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Characterizing *Neisseria meningitidis* in Southern Vietnam between 2012 and 2021: A predominance of the chloramphenicol-resistant ST-1576 lineage

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ABSTRACT

Objectives: Our goal was to describe Invasive Meningococcal Disease (IMD) in Southern Vietnam over the last 10 years. We characterized 109 *Neisseria meningitidis* strains in Southern Vietnam isolated between 1980s to 2021, that were collected from IMD (n = 44), sexually transmitted infections (n = 2), and healthy carriage (n = 63).
Methods: IMD were confirmed by bacterial culture and/or real-time polymerase chain reaction at the national reference laboratory in Pasteur Institute of Ho Chi Minh City (PIHCM). Antimicrobial resistance was determined on 31 IMD and two sexually transmitted infection isolates with E-test for chloramphenicol (CHL), penicillin (PEN), ciprofloxacin (CIP), ceftriaxone (CRO), and rifampicin (RIF). Sequencing was performed for analyzing of multilocus-sequence-typing (MLST), *porA, fetA*, and antibiotic resistance genes, including *gyrA, penA*, and *rpoB*.
Results: The incidence rate during this period was 0.02 per 100,000 persons/year. Serogroup B accounted for over 90% of cases (50/54). ST-1576 were mainly responsible for IMD, 27/42 MLST profiles, and associated with CHL resistance. Resistance was prevalent among IMD isolates. Thirteen were resistant to CHL (minimum inhibitory concentration [MIC] ≥16 mg/l), 12 were intermediate to PEN (MIC between 0.19 and 0.5 mg/l), and five were CIP-resistant (MIC between 0.19 and 0.5 mg/l). Particularly, one was non-susceptible to CRO (MIC at 0.125 mg/l), belonging to ST-5571 lineage. The resistance was due to carrying resistant alleles of *penA* and *gyrA* genes, and *catP* gene. Notably, seven isolates were resistant/non-susceptible to two or more antibiotics.
Conclusion: Our results suggest the persistence of the circulating ST-1576 in Southern Vietnam, with a spread of antimicrobial resistance across the community.

Introduction

Invasive meningococcal diseases (IMD), caused by *Neisseria meningitidis* (Nm*)*, remains a significant global public health concern worldwide. It is estimated that at least 1.2 million cases occur annually, with three peaks of incidence among infants under the age of 1 year among adolescents and among older adults beyond 75 years of age [1]. The death rate for IMD may range from 4.1% to 20.0% [2,3].

Nm is classified into 12 serogroups based on the capsules of polysaccharide it possesses, and six of these serogroups, namely A, B, C, W, X, and Y, account for more than 90% of IMD cases worldwide [1]. The prevalence of serogroups varies both geographically and temporally [4], and the use of vaccines plays a significant role in shaping the circulating serogroups in a particular area [2]. For instance, serogroup A (NmA) was predominant and caused multiple epidemics in the meningitis belt in the sub-Sahara region for many decades. However, with the introduction of the MenAfriVac in 2010, the incidence of NmA has significantly decreased in this region [5]. Currently, NmB is prevalent not only in IMD cases but in carriages across the North American, Europe, Australia, and the Asia-Pacific region. NmC, NmW, and NmY have been observed circulating in specifically geographic regions, such as NmC in China and Mexico, NmW in Africa, and NmY in Northern Europe and the United States [2,6].

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In Southern Vietnam, IMD has long been a mandatory notifiable disease. The first cases were notified as early as the 1950s, with 12 cases reported between 1950 and 1965. Subsequently, two major outbreaks occurred in Southern Vietnam. The first outbreak took place in 1971-1973, resulting in 930 cases. Meanwhile, the second one occurred between 1976 and 1979, which saw a high incidence of 20/100,000 persons in 1977, affecting 7000 cases. The serogroup responsible for the former epidemic was unknown, while in the latter epidemic NmC merged as the primary cause, presenting up to 96% of the reported cases [7]. Since the 1980s, the number of IMD cases has significantly declined and become sporadic, with a total of 29 laboratory-confirmed cases documented between the 1980s and 2011. Of these, 27 were attributed to NmB while two cases were caused by NmC.

