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Original article

Clinical features and antibiotic resistance in pediatric pneumococcal meningitis in Southern Vietnam, 2012–2023: A multicenter retrospective study

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ABSTRACT

Background: *Streptococcus pneumoniae* is a major cause of morbidity and mortality in children. This study investigates the clinical features, cerebrospinal fluid (CSF) findings, serotype distribution, and antibiotic resistance patterns in Vietnamese children aged 1–59 months with pneumococcal meningitis (PM).

Methods: This retrospective study (2012–2023) was conducted at two tertiary pediatric hospitals in Ho Chi Minh City. CSF samples from probable bacterial meningitis (PBM) cases were analyzed using biochemistry, culture, and real-time polymerase chain reaction (rt-PCR). Serotyping and antibiotic-resistance genes were identified using quadriplex rt-PCR.

Results: Among 2922 PBM cases, 155 (5.3 %) were confirmed as PM. Of these, 58.7 % occurred in children under one year and 62.6 % during the rainy season. Fever (98.1 %) and vomiting (67.7 %) were the most common symptoms. Infants under 12 months frequently exhibited nonspecific signs like convulsions (48.4 %) and bulging fontanels (34.1 %), while older children displayed classic symptoms such as neck stiffness (32.4 %) and behavioral changes (26.5 %). CSF analysis revealed turbid appearance, WBC ≥ 100 cells/mm³ (85.2 %), and protein ≥ 1 g/L (60.0 %) ($p < 0.05$). Severe outcomes were noted in 17.4 % of PM cases, with a 3.2 % fatality rate. The 13-valent pneumococcal conjugate vaccine (PCV13) serotypes caused 81.3 % of confirmed cases, predominantly serotypes 6 A/B (34.8 %) and 19 F (20.0 %). Among 137 isolates tested, high prevalence rates were observed for the *pbp2b* (68.6 %), *mef(A)/erm(B)* (93.4 %), and *tetM* (92.0 %) resistance genes. Additionally, 61.3 % of isolates showed multiple resistance genes, particularly in serotypes 6 A/B, 23 F, 9 V, and 13. Antibiotic resistance in non-PCV13 serotypes increased over time.

Conclusions: PM in Vietnamese children presents age-specific clinical presentations and is predominantly caused by highly resistant PCV13 serotypes. The rising resistance in non-PCV13 serotypes poses a formidable challenge in managing pneumococcal infections. These findings emphasize the urgent need for PCV introduction in the national immunization program and ongoing resistance surveillance.

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Key points

This study examines pneumococcal meningitis in Vietnamese children under five, emphasizing clinical features, serotype distribution, and antibiotic resistance patterns over 12 years, underscoring evolving resistance in PCV13 serotypes and the urgent need to introduce PCVs into the national immunization program.

Introduction

Streptococcus pneumoniae remains a prominent global health concern, particularly as a leading cause of morbidity and mortality among children under five years old [1]. According to the World Health Organization (WHO), pneumococcal infections lead to nearly 294,000 deaths annually in this age group, representing about 5 % of all child deaths worldwide [2]. Despite advancements in antibiotic treatments and widespread vaccination programs, pneumococcal meningitis (PM) continues to pose severe risks, including high mortality rates. Complications such as behavioral and intellectual disorders, hearing loss, and neurological deficits remain prevalent, especially in low- and middle-income countries where mortality rates can reach up to 26 % [3].

Early and accurate diagnosis of PM is essential for effective management and improved patient survival. Typically, diagnosis involves performing a lumbar puncture to identify bacteria or bacterial antigens in the cerebrospinal fluid (CSF) [4]. However, the efficacy of CSF cultures can be compromised by prior antibiotic treatment [5,6]. Although more sensitive molecular techniques like real-time polymerase chain reaction (rt-PCR) improve detection rates, particularly in patients who have received antibiotics, such resources are often inaccessible in under-resourced settings [7]. Additionally, with decreasing disease incidence due to vaccination, many clinicians are less familiar with the clinical presentation of PM, complicating timely and accurate diagnoses. Moreover, a rise in pneumococcal antimicrobial resistance complicates treatment options, often leading to delays in diagnosis and appropriate treatment, particularly in urgent care scenarios [8].

In Vietnam, the introduction of pentavalent vaccine (DTP-HepB-Hib) into the national expanded program on immunization (NEPI) since 2011 has shifted the epidemiology of bacterial meningitis. *S. pneumoniae* has emerged as predominant pathogen, with an escalating trend in antibiotic resistance and the proliferation of multidrug-resistant organisms, despite the availability of the 10-valent of pneumococcal conjugate vaccine (PCV10) and the 13-valent PCV (PCV13) in the private sector in 2014 and 2019, respectively [9]. However, data on PM in Vietnamese children, particularly regarding clinical characteristics, CSF findings, serotype distribution and antibiotic resistance genes patterns, remain limited [10–12].

This study aims to address these gaps by focusing on two main objectives: (1) to describe the clinical and laboratory characteristics of PM in children under five years old treated at pediatric hospitals in Southern Vietnam from 2012 to 2023, including serotype distribution and risk factors for severe diseases, and (2) to examine trends in antibiotic resistance genes over time across different PCV implementation periods. These findings provide critical insights into the current epidemiology and clinical aspects of PM, as well as the circulating serotypes and antibiotic resistance genes. This information is essential for early diagnostic, effective treatment, and prioritizing vaccine introduction strategies, and enhancing antimicrobial resistance surveillance efforts.

