JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Analysis of bacteriological characteristics of nosocomial infections in preterms based on Gram-negative infection factors

Junjie Xiang[1 #], Yuanxin Kuang[1 #], Qian Cheng[1], Keqian Zhang[1], Xindie Hu[1], Binghong Zhang[1], Yanyan Shang[1], Zhiyue Deng[1,2]

[1] *Department of Neonatology, Renmin Hospital of Wuhan University, Wuhan, China*
[2] *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*

# Authors contributed equally to this work, and should be considered co-first authors.

Abstract

Introduction: Nosocomial infections (NIs) in the neonatal intensive care unit (NICU) are a significant cause of disability or death in preterm infants. This study focused on the bacteriological characteristics of NIs in preterm infants based on blood culture, and the factors related to Gram-negative bacterial infection.

Methodology: This retrospective study was conducted at the NICU in Renmin Hospital of Wuhan University from January 2020 to June 2024. The patients were divided into Gram-negative and Gram-positive bacteria groups based on Gram staining results. Logistic regression analysis was performed to identify the factors influencing Gram-negative bacterial infection, and the diagnostic value was evaluated based on the area under the receiver operating characteristic curve (AUC).

Results: A total of 54 strains (54/95, 56.84%) of Gram-positive bacteria and 41 strains (41/95, 43.16%) of Gram-negative bacteria were detected in the blood cultures of the 95 patients diagnosed with late-onset sepsis. The proportion of Gram-negative bacteria in the past 5 years exhibited a trend of initially decreasing and then increasing. Use of mechanical ventilation, decreased monocyte count, and elevated lactate levels were identified as factors predicting Gram-negative bacterial infection ($p < 0.05$). The AUC values of these factors for predicting Gram-negative bacterial infection were 0.56 ($p < 0.05$), 0.71 ($p < 0.05$), and 0.71 ($p < 0.05$), respectively.

Conclusions: The proportion of Gram-negative bacterial infections showed a trend of initially decreasing and then increasing from 2020 to 2024. The use of mechanical ventilation, decreased monocyte counts, and elevated lactate levels were factors influencing Gram-negative bacterial infection.

Key words: nosocomial infections; sepsis; preterms; bacteria.

J Infect Dev Ctries 2026; 20(8):1217-1224. doi:10.3855/jidc.21965

(Received 17 June 2025 – Accepted 11 February 2026)

Introduction

Nosocomial infection (NI), also referred to as healthcare-associated infection (HAI), was defined by the World Health Organization (WHO) as an infection occurring ≥ 48 hours after hospital admission, and was not present or incubating at the time of admission [1]. With the continuous improvement of medical technologies and management, the gestational age at which preterm infants in the neonatal intensive care unit (NICU) are treated has been decreasing, and the survival rate is continually rising. Preterm infants are hosts with compromised immune function, requiring prolonged hospitalization, frequently undergoing invasive procedures, and receiving long-term broad-spectrum antibiotics and intravenous nutrition [2,3]. As a result, the risk of NIs in preterm infants is correspondingly high. Infections related to the NICU significantly increase resource utilization, hospital costs, and the length of hospitalization in the NICU [4]. Additionally, large cohort studies have shown that neonatal infections in low-birth-weight infants are associated with adverse early neurodevelopmental and growth outcomes in children [5]. Currently one of the major challenges in NICU management worldwide is how to prevent and control HAIs.

NIs in the NICU are primarily bloodstream infections. Bloodstream infections in extremely low birth weight (ELBW) infants (< 1,000 g) are associated with more than a two-fold increase in mortality, prolonged hospitalization by 9–27 days, and significantly worsened neurodevelopmental outcomes [6]. Bloodstream infections are primarily bacterial in nature and can be classified into Gram-positive and Gram-negative bacterial infections. Gram-negative bacterial infections are more critical than Gram-positive bacterial infections. Gram-negative bacteria possess an outer membrane covered by a capsule or slime layer, which can conceal surface antigens and trigger the body's immune response. The mortality rate of neonatal sepsis caused by Gram-negative bacteria is significantly

higher than that caused by Gram-positive bacteria [7]. In an observational study, the incidence of Gram-negative invasive bacterial infections in infants increased between 2011/2012 and 2018/2019 in England, driven mainly by an increase in late-onset infections [8]. Therefore, early identification of NIs caused by Gram-negative bacteria is of significant clinical importance.

