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Clinical characteristics, laboratory findings, and etiology of neonatal bacterial meningitis at a tertiary children's hospital in Vietnam

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Abstract:

Neonatal bacterial meningitis (NBM) is a significant disease associated with substantial mortality worldwide, prolonged hospitalisation, and increased sequelae. This study aims to investigate clinical, paraclinical and etiological characteristics of neonatal bacterial meningitis at the Neonatal Intensive Care Department, Neonatal Care Centre, Vietnam National Children's Hospital. A total of 123 newborn patients (68 boys and 55 girls) <28-days old diagnosed with NBM were admitted from May 2021 to October 2023. Clinical manifestations were various and nonspecific. Lethargy, hypotonia, respiratory distress requiring mechanical ventilation, hypotension, abdominal distension, and dirty gastric fluid were more prevalent in preterm neonates, while irritability/stimulation, hypertonia, feeding difficulties were predominant in term neonates. Statistically significant differences between term and preterm infants were observed in leucocytosis, thrombocytopenia, and coagulation disorders. Positive blood cultures, cerebrospinal fluid cultures, and cerebrospinal fluid polymerase chain reaction (PCR) account for 82.5, 19.5, and 40%, respectively. The most common aetiologies in preterm infants were *Klebsiella pneumoniae, Serratia marcescens*, and *Escherichia coli*. The term infant group included Group B *Streptococcus* (GBS), *Escherichia coli*, and *Elizabethkingia meningoseptica*. GBS and *Escherichia coli* mainly cause early-onset neonatal bacterial meningitis (ENBM). In contrast, late-onset neonatal bacterial meningitis (LNBM) had more *Klebsiella pneumoniae* and *Serratia marcescens.* The highest mortality rate in the study was associated with *Klebsiella pneumoniae* and *Staphylococcus aureus*. In conclusion, the clinical features of NBM are diverse and nonspecific. Diagnostic tests included a complete blood count test, C-reactive protein (CRP), cerebrospinal fluid (CSF) analysis, blood and CSF culture. The common bacterial aetiology found in the study was GBS, *K. pneumoniae* and *E. coli.*

Keywords: **bacterial meningitis, infection, neonate.**

Classification number: **3.2**

1. Introduction

Neonatal bacterial meningitis (NBM) is an inflammation of the meninges due to bacterial invasion in neonates under 28 days of age. It is a common infectious disease that results in severe clinical conditions, high mortality rates, prolonged hospital stays, and long-term complications in neonates. In the UK, the annual incidence rate of NBM is 0.38 per 1000 live births, while the incidence rate of viral meningitis is 0.83 per 1000 live births [1, 2]. Other reports estimate the incidence of NBM to be 0.3 per 1000 live births based on culture results. The rate of positive cultural results is meagre; only 30 to 50% of neonatal sepsis in the Neonatal Intensive Care Department perform lumbar puncture, and about 75% of these evaluations after the initiation of broad-spectrum antibiotics [3].

A Canadian study reported an incidence rate of neonatal meningitis ranging from 2.2 to 3.5 per 1000 cases treated in the Neonatal Intensive Care Department over seven years [4]. In developing countries, the incidence rate of NBM is higher, ranging from 0.8 to 6.1%, with a mortality rate of up to 58%. The actual disease incidence may be higher due to limited epidemiological investigations in many rural and developing areas [3, 5].

In Vietnam, NBM remains a prevalent central nervous system infection in neonates. Some studies on NBM in neonates at the Vietnam National Children's Hospital have shown diverse and nonspecific clinical symptoms, as well as differences in the causative bacteria compared to many

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studies worldwide, and a high mortality rate ranging from 7 to 20% [6, 7]. Research by N.H. Tam, et al. (2021) [8] at Children's Hospital 1 showed that the rate of neonatal meningitis in sepsis cases was 22.4%. Similarly, D.V. Thuc, et al. (2021) [9] at Hai Phong Children's Hospital showed that neonatal meningitis rates were 21.7%, and the sequelae rate was 7.2%. P.T. Phuong (2020) [10] at Thanh Hoa Children's Hospital documented a sequelae rate of 9.38% and a severe case discharge rate of 6.25%.

According to a study at the Neonatal Care Centre in Vietnam National Children's Hospital from 2000 to 2004, the rate of NBM accounted for 1.05% of the total number of hospitalised, the mortality rate was 19.8% and the rate of sequelae was 12.3% [7]. Vietnam's research was conducted at different medical facilities over a short period and with a small number of patients. However, the NBM rate was still high, with severe sequelae and high mortality rates.

The diagnosis of NBM largely relies on common clinical characteristics and basic ancillary tests. The clinical manifestation of NBM in neonates is not specific, so cerebrospinal fluid (CSF) analysis is the gold standard for diagnosing NBM. However, CSF culture results could take longer, and pre-antibiotic administration could increase the rate of false negatives. This research aims to evaluate the clinical and laboratory features of NBM and investigate the changes and differences in the aetiology compared to studies worldwide.

