistance rates were observed across both Gram-negative and Gram-positive pathogens (Table 2A–B). Among Gram-negative isolates, *Klebsiella aerogenes* demonstrated high resistance to cefotaxime (84.0%), cefepime (83.5%), ampicillin (86.0%), and linezolid (93.3%), while *Klebsiella pneumoniae* showed complete resistance to clindamycin and linezolid. *Pseudomonas aeruginosa* demonstrated complete resistance to ampicillin, clindamycin, and linezolid. Among Gram-positive organisms, *Staphylococcus epidermidis* demonstrated complete resistance to cefepime, while *Enterococcus faecalis* showed high resistance to meropenem (91.6%) and complete resistance to tobramycin. Overall, resistance was most pronounced against β-lactams and selected reserve antibiotics within the studied NICU cohort.

Pseudomonas aeruginosa demonstrated high resistance rates including 100% resistance to ampicillin, clindamycin, and linezolid. *Klebsiella aerogenes* showed 100% resistance to tobramycin and high resistance (93.3%) to linezolid. *Klebsiella pneumoniae* exhibited complete resistance to clindamycin and linezolid. *Acinetobacter baumannii* demonstrated complete resistance to cefepime.

Among Gram-positive organisms, *Staphylococcus aureus* showed 100% resistance to piperacillin, while *Staphylococcus epidermidis* was fully resistant to cefepime. *Enterococcus faecalis* showed complete resistance to tobramycin and high resistance to meropenem (91.6%).

Overall, the highest resistance rates were observed among β-lactam antibiotics and selected reserve agents, including carbapenems and glycopeptides, within the studied cohort.

DISCUSSION

This study describes the evolving microbiological and antimicrobial resistance landscape of neonatal sepsis in a tertiary NICU in Western Ukraine during 2023–2025. The main findings include a shift in pathogen distribution from predominantly Gram-positive organisms toward Gram-negative bacteria, a high prevalence of multidrug-resistant (MDR) and ESKAPE pathogens, and markedly elevated resistance rates to commonly used and reserve antibiotics. These findings should be interpreted as descriptive and context-specific to a tertiary referral NICU population.

The persistent predominance of coagulase-negative staphylococci, particularly *Staphylococcus haemolyticus*, is consistent with global NICU epidemiology, where these organisms represent leading causes of late-onset sepsis in preterm and critically ill neonates [17–18]. The identification of methicillin-resistant strains reflects increasing antimicrobial selection pressure in hospital settings with frequent empirical use of broad-spectrum antibiotics [19]. Similar shifts toward clinically relevant MDR coagulase-negative staphylococci have been reported in NICU populations worldwide, highlighting their transition from contaminants to significant opportunistic pathogens in vulnerable hosts [20].

A key observation in this study is the increased proportion of Gram-negative organisms, including *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. These pathogens are recognized members of the ESKAPE group and are globally associated with healthcare-associated antimicrobial resistance [21]. Their presence across bloodstream, cerebrospinal fluid, and respiratory specimens suggests sustained nosocomial circulation within the NICU environment rather than isolated sporadic infections. Similar epidemiological shifts have been described in healthcare systems under structural stress, where infection prevention challenges may facilitate dissemination of MDR Gram-negative organisms [22]. However, causal inference cannot be established based on the present dataset.

The detection of ESBL-producing *Klebsiella* spp. and non-fermenting Gram-negative bacilli with reduced susceptibility to carbapenems is of particular clinical concern. These pathogens are well documented causes of severe neonatal infections, including sepsis and meningitis, and are associated with limited therapeutic options and increased mortality risk in NICU settings [23–24]. Their increasing representation in this cohort likely reflects a combination of horizontal transmission and antimicrobial selection pressure within a high-risk population exposed to broad empirical antibiotic regimens.

Polymicrobial bloodstream and CSF infections were frequently observed and may reflect advanced colonization patterns in critically ill neonates. Such infections, particularly when involving MDR organisms, are associated in the literature with increased clinical complexity, delayed pathogen-directed therapy, and prolonged hospitalization [25]. In this context, polymicrobial growth should be considered a

marker of severe disease and complex NICU microbiological ecology rather than a distinct etiological entity.