Blood culture is currently considered the gold standard for the diagnosis of bloodstream infections in clinical practice; however, it has limitations related to time sensitivity. Generally, preliminary blood culture results require about 48–72 hours after blood samples are taken. Therefore, until the results are available, doctors and nurses can only rely on the patient's clinical symptoms, medical history, and immediate blood gas results to make empirical treatment decisions. Although pathogenic next-generation sequencing (NGS) testing offers advantages over blood culture in terms of timeliness, it is not yet widely used due to factors such as cost, especially in developing countries. Meanwhile, inappropriate antibiotic use contributes to the development and clinical significance of bacterial antibiotic resistance [9].

This study was a retrospective analysis of preterm infants with a first episode of positive blood culture during hospitalization at Renmin Hospital of Wuhan University from January 2020 to June 2024. This study aimed to investigate the distribution and change of bacterial species in positive blood cultures, and their corresponding clinical characteristics. In addition, it sought to identify one or more statistically significant risk factors that can assist in preliminarily determining the type of bacteria causing bloodstream infections before blood culture results are available, thereby helping doctors and nurses to prepare appropriate countermeasures in advance.

Methodology

Patient population

This study was a retrospective cohort study conducted at Renmin Hospital of Wuhan University, a tertiary teaching hospital in Wuhan, Hubei Province, China. All patients were recruited from the NICU.

The inclusion criteria were as follows: (1) infants aged ≤ 28 days who were admitted to the NICU between January 2020 and June 2024, with a hospital stay of ≥ 48 hours; (2) premature birth, defined as a gestational age of < 37 weeks; (3) infants clinically diagnosed with late-onset sepsis, with the first blood culture result indicating bacterial growth.

The exclusion criteria were as follows: (1) infants with blood culture results indicating mixed infections; (2) infants with incomplete clinical data; (3) infants with preexisting disease, congenital malformations, and genetic metabolic diseases. Duplicate bacterial isolates from the same patient were excluded to avoid analytical bias. Only the first isolate from the first episode of bloodstream infection during NICU hospitalization was included.

This study received approval from the Medical Ethics Committee of Renmin Hospital of Wuhan University (No. WDRY2023-K098). All patient data were anonymized during the data collection and analysis process. Given the retrospective nature of the study and the use of anonymized data, the requirement for written informed consent was waived by the ethics committee (Approval No. WDRY2023-K098(X01)), as the study did not involve patient privacy and posed no additional risk to the rights or welfare of the infants.

Microbiological analysis

Blood cultures were processed using standard microbiological techniques. Blood samples were inoculated into BD BACTEC™ Peds Plus™/F culture vials (Becton, Dickinson and Company, New Jersey, USA) and incubated in an automated blood culture system. Bacterial identification was performed using the VITEK MS automated system (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing was conducted using the VITEK 2 system (bioMérieux, Marcy-l'Étoile, France). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2022 edition) [10].

Data collection

The data extracted from the medical record system included: (1) demographic-related data, such as gender, gestational age, birth weight, age at infection onset, corrected gestational age and weight at the time of infection, urine output, body temperature; (2) vital signs and clinical manifestations at the time of infection, such as heart rate, response, skin color and condition, respiratory status; (3) relevant procedures performed within 7 days prior to the infection, including the use of mechanical ventilation and the presence of a peripherally inserted central catheter (PICC); (4) results of blood cultures, complete blood counts, and blood gas analyses obtained from the same batch at the time of suspected infection.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp,

Armonk, NY, USA) and R software (version 4.3.1). Firstly, the distribution of bacterial species in blood culture results for all infants was statistically analyzed, along with the number of infections and trends over the years. Then, univariate regression analysis was used to select variables with statistical significance ($p < 0.1$) between the two groups. Comparisons between groups were conducted using the independent-samples t test or Mann–Whitney U test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. These variables were further analyzed using multivariable backward stepwise logistic regression analysis to identify the final statistically significant variables ($p < 0.05$). Receiver operating characteristic (ROC) curves were plotted using R software, and the area under the curve (AUC) was calculated to validate the reliability of the binary logistic regression analysis results and to evaluate predictive capability.

Results

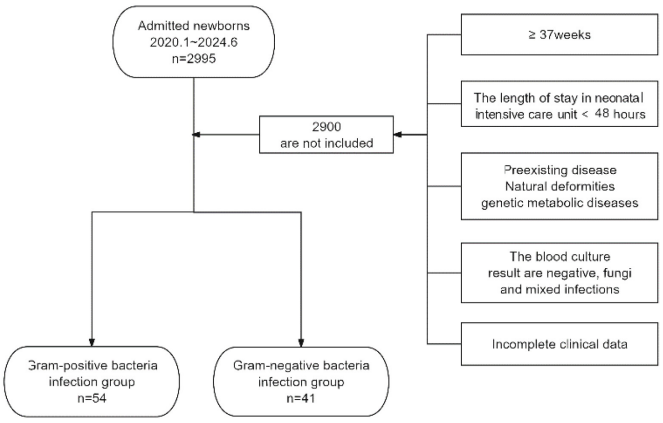
Bacterial distribution and trends

This study identified 95 premature infants that met the above criteria from a total of 2,995 newborns admitted. Among them, 41 infants had their first blood culture results positive for Gram-negative bacteria, while 54 infants were positive for Gram-positive bacteria. They were accordingly divided into the Gram-negative bacterial infection group (n = 41), and the Gram-positive bacterial infection group (n = 54). The flowchart of this selection process is shown in Figure 1.