Objective: Bacterial meningitis in newborns is one of the leading infectious diseases, resulting in severe sequelae and high mortality rates among newborns hospitalised at the Neonatal Care Centre, Vietnam National Children's Hospital. Therefore, the authors conducted this study to investigate the clinical and paraclinical characteristics and bacterial aetiology of the group of newborns hospitalised and treated at our unit. The findings aim to enhance the quality of treatment and care, reduce the rates of sequelae and mortality caused by bacterial meningitis, and provide evidence-based recommendations for colleagues in the field. This article focuses on clinical, paraclinical, and bacterial aetiology. The treatment and assessment of neurological complications will be discussed in a separate section.

2. Subjects and methods

2.1. Subjects

During the study period, we included 123 children aged under 28 days old who were diagnosed with NBM.

Selection criteria: Neonates meeting one of the following criteria: (a) isolation of a bacterial pathogen from CSF culture; (b) isolation of a bacterial pathogen from blood culture with CSF pleocytosis (≥21 cells/mm^3) and CSF protein >1 g/l (within three days of lumbar puncture procedure); (c) isolation of a bacterial pathogen from CSF PCR [11, 12].

Written informed consent was obtained from all participants' parents or legal guardians. This study was approved by the Ethics Committee in Medical Research of the Vietnam National Children's Hospital under Decision No. 1333/Vietnam National Children's Hospital-TRICH. The study was conducted in accordance with the Declaration of Helsinki.

Exclusion criteria: Patients with CSF test results indicating other aetiologies, such as viral or fungal infections. Patients whose parents or guardians did not consent to participate in the study.

2.2. Methods

Study design: This prospective, cross-sectional descriptive study was conducted from May 2021 to October 2023 at Neonatal Care Centre, Vietnam National Children's Hospital - one of the largest hospitals in Vietnam with 180 beds in total (80 beds in Neonatal Intensive Care Department) and approximately 4,000 neonatal patients treated annually.

Data acquisition: Data were collected on demographic, clinical, and subclinical characteristics.

General characteristics: gender, gestational age, birth weight, maternal risk factors, and invasive procedures. Diagnosis made in the first three days of life or between days 4 and 28 of life was defined as early-onset and late-onset neonatal meningitis, respectively.

Clinical characteristics: changes in body temperature, neurological symptoms, respiratory symptoms, circulatory symptoms, gastrointestinal symptoms, or jaundice.

Subclinical findings: complete blood count (CBC), C-reactive protein (CRP), CSF (protein, glucose, cells, culture, PCR), blood culture, CSF culture, and CSF multiplex real-time PCR assays (Tests according to ISO 15189:2020).

Statistical analysis: A convenience sampling method was used to determine the sample size. Statistical analyses were conducted using SPSS software (version 26.0). Categorical variables were presented as frequencies and percentages, while continuous variables were described using the median and interquartile range (IQR). A two-sided p-value of less than 0.05 was considered statistically significant.

3. Results

From May 2021 to October 2023, data were collected from 123 neonates diagnosed with NBM at the Neonatal Intensive Care Department, Neonatal Care Centre, Vietnam National Children's Hospital. The cohort included 68 boys (55.3%) and 55 girls (44.7%), with preterm neonates (<37 weeks of gestation) accounting for 54.5% (67/123).

Preterm neonates (<37 weeks of gestation) and those with low birth weight (<2500 grams) were more likely to develop LNBM compared to ENBM (Table 1). Risk factors for NBM included maternal infections (30.9%) and prolonged labour (7.3%). Of the 123 patients, 104 (84.6%) were transferred from frontline facilities, and 95 of these (91.3%) had received antibiotics prior to lumbar puncture.

Table 1. Baseline characteristics of the study population.

Characteristics		Total (n=123)	<37 weeks (n=67)	≥37 weeks (n=56)
Birth weight	<2500 g	67 (54.5)	61 (91.0)	6 (10.7)
	≥2500 g	56 (45.5)	6 (9.0)	50 (89.3)
Classify	ENBM	29 (23.6)	14 (20.9)	15 (26.8)
	LNBM	94 (76.4)	53 (79.1)	41 (73.2)
Risk factors	Maternal infections	38 (30.9)	21 (31.3)	17 (30.4)
	Prolonged labour	9 (7.3)	5 (7.5)	4 (7.1)
Interventions at the local hospital	Antibiotics	95 (77.2)	56 (83.6)	39 (69.6)
	Mechanical ventilation (MV)	65 (62.5)	58 (93.5)	7 (16.7)
	Central catheter	18 (17.3)	16 (25.8)	2 (4.8)
Time treatment (days) Mean±SD (min - max)	MV duration	6.35±6.35 (1-30)		
	Length of hospital stay	6.39±6.57 (1-30)		
	Duration of an indwelling catheter	1.03±2.92 (1-17)		

Values are given as n (%); SD: Standard deviation; LNBM: Late-onset neonatal bacterial meningitis; ENBM: Early-onset neonatal bacterial meningitis.

