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CHARACTERISTICS OF GRAM-NEGATIVE NEONATAL SEPSIS AND ASSOCIATED RISK FACTORS AT BACH MAI HOSPITAL

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ABSTRACT

Objective: To describe the clinical and laboratory characteristics of Gram-negative neonatal infections and to identify risk factors associated with multidrug-resistant (MDR) Gram-negative infections at the Pediatric Center, Bach Mai Hospital, during 2023–2025.

Methods: A cross-sectional study combined with a case–control design was conducted among neonates (<28 days old) diagnosed with neonatal sepsis. The case group included 60 infants with culture-confirmed MDR Gram-negative infections, and the control group consisted of 180 infants with negative cultures. Clinical manifestations, laboratory findings, and potential risk factors were collected and statistically analyzed.

Results: Among 60 cases of Gram-negative neonatal infections, late-onset sepsis accounted for 78.9%, while early-onset sepsis accounted for 21.1%. Klebsiella pneumoniae was the most common pathogen (42.3%). The predominant clinical features were respiratory distress (83.3%), tachypnea ≥60 breaths/min (75%), chest retraction (73.3%), SpO_2 <90% (56.7%), poor feeding (56.7%), and jaundice (35%). Laboratory abnormalities included leukocytosis (43.3%), thrombocytopenia (40%), hypoalbuminemia (66.7%), elevated CRP (78.3%), and coagulopathy(70%).

Significant risk factors for MDR Gram-negative infection ($p < 0.05$) included age at admission ≥7 days, late-onset sepsis, bag-mask ventilation or re-intubation, blood transfusion, parenteral nutrition, umbilical or central line catheterization, invasive mechanical ventilation ≥7 days, use of vasopressors, and exposure to ≥2 antibiotics or antibiotic regimen changes during treatment.

Conclusion: Gram-negative neonatal infections, particularly those caused by *Klebsiella pneumoniae*, are common and mainly associated with late-onset sepsis. Respiratory symptoms predominate, often accompanied by hematologic, biochemical, and coagulation abnormalities. Early identification and close monitoring of high-risk neonates, adherence to aseptic techniques, effective infection control, and rational antibiotic use are essential to improve outcomes and reduce mortality.

Keywords: Neonatal sepsis, Gram-negative bacteria, risk factors, multidrug resistance.

1. INTRODUCTION

Neonatal sepsis is an inflammatory condition involving one or more organs caused by infection during the neonatal period. It has a high mortality rate, ranking second only to respiratory distress syndrome, especially among preterm infants [1]. In recent years, antibiotic resistance among bacteria has increased markedly and become a critical global concern. A study by Dinh Thi Thuy Ha on the treatment of multidrug-resistant (MDR) Gram-negative infections at Dong Nai General Hospital reported that 35.8% of empirical antibiotic regimens were inappropriate, and 64.9% of patients required modification of therapy after susceptibility results due to poor clinical response or disease progression [2]. In 2021, the World Health Organization (WHO) published a list of priority antibiotic-resistant pathogens, emphasizing MDR Gram-negative bacteria such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterobacteriaceae, causing severe infections such

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as pneumonia and sepsis in hospitalized patients [3]. Risk factors for Gram-negative neonatal sepsis (GNNS) are often related to clinical care and invasive interventions. At the Pediatric Center, Bach Mai Hospital, thousands of neonates are admitted annually, with a high proportion of bacterial isolates being MDR pathogens. However, no prior research has examined GNNS at this hospital. Therefore, this study aimed to describe the clinical and laboratory characteristics of Gram-negative neonatal infections and identify risk factors for MDR Gram-negative infections at the Pediatric Center, Bach Mai Hospital.

2. METHODS

2.1. Study Period and Setting

This study was conducted from June 2023 to June 2025 at the Pediatrics Center, Bach Mai Hospital

2.2. Study Subjects

- Inclusion criteria:

+ Neonates aged less than 28 days who were clinically diagnosed with neonatal sepsis

- Exclusion criteria: (1) Neonates whose parents or legal guardians did not provide consent to participate in the study, (2) Cases in which treatment was voluntarily discontinued, or the patient was transferred to another hospital, (3) Medical records lacking essential information.