Bacterial species and distribution trends of NIs in premature infants

Among the 95 infants diagnosed with late-onset sepsis, the blood culture results showed 54 strains of Gram-positive bacteria (54/95, 56.84%) and 41 strains of Gram-negative bacteria (41/95, 43.16%). The predominant Gram-positive bacteria were *Staphylococcus epidermidis* (34/54, 62.96%), *Enterococcus faecalis* (7/54, 12.96%), and *Staphylococcus aureus* (6/54, 11.11%); the predominant Gram-negative bacteria were *Escherichia coli* (14/41, 34.17%) and *Klebsiella pneumoniae* (14/41, 34.17%). The specific distribution of pathogens, the number of cases, and the composition ratios are shown in Table 1.

Figure 1. Patients' selection and enrollment.



NICU: neonatal intensive care unit.

Table 1. Distribution of bacterial species isolated from blood cultures in the study population.

Bacteria	n	Proportion (%)
Gram-positive bacteria		
Staphylococcus epidermidis	34	35.78
Enterococcus faecalis	7	7.37
Staphylococcus haemolyticus	6	6.32
Enterococcus faecium	2	2.11
Bacillus cereus	1	1.05
Staphylococcus xylosus	1	1.05
Staphylococcus hominis	1	1.05
Stenotrophomonas maltophilia	1	1.05
Staphylococcus warneri	1	1.05
Gram-negative bacteria		
Escherichia sp.	14	14.74
Klebsiella pneumoniae	14	14.74
Enterobacter cloacae	4	4.21
Klebsiella aerogenes	2	2.11
Pseudomonas putida	1	1.05
Citrobacter freundii	1	1.05
Enterobacter hormaechei	1	1.05
Achromobacter xylosoxidans	1	1.05
Serratia marcescens	1	1.05
Proteus mirabilis	1	1.05
Pseudomonas aeruginosa	1	1.05
Overall total	**95**	**100**

The overall number of detected pathogens showed fluctuating changes over the previous 5 years, with the highest number of infection cases in 2021, followed by a decreasing trend each subsequent year. Among them, the proportion of Gram-negative bacteria first decreased and then increased. The trend changes are illustrated in Figure 2.

Figure 2. Percentage and count of Gram-negative/Gram-positive bacteria by year

Analysis of clinical data of premature infants with NIs

The results of the univariate logistic regression analysis showed that infants with Gram-negative bacterial infections had a lower corrected gestational age at the time of infection. Additionally, there was a higher proportion of mechanical ventilation usage, apnea, and tachycardia in the 7 days prior to infection among those with Gram-negative bacterial infections, and the differences were statistically significant ($p < 0.1$). There were no statistically significant differences in gender, gestational age, birth weight, age at infection, and duration of PICC use at the time of infection between the two groups. The laboratory examination results at the initial stage of infection showed that infants with Gram-negative bacterial infections had lower monocyte percentages and counts, but higher

Table 2. Univariate logistic regression analysis of factors associated with Gram-negative bacterial infection.