3.1. Clinical characteristics of bacterial meningitis neonates hospitalised

Lethargy, hypotonia, respiratory distress requiring mechanical ventilation, hypotension, and abdominal distension were more common in preterm neonates. Conversely, fever, irritability/stimulation, hypertonia, and reduced sucking/poor feeding were more frequently observed in term neonates (Table 2).

Table 2. Clinical characteristics of bacterial meningitis neonates.

Symptoms	Total (n=123)	<37 weeks (n=67)	≥37 weeks (n=56)	P
Fever	59 (48.0)	12 (17.9)	47 (83.9)	**<0.01***
Lethargy	55 (44.7)	38 (56.7)	17 (30.4)	**<0.01***
Irritability/stimulation	14 (11.4)	1 (1.5)	13 (23.2)	**<0.01***
Seizures	16 (13.0)	2 (3.0)	14 (25)	**<0.01****
Bulging fontanel	9 (7.3)	3 (4.5)	6 (10.7)	>0.05
Hypertonia	15 (12.2)	3 (4.5)	12 (21.4)	**<0.01***
Hypotonia	10 (8.1)	10 (14.9)	0	**<0.01***
Tachypnoea	2 (1.6)	1 (1.5)	1 (1.8)	>0.05
Grunting	10 (8.1)	1 (1.5)	9 (16.1)	>0.05
Intercostal retractions	2 (1.6)	2 (3.0)	0	>0.05
Apnoea	9 (7.3)	8 (11.9)	1 (1.8)	>0.05
Hypoxemia	3 (2.4)	1 (1.5)	2 (3.6)	>0.05
MV	54 (43.9)	46 (68.7)	8 (14.3)	**<0.01***
Tachycardia	4 (3.3)	2 (3.0)	2 (3.6)	>0.05
Bradycardia	1 (0.8)	1 (1.5)	0	>0.05
Hypotension	18 (14.6)	14 (20.9)	4 (7.1)	**0.03****
CRT >3 s	20 (16.3)	14 (20.9)	6 (10.7)	>0.05
Inotropes	22 (17.9)	16 (13)	6 (10.7)	>0.05
Feeding intolerance	30 (24.4)	26 (38.8)	4 (7.1)	**0.014****
Vomiting	10 (8.1)	5 (7.5)	5 (8.9)	>0.05
Diarrhoea	5 (4.1)	1 (1.5)	4 (7.1)	>0.05
Reduced sucking/poor feeding	37 (30.1)	13 (19.4)	24 (42.9)	**0.005***
Abdomen distension	32 (26.0)	23 (34.3)	9 (16.1)	**0.02****
Hepatomegaly	12 (9.8)	9 (13.4)	3 (5.4)	>0.05
Jaundice	44 (35.8)	24 (35.8)	20 (35.7)	>0.05
Petechia	15 (12.2)	12 (17.9)	3 (5.4)	>0.05
Sclerema	8 (6.5)	7 (10.4)	1 (1.8)	>0.05

*: Chi-square test; **: Fisher's exact test, P-values indicating statistical significance are given in bold font, Values are given as n (%); CRT: Capillary refill time; MV: Mechanical ventilation.

3.2. Laboratory characteristics of bacterial meningitis neonates

Leucocytosis, platelets count <$100x10^9$/l, anaemia, and coagulation disorders showed statistically significant differences between the preterm and term infant groups (Table 3).

Table 3. Blood tests of bacterial meningitis neonates.

Characteristics	Median (IQR) min-max		<37 weeks (n=67)	≥37 weeks (n=56)	p
WBC (10^9/l)	13,3 (15,0) (1.45-57.6)	<$5x10^9$/l	10 (14.9)	7 (12.5)	>0.05
		5-<25	39 (58.2)	41 (73.2)	
		≥25	***18 (26.9)***	8 (14.3)	
PLT (10^9/l)	110 (241) (2-859)	<$100x10^9$/l	48 (71.6)	12 (21.4)	**<0.01***
		≥$100x10^9$/l	19 (28.4)	***44 (78.6)***	
Haemoglobin (g/l)	126.1±25.4 (70-208)	<60	0	0	>0.05
		60-<80	3 (4.5)	0	
		80-<120	29 (43.3)	17 (30.4)	
		≥120	35 (52.2)	39 (69.6)	
CRP (mg/l)	92.6 (100.9) (1.55-359.9)	<6	5 (7.5)	2 (3.6)	>0.05
		6-20	8 (11.9)	5 (8.9)	
		>20	54 (80.6)	49 (87.5)	
		92.6 (100.9)	83.7 (106.2)	109.3 (103.1)	
Coagulopathy			***31 (46.3)***	10 (17.9)	**0.001***
DIC			1 (1.5)	1 (1.8)	>0.05

*: Fisher's exact test; P-values indicating statistical significance are given in bold font. Values are given as n (%); SD: Standard deviation; PLT: Platelet; WBC: White blood cells; DIC: Disseminated intravascular coagulation; CRP: C-reactive protein.