2.3. Research Design

A descriptive cross-sectional study combined with a case–control design was applied

For the descriptive cross-sectional component, the sample size was calculated using the formula for a single population proportion:

$$n = Z^2_{1-\alpha/2} \frac{p(1-p)}{d^2}$$

Where:

+ n: minimum required sample size

+ α = 0.05 (significance level)

+ $Z_{1-\alpha/2}$ = 1.96 (corresponding to 95% confidence interval)

+ d (desired precision) = 0.05

+ p = 0.041 based on the prevalence of multidrug-resistant Enterobacter cloacae reported by Tran Luong Nhan and Nguyen Thi Quynh Nga at the Neonatal Department, Hanoi Obstetrics and Gynecology Hospital (2024)[4].

The minimum required sample size was 60 neonates with Gram-negative bacterial sepsis confirmed by culture. The case group included all neonates infected with multidrug-resistant Gram-negative bacteria. The control group included neonates with clinical sepsis but negative culture results, matched to the case group by gestational age and birth weight. The study used a 1:3 case-to-control ratio.

2.4. Data Collection

- Sex: Male/Female. Gestational age: Number of completed gestational weeks at birth. Postnatal age at admission, birth weight, and time of onset of neonatal infection. Clinical symptoms were collected through clinical examination and review of medical records.

- Medical history included: referral source, antibiotic use, and prior invasive procedures performed before admission. Maternal history: mode of delivery, maternal diseases, and infections during pregnancy. Clinical manifestations of neonatal infection comprised: Temperature instability, Respiratory signs, Circulatory abnormalities, Neurological, gastrointestinal, and skin–mucosal symptoms Laboratory investigations included: Blood tests such as complete blood count (red blood cells, white blood cells, platelets) Albumin, C-reactive protein (CRP), glucose, total calcium, prothrombin time (PT%) activated partial thromboplastin time (APTT), and fibrinogen. Results of culture from clinical specimens. Invasive procedures: endotracheal intubation, site of intubation, reintubation, and invasive mechanical ventilation. Other procedures: umbilical venous or arterial catheterization, arterial blood pressure monitoring. Treatment methods: Antibiotic therapy: number of antibiotics used and any changes in antibiotic regimen. Supportive treatment: blood transfusion, surfactant administration, parenteral nutrition, and vasopressor use.

2.5. Statistical Analysis

Data were analyzed using SPSS version 25.0. Frequencies and percentages were calculated for qualitative variables. The differences between proportions were compared using the Chi-square (χ^2) test. Fisher's exact test was applied when the expected cell counts were <5 to determine the association between clinical and laboratory features of neonatal sepsis. Univariate analysis was performed to explore the relationship between potential risk factors and neonatal Gram-negative infection. Multivariate logistic regression analysis was subsequently applied to identify independent predictors associated with multidrug-resistant Gram-negative bacterial infection.

2.6. Ethical Considerations

The study complied with the principles of biomedical research ethics. The research protocol was approved by the Ethics Committee of Bach Mai Hospital under Decision No. 7331/QĐ-BM

3. RESULTS

Table 1. Distribution of Gram-Negative Bacterial Species Among Study Subjects

Bacterial species	Early-onset sepsis (n=15)	Late-onset sepsis (n=56)	Total (n=71)
	n (%)	n (%)	n (%)
Klebsiella pneumoniae	7 (23.3%)	23 (76.7%)	30 (42.3%)
E.coli	1 (12.5%)	7 (87.5%)	8 (11.3%)
Smaltophilia	4 (44.4%)	5 (55.6%)	9 (12.7%)
Acinobacter baumannii	0	8 (100%)	8 (11.3%)
Pseudomonas aeruginosa	1 (14.3%)	6 (85.7%)	7 (9.9%)
Enterobacter	1 (16.7%)	5 (83.3%)	6 (8.5%)
Other Gram- negative bacterial	1 (33.3%)	2 (66.7%)	3 (4.2%)

Late-onset neonatal sepsis (LONS) accounted for the majority (78.9%), whereas early-onset neonatal sepsis (EONS) represented only 21.1%. *Klebsiella pneumoniae* was the most frequent pathogen (42.3%), followed by *S. maltophilia* (12.7%), *A. baumannii* (11.3%), *E. coli* (11.3%), *P. aeruginosa* (9.9%), and *Enterobacter spp.* (8.5%), and other Gram-negative bacteria (4.2%).