Variables	β	SE	Z	*p*	OR (95%CI)
GA	- 0.10	0.08	- 1.32	0.186	0.90 (0.77 ~ 1.05)
Weight	- 0.09	0.45	- 0.21	0.834	0.91 (0.38 ~ 2.19)
Age of infection	- 0.01	0.02	- 0.65	0.514	0.99 (0.95 ~ 1.03)
Corrected GA of infection	- 0.21	0.10	- 2.09	**0.037**	0.81 (0.66 ~ 0.99)
Gender					
Female					1.00 (Reference)
Male	- 0.17	0.42	- 0.42	0.675	0.84 (0.37 ~ 1.90)
Output	- 0.00	0.00	- 0.55	0.582	1.00 (0.99 ~ 1.00)
T_{max}	- 0.05	0.77	- 0.06	0.952	0.95 (0.21 ~ 4.31)
Mechanical ventilation					
N					1.00 (Reference)
Y	2.21	1.10	2.00	**0.045**	9.09 (1.05 ~ 78.75)
PICC					
N					1.00 (Reference)
Y	- 0.12	0.42	- 0.29	0.771	0.89 (0.39 ~ 2.01)
Apnea					
N					1.00 (Reference)
Y	0.80	0.45	1.79	**0.074**	2.23 (0.93 ~ 5.39)
Jaundice					
N					1.00 (Reference)
Y	- 0.23	0.46	- 0.49	0.623	0.80 (0.32 ~ 1.96)
Mottled skin					
N					1.00 (Reference)
Y	0.48	0.42	1.15	0.252	1.62 (0.71 ~ 3.69)
Tachycardia					
N					1.00 (Reference)
Y	1.41	0.46	3.07	**0.002**	4.10 (1.67 ~ 10.12)
WBC	- 0.07	0.05	- 1.60	0.109	0.93 (0.85 ~ 1.02)
RBC	0.08	0.10	0.79	0.429	1.08 (0.89 ~ 1.31)
Hb	- 0.01	0.01	- 0.76	0.448	0.99 (0.97 ~ 1.01)
PLT	- 0.00	0.00	- 0.76	0.445	1.00 (0.99 ~ 1.00)
Neutrophil count	- 0.07	0.05	- 1.28	0.199	0.94 (0.85 ~ 1.04)
Lymphocyte count	- 0.09	0.13	- 0.70	0.486	0.91 (0.71 ~ 1.18)
Monocyte count	- 0.66	0.34	- 1.96	**0.050**	0.51 (0.27 ~ 0.99)
Neutrophil ratio	0.00	0.01	0.15	0.877	1.00 (0.98 ~ 1.02)
Lymphocyte ratio	0.01	0.01	0.68	0.496	1.01 (0.98 ~ 1.04)
Monocyte ratio	- 0.06	0.03	- 1.87	**0.062**	0.94 (0.88 ~ 1.00)
CRP	0.01	0.01	2.72	**0.006**	1.01 (1.01 ~ 1.03)
PCT	0.10	0.03	2.96	**0.003**	1.10 (1.03 ~ 1.17)
SAA	0.01	0.00	2.83	**0.005**	1.01 (1.01 ~ 1.01)
pH	- 6.67	2.54	- 2.63	**0.009**	0.00 (0.00 ~ 0.18)
pO_2	- 0.00	0.00	- 0.60	0.546	1.00 (0.99 ~ 1.01)
pCO_2	0.00	0.02	0.23	0.820	1.00 (0.97 ~ 1.04)
SpO_2	0.00	0.05	0.01	0.989	1.00 (0.91 ~ 1.10)
Na^+	- 0.05	0.05	- 1.02	0.307	0.95 (0.86 ~ 1.05)
K^+	0.37	0.35	1.06	0.290	1.45 (0.73 ~ 2.86)
Ca^{2+}	- 2.52	1.60	- 1.58	0.114	0.08 (0.00 ~ 1.84)
Lactate	0.86	0.27	3.23	**0.001**	2.37 (1.40 ~ 4.00)
HCO_3^-	- 0.21	0.07	- 2.94	**0.003**	0.81 (0.70 ~ 0.93)
BE	- 0.17	0.06	- 2.98	**0.003**	0.85 (0.76 ~ 0.94)

The **bold text** in the *p* value column indicates statistically significant results ($p < 0.1$). GA: gestational age; Output: urine volume in the last 24 hours at the time of infection; Tmax: the highest temperature in the last 24 hours at the time of infection; Mechanical ventilation: mechanical ventilation was used for 7 days prior to infection; PICC: peripherally inserted central catheter; WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; PLT: platelet count; CRP: C-reactive protein; PCT: procalcitonin; SAA: serum amyloid A; pH: potential of hydrogen; pO_2: partial pressure of oxygen; pCO_2: partial pressure of carbon dioxide; SpO_2: saturation of peripheral oxygen; BE: base excess.