Low CSF glucose levels showed a statistically significant difference between the preterm and term infant groups (Table 4).

Table 4. Cerebrospinal fluid characteristics in bacterial meningitis neonates.

Characteristics	Mean±SD (min-max)		<37 weeks (n=67)	≥37 weeks (n=56)	p
Cells (cells/mm^3)	338 (1933) (11-9974)	<21	1 (1.5)	1 (1.8)	**<0.01***
		21-1000	55 (82.1)	25 (44.6)	
		>1000	11 (16.4)	30 (53.6)	
Protein (g/l)	1.94 (1.76) (0.66-50)	<1 g/l	4 (6.0)	6 (10.7)	>0.05
		≥1 g/l	63 (94.0)	50 (89.3)	
Glucose (mmol/l)	2.0 (1.0) (0.01-38.4)	<2.2	36 (53.7)	15 (26.8)	**0.003****
		≥2.2	31 (46.3)	41 (73.2)	

*: Phi and Cramer's test; **: Chi-square test; P-values indicating statistical significance are given in bold font. Values are given as n (%); SD: Standard deviation.

Blood culture results had the highest positive rate at 82.1%, followed by CSF multiplex real-time PCR technique (Fig. 1).

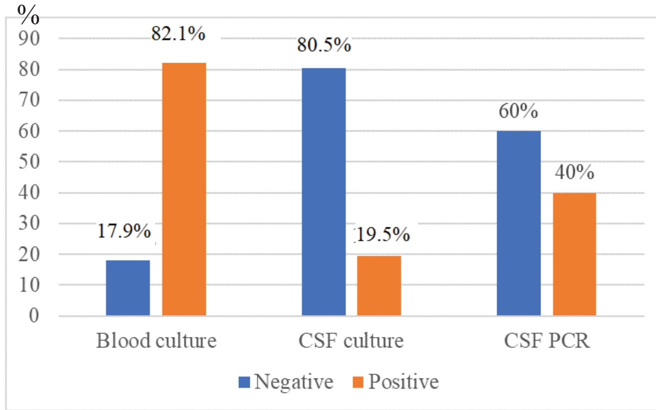

Fig. 1. Comparison of blood culture, cerebrospinal fluid culture, and cerebrospinal fluid multiplex real-time polymerase chain reaction technique results.

3.3. Aetiologies characteristics in bacterial meningitis neonates

E. coli, *K. pneumoniae*, and *S. marcescens* were the three most common pathogens in preterm neonates' blood cultures. However, GBS and *E. coli* were predominant in term neonates (Table 5).

Table 5. Blood culture results.

Bacterials	Blood culture results	<37 weeks (n=67)	≥37 weeks (n=56)	Total (n=123)
Gram positive	GBS	3 (4.5)	12 (21.4)	15 (12.2)
	S. aureus	3 (4.5)	5 (8.9)	8 (6.5)
	Streptococcus gallolyticus	2 (2.9)	0	2 (1.6)
Gram negative	*K. pneumoniae*	28 (41.8)	3 (5.4)	31 (25.2)
	S. marcescens	12 (17.9)	0	12 (9.8)
	E. coli	8 (11.9)	6 (10.7)	14 (11.4)
	E. meningoseptica	4 (6.0)	5 (8.9)	9 (7.3)
	Enterobacteraceae	3 (4.5)	1 (1.8)	4 (3.3)
	Stenotrophomonas maltophilia	1 (1.5)	1 (1.8)	2 (1.6)
	Acinetobacter baumannii (AC)	1 (1.5)	1 (1.8)	2 (1.6)
	Raoultella planticola	1 (1.5)	0	1 (0.8)
	Proteus mirabilis	0	1 (1.8)	1 (0.8)
Negative		1 (1.5)	21 (37.5)	22 (17.9)

Values are given as n (%); GBS: Group B streptococcus.

Of the neonates, 32.2% (18/56) term neonates had positive CSF cultures, with GBS and *E. meningoseptica* being the primary pathogens. Among the preterm group, positive CSF culture accounted for 9% (6/67) (Table 6).

Table 6. Cerebrospinal fluid culture results.

Bacterials	Cerebrospinal fluid results	<37 weeks (n=67)	≥37 weeks (n=56)	Total (n=123)
Negative		61 (91.1)	38 (67.8)	99 (80.5)
Positive		6 (9)	18 (32.2)	24 (19.5)
Gram positive	GBS	1 (1.5)	**7 (12.5)**	**8 (6.5)**
	S. gallolyticus	1 (1.5)	0	1 (0.8)
Gram negative	*K. pneumoniae*	2 (2.9)	1 (1.8)	3 (2.4)
	E. coli	1 (1.5)	3 (5.4)	4 (3.3)
	E. meningoseptica	1 (1.5)	**6 (10.7)**	**7 (5.7)**
	Enterococcus faecium	0	1 (1.8)	1 (0.8)

GBS: Group B streptococcus.