Methods

Study design and surveillance setting

A retrospective study, multicenter sentinel surveillance was conducted across two tertiary-level children's hospitals in Vietnam from 2012 to 2023. Hospitals were selected based on two criteria: (1) possession of adequate research capabilities and laboratory resources necessary for bacterial culture, and (2) demonstrated commitment to and capacity for sustained research participation. Children's Hospitals 1 and 2, major pediatric centers in southern Vietnam with a combined capacity of over 1000 beds, were central to the surveillance efforts. These hospitals are pivotal in providing pediatric care and serve as training centers for medical professionals specializing in pediatrics [13]. The study periods were divided into pre-PCV (2012–2014), PCV10 (2015–2019), and PCV13 (2020–2023) eras.

Study population and case definition

The study population comprised Vietnamese children aged one to 59 months who were admitted with fever and suspected bacterial meningitis to participating hospitals.

Inclusion criteria: case was identified based on the WHO case definition for bacterial meningitis [14]: (1) acute onset of fever (> 38.5°C rectal or 38°C axillary); (2) one of the following clinical symptoms or signs: headache, convulsions, altered consciousness, neck stiffness, behavioral changes, vomiting, or bulging fontanel; (3) CSF examination showing at least one of the following findings: the turbid appearance, or leukocytosis (> 100 cells/mm3), or protein (> 1 g/L), or leukocytosis (10–100 cells/mm3) and protein (> 0.45 g/L), or leukocytosis (10–100 cells/mm3) and glucose (< 2.2 mmol/L); (4) detection of *S. pneumoniae* in the CSF via rt-PCR. Cases meeting criteria (1), (2), and (3) were classified as probable bacterial meningitis (PBM). A PBM case meeting criterion (4) were considered a confirmed PM case.

Exclusion criteria: Patients diagnosed with meningitis caused by *Neisseria meningitidis*, *Haemophilus influenzae*, viruses, *Mycobacterium tuberculosis*, or fungi were excluded.

Clinical outcomes

Clinical outcomes were assessed at hospital discharge and classified into five categorized: (1) death, (2) discharge upon family request, (3) referral to other healthcare facilities, (4) development of sequelae, and (5) recovery. Among these, outcomes 1–4 were considered severe disease, while recovery was classified as non-severe. This classification was specifically designed for the multivariate analysis to identify risk factors associated with severe disease.

Serotyping and antibiotic resistance gene detection

CSF specimens from suspected cases underwent microscopy, bacterial culture, and rapid diagnostic tests such as BinaxNOW and Pastorex Meningitis antigen rapid latex agglutination [9]. CSF analysis included turbidity, white blood cell (WBC) count, and glucose and protein levels [15]. Residual CSF specimens were sent to the National Reference Laboratory at the Pasteur Institute of Ho Chi Minh City (PI HCMC) for PM confirmation and serotyping.

We identified pneumococcal serotypes using two methods: multiplex conventional PCR for 40 serotypes in CSF samples collected between 2012 and 2017 and sequential direct triplex rt-PCR approach for 21 serotypes for those from 2018 to 2023 [14,16]. This included all 13 serotypes in the PCV13, along with eight additional important serotypes or serogroups [16,17].