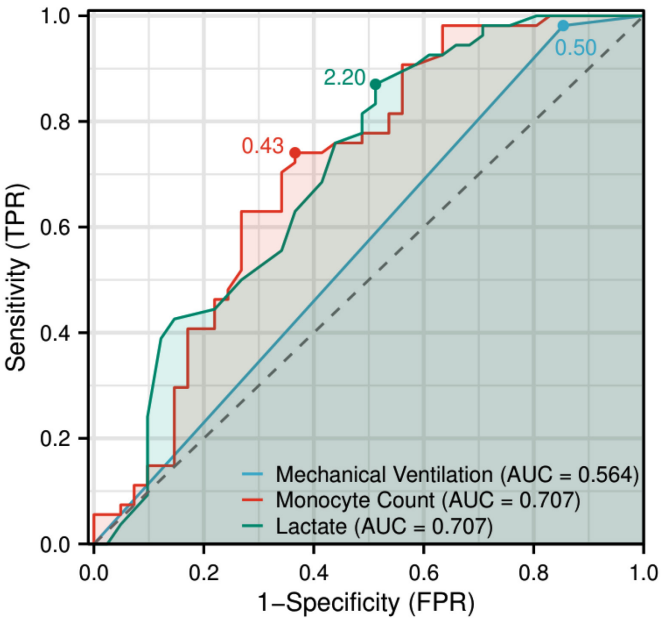
levels of inflammatory markers, including C-reactive protein (CRP), procalcitonin (PCT), and serum amyloid A (SAA). Blood gas analysis revealed that infants with Gram-negative bacterial infections had lower pH values, standard bicarbonate levels, and base excess, but higher lactate levels, compared with those with Gram-positive bacterial infections. The differences were statistically significant ($p < 0.1$). The analysis of clinical data is shown in Table 2.

Multifactorial analysis to determine the influencing factors of Gram-negative bacterial infections

The variables showing statistically significant differences in the univariate logistic regression analysis were entered into a multivariable backward stepwise logistic regression model. The presence of Gram-negative bacterial infection was defined as the dependent variable, and a backward elimination method was applied for variable selection. The results showed that among the above variables, mechanical ventilation usage in the 7 days prior to infection, low monocyte count, and high lactate levels were independent predictors of Gram-negative bacterial infections ($p < 0.05$; Table 3).

Among the identified factors, mechanical ventilation use within 7 days prior to infection was treated as a categorical variable, while monocyte count and lactate levels were treated as continuous variables. The AUC values for the three factors were calculated after plotting the ROC curve. The AUC for mechanical ventilation use within 7 days prior to infection in predicting Gram-negative bacterial infection was 0.564 (95% CI: 0.51–0.62), with a specificity of 15% and a sensitivity of 98%. The AUC value for monocyte count in predicting Gram-negative bacterial infection was 0.707 (95% CI: 0.60–0.82), with a specificity of 37% and a sensitivity of 26%. The AUC value for lactate levels was 0.707 (95% CI: 0.60–0.82), with a specificity of 49% and a sensitivity of 87%. The ROC curve is shown in Figure 3.

Figure 3. ROC curves and AUC values of mechanical ventilator use, monocyte count, and lactate for predicting Gram-negative bacterial infection.



Discussion

Neonatal sepsis is one of the major challenges faced in neonatology, and, along with prematurity, accounts for a substantial proportion of deaths among children under 5 years of age worldwide. Research has indicated that compared to Gram-positive bacteria, Gram-negative bacteria are associated with more severe complications, such as necrotizing enterocolitis, meningitis, and cholestasis; resulting in higher mortality rates [11]. The pathogenic bacteria isolated from intubated and mechanically ventilated infants primarily consist of Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Serratia marcescens* [12], which is consistent with the research findings in this study. Nevertheless, variations

Table 3. Multivariable backward stepwise logistic regression analysis identifying independent predictors of Gram-negative bacterial infection.

Variables	β	SE	Z	*p*	OR (95%CI)
Corrected GA of Infection	- n0.24	0.15	- 1.63	0.103	0.79 (0.59 ~ 1.05)
Mechanical ventilation					
N					1.00 (Reference)
Y	3.02	1.29	2.34	**0.019**	20.40 (1.63 ~ 254.96)
Monocyte Count	- 1.26	0.55	- 2.29	**0.022**	0.28 (0.10 ~ 0.84)
CRP	0.01	0.01	1.88	0.060	1.01 (1.00 ~ 1.03)
PCT	0.06	0.04	1.43	0.154	1.07 (0.98 ~ 1.16)
Lactate	0.81	0.34	2.36	**0.018**	2.26 (1.15 ~ 4.44)
BE	- 0.11	0.08	- 1.38	0.167	0.90 (0.77 ~ 1.05)

The **bold text** in the *p* value column indicates statistically significant results ($p < 0.05$). GA: gestational age; Mechanical ventilation: mechanical ventilation was used for 7 days prior to infection; CRP: C-reactive protein; PCT: procalcitonin; BE: base excess.

in the relative prevalence of specific pathogens across regions may reflect differences in local antimicrobial prescribing practices, infection control strategies, and baseline resistance patterns [13].