Cerebrospinal fluid polymerase chain reaction samples tested positive accounted for 32/80 (40%) and the preterm infant group predominantly found GBS and *E. coli* (Table 7).

Table 7. Results of cerebrospinal fluid polymerase chain reaction.

Results	<37 weeks (n=31)	≥37 weeks (n=49)	Total (n=80)
Negative	28 (90.3)	20 (40.8)	48 (60.0)
GBS	2 (6.5)	**27 (55.1)**	**29 (36.2)**
E. coli	1 (3.2)	2 (4.1)	3 (3.8)

The primary pathogens causing NBM in the preterm infant group were *K. pneumoniae, E. coli,* and *S. marcescens*. In the term infant group, GBS, *E. coli*, and *E. meningoseptica* were the predominant aetiologies (Table 8).

Table 8. Bacterial pathogens according to gestational age.

Bacterials	Pathogens	<37 weeks (n=67)	≥37 weeks (n=56)	Total (n=123)
Gram positive	GBS	4 (6.0)	**29 (51.8)**	**33 (26.9)**
	S. aureus	3 (4.5)	5 (8.9)	8 (6.5)
	S. gallolyticus	2 (2.9)	0	2 (1.6)
Gram negative	*K. pneumoniae*	**28 (41.8)**	3 (5.3)	**31 (25.2)**
	S. marcescens	**12 (17.9)**	0	12 (9.8)
	E. coli	**8 (11.9)**	**8 (14.3)**	**16 (13.0)**
	E. meningoseptica	4 (6.0)	**6 (10.7)**	10 (8.1)
	Enterobacteraceae	3 (4.5)	2 (3.6)	5 (4.1)
	AC	1 (1.5)	1 (1.8)	2 (1.6)
	S. maltophilia	1 (1.5)	1 (1.8)	2 (1.6)
	R. planticola	1 (1.5)	0	1 (0.8)
	P. mirabilis	0	1 (1.8)	1 (0.8)

GBS and *E. coli* were the leading causes of ENBM, while LNBM was associated with *K. pneumoniae* (Table 9).

Table 9. Bacterial pathogens according to classification.

Bacterials	Pathogens	ENBM n=29	LNBM n=94	Total n=123
Gram positive	GBS	**11 (37.9)**	**22 (23.4)**	33 (26.9)
	S. aureus	3 (10.3)	5 (5.3)	8 (6.5)
	S. gallolyticus	0	2 (2.1)	2 (1.6)
Gram negative	*E. coli*	**5 (17.2)**	**11 (11.7)**	16 (13.0)
	K. pneumoniae	**4 (13.8)**	**27 (28.7)**	31 (25.2)
	E. meningoseptica	2 (6.9)	8 (8.5)	10 (8.1)
	S. marcescens	2 (6.9)	10 (10.7)	12 (9.8)
	Enterobacteriaceae	1 (3.5)	4 (4.3)	5 (4.1)
	S. maltophilia	0	2 (2.1)	2 (1.6)
	AC	0	2 (2.1)	2 (1.6)
	P. mirabilis	1 (3.5)	0	1 (0.8)
	R. planticola	0	1 (1.1)	1 (0.8)

Values are given as n (%); SD: Standard deviation; LNBM: Late-onset neonatal bacterial meningitis; ENBM: Early-onset neonatal bacterial meningitis.

K. pneumoniae and *S. aureus* were the leading causes of mortality (Table 10).

Table 10. Bacterial pathogens according to treatment outcome.

Bacterials	Pathogens	Mortality (n=21)	Survivor (n=102)	Total (n=123)
Gram positive	GBS	2 (9.5)	31 (30.4)	33 (26.9)
	S. aureus	**3 (14.3)**	5 (4.9)	8 (6.5)
	S. gallolyticus	0	2 (2.0)	2 (1.6)
Gram negative	*K. pneumoniae*	**9 (42.9)**	22 (21.6)	31 (25.2)
	E. coli	2 (9.5)	14 (13.7)	16 (13.0)
	S. marcescens	1 (4.8)	11 (10.8)	12 (9.8)
	E. meningoseptica	2 (9.5)	8 (7.9)	10 (8.1)
	Enterobacteriaceae	2 (9.5)	3 (2.9)	5 (4.1)
	S. maltophilia	0	2 (2.0)	2 (1.6)
	AC	0	2 (2.0)	2 (1.6)
	P. mirabilis	0	1 (0.9)	1 (0.8)
	R. planticola	0	1 (0.9)	1 (0.8)

Values are given as n (%).