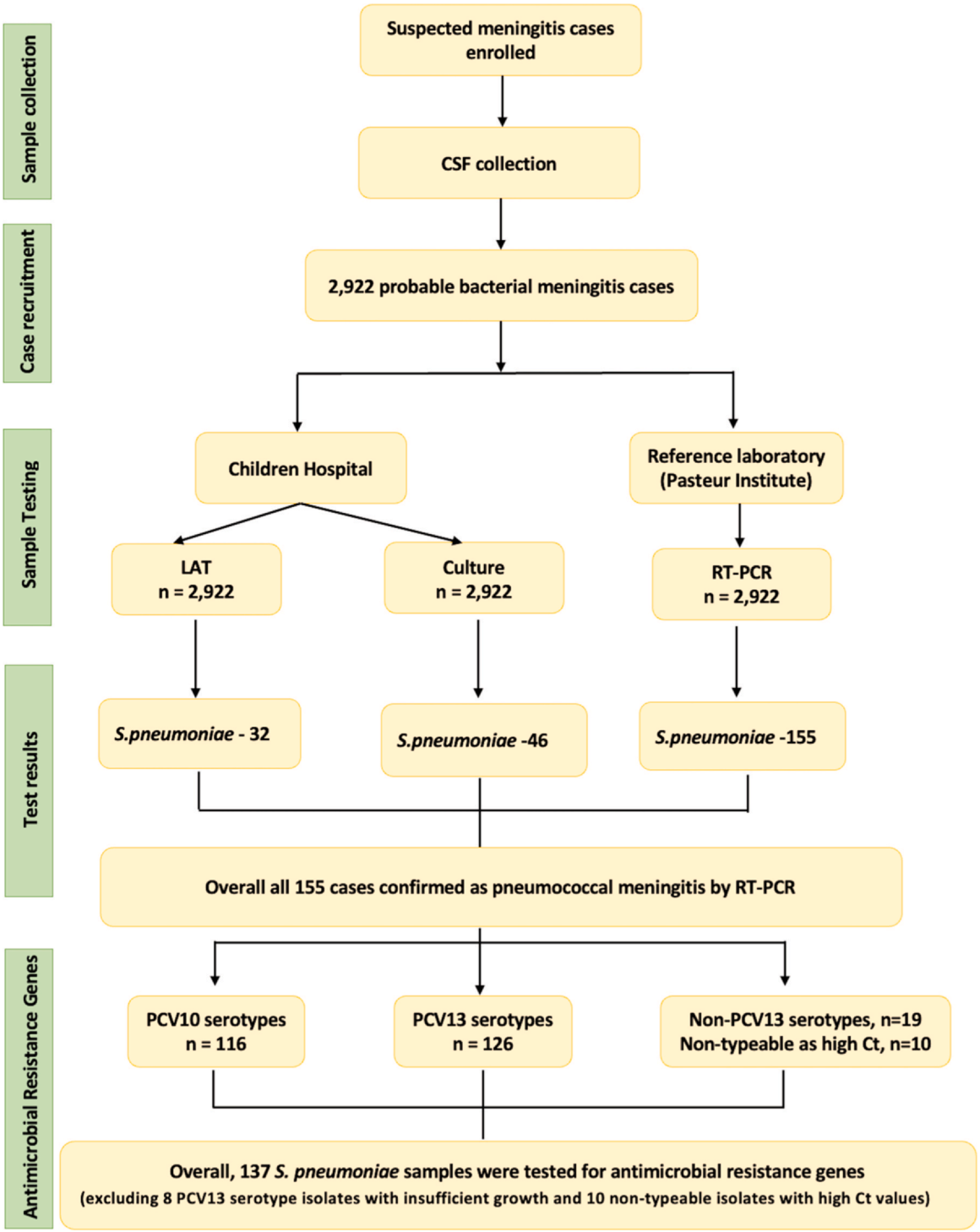

Fig. 1. Summary of pneumococcal meningitis case recruitment, sample testing, serotypes, and antibiotic resistance genes results from 2012 to 2023.

We employed a quadridplex rt-PCR approach to identify genetic markers predictive of antibiotic resistance (ABR) in PM cases. This method targeted four specific ABR genes: pnenicillin-binding protein 2b (*pbp2b*, wild-type) to determine penicillin susceptibility, erythromycin ribosme methylation (*ermB)* and macrolide efflux gene A (*mef(A))* for macrolide resistance, and tetracycline resistance gene M (*tetM)* for tetracycline resistance. We included samples that tested positive for *S. pneumoniae,* while those that were non-typeable due to low DNA concentration (typically with Cycle Threshold above 30) were excluded. A total of 137 CSF samples were subjected to rt-PCR detection of these ABR genes.

The quadriplex reaction mixture consisted of 12.5 µL of 2X Mastermix PerfeCTA qPCR ToughMix Low ROX (QuantaBio, USA), appropriate primers and probes concentrations (IDT, USA), 2 µL of template, and molecular-grade water adjusted to a total volume of 25 µL. The primers and probe concentrations, utilizing four different reporting dyes (ROX, FAM, HEX, and CY5), were determined based on established methodologies [18]. The reactions followed a thermal

Table 1
Demographic characteristics and CSF findings of pneumococcal meningitis.

Characteristics	Pneumococcal Meningitis Case (N = 155) n (%)
Gender	
Male	85 (54.8)
Female	70 (45.2)
Age in months, median (IQR))	9 (5–21)
Age group (in months categorized)	
2–11	91 (58.7)
12–23	34 (21.9)
24–59	30 (19.4)
Permanent Residence	
Southeastern	85 (58.8)
Southwestern (Mekong River)	37 (23.9)
Other	33 (21.3)
Seasonality	
Dry season	58 (37.4)
Rainy season	97 (62.6)
CSF appearance	
Clear	76 (49.0)
Turbid	79 (51.0)
WBC count (cells/mm^3)	
10–100	23 (14.8)
≥ 100	132 (85.2)
Protein (g/L)	
< 1	62 (40.0)
≥ 1	93 (60.0)
Glucose (mmol/L)	
< 2.2	3 (1.9)
≥ 2.2	152 (98.1)

Abbreviation: PM, pneumococcal disease; rt-PCR, real-time polymerase chain reaction; CSF, cerebrospinal fluid; WBC, White blood cell.

cycling profile of 55°C for 5 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Statistical analysis

Data analysis included Chi-squared or Fisher's exact test for comparisons of clinical characteristics and treatment outcome stratified by age group, as well as serotype distribution across different PCV eras. Multivariate logistic regression with forward selection (p-value ≤ 0.25) based on the Akaike Information Criterion (AIC) identified predictors of severe outcomes. A 2-tailed p-value ≤ .05 was considered significant. All analyses were conducted using Stata version 18.0 and R version 4.4.1.

Results

Demographic, clinical characteristics, and CSF findings

Between 2012 and 2023, 2922 children with PBM were enrolled. Of these, 32 and 46 PM cases were identified via the Latex Agglutination test and bacterial culture, respectively, representing about one-third of the 155 cases confirmed by rt-PCR (Fig. 1). PM cases were more prevalent in males (54.8 %) than females (45.2 %), with a median age of 9 months (interquartile range [IQR]: 5–21 months). Most cases (58.7 %) occurred in children under one year, with 58.8 % from the southeastern region and 62.6 % during the rainy season (Table 1).

In the 155 confirmed PM cases, the most common symptoms were fever (98.1 %) and vomiting (67.7 %). Among 91 infants under 12 months, frequent presentations included nonspecific symptoms such as convulsions (48.4 %) and bulging fontanels (34.1 %). In 34 older children (12–23 months), classic signs like neck stiffness (32.4 %) and behavioral changes (26.5 %) were often exhibited. Headaches were prominent in children aged two years and older (19/30, 63.3 %). Statistical analyses indicated significant differences in symptoms such as bulging fontanel, neck stiffness, and headaches across age groups ($p < 0.05$). The overall severe outcome rate for PM was 17.4 % (27/155; 95 % CI, 11.4 %–24.3 %), with case fatality, neurological sequelae, and motor impairments documented at rates of 3.2 %, 5.8 %, and 1.9 %, respectively (Table 2).