It is worth noting to emphasize the significance of characterizing meningococci associated with IMD, including both isolates and clinical samples. Such characterization provides valuable information for the management of meningococcal diseases in public health. A concise description of meningococcal diversity, which relies on molecular techniques like sequencing, assists in tracing the spread of the pathogen. Multilocus sequence typing (MLST) has emerged as the "gold-standard" approach for *Neisseria* pathogen characterization and has greatly contributed to the epidemiology of other transmitted diseases. By utilizing the nucleotide sequence-based method, MLST enables the definition, tracing and intervention against various outbreaks, thus enhancing IMD surveillance and pathogenic biology [8].

Another integral part for describing meningococcal characteristics is typing the immunogenic outer-membrane proteins. Protein porin A (PorA) is a component of several MenB vaccines [9,10] and its typing provides crucial information not only for assessing the strain coverage by protein-based MenB vaccines but also for detailed epidemiological analysis of the bacteria.

Recently, there has been a global increase in the prevalence of meningococcal strains resistant to penicillin (PEN), ciprofloxacin (CIP), or rifampicin (RIF). These resistant and non-susceptible phenotypes are associated with specific modifications in *penA, gyrA,* or *rpoB*, respectively. Sequencing defined fragments of these genes and allowed the analysis of mutations at effective sites which influence the antibiotic resistance, as well as the identification of horizontal gene transfer among *Neisseria* species [6].

We hereby aimed to describe the characteristics of IMD in Southern Vietnam between 2012 and 2021. Additionally, we described molecular characteristics of Nm obtained from patients with IMD from the 1980s to 2020, as well as carriage isolates collected from healthy recruits in the same geographical region from 2012 to 2014 [11]. Information obtained from this analytic work provided essential insights into the epidemiology and characteristics of Nm strains in Southern Vietnam and their potential to affect individual-level and population-level health.

Materials and methods

Surveillance system in Southern Vietnam

The surveillance system for IMD in Southern Vietnam was established, requiring mandatory reporting of suspected cases to the Pasteur Institute of Ho Chi Minh City (PIHCMC), a subnational public health institute. Upon receiving specimens and isolates, they underwent species identification and determination of serogroups. The current national surveillance guidelines, updated in 2012 [12], define a suspected case as any individual with fever and at least one of the following signs: headache, bulging fontanel (children under 1 year of age), vomiting, neck stiffness, altered or reduced consciousness, photosensitivity, appearing petechial or purpuric rash, and manifestation of septic shock. A confirmed case is defined as a suspected case that meets at last one of the following criteria: positive for polymerase chain reaction (PCR) or bacterial culture from blood, cerebrospinal fluid, or petechial swabs.

Nm was identified through conventional and molecular methods. For bacterial culture, specimens were streaked on blood or chocolate agar plate, made by supplying 5% sheep blood to Columbia base agar (Oxoid, Hants, UK). The isolates were then subjected to biochemical testing with API NH Kit (bioMerieux, Marcy-L'Etoile, France). For molecular method, DNA was extracted from specimens using QIAamp DNA mini Kit (Qiagen, Hilden, Germany). We applied real-time PCR (rt-PCR) detecting the *sodC* gene of Nm, using the PerfeCTa qPCR TouchMix (Quanta Biosciences, Gaitherburg, USA), primers, and probes from IDT (Coralville, USA) and running on ABI7500 fast instrument (Applied Biosystems, Waltham, USA). This protocol was according to the standard methods of the World Health Organization [1]. Serogroup A, B, C, W, X, or Y were determined by latex agglutination using Wellcogen Bacterial Kit (Remel, Kent, UK) and/or by serogroup-specific monoplex rt-PCR as mentioned above.

Clinical samples and bacterial isolates

We enrolled 109 meningococcal strains for the characterization, including 44 IMD, two STI, and 63 from healthy carriers. Of the IMD, 11 isolates were from the 1980s and 2003; 20 isolates and 13 clinical samples from 2012-2021. The specimens were selected for sequencing based on their rt-PCR cycle-threshold (Ct) value below 30. The two STI isolates, NmB and NmY, were from adult patients-cases who presented with urethritis at a hospital in Ho Chi Minh City in 2019. For carriage, the isolates were obtained from healthy military recruits in Southern Vietnam between 2012-2014, as part of the previous study [11].