4. Discussion

We studied 123 neonates with neonatal bacterial meningitis (NBM), comprising 68 boys and 55 girls, resulting in a male-to-female ratio of 1.24:1. Preterm neonates accounted for a higher proportion of cases. Our findings are consistent with previous studies indicating that preterm neonates are at an increased risk of developing NBM [13]. However, in our study, late-onset NBM (LNBM) accounted for a higher proportion in the preterm group, likely due to the high rate (84.6%) of participants being transferred from lower-level facilities. These facilities often perform invasive procedures, thereby increasing the risk of LNBM (Table 1). Additionally, 91.3% of patients had received antibiotics before lumbar puncture, which influenced CSF bacterial culture results, leading to an increased rate of false negatives.

The risk factors for NBM were that 30.9% of patients had mothers with pre-existing and perinatal infectious diseases. Moreover, prolonged labour is also a risk factor for NBM.

The symptoms of NBM are often subtle and nonspecific, leading to misdiagnosis or delayed diagnosis. Fever accounted for 48%, predominantly in term neonates ($p<0.01$), and hypothermia was in 11.9% of mainly preterm neonates (Table 2). Previous studies have reported fever as a common systemic symptom, occurring in approximately 92-100% of cases [14, 15]. Respiratory symptoms were present in 64.9%, with 43.9% requiring MV, mainly in the preterm group (Table 2). V. Nizet, et al. (2016) [16] found that 41% of neonates had respiratory distress, including tachypnoea, grunting, intercostal retractions, and bradypnea, indicative of neonatal infection.

Severely infected neonates may develop septic shock, presenting with arrhythmias, hypotension, prolonged capillary refill time, cyanosis, and the need for inotropic support, primarily affecting preterm neonates (Table 2).

Neurological symptoms in NBM are challenging to assess but are often highly diagnostic. In our study, we observed seizures in 44.7% of cases, irritability/crying in 11.4%, convulsions in 13%, hypertonia in 12.2%, and bulging fontanelles in 7.3%. Seizures were more prevalent in the preterm group (30.9%) compared to the term group (13.8%), whereas convulsions and hypertonia were more frequent in term neonates ($p<0.01$). Seizures have been reported as a specific symptom for diagnosing NBM, with studies estimating their prevalence at 42.8%, particularly in preterm infants. Bulging fontanelles and convulsions are also recognised as key diagnostic indicators of NBM [3, 16-19].

Additionally, convulsions are commonly associated with GBS meningitis and are a late-presenting symptom as well as a prognostic factor for later complications in NBM [15]. N. Mehta, et al. (2022) [20] studied 303 neonates with neonatal sepsis. They found that 8.9% (27/303) of neonates had seizures, with 60% caused by bacterial meningitis and 85% (23/27) having at least one epileptic seizure on electroencephalography during the follow-up period up to 24 months of age. Seizures were associated with epilepsy and delayed motor development in NBM [20].

In our research, poor breastfeeding or suckling was noted in 30.1% of cases, vomiting in 8.2%, abdominal distension in 26%, diarrhoea in 4.1%, and abnormalities in gastric fluid in 24.4%. In preterm neonates, abnormalities in gastric fluid and abdominal distension were the most common symptoms, whereas poor breastfeeding or suckling was more prevalent in term neonates ($p<0.05$) (Table 3). M. Xu, et al. (2019) [15] reported similar findings, with poor breastfeeding in 24.9%, vomiting in 7%, abdominal distension in 17%, and diarrhoea in 14.9%.

Jaundice was observed in 35.8% of neonates, with similar prevalence between term and preterm groups. Jaundice is a common condition in neonates, often physiological, but in some cases indicative of pathological conditions. Clinicians should consider infection as a potential cause of jaundice and conduct further diagnostic evaluations to avoid missing underlying infections.

A study involving 1,763 jaundiced neonates reported that 131 had infections, with urinary tract infections and sepsis accounting for 77.9 and 16.8% of cases, respectively, primarily caused by *K. pneumoniae* and *E. coli* [21]. Petechia (11.9%) and scleroderma (6.5%) were less common in clinical practice, mainly observed in the preterm group, and indicative of severe infections in late-stage neonates.

We found that 17 out of 123 (13.8%) patients had WBC <5x10^9/l, while 21.1% had WBC ≥25,000x10^9/l. Thrombocytopenia was 48.8% (60/123), with the preterm group having significantly higher compared to the full-term group (p<0.01) (Table 4). Thrombocytopenia is an essential consideration as a prognostic factor in both neonatal and adult meningitis [22, 23]. One study identified thrombocytopenia as a level 3 factor in the prognostic classification of neonatal meningitis [24]. Thrombocytopenia may be seen in the setting of viral or fungal meningitis. However, thrombocytopenia combined with increased CRP and leucocytosis usually suggests a bacterial aetiology.