Table 2. Characteristics of study subjects according to referral source, birth weight, and timing of neonatal sepsis

Characteristics	Case group		Control group		Total (n=240)		p
	n	%	n	%	n	%	
Referral source							
From a lower-level hospital	22	36.7	45	25	67	27.9	< 0.05
From Obstetrics	35	58.3	134	74.4	169	70.4	
From home	3	5	1	0.6	4	1.7	
Birth weight							
< 2500g	53	88.3	142	78.9	195	81.3	> 0.05
≥ 2500g	7	11.7	38	21.1	45	18.8	
Type of neonatal sepsis							
Early-onset sepsis	13	21.7	154	85.6	167	69.6	< 0.001
Late-onset sepsis	47	78.3	26	14.4	73	30.4	

The proportion of neonates referred from lower-level hospitals in the case group was 36.7%, higher than that in the control group (25.0%). Conversely, most neonates in the control group were admitted from the Bach Mai Hospital Obstetrics Department (74.4%). The case group included 13 neonates with early-onset sepsis (21.7%) and 47 neonates with late-onset sepsis (78.3%). There was a statistically significant difference between the two groups in terms of referral source and timing of neonatal sepsis ($p < 0.05$)

Table 3. Characteristics of respiratory distress symptoms according to age at hospital admission

Symptoms	Age at admission						p
	≥ 7 days		< 7 days		Total		
	n	%	n	%	n	%	
Tachypnea							
Yes	5	50	40	80	45	75	0.046
No	5	50	10	20	15	25	
Apnea							
Yes	2	20	32	64	34	56.7	0.01
No	8	80	18	36	26	43.3	
Grunting							
Yes	2	20	25	50	27	45	> 0.05
No	8	80	25	50	33	55	
Chest retraction							
Yes	4	40	40	80	44	73.3	0.009
No	6	60	10	20	16	26.7	
Cyanosis							
Yes	3	30	29	58	32	53.3	> 0.05
No	7	70	21	42	28	46.7	
Oxygen saturation (SpO_2)							
< 90%	2	20	32	64	34	56.7	0.01
≥ 90%	8	80	18	36	26	43.3	

Common respiratory symptoms among neonates included tachypnea (75%), apnea (56.7%), chest retraction (73.3%), grunting (45.0%), cyanosis (53.3%), and oxygen saturation <90% (56.7%). There were statistically significant differences between groups in the rates of tachypnea ($p = 0.046$), apnea ($p = 0.01$), chest retraction ($p = 0.009$), and low oxygen saturation ($p = 0.01$). Differences in grunting and cyanosis were not statistically significant ($p > 0.05$).

Table 4. Hematological, biochemical, and coagulation characteristics of the study subjects

Sepsis Character-istics	Neonatal						p
	Early-onset sepsis		Late-onset sepsis		Total (n=60)		
	n	%	n	%	n	%	
WBC count							
Increased	5	50	21	42	26	43.3	> 0.05
Normal	4	40	21	42	25	41.7	
Decreased	1	10	8	16	9	15	
Platelet count (PLT)							
Normal	9	0	27	54	36	60	0.034
Decreased	1	10	23	46	24	40	
C-reactive protein (CRP)							
Increased	7	53.8	40	85.1	47	78.3	0.015
Normal	6	46.2	7	14.9	13	21.7	
Coagulation disorders							
Yes	40	75.5	2	28.6	42	70	0.011
No	13	24.5	5	71.4	18	30	

The proportion of thrombocytopenia in the late-onset sepsis group was higher than in the early-onset group, 46%, showing a statistically significant difference (p = 0.034). CRP levels were observed in 85.1% of neonates with late-onset sepsis, compared with 53.8% in the early-onset group, with a statistically significant difference (p < 0.05). Coagulation disorders were more frequent in the early-onset sepsis group 75.5% and the difference was statistically significant (p < 0.05).