Among examined CSF samples (Tables 1), 51.0 % (79/155) showed a turbid appearance. There were significant differences in WBC count and protein levels between PM and non-PM cases ($p < 0.05$), with elevated levels observed in PM cases: WBC ≥ 100 cells/mm³ (85.2 %) and protein ≥ 1 g/L (60.0 %). Low glucose levels (< 2.2 mmol/L) were noted in 1.9 % of PM cases.

Prognostic risk factors and signs predicting for severe pneumococcal meningitis

Multivariate analysis identified several significant risk factors associated with severe PM, including serotype 6 A/B (aOR: 2.8, 95 %

Table 2
Clinical characteristics, outcomes, and sequelae by age group.

Variables	PM (N = 155) n (%)	1–11 months (N = 91) n (%)	12–23 months (N = 34) n (%)	24–59 months (N = 30) n (%)	p-value
Symptoms					
Fever	152 (98.1)	89 (97.8)	33 (97.1)	30 (100)	1.000
Headache	22 (14.2)	0 (0)	3 (8.8)	19 (63.3)	< 0.001
Convulsion	74 (47.7)	44 (48.4)	20 (58.8)	10 (33.3)	0.123
Altered consciousness	39 (25.2)	23 (25.3)	5 (14.7)	11 (36.7)	0.130
Neck stickness	27 (17.4)	9 (9.9)	11 (32.4)	7 (23.3)	0.008
Behavioral change	38 (24.5)	19 (20.9)	9 (26.5)	10 (33.3)	0.371
Vomiting	105 (67.7)	59 (64.8)	25 (73.5)	21 (70.0)	0.624
Bulging fontanel	32 (20.7)	31 (34.1)	1 (2.9)	0 (0)	< 0.001
Treatment Outcome					
Death	5 (3.2)	3 (3.3)	1 (2.9)	1 (3.3)	0.860
Discharge upon family request	2 (1.3)	2 (2.2)	0	0	
Referral to other healthcare facilities	8 (5.2)	7 (7.7)	1 (2.9)	0	
Sequelae	12 (7.7)	6 (6.6)	3 (8.8)	3 (10.0)	
Recovery	128 (82.6)	73 (80.2)	29 (85.3)	26 (86.7)	
Sequelae					
Neurological sequelae^{+}	9 (5.8)	4 (4.4)	3 (8.8)	2 (6.7)	0.755
Motor Impairment^{++}	3 (1.9)	2 (2.2)	0 (0)	1 (3.3)	
Severe outcome	27 (17.4)	18 (19.8)	5 (14.7)	4 (13.3)	0.732

Abbreviation: PM, pneumococcal disease.
Neurological sequelaes^{+} (9): Brain abscess (5), hydrocephalus (4).
Motor impairments^{++} (3): limb weakness (3).

Table 3
Multivariate analysis of prognostic risk factors and signs predicting severe of pneumococcal meningitis in children under five years.

Risk Factors	Non severe (N = 128) n (%)	Severe (N = 27) n (%)	Univariate analysis		Multivariate analysis	
			OR (95 % CI)	p-value	aOR (95 % CI)	p-value
Sex: Male	66 (51.6)	19 (70.4)	2.2 (0.9–5.5)	0.079	2.0 (0.8–5.2)	0.149
Aged 1–11 months	73 (57.0)	18 (66.7)	1.6 (0.5–5.2)	0.430		
Aged 12–23 months	29 (22.7)	5 (18.5)	1.1 (0.3–4.6)	0.875		
Rainy season	77 (60.2)	19 (70.4)	1.6 (0.6–3.9)	0.323		
Southwestern Vietnam	30 (23.4)	7 (25.9)	1.1 (0.4–3.0)	0.783		
Serotype 6 A/B	41 (32.0)	13 (48.2)	2.0 (0.8–4.6)	0.114	2.8 (1.1–7.4)	0.037
Serotype 19 F	27 (21.1)	4 (14.8)	0.7 (0.2–2.0)	0.461		
Serotype 23 F	14 (10.9)	1 (3.7)	0.3 (0.1–2.5)	0.272		
Serotype 14	12 (9.4)	3 (11.1)	1.2 (0.3–4.6)	0.782		
Serotype 19 A	5 (3.9)	3 (11.1)	3.1 (0.7–13.7)	0.141	5.4 (1.1–27.7)	0.040
Serotype 15 A/F	4 (3.1)	3 (11.1)	3.8 (0.8–18.4)	0.089	7.1 (1.3–38.4)	0.022
Multiple resistant genes	70 (54.7)	14 (51.9)	0.9 (0.4–2.0)	0.788		
Signs Predicting	**Non severe (N = 128) n (%)**	**Severe (N = 27) n (%)**	**Univariate analysis OR (95 % CI)**	**p-value**	**Multivariate analysis aOR (95 % CI)**	**p-value**
Sex: Male	66 (51.6)	19 (70.4)	2.2 (0.9–5.5)	0.079	2.5 (1.0–6.4)	0.054
Convulsion	56 (43.8)	18 (66.7)	2.6 (1.1–6.2)	0.034	2.7 (1.1–6.6)	0.033
Altered consciousness	28 (21.9)	11 (40.7)	2.5 (1.1–5.9)	0.044		
Neck stiffness	19 (14.8)	8 (29.6)	2.4 (0.9–6.3)	0.071	2.2 (0.8–6.1)	0.119
Behavioural change	27 (21.1)	11 (40.7)	2.6 (1.1–6.2)	0.035		
Vomitting	88 (68.8)	17 (63.0)	0.8 (0.3–1.8)	0.559		
Bulging fontanel	24 (18.8)	8 (29.6)	1.8 (0.7–4.7)	0.209	2.4 (0.9–6.5)	0.096
CSF turbid appearance	63 (49.2)	16 (59.3)	1.5 (0.6–3.5)	0.345		
WBC count: ≥ 100 (cells/mm3)	109 (85.2)	23 (85.2)	1.0 (0.3–3.2)	0.997		
Protein ≥ 1 g/L	79 (61.7)	14 (51.9)	0.7 (0.3–1.5)	0.343		
Glucose < 2.2 mmol/L	2 (1.6)	1 (3.7)	2.4 (0.2–27.7)	0.477		