Our study showed a high CRP value median of 99.5±73.6 (ranging from 1.55-359.9 mg/l), with 83.7% of patients having CRP-values >20 mg/l, and only 5.7% presenting with normal CRP-values (<6 mg/l) (Table 3). Our results were higher than those reported by other authors, with 50% of patients having increased CRP and the highest value being 48 mg/l, as our study population consisted of patients with confirmed diagnoses of neonatal meningitis with positive culture results [10].

Lumbar puncture remains a mandatory test and the gold standard for diagnosing NBM. In our research, the average CSF cell count was 1089.9±1777.6 cells/mm^3 (ranging from 11 to 9974 cells/mm^3), with a significant difference between the term and preterm groups (p<0.01). A third of patients (33.3%) had very high CSF cell counts (>1000 cells/mm^3), while 1.6% had average values (<21 cells/mm^3). H.P. Garges, et al. (2006) [25] showed that WBC in CSF has a high sensitivity of 97% but a specificity of only 11%, and the WBC cutoff upper limit of 21 cells/mm^3 has a sensitivity of 79% and a specificity of 81% [25]. Another study on 9111 neonates (≥34 weeks gestation) diagnosed with NBM using a WBC cutoff over 20 cells/mm^3 showed a 13% missed diagnosis of NBM [26]. We found in CSF that 91.8% of patients had protein levels ≥1 g/l, and 8.2% had protein levels <1 g/l, with protein levels average of 3.08±4.75 (ranging from 0.66-50 g/l). Moreover, abnormal glucose levels were in 29.3% of preterm neonates and 13.2% of term neonates (p<0.01).

Positive blood cultures were identified in 82.1% (101/123 of patients, while 19.5% (24/123) had positive CSF cultures. The aetiology identified in blood cultures consisted of 12 types, with 2/3 being gram-negative bacteria. The most commonly isolated pathogens were *K. pneumoniae* (25.2%), GBS (12.2%), followed by *E. coli* (11.3%) and *S. marcescens* (9.7%). Other less frequently encountered pathogens included *S. aureus* and *E. meningoseptica*. Gram-negative bacteria such as *K. pneumoniae*, *S. marcescens*, and *E. coli* were predominant in the preterm group. In contrast, gram-positive bacteria, including GBS and *S. aureus*, were more prevalent in the term group (Tables 6 and 7).

N.T.N. Tu, et al. (2022) [27] evaluated sepsis in term neonates at Vietnam National Children's Hospital, reporting that gram-negative bacteria in blood cultures accounted for 51.7% of cases, gram-positive bacteria accounted for 35.3%, and the remaining were fungi. The most commonly isolated gram-negative bacteria were *K. pneumoniae* and *E. coli*, while *S. aureus* and GBS were the predominant gram-positive bacteria. Our findings align with this research, conducted at the same site, while also identifying a broader range of bacterial pathogens, AC, *S. gallolyticus*, *P. mirabilis*, *R. planticola*, or the *Enterobacteriaceae* genus.

Compared to blood culture, the rate of CSF culture is much lower at 24/123 (19.5%) due to the high rate of antibiotic use prior to lumbar puncture. In our study, 95/123 (77.2%) of patients had received antibiotics before admission to the Neonatal Intensive Care Department. According to J.T. Kanegaye, et al. (2001) [28], when using a dose of 50 mg/kg third-generation cephalosporin, 3/9 CSF specimen cultures with *Neisseria meningitidis* were negative within 1 hour, with the earliest negative result occurring at 15 minutes and all being negative after 2 hours. *Streptococcus pneumoniae*, the first negative CSF culture, occurred after 4.3 hours, with 5 out of 7 cultures being negative from 4 to 10 hours after starting intravenous antibiotics [28].

The bacteria identified in the CSF cultures were GBS (6.5%), *E. meningoseptica* (4.9%), *E. coli*, *K. pneumoniae*, *S. gallolyticus*, and *E. faecium*. H. Boskabadi, et al. (2020) [19] studied 468 suspected neonatal infections 233 (50%) cases required lumbar puncture, 148 (63.5%) had negative CSF cultures, and 85 (36.5%) had positive CSF cultures. Positive blood culture results in 80% LNBM, and *K. pneumoniae* and *E. aerogenes* were the most common pathogens causing meningitis.

In our research, using multiplex real-time PCR on 80 CSF samples, 40% (32/80) returned positive results, with GBS detected in 29/32 cases and *E. coli* in 3/32 cases. Among these, 23/80 patients had positive CSF PCR but negative CSF culture results, and 17/80 PCR samples were positive, but both blood and CSF cultures were negative. In our study, we used a multiplex real-time PCR technique to detect seven types of bacteria, including *E. coli K1*, GBS, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. Therefore, the detection rate was lower than that of blood culture but higher than that of CSF culture.