Recent international studies over the past decade have reported shifts in neonatal sepsis pathogen profiles, with an increasing concern regarding multidrug-resistant (MDR) Gram-negative organisms and progressively limited therapeutic options [14]. Evidence from low- and lower-middle-income countries also indicates that Gram-negative neonatal sepsis is frequently caused by Enterobacterales (e.g., *Escherichia coli*, *Klebsiella* spp.) and other Gram-negative pathogens, with substantial antimicrobial resistance reported across settings [15]. These trends are largely attributed to widespread use of broad-spectrum antibiotics, prolonged NICU hospitalization, and increased survival of extremely preterm infants. Therefore, early identification of neonatal sepsis caused by these two types of bacteria helps in the early selection of more effective antibiotics, which is beneficial for improving the prognosis of affected children and reducing the occurrence of bacterial resistance.

This study found that the use of mechanical ventilation was an independent predictor of Gram-negative bacterial infections. Infants who use mechanical ventilation often expose their previously relatively sterile lower respiratory tracts to the external environment after intubation. This can lead to an inability to effectively cough, reduced ciliary clearance function, and the shedding of biofilms that can obstruct small airways, resulting in ventilator-associated pneumonia, which may progress to severe conditions such as neonatal sepsis [16,17]. Previous studies have shown that 20% of newborns with late-onset sepsis underwent intubation or mechanical ventilation after delivery. Compared to infants who did not undergo intubation or mechanical ventilation, those who did had an 8.308-fold increased risk of developing late-onset sepsis [18]. The most commonly isolated pathogens in the neonatal population were Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* [19]. In a retrospective study on neonatal sepsis caused by Gram-negative bacteria, Nordberg *et al.* [20] found that the duration of ventilatory support for infants with late-onset sepsis caused by Gram-negative bacteria was significantly greater than that in the uninfected group. This is similar to the findings in this study. Therefore, if there is a high suspicion of sepsis in the patient, reviewing the history of mechanical ventilation use can help make an empirical judgment regarding Gram-positive or Gram-negative bacteria before blood culture results are available, thus allowing for early prevention.

This study indicates that lactate is an influencing factor for Gram-negative bacterial infections. Lactate is formed from pyruvate in the cytosol and is part of glycolysis. Pyruvate is oxidized in the presence of sufficient oxygen, and enters the tricarboxylic acid (TCA) cycle. Patients with sepsis experience various microcirculatory disturbances, cellular metabolic imbalances, ischemia, and anaerobic metabolism due to hypoxia. Under conditions of tissue hypoxia or inadequate perfusion, pyruvate is first converted to lactate, leading to elevated lactate levels [21,22]. Elevated lactate levels are early predictive factors for mortality in children with sepsis and septic shock [23]. Yilmaz *et al.* [24] assessed the lactate levels of 65 children with septic shock upon admission to the pediatric intensive care unit (PICU) and found that non-survivors had significantly higher lactate levels at the time of PICU admission compared to survivors. Additionally, studies have found that elevated blood lactate levels in children are associated with poor outcomes in septic shock [23]. In this study, the lactate levels were closely associated with Gram-negative bacterial infections. Therefore, early monitoring of lactate levels can help assess the nature of the bacteria, enabling timely and appropriate treatment, which may improve the prognosis of neonatal sepsis.

In this study, there was a decrease in monocyte count associated with Gram-negative bacterial infection. Monocytes are a key component of the innate immune system and play a crucial role in coordinating inflammation and the initial response to infection [25]. After septic infection, circulating monocytes infiltrate the infected tissues and differentiate into macrophages and dendritic cells (DCs) to perform their effector functions, which include pro-inflammatory and anti-inflammatory activities, antigen presentation, and tissue remodeling [26]. Liang *et al.* [3] found that the monocyte levels in newborns who died within 7 days after the onset of sepsis were significantly lower. This finding is similar to a study that identified predictors of prognosis in neonatal sepsis, which indicated that low monocyte counts are associated with poorer survival rates. To our knowledge, this study is among the first to identify a decrease in monocyte count associated with Gram-negative bacterial infections. Therefore, monitoring monocyte counts may assist clinicians in the early differentiation of bacterial pathogens in neonatal sepsis infections and implement therapeutic interventions to improve outcomes.

This study provided a comprehensive comparison of Gram-negative and Gram-positive bacterial infections from multiple perspectives, including baseline characteristics, clinical manifestations, and laboratory tests of infants. The study identified that the combination of mechanical ventilation use, decreased monocyte count, and elevated lactate levels has high predictive value for assessing Gram-negative bacterial infections. Due to the low positivity rate of blood cultures in clinical practice, early prediction of Gram-negative bacterial infections using clinically relevant information from patients has significant implications for antibiotic selection. This approach may help improve treatment outcomes and prognosis for the infants while also reducing the emergence of antibiotic-resistant bacteria.