Table 5. Association between sex, age at admission, and onset time of sepsis with Gram-negative neonatal sepsis

Factors	Case group (n= 60)		Control group (n= 180)		OR (95%CI)	p
	n	%	n	%		
Sex						
Male	35	58.3	93	51.7	1.31 (0.726 – 2.364)	0.370
Female	25	41.7	87	48.3		
Age at admission						
≥ 7 days	10	16.7	8	4.4	4.30 (1.611 – 11.475)	0.002
<7 days	50	83.3	172	95.6		
Onset time of neonatal sepsis						
Early-onset sepsis	13	21.7	154	85.6	0.047 (0.022 – 0.098)	< 0.001
Late-onset sepsis	47	78.3	26	14.4		

In the case group, 58.3% were male, compared with 51.7% in the control group, with no statistically significant difference (p = 0.37). Regarding the age at admission, neonates admitted at ≥7 days accounted for 16.7% in the case group and 4.4% in the control group, showing a statistically significant difference (p = 0.002). Early-onset sepsis accounted for 69.6% of all cases. There was a statistically significant difference between the case and control groups in terms of the onset time of sepsis (p < 0.001).

Table 6. Association between antibiotic modification, blood transfusion, and intubation status at admission with Gram-negative neonatal sepsis

Factors	Group				OR (95% CI)	p
	Case group		Control group			
	n	%	n	%		
Antibiotic modification						
Yes	47	78.3	39	21.7	13.07 (6.43 – 26.57)	< 0.001
No	13	21.7	141	78.3		
Blood transfusion						
Yes	44	73.3	69	38.3	4.424 (2.318 – 8.443)	< 0.001
No	16	26.7	111	61.7		
Intubation at admission						
Yes	34	56.7	72	40	1.962 (1.086 – 3.543)	< 0.05
No	26	43.3	108	60		

In the case group, 78.3% of neonates required antibiotic modification, compared with 21.7% in the control group. The proportion of infants who were intubated at admission was higher in the case group (56.7%), showing a statistically significant difference. Blood transfusion was performed in 73.3% of cases, with a highly significant association (p < 0.001).

4. DISCUSSION

4.1. Distribution of Gram-Negative Bacterial Species

Late-onset neonatal sepsis accounted for the majority of cases (78.9%). Klebsiella pneumoniae was the most frequently isolated pathogen, representing

42.3% of all Gram-negative infections. *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and other *Gram-negative bacilli* were identified as the main causative agents of late-onset neonatal sepsis.

The findings of this study are consistent with a 2023 investigation on bacterial etiology conducted at the National Children's Hospital, which reported that Gram-negative bacteria were the predominant pathogens responsible for both overall and late-onset neonatal sepsis, with *Klebsiella pneumoniae* being the most common isolate. The proportion of Gram-negative and Gram-positive bacteria in early-onset neonatal sepsis was approximately equivalent [5].

4.2. Characteristics by Referral Source, Birth Weight, and Timing of Neonatal Sepsis

These results are consistent with the observation that neonates referred from lower-level healthcare facilities often present in more severe conditions, experience longer transfer times, and have a higher risk of infection compared to those delivered and transferred directly from the hospital's obstetrics department. This difference emphasizes the importance of infection control practices and safe patient transfer protocols. Our findings align with studies conducted at hospitals in Menoufia and El Kalyoubia (2019), which reported that low-birth-weight and preterm infants were 3–10 times more likely to develop neonatal sepsis than term infants, due to an immature immune system and prolonged hospitalization. Low Apgar scores and intrapartum complications, such as birth asphyxia, premature rupture of membranes, and prolonged labor, were also identified as significant risk factors [6]. Similarly, a 2022 study by Duong Quoc Truong at Thai Nguyen Central Hospital found that prematurity was a significant risk factor for neonatal sepsis, as immune responses in this age group are not fully developed. In their study, 63.2% of neonatal sepsis cases occurred in preterm infants, and neonatal sepsis was more common among infants weighing <2500 g than among those of normal birth weight[7]. Late-onset neonatal sepsis is frequently associated with hospital-acquired infections, invasive procedures, and prolonged hospital stays. Consequently, late-onset sepsis has been shown to correlate strongly with multidrug-resistant Gram-negative bacteria such as Klebsiella, Enterobacter, and Acinetobacter. Therefore, strict infection control, minimizing unnecessary invasive procedures, and judicious antibiotic selection are essential to effectively managing late-onset Gram-negative neonatal sepsis.