Antimicrobial susceptibility testing (AST)

AST was performed on the 33 pathogenic isolates using E-test strips (Liofilchem, Abruzzi, Italy) and Muller-Hinton agar plates (Oxoid, Hants, UK) supplemented with 5% sheep blood. Minimum inhibitory concentration (MIC) was determined for five antibiotics: PEN, CIP, RIF, CRO, and CHL. The susceptible, intermediate, and resistant categories were interpreted according to the recommendations of the European Monitoring Group on Meningococci (EMGM) [13]. Specifically, the intermediate category for PEN was defined as MIC values between 0.125 and 1 mg/l.

MLST and genotyping

We performed sequencing on 42 out of 44 pathogenic and 63 carriage strains. Two IMD isolates collected in 2021 were characterized for serogroup and AST only due to resource limitations. The sequencing approach targeted the seven housekeeping genes used in MLST [8,14], using either 3130 or 3130xl capillary DNA analyzer (Applied Biosystems, Waltham, USA). Additionally, *porA* and *fetA* genes were sequenced for fine-typing [9,15]. We analyzed three antimicrobial resistant genes, namely *rpoB, gyrA*, and *penA*, responsible for RIF, CIP, and PEN resistance, respectively [16–18]. The resulting sequences were edited and assembled using Chromas v2 and Bioedit v7.5 software. Allele numbers and sequence profiles were obtained through the *Neisseria* MLST database (https://pubmlst.org/organisms/neisseria-spp).

We conducted whole genome sequencing (WGS) for 13 IMD isolates selected based on their resistance to CIP, PEN, CRO, or CHL and two STI isolates. The WGS was performed using Miseq System and the Nextera XT Kit v2 (Illumina, San Diego, USA) following the standard protocol, running 300 cycles on Miseq System to generate 300bp paired-end reads. The generated reads were then subjected to *de novo* assembly using Spades v.3.13 [19]. The resulting assemblies were submitted to both the MLST database and National Center for Biotechnology Information (NCBI) Genebank under BioProject IDs: PRJNA671557 and PRJNA672782. To build a phylogenetic network, we used a gene-by-gene approach through the Genome Comparator tool on the PubMLST, then visualized with Splitstree v4 [20].

NmB comparison

Isolates from Vietnam were grouped with goeBURST [21] to determine their genetic relationships, utilizing MLST profiles obtained from the database accessed in March 2023 and defined to group at single and double locus variants. To further assess the genetic relatedness, we compared the NmB in Vietnam to those in countries in Asia. We conducted a search of the PubMLST *Neisseria* database (accessed Mar-14-2023) for all NmB genomes in Asia, yielding 370 genomes. A neighbor-joining tree was generated using concatenated nucleotide sequences of the 1605 loci of the cgMLST, then being annotated and visualized with iTOL [22].

Results

Description of confirmed Invasive Meningococcal Disease cases in Southern Vietnam

From 2012-2021, 54 IMD cases were confirmed at the national reference laboratory; of those, only 20 meningococcal isolates were collected. NmB was the most common, accounting for 93% (50/54) of the confirmed cases, and it was the only serogroup detected since 2013. NmC was detected in four cases over the 10-year study period.

The incidence rate annually averaged 0.02 per 100,000 inhabitants per year in this period. Male patients constituted 70% of the confirmed cases (38 out of 54). Age distribution revealed that 17 cases were under the age of five, three cases were in 5-17, 25 cases were in 18-24, and four cases were above 25 years of age. The majority of confirmed cases (42 out of 54) presented with clinical manifestations consistent with meningitis. Ten of those were associated with sepsis, while 12 cases presented with septicemia alone.

Antimicrobial susceptibility testing

All 20 IMD isolates from 2012-2021 period were non-susceptible/resistant to CRO, PEN, CIP, or CHL. Of these, 13 were resistant to CHL (MIC values ranging between 16 and 256 mg/l), 11 were intermediate to PEN (MIC: from 0.19 to 0.38 mg/l), five were resistant to CIP (MIC: between 0.19 and 0.5 mg/l), and one isolate was non-susceptible to CRO, with MIC value at 0.125 mg/l. Notably, seven of which were multiple-resistant as being resistant/non-susceptible to at least two different antibiotics. Meanwhile, those between the 1980s and 2003 were resistant to CHL only, with four isolates having MIC ≥32 mg/l. The STI NmB was intermediate to PEN (MIC at 0.5 mg/l) and resistant to CIP (MIC at 0.094 mg/l) (Table 1).