However, several limitations of this study should be acknowledged. First, this was a single-center retrospective study, which may limit the generalizability of the findings and introduce potential selection and information bias. Second, the sample size was relatively small, particularly when stratified by pathogen type, which may have reduced the statistical power to detect additional predictive factors. Third, although multiple clinical and laboratory variables were analyzed, other potentially relevant factors, such as prior antibiotic exposure, duration of invasive procedures, and maternal or perinatal risk factors, were not fully evaluated. In addition, due to the retrospective design, causal relationships cannot be established, and the identified predictors should be interpreted as associations rather than definitive determinants. Therefore, multicenter, large-scale prospective studies are warranted to further validate the discriminatory value and clinical applicability of these predictors for Gram-negative bacterial sepsis in newborns.

Conclusions

The predominant pathogens in neonates with sepsis in this study were *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Escherichia coli*. The proportion of Gram-negative bacterial infections showed a trend of decline in the first year, followed by an increase from 2020 to 2024. The use of mechanical ventilation, decreased monocyte count, and elevated lactate levels were influencing factors for Gram-negative bacterial infections. The combined use of mechanical ventilation, decreased monocyte count, and higher lactate levels had high discriminatory value for Gram-negative bacterial infections.

Acknowledgements

The authors thank the NICU department of Renmin Hospital of Wuhan University for providing the original dataset.

Funding

This work was supported by Key Laboratory Open Project of Hubei Province (No. 2022KFH023). The funding body did not take part in the design of the study; collection, analysis and interpretation of the data; or manuscript writing.

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki, and was approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University (NO. WDRY2023-K098-X01). Given the retrospective nature of the study and the use of fully anonymized data, the requirement for informed consent was waived by the ethics committee.

Corresponding authors

Yanyan Shang, MNS.
Department of Neonatology, Renmin Hospital of Wuhan University,
Ziyang Road Wuchang District, No. 99 Jiefang Road, Wuhan, Hubei 430060, PR China.
Tel: +8618627887467
Fax: 027-88042292
Email: rm001178@whu.edu.cn

Binghong Zhang, PhD.
Department of Neonatology, Renmin Hospital of Wuhan University,
Ziyang Road Wuchang District, No. 99 Jiefang Road, Wuhan, Hubei 430060, PR China.
Tel: +8613886009855
Fax: 027-88042292
Email: zbhong6288@163.com

Conflict of interest

No conflict of interest is declared.

References

1. World Health Organization (2016) Guidelines on core components of infection prevention and control programmes at the national and acute health care facility level. Geneva: World Health Organization. Available: https://www.who.int/publications/i/item/9789241549929. Accessed: 1 November 2016.
2. Zhang Y, Li S, Li Y, Zheng J, Dong Y (2024) Analysis and risk factors of deep vein catheterization-related bloodstream infections in neonates. Medicine (Baltimore) 103: e37184. doi: 10.1097/MD.0000000000037184.
3. Liang X, Luo H, Chen X, Yu X, Yan Q (2023) Prognostic value of blood inflammatory parameters for predicting mortality in neonates with sepsis. Int Immunopharmacol 123: 110780. doi: 10.1016/j.intimp.2023.110780.
4. Donovan EF, Sparling K, Lake MR, Narendran V, Schibler K, Haberman B, Rose B, Meinzen-Derr J; Ohio Perinatal Quality Collaborative. (2013) The investment case for preventing