Respiratory isolates from mechanically ventilated neonates were dominated by *Pseudomonas aeruginosa* and *Klebsiella* spp., supporting the role of ventilator-associated colonization and biofilm formation in sustaining MDR reservoirs. Endotracheal colonization is a recognized precursor of ventilator-associated pneumonia and secondary bloodstream infection in NICU patients, particularly in prolonged mechanical ventilation [26]. The observed distribution therefore likely reflects device-associated colonization dynamics within intensive care settings.

The highest clinical burden was observed in fatal cases of neonatal sepsis, where MDR and ESKAPE pathogens were frequently identified, predominantly ESBL-producing *Klebsiella* spp. in polymicrobial combinations. These findings are consistent with evidence that ESKAPE pathogens are disproportionately associated with adverse outcomes in critically ill neonates due to delayed effective therapy and restricted antimicrobial options [21, 27]. However, given the retrospective and descriptive design of this study, associations with mortality should be interpreted cautiously.

Antimicrobial susceptibility testing demonstrated high resistance rates across multiple antibiotic classes, including β-lactams, aminoglycosides, carbapenems, and glycopeptides. These patterns reflect a highly selected hospital population in a tertiary referral NICU and are consistent with global trends of increasing resistance among neonatal pathogens [14]. Of particular concern is the high resistance observed in *Klebsiella* spp. and *Acinetobacter baumannii*, organisms known for rapid acquisition of resistance determinants and extensive horizontal gene transfer.

From a clinical perspective, these findings support the need for continuous local surveillance of NICU pathogens and periodic revision of empirical antibiotic protocols based on up-to-date microbiological data. Early targeted therapy guided by microbiological diagnostics and antimicrobial stewardship principles, including timely de-escalation, remains essential to optimize outcomes while minimizing selection pressure. Strengthening infection prevention and control measures, including hand hygiene compliance, environmental decontamination, and device-related infection prevention strategies, is also critical to limit further spread of MDR organisms.

The observed microbiological shifts should be interpreted within the broader context of healthcare system strain during the study period. Similar patterns have been reported in other settings experiencing healthcare disruption, where infection control challenges and increased antimicrobial utilization may contribute to changes in nosocomial pathogen ecology [27]. However, the present study was not designed to assess causal relationships between systemic factors and microbiological outcomes.

Finally, this study has limitations inherent to its retrospective single-center design and relatively small sample size, which limit generalizability. Additionally, incomplete availability of detailed clinical severity stratification restricted adjustment for potential confounders. Therefore, the findings should be

considered descriptive and hypothesis-generating, providing a basis for future prospective multicenter studies.

CONCLUSIONS

Neonatal sepsis in this tertiary NICU demonstrated a progressive increase in mortality during 2023–2025, accompanied by a rising proportion of Gram-negative and multidrug-resistant (MDR), including ESKAPE, pathogens. The etiological landscape remained dominated by coagulase-negative staphylococci, particularly *Staphylococcus haemolyticus*, while an increasing contribution of Gram-negative bacilli, including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, was observed.

High levels of antimicrobial resistance to cephalosporins, carbapenems, and aminoglycosides were recorded, which substantially limited the effectiveness of empirical therapeutic regimens in this cohort.

These findings underscore the importance of continuous local microbiological surveillance and regular updating of empirical antibiotic protocols in NICU settings. Strengthening antimicrobial stewardship and infection prevention and control measures is essential to mitigate the spread of multidrug-resistant organisms and to support optimized management of neonatal sepsis.

Abbreviations

AMR
antimicrobial resistance
ATCC
American Type Culture Collection
CSF
cerebrospinal fluid
ESBL
extended–spectrum beta–lactamase
EUCAST
European Committee on Antimicrobial Susceptibility Testing
ICU
intensive care unit
LOS
late–onset sepsis
MDR
multidrug–resistant
NICU
neonatal intensive care unit
SD

standard deviation

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of Bukovinian State Medical University (protocol #4 18.12.2025).

Due to the retrospective observational design and use of anonymized clinical and microbiological data, the requirement for informed consent was waived by the ethics committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to institutional data protection policies but are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

K.R. and L.K. performed data analysis and wrote the main manuscript text. O.K. supervised the study and critically revised the manuscript. O.V., K.V., and V.K. contributed to data collection and investigation. K.R. prepared the figures and tables. All authors reviewed and approved the final manuscript.