MLST characterization and genotyping

Analysis of 42 MLST profiles of Nm isolates causing IMD revealed 20 different STs, 15 of which were unidentified previously. Eight of these ST were associated with specific CC, including four NmB in hyper-invasive CC41/44, two NmC and one NmB in CC4821, and one NmB in CC162. The most prevalent ST was ST-1576 lineage, which was found in 27 strains, and all belonged to NmB. PorA VR1/VR2 was classified into 13 different subtypes. PorA of P1.19,15 was the most popular, representing in 15 strains. Three NmC expressed subtypes of P1.5-1,2-2 (two isolates) and P1.7-4,13-20. FetA was represented by nine variants, of which F4-6 and F1-5 were commonly identified with 14 and nine trains, respectively. Genotype of B:P1.19,15:F4-6;ST-1576 was dominant and found in nine strains among while three strains of ST-4821 complex displayed solely one genotype as P1.5-1,2-2:F5-8 (Table 1).

For the carriage, 63 MLST profiles were categorized into 17 different STs and they belonged to five defined CCs, including CC41/44 (n = 10), CC1136 (n = 8), CC4821 (n = 9), CC175 (n = 1), and CC32 (n = 1). NmB isolates showed high diversity, they were found in five CC but mainly in ST-1576 derivatives that are not assigned to a known clonal complex (21). NmC strains belonged to two CC: ST-41/44 and ST-4821 complex, found in five and seven isolates, respectively. NmNG strains were mainly associated with CC1136 (n = 6) and ST-1576 lineage (n = 9). The genotypes of B:P1.19,15:F4-6:ST-1576 (n = 16), C:P1.5-1,2-2:F5-8:ST-4821 (n = 9), NG:P1.12-24,13-1:ST-1576 (n = 7), and NG:P1.18-4,25:F4-1:ST-1136 (n = 6) were most common in carriage isolates (Table 2).

Antimicrobial resistance gene typing

For IMD, isolates resistant to CIP harbored *gyrA*8 or *gyrA*380 alleles with the mutation at T91I while all CIP-susceptible strains had wild-type (WT) *gyrA*. There were four alleles of *penA* that had the mutations F504L, A510V, I515V, H541N, and I566V, including *penA*7, *penA*587, *penA*939, and *penA*940, and they were from the PEN-intermediate isolates. At the same time, all *rpoB* alleles were relevant to the susceptible type. To identify the gene that was responsible for CHL resistance, we submitted the WGS assemblies to the Resfinder (https://cge.food.dtu.dk/services/ResFinder/). We found that all CHL-resistant isolates contained a 624 bp fragment of chloramphenicol acetyltransferase (*catP*) gene, origin from transposon *Tn4451* of *Clostridium perfringens*.

Among carriage isolates, we identified 21 out of 63 bearing either *gyrA*8 or *gyrA*269, which had mutations at T91I or D95N, respectively. While *rpoB* was identified six different alleles but all were WT.

Genetic relationships between isolates in Southern Vietnam

There was a close relationship between NmB causing IMD in Southern Vietnam, as indicated by their MLST profiles and the clustering pattern in the goeBURST. The majority of these isolates belonged to ST-1576 and its derivatives (single locus variants or double locus variants), comprising of 10 different ST: ST-1576 as the founder, ST-13860, ST-11013, ST-11005, ST-11006, ST-12692, ST-14737, ST-14807, ST-15186, and ST-15565.

Analyzing core-genome MLST, the ST-1576 lineage stood far apart from other strains in Vietnam (Figure 1). In comparison to other NmB strains in Asia, the CHL-resistant ST-1576 also formed a distinct clade (Figure 2).

We observed an association between AST and ST-1576 lineage. All IMD ST-1576 isolates were resistant to CHL, except one isolate ST-1576 collected in 1986 was susceptible to CHL. Genotypically, this isolate, typed as B:P1.5,2:F1-5:ST-11013, differed from the rest within the lineage and placed a distinct position (Figure 1). Five isolates, typed as B:P1.7-2,13-3:F1-5:ST-15186, B:P1.19,15:F4-6:ST-13860 (n = 2), B:P1.19,15:F4-6:ST-11006, and B:P1.7/16-83:F5-2:ST-15565 were additionally resistant to CIP and/or intermediate to PEN (Table 1). For carriage ST-1576 isolates, the CHL resistance was found in both NmB and NmNG isolates.