NICU-associated infections. Am J Perinatol 30: 179–184. doi: 10.1055/s-0032-1322516.
5. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, Higgins RD; National Institute of Child Health and Human Development Neonatal Research Network (2004) Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA 292: 2357–2365. doi: 10.1001/jama.292.19.2357.
6. Hadfield BR, Cantey JB (2021) Neonatal bloodstream infections. Curr Opin Infect Dis 34: 533–537. doi: 10.1097/QCO.0000000000000764.
7. Song WS, Park HW, Oh MY, Jo JY, Kim CY, Lee JJ, Jung E, Lee BS, Kim KS, Kim EA (2022) Neonatal sepsis-causing bacterial pathogens and outcome of trends of their antimicrobial susceptibility a 20-year period at a neonatal intensive care unit. Clin Exp Pediatr 65: 350–357. doi: 10.3345/cep.2021.00668.
8. Hallmaier-Wacker LK, Andrews A, Hope R, Demirjian A, Lamagni TL, Collin SM (2023) Incidence of infant Gram-negative invasive bacterial infections in England, 2011-2019: an observational study using population-wide surveillance data. Arch Dis Child 108: 762–767. doi: 10.1136/archdischild-2023-325569.
9. Lemiech-Mirowska E, Kiersnowska ZM, Michałkiewicz M, Depta A, Marczak M (2021) Nosocomial infections as one of the most important problems of healthcare system. Ann Agric Environ Med 28: 361–366. doi: 10.26444/aaem/122629.
10. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN (2021) Overview of changes to the Clinical and Laboratory Standards Institute performance standards for antimicrobial susceptibility testing, M100, 31st Edition. J Clin Microbiol 59: e0021321. doi: 10.1128/JCM.00213-21.
11. Coggins SA, Glaser K (2022) Updates in late-onset sepsis: risk assessment, therapy, and outcomes. Neoreviews 23: 738–755. doi: 10.1542/neo.23-10-e738.d.
12. Yadav P, Yadav SK (2022) Progress in diagnosis and treatment of neonatal sepsis: a review article. JNMA J Nepal Med Assoc 60: 318–324. doi: 10.31729/jnma.7324.
13. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis 13: 1057–1098. doi: 10.1016/S1473-3099(13)70318-9.
14. Ferreira ICDS, Machado ICB, Menezes RP, Jesus TA, Lopes MSM, Araújo LB, Ferreira DMLM, Röder DVDB (2025) Challenges and trends in Gram-negative bacterial infections in critically neonates: a seven-and-a-half-year observational study. Am J Infect Control 53: 13–21. doi: 10.1016/j.ajic.2024.08.004.
15. Wen SCH, Ezure Y, Rolley L, Spurling G, Lau CL, Riaz S, Paterson DL, Irwin AD (2021) Gram-negative neonatal sepsis in low- and lower-middle-income countries and WHO empirical antibiotic recommendations: a systematic review and meta-analysis. PLoS Med 18: e1003787. doi: 10.1371/journal.pmed.1003787.
16. Dang J, He L, Li C (2023) Risk factors for neonatal VAP: a retrospective cohort study. Exp Biol Med (Maywood) 248: 2473–2480. doi: 10.1177/15353702231220673.
17. Fernández-Barat L, López-Aladid R, Torres A (2020) Reconsidering ventilator-associated pneumonia from a new dimension of the lung microbiome. EBioMedicine 60: 102995. doi: 10.1016/j.ebiom.2020.102995.
18. Aydiko A, Gultie T, Abebe GF, Ginbeto T, Ukke GG (2022) Determinants of late-onset neonatal sepsis among neonates admitted to the neonatal intensive care unit of Arba-Minch general hospital, southern Ethiopia. PLoS One 17: e0279622. doi: 10.1371/journal.pone.0279622.
19. Howroyd F, Chacko C, MacDuff A, Gautam N, Pouchet B, Tunnicliffe B, Weblin J, Gao-Smith F, Ahmed Z, Duggal NA, Veenith T (2024) Ventilator-associated pneumonia: pathobiological heterogeneity and diagnostic challenges. Nat Commun 15: 6447. doi: 10.1038/s41467-024-50805-z.
20. Nordberg V, Iversen A, Tidell A, Ininbergs K, Giske CG, Navér L (2021) A decade of neonatal sepsis caused by Gram-negative bacilli-a retrospective matched cohort study. Eur J Clin Microbiol Infect Dis 40: 1803–1813. doi: 10.1007/s10096-021-04211-8.
21. Garcia-Alvarez M, Marik P, Bellomo R (2014) Sepsis-associated hyperlactatemia. Crit Care 18: 503. doi: 10.1186/s13054-014-0503-3.
22. Hernandez G, Bellomo R, Bakker J (2019) The ten pitfalls of lactate clearance in sepsis. Intensive Care Med 45: 82–85. doi: 10.1007/s00134-018-5213-x.
23. Scott HF, Brou L, Deakyne SJ, Kempe A, Fairclough DL, Bajaj L (2017) Association between early lactate levels and 30-day mortality in clinically suspected sepsis in children. JAMA Pediatr 171: 249–255. doi: 10.1001/jamapediatrics.2016.3681.
24. Yilmaz A, Kaya N, Gonen I, Uygur A, Perk Y, Vural M. (2023) Evaluating of neonatal early onset sepsis through lactate and base excess monitoring. Sci Rep 13: 14837. doi: 10.1038/s41598-023-41776-0.
25. Xia X, Wang Y, Xie M, Qiu S, Zhou J (2022) Elevated neutrophil-to-monocyte ratio as a prognostic marker for poor outcomes in neonatal sepsis. Heliyon 8: e11181. doi: 10.1016/j.heliyon.2022.e11181.
26. Shi C, Pamer EG (2011) Monocyte recruitment during infection and inflammation. Nat Rev Immunol 11: 762–774. doi: 10.1038/nri3070.