Abbreviation: CSF, cerebrospinal fluid; WBC, white blood cell; PCV, pneumococcal conjugate vaccine; aOR, adjusted odds ratio; CI, confidence interval.

CI: 1.1–7.4, p = 0.037), serotype 19 A (aOR: 5.4, 95 % CI: 1.1–27.7, p = 0.040), serotype 15 A/F (aOR: 7.1, 95 % CI: 1.3–38.4, p = 0.022). Convulsions, with an aOR of 2.7 (95 % CI: 1.1–6.6, p = 0.033), were a predictive sign of severe PM cases (Table 3).

Serotyping

Among the 155 PM cases, 74.8 % were caused by serotypes included in PCV10, 80.0 % in Pneumosil, 81.3 % in PCV13 and PCV15, and 83.2 % in PCV20. The most prevalent serotypes were 6 A/B (34.8 %) and 19 F (20.0 %). Non-PCV13 serotypes accounted for 12.3 % of isolates, while 6.5 % were unknown due to low concentration of pathogens in the specimens (high Ct values). Age-specific distribution indicated that serotypes 6 A/B, 19 F, 14, and 23 F were predominant in children under two years, whereas 6 A/B, 19 F, 19 A, and 15 A/F dominated in those aged 2–5 years (Fig. 2A). Pneumococcal meningitis cases declined across PCV implementation periods, decreasing by 68 % from 97 cases in the PCV10 era (2015–2019) to 31 cases in the PCV13 era (2020–2023). In contrast, the proportion of non-PCV13 serotype cases increased significantly from 3.7 % to 15.5 %, ultimately reaching 41.9 % across all three PCV eras (p < 0.05), as shown in Fig. 2B.

Antibiotic resistance genes

Genotypic analysis of antibiotic resistance among 137 *S. pneumoniae* isolates identified a high prevalence of the *pbp2b*, *mef(A)/erm (B)*, and *tetM genes*, detected in 68.6 %, 93.4 %, and 92.0 % of samples, respectively. Additionally, 61.3 % of isolates carried multiple resistance genes (*pbp2b*, *mef(A)/erm(B)* and *tetM*) (Fig. 3A). These resistance-associated genes were distributed across all serotypes, with a higher prevalence in serotype 6 A/B. Muliple resistance genes were most frequently observed in serotypes 6 A/B, 23 F, 9 V, 11 A/D, and 13, with the rate exceeding 80 % among the tested isolates (Table 4).

The rate of the altered *pbp2b* gene, associated with penicillin resistance, remained stable at approximate 65 % throughout the study period. However, its presence among PCV13 serotypes decreased by 20.4 % from pre-PCV to PCV13 era, while non-PCV13 serotypes exhibited an increasing trends (p < 0.05) (Fig. 3B and C.1). Similarly, the proportion of *mef(A)/erm(B)* and *tetM* genes–linked to macrolide and tetracycline resistance–declined by 29.2 % in PCV13 serotypes but increased by 37.9 % and 27.6 %, respectively, among non-PCV13 serotypes (p < 0.05) (Fig. 3C.2 and C.3).

Discussion

Pneumococcal meningitis is a severe invasive disease requiring timely detection and intervention to reduce mortality and neurological complications. Diagnosing PM in children poses unique challenges due to age-related differences in clinical presentation. This study aimed to elucidate the clinical presentations, paraclinical characteristics, serotype distribution, and antibiotic resistance genes of PM in children under five years in southern Vietnam, particularly before PCV introduction into the NEPI.

Our findings reveal significant age-related variations in symptoms. While non-specific signs, such as convulsions and bulging fontanels, are more prevalent in infants, older children tend to exhibit more classical symptoms like neck stiffness and behavioral changes, mirroring findings from diverse geographic regions including China, India, and several European countries [4,19–23]. This variation highlights the necessity for heightened vigilance, early recognition and appropriate empirical treatment, especially in younger children where typical symptoms may be absent. Convulsion is associated with a significantly higher risk of severe outcomes (p < 0.05) and may necessitate a cautious prognosis.