4.3. Characteristics of Respiratory Symptoms

The findings of this study are consistent with those of Nguyen Thi Nguyen Thao (2025), who reported that 90.2% of neonates exhibited respiratory abnormalities, including tachypnea (82.4%), apnea (27.5%), chest retraction (5%), cyanosis (66.7%), grunting (54.9%), and pulmonary rales (25.5%)[8]. In early-onset neonatal sepsis, severe respiratory distress is often the predominant clinical manifestation, characterized by rapid breathing, apneic episodes, and reduced oxygen saturation. Chest retraction reflects significant respiratory compromise in neonates. These symptoms were more frequently observed among infants admitted within the first few days of life, possibly due to maternal infection or prematurity-related vulnerability.

4.4. Laboratory Characteristics of Multidrug-Resistant Gram-Negative Infections

The results showed leukocytosis in 43.3% of cases, thrombocytopenia in 40%, hypoalbuminemia in 66.7%, and elevated C-reactive protein levels in 78.3%. Coagulation disorders were observed in 70% of the neonates. A study by Ho Thi Phuong Thanh, conducted in the Neonatal Intensive Care Unit of Nghe An Obstetrics and Pediatrics Hospital, reported leukopenia in 22.9% of cases, leukocytosis in 14.3%, thrombocytopenia in 34.3%, and elevated CRP in 68.6% of patients [9]. Similarly, Doan Thi Thanh Binh et al. found that laboratory findings alone were insufficient for diagnosing neonatal sepsis, with elevated CRP (>6 mg/L) observed in only 52.4% of cases and abnormal white blood cell counts in 25% of cases, compared with normal limits[10]. CRP elevation was predominant in both groups, but notably higher among late-onset sepsis cases. These findings suggest that CRP serves as a valuable marker for differentiating between early- and late-onset sepsis, with a clear upward trend in late-onset infections. Other biochemical parameters, such as glucose, total calcium, and albumin levels, showed no significant correlation with the timing of sepsis onset, indicating their limited value in stratifying neonatal sepsis by onset period.

4.5. Association Between Sex, Age at Admission, and Onset of Sepsis with Gram-Negative Neonatal Sepsis

The distribution of sex was relatively balanced between the two groups. Neonates admitted at ≥7 days of age had a higher risk of Gram-negative neonatal sepsis. Early-onset sepsis accounted for 69.6% of all cases, of which the majority (85.6%) belonged to the control group. A statistically significant difference in the timing of sepsis onset was observed between the two groups.

These findings are consistent with a 2023 study by Nguyen Thi Quynh Nga et al. at the National Children's Hospital, which demonstrated that early-onset neonatal sepsis was associated with higher rates of maternal factors such as prolonged rupture of membranes (>18 hours), fever within one week before delivery, and genital infections [5]. This suggests that early-onset sepsis is primarily related to vertical transmission from mother to infant. In contrast, late-onset sepsis was predominantly associated with hospital-related risk factors, such as invasive procedures, catheterization, prolonged mechanical ventilation, and infection with multidrug-resistant Gram-negative organisms, resulting in a higher incidence in the case group. These observations underscore the critical importance of stringent infection control measures and prevention of multidrug-resistant pathogens to reduce the rate of late-onset neonatal sepsis.

4.6. Association Between Antibiotic Modification, Blood Transfusion, and Clinical Status at Admission with Gram-Negative Neonatal Sepsis

Antibiotic modification was strongly associated with Gram-negative neonatal sepsis, reflecting the burden of severe infection, extensive antimicrobial resistance, or inappropriate initial antibiotic selection. Blood transfusion and endotracheal intubation at admission were identified as significant risk factors for Gram-negative infection. These findings highlight the role of invasive procedures, such as blood transfusions and airway intubation, in facilitating Gram-negative neonatal sepsis, possibly due to the severity of illness and exposure to hospital-acquired pathogens, particularly multidrug-resistant Gram-negative bacteria.

5. CONCLUSION

Gram-negative neonatal infections, particularly those caused by Klebsiella pneumoniae, are frequent and mainly associated with late-onset sepsis. Respiratory manifestations dominate, accompanied by hematologic, biochemical, and coagulation disturbances. Early detection, careful monitoring of high-risk neonates, strict aseptic practices, and rational antibiotic use are vital to improve clinical outcomes and reduce mortality.

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