S.M. Morrissey, et al. (2017) [29] analysed 859 CSF samples from patients diagnosed with meningitis aged 7-90 days; 17/22 PCR samples were positive for GBS but had negative CSF cultures and concluded that PCR is beneficial for diagnosing meningitis neonates, especially for GBS [29]. Other research with 24/133 (18%) positive samples, including 10/24 *E. coli*, 6/24 GBS, and 3/24 *L. monocytogenes*. Only 5/24 patients had positive CSF culture results for the same bacteria detected by PCR [17].

In our research, two cases had CSF cell counts <21 cells/mm^3, and three had CSF protein values <1 g/l despite positive CSF culture results. This is consistent with the observations by H.P. Garges, et al. (2006) [25] on 95 neonates with positive CSF culture, where 12 neonates (12.6%) had cell counts <21 cells/mm^3. The study emphasised that relying solely on biochemical cell count criteria for diagnosing NBM may still miss some cases where neonates had normal CSF but a positive CSF culture.

A retrospective study of 231 NBM from 2009 to 2020 comprised 128 (boys 55.4%), 72 (31.2%) preterm neonates, and 48 (20.8%) cases of ENBM. The most common pathogens were *E. coli* (39.0%) and GBS (22.1%). Gram-negative bacteria were prevalent in preterm neonates compared to term neonates. GBS was prevalent in term neonates, while *K. pneumoniae* and *E. cloacae* were more common in preterm neonates. gram-positive bacteria were more frequently associated with ENBM compared to LNBM. Both *E. coli* (46.3 and 30.9%) and GBS (29.8 and 13.6%) showed a significant increase, while *Enterococcus* (3.3 and 12.7%) showed a significant decrease in the period 2015-2020 compared to 2009-2014.

Our findings revealed a broader diversity of pathogenic bacterial groups compared to other studies. In addition to the common pathogens such as GBS, *E. coli*, and *K. pneumoniae*, other pathogens, such as *E. meningoseptica* and *S. marcescens*, had a significant prevalence. The Neonatal Care Centre, Vietnam National Children's Hospital is a leading neonatal treatment facility in northern Vietnam, catering to severely ill neonates referred from lower-level hospitals. Prolonged hospitalisation and interventions such as mechanical ventilation, intravenous nutrition, and central catheters contribute to high infection rates, including sepsis and NBM.

For ENBM, GBS and *E. coli* remain the most common pathogens, while LNBM frequently involves GBS, *E. coli*, *K. pneumoniae*, and *S. marcescens*. These findings align with international studies, which identify GBS as the leading cause of NBM. However, in our study, pathogens such as *L. monocytogenes* and *N. meningitidis* were not encountered, possibly due to the small sample size. A study by W.E. Naggar, et al. (2019) [4] in Canada over six years showed that the primary pathogens causing neonatal sepsis in the neonatal intensive care unit were GBS and *E. coli*.

The mortality rate of the paediatric group in the study was 17.1% (21/123). This result is consistent with the findings of many authors regarding the mortality rate of NBM, which ranges from 5-20% and has not changed significantly over the past 20 years. N.T. Thanh (2004) [7] reported a mortality rate of 19.8% at Vietnam National Children's Hospital. In our study, the most common causative agent associated with mortality in NBM was gram-negative, predominantly caused by *K. pneumoniae*, while gram-positive pathogens, particularly *S. aureus*, had a higher mortality rate. On the other hand, GBS and *E. coli* were associated with higher survival rates. M.Y. Oncel, et al. (2024) [30] concluded that the main causative agents were gram-positive bacteria (54.5%), with the predominant group being coagulase-negative staphylococci (25.3%) among 634 deceased neonates.

5. Conclusions

Bacterial meningitis is a severe condition in neonates treated at the Neonatal Care Centre of the Vietnam National Children's Hospital. The disease predominantly affects male neonates, preterm infants, and those with low birth weight. Clinical presentations often include severe infection symptoms, with a high prevalence of fever, respiratory failure, and lethargy.

Paraclinical characteristics of neonatal bacterial meningitis: NBM in neonates frequently presents with leucocytosis, platelet counts <100×10^9/l, anaemia, and coagulation disorders, with significant differences between preterm and term groups.

Regarding the bacterial aetiology, our study shows that GBS, *K. pneumoniae*, and *E. coli* were the most common pathogens identified. In preterm neonates, *K. pneumoniae*, *S. marcescens*, and *E. coli* were prevalent, while term neonates were more commonly affected by GBS, *E. coli*, and *E. meningoseptica*. Early-onset NBM was primarily caused by GBS and *E. coli*, whereas late-onset cases also frequently involved *K. pneumoniae* and *S. marcescens*. Notably, our study highlighted a greater diversity of bacterial species compared to many other studies.

6. Study limitations

Our study had a small sample size and was conducted at a single Neonatal Intensive Care Department, which limits its ability to provide a comprehensive assessment of neonatal bacterial meningitis in Vietnam today. Further prospective studies are necessary, along with evidence from the general population and neonates, who bear the highest disease burden, to gather data on the epidemiology, clinical presentation, and management of the disease.

CRediT author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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