The superior detection rate of rt-PCR (5.3 %) over culture (1.6 %) and latex agglutination tests (1.1 %) aligns with other studies, emphasizing the need for advanced diagnostic tools in clinical settings [24]. However, its routine implementation is limited by cost and infrastructure constraints [25,26]. CSF culture, the traditional diagnostic standard, demonstrates a positive result rate of only 4–70 % [26–28]. The low culture positivity in our study may be attributed to

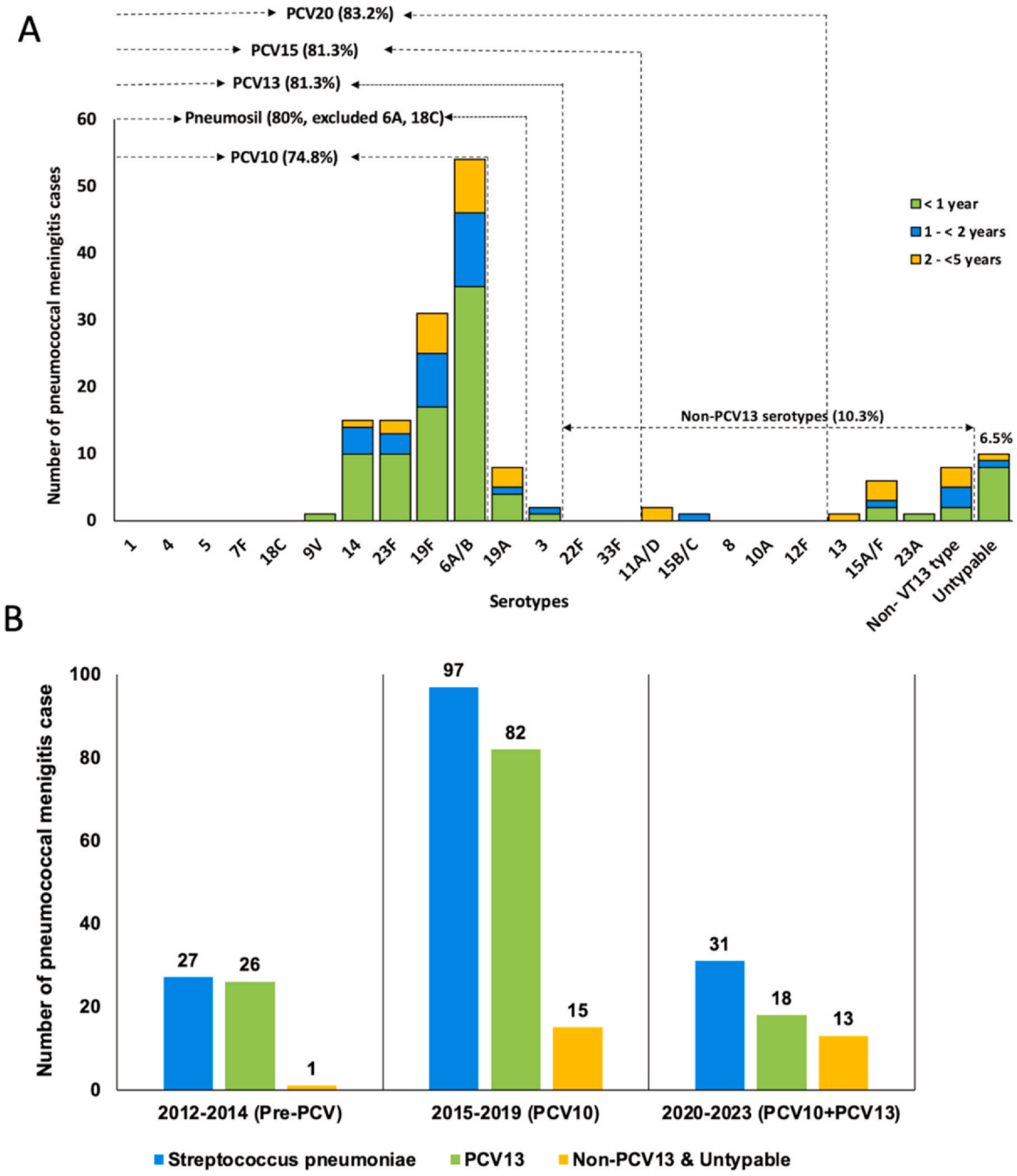

Fig. 2. Serotype distribution of pneumococcal meningitis cases from 2012 to 2023. (A) Serotype distribution of pneumococcal meningitis cases by age groups. (B) Serotype distribution of pneumococcal meningitis cases by PCV eras.

prior antibiotic use before lumbar puncture, highlighting the need for a comprehensive diagnostic approach that integrates clinical judgment and empirical treatment to prevent missed diagnoses.

We identified high prevalence rates of resistance-associated genes, including *pbp2b* (68.6 %), *mef(A)/erm(B)* (93.4 %), and *tetM* (92.0 %). Mutations in *pbp2b* have been shown to reduce the binding affinity of *pbp2b* for β-lactam antibiotics, leading to decreased susceptibility or outright resistance, particularly to penicillin [29,30]. These findings align with global trends but exceed resistance rates reported in some Western, Asian, and African countries [10,19,31–33]. Our results reinforce and are consistent with current clinical practice in Vietnam and globally, where clinicians have selected third-generation cephalosporins, such as ceftriaxone or cefotaxime, as the first-line treatment for pneumococcal meningitis in regions with high penicillin resistance [34,35]. Additionally, despite the fact that these antibiotics have not been indicated for pneumococcal meningitis, our study found high rates of resistance-associated genes for penicillin, macrolides, and tetracyclines. This may suggest widespread use of these antibiotics for common acute illnesses, particularly in children. This phenomenon is quite prevalent in Vietnam, as documented in previous studies [36–38]. Public health campaigns are essential to promote rational antibiotic use and to ongoing effective surveillance systems to monitor the spread of resistant strains.