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Table 1

Table 1 is not available with this version

Figures

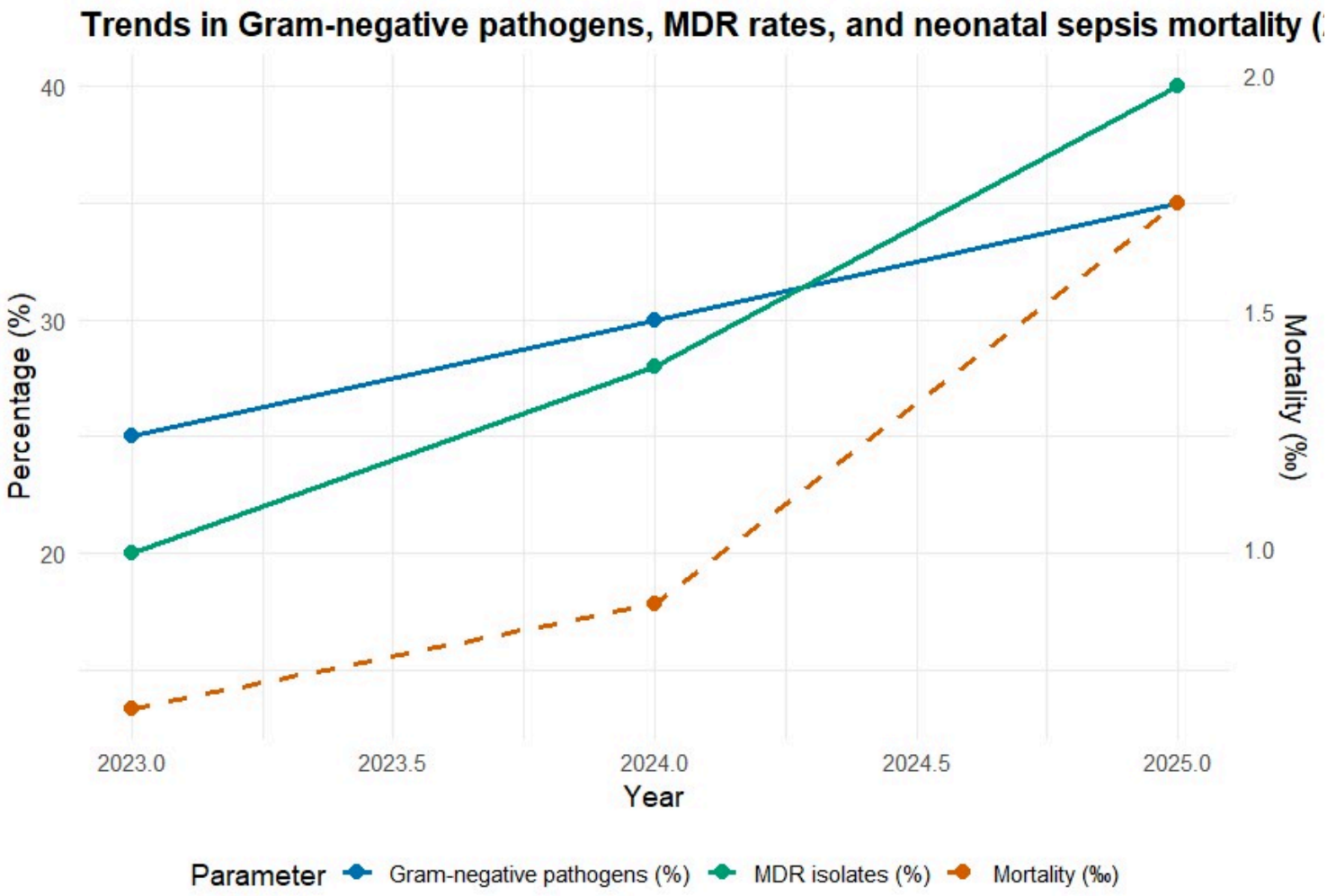


Figure 1

Trends in Gram-negative pathogens, MDR rates, and neonatal sepsis mortality (2023–2025).

illustrates temporal trends observed in the NICU cohort during 2023–2025. The proportion of Gram-negative pathogens increased from approximately 25% in 2023 to 35% in 2025. During the same period, the proportion of multidrug-resistant (MDR) isolates rose from 20% to 40%. Neonatal sepsis-associated

mortality also increased, from 0.7‰ in 2023 to 1.8‰ in 2025. These trends occurred concurrently within the studied cohort; however, the figure is descriptive and does not imply causal relationships between pathogen distribution, antimicrobial resistance, and mortality outcomes