Pneumosil, PCV13 & PCV15 and PCV20 serotypes accounted for 80.0 %, 81.3 %, and 83.2 % of PM cases, respectively. Additionally, over 80 % of vaccine-covered serotypes, such as 6 A/B, 23 F, and 9 V, carry multiple drug-resistance genes. Despite PCVs being available only in

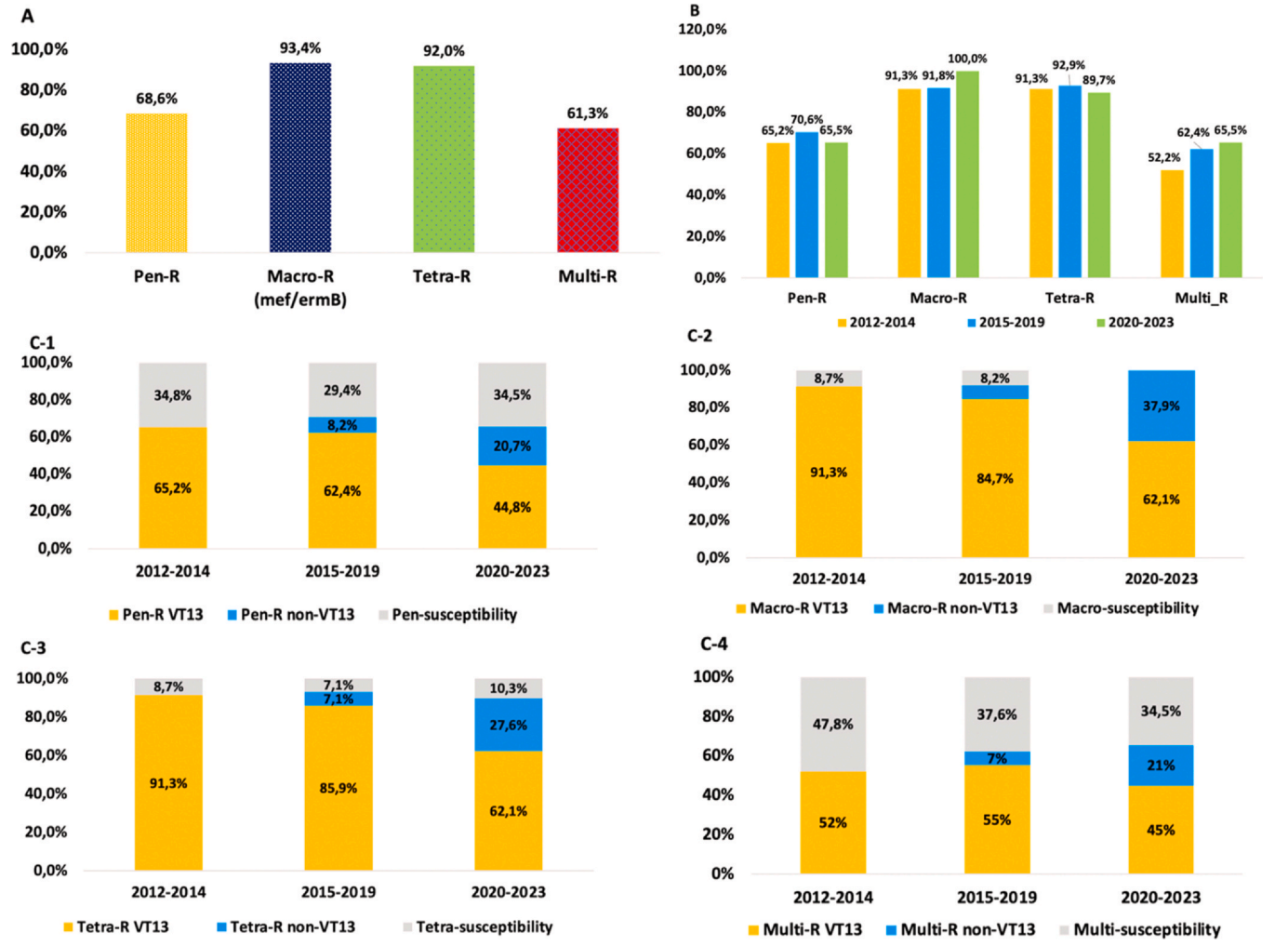

Fig. 3. Antibiotic resistance genes in 137 pneumococcal meningitis samples from children under five years in Southern Vietnam, 2012–2023. (A) Prevalence of resistance–associated genes for penicillin, macrolide, tetracycline, and multiple resistance genes during the study period. (B) Prevalence of these genes by PCV Eras (pre-PCV10: 2012–2014; PCV10: 2015–2019; and PCV13: 2020–2023). (C.1) Penicillin-resistant genes by serotypes, showing a decline from 65.2 % to 44.8 % in PCV13 serotypes and an increase from 0 % to 20.7 % in non-PCV13 serotypes. (C.2), (C.3), (C.4) Macrolide, Tetracycline, and Multiple resistance genes by serotypes, respectively. Antibiotic-resistance genes emerged more frequently in non-PCV13 serotypes in the PCV10 and PCV13 eras.

Table 4
Resistance genes rate in different serotypes among 137 *S.pneumoniae* isolates.

Serotypes	No. Isolate n (%)	Penicillin resistance gene (*pbp2*) n (%)	Macrolide resistance gene (*mef*) n (%)	Macrolide resistance gene (*ermB*) n (%)	Macrolide resistance gene (mef/*ermB*) n (%)	Tetracycline resistance gene (*tetM*) n (%)	Multidrug resistance genes n (%)
PCV10 serotypes	110 (80.3)	77 (70.0)	38 (34.5)	98 (89.1)	102 (92.7)	104 (94.5)	68 (61.8)
9 V	1 (0.7)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)
14	15 (13.6	10 (66.7)	6 (40)	14 (93.3)	14 (93.3)	15 (100)	9 (60)
19 F	28 (25.5)	5 (17.9)	24 (85.7)	23 (82.1)	26 (92.9)	28 (100)	3 (10.7)
23 F	14 (10.2)	13 (92.9)	1 (7.1)	13 (92.9)	13 (92.9)	13 (92.9)	12 (85.7)
6 A/B	52 (47.3)	48 (92.3)	7 (13.5)	47 (90.4)	48 (92.3)	47 (90.4)	43 (82.7)
PCV13 serotypes	119 (86.9)	81 (68.1)	46 (38.7)	106 (89.1)	111 (93.3)	112 (94.1)	72 (60.5)
6 A/B	52 (47.3)	48 (92.3)	7 (13.7)	47 (90.4)	48 (92.3)	47 (90.4)	43 (82.7)
3	2 (1.5)	1 (50)	2 (100)	1 (50)	2 (100)	1 (50)	1 (50)
19 A	7 (5.1)	3 (42.9)	6 (85.7)	7 (100)	7 (100)	7 (100)	3 (42.9)
Non-PCV13 serotypes	18 (13.1)	13 (72.2)	4 (22.2)	14 (77.8)	17 (94.4)	14 (77.8)	12 (66.7)
11 A/D	2 (1.5)	2 (100)	0	2 (100)	2 (100)	2 (100)	2 (100)
13	1 (0.7)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)
15 A/F	6 (4.4)	5 (83.3)	0	5 (83.3)	5 (83.3)	5 (83.3)	4 (66.7)
23 A	1 (0.7)	0	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
Non-VT13 type	8 (5.8)	5 (62.5)	3 (42.9)	5 (62.5)	8 (100)	5 (62.5)	5 (62.5)

Abbreviation: pbp2b, penicillin-binding protein; mef, macrolide efflux factor; ermB, erythromycin-resistance; tetM, tetracycline-resistance; PCV, pneumococcal conjugate vaccine; VT, vaccine-type.

private healthcare, the number of PM cases declined by 68 %, from 97 cases (2015–2019) to 31 cases (2020–2023). Our findings align with global and regional assessments from 2000 to 2015, demonstrating that PCV introduction into NEPI has significantly reduced morbidity and mortality [39]. These results provide strong evidence for policymakers to prioritize the introduction of the most appropriate PCVs into NEPI, considering factors such as serotype coverage, disease severity, and vaccine cost-effectiveness to enhance accessibility, reduce disease burden, and mitigate antimicrobial resistance.

This study has limitations, including the lack of data on prior antibiotic use and antimicrobial susceptibility testing, which could have provided deeper insights into resistance patterns. Additionally, surveillance was conducted in two pediatric hospitals in Ho Chi Minh City, which are central-level facilities that primarily manage severe or complicated cases, potentially leading to underestimation of local disease burden and resistance patterns. However, since most invasive pneumococcal meningitis cases are severe, provincial-level hospitals in the southern region typically refer such cases to these two facilities. Despite these limitations, this study provides critical insights into pediatric pneumococcal meningitis, contributing to the strategic planning for early diagnosis, optimal treatment, vaccine implementation, and antibiotic stewardship policies.

Conclusion

In Southern Vietnam, pneumococcal meningitis exhibits distinct age-dependent clinical manifestations, underscoring the need for timely diagnosis through clinical assessment and CSF analysis. Most cases were caused by PCV13 serotypes, which exhibited high prevalence of resistance-associated genes for penicillin, macrolides and tetracycline, along with a notable increase in resistance among non-PCV13 serotypes over the past 12 years. These findings emphasize the urgent need to introduce PCV into the NEPI, strengthen surveillance, and enforce antibiotic stewardship to monitor evolving serotypes and resistance trends, ultimately protecting pediatric health.

Author contributions

H.C.T., T.V.P., and Thuong.V.N. conceptualized and designed the study. H.T.N., Trung.V.N., and T.V.H. were responsible for data acquisition and management. T.V.P. and D.T.T.V. conducted the laboratory analyses. H.C.T. drafted the initial version and prepared the manuscript. H.C.T., N.S., and Thuong. V.N. provided input on the study interpretation and critically reviewed the manuscript. All authors reviewed and approved the final version.

Ethical considerations

The study was approved by the Institutional Review Boards of the Pasteur Institute in Ho Chi Minh City (approval Ref: 3723/GCN-PAS) on October 29, 2024, and adhered to the ethical principles of the 2013 Declaration of Helsinki.

Disclaimer statement

The findings and conclusions presented in this publication are those of the author and do not necessarily represent the official stance of the Vietnamese Ministry of Health or the Pasteur Institute in Ho Chi Minh City.

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Data availability

The data produced and analyzed in this study are not publicly accessible due to confidentiality and privacy restrictions. However, they can be obtained from the corresponding author upon reasonable request.

Abbreviations

ABR, antibiotic resistance; **AIC**, Akaike information criterion; **AOR**, adjusted odds ratio; **CI**, confidence interval; **CSF**, cerebrospinal fluid; **DTP-HepB-Hib**, diphtheria-pertussis-tetanus-hepatitis B-haemophilus influenzae type b vaccine; **ermB**, erythromycin ribosome methylation gene B; **IQR**, interquartile range; **mefA**, macrolide efflux gene A; **NEPI**, national expanded program on immunization; **PBM**, probable bacterial meningitis; **pbp2b**, penicillin-binding protein 2b gene; **PCV**, pneumococcal conjugate vaccine; **PI HCMC**, Pasteur Institute in Ho Chi Minh City; **PM**, pneumococcal meningitis; **Rt-PCR**, real-time polymerase chain reaction; **tetM**, tetracycline resistance gene M; **WBC**, white blood cell; **WHO**, World Health Organization

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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