To assess the prevalence of the *catP* gene among Nm, we utilized the *in-silico* PCR tool on the PubMLST with two primers from the prior work [23]. The expected product length was set at 1209pb. A search in The Genomes database search, limited to *N. meningitidis* species and total length ≥ 1 Mbp, resulted in 36,518 genomes. No mismatches were allowed for both primers, and the product length was set between 1200 pb and 1500 pb to match the length of the original *catP* gene (1472bp) (GenBank accession number AF031037). Our findings revealed that only the genomes of ST-1576 and its derivatives were positive, generating the expected 1209 pb products. Notably, the genome of NmB ST-8434, as a singleton, from Bangladesh (PubMLST ID: 94068) also contained the *catP* gene. However, this strain clustered with the same group as ST-1576 at the level of 1605 loci of cgMLST (Figure 2).

Discussion

We observed a consistent predominance of NmB among IMD cases in Southern Vietnam during the last decade, aligning with trends observed in other Asian countries. A striking finding in this study is the significant

Table 1
The genotypes and resistance profile of pathogenic meningococcal strains in Southern Vietnam by time.

# of isolates	Serogroup	CC	ST	PEN	CRO	RIF	CIP	CHL	*penA*	*rpoB*	*gyrA*	PorA VR1/VR2	FetA	Comments
1	B	ST-4821	15710	0.25/I	0.002/S	<0.5/S	<0.03/S	<2/S	F504L, A510V, I515V, H541, I566V (*penA939*)	WT	WT	5-1/2-2	F5-8	
2	C		11003	0.38/I	0.002/S	<0.5/S	<0.03/S	<2/S	NA	WT	WT	5-1/2-2	F5-8	
1	B	ST-41/44	6058	0.25/I	0.002/S	<0.5/S	<0.03/S	<2/S	NA	NA	NA	18-1/34	F1-5	
2	B		303	<0.06/S	0.002/S	<0.5/S	<0.03/S	<2/S	WT	WT	WT	19/15	F1-5	
1	B		11014	<0.06/S	0.002/S	<0.5/S	<0.03/S	<2/S	WT	WT	WT	22-1/14	F5-2	
1	B	Not assigned, linked to	15186	0.25/I	0.002/S	<0.5/S	<0.03/S	>16/R	F504L, A510V, I515V, H541, I566V (*penA939*)	WT	WT	7-2/13-2	F1-5	MR
1	B	ST-1576	1576	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	NA	WT	WT	5-1/2-19	F4-6	
6	B		1576	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	WT	WT	WT	19/15	F4-6	
1	B		1576	<0.06/S	0.003/S	<0.5/S	<0.03/S	>16/R	WT	WT	WT	22-25/14	F4-6	
1	B		1576	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	NA	NA	NA	19/15	F5-137	
2	B		1576	<0.06/S	0.003/S	<0.5/S	<0.03/S	>16/R	WT	WT	WT	12-24/ 13-1	F4-6	
1	B		1576	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	WT	WT	WT	19/15	F1-5	
1	B		13860	0.25/I	0.002/S	<0.5/S	0.19/R	>16/R	F504L, A510V, I515V, H541N, I566V (*penA7)*	WT	T91I (*gyrA 8)*	19/15	F4-6	MR
1	B		13860	0.125/I	0.002/S	<0.5/S	0.19/R	>16/R	WT	WT	T91I	19/15	F4-6	MR
1	B		11005	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	WT	WT	WT	19/15	F1-5	
1	B		11006	0.38/I	0.002/S	<0.5/S	<0.03/S	>16/R	F504L, A510V, I515V, H541N, I566V (*penA587*)	WT	WT	19/15	F4-6	MR
1	B		11013	<0.06/S	0.006/S	<0.5/S	<0.03/S	<2/S	WT	WT	WT	5/2	F1-5	
1	B		14807	<0.06/S	0.002/S	<0.5/S	<0.03/S	>16/R	NA	NA	NA	5-1/2-2	F1-5	
1	B		1576									7-2/16-83	F5-2	CSF
1	B		1576									22-25/14	F1-7	CSF
1	B		1576									NA	NA	CSF
1	B		12962									20/23-6	F1-91	Blood
1	B		13860									NA	F4-6	CSF
1	B		13860									NA	NA	CSF
1	B		13860									19/15	F4-6	CSF
1	B		14737									22-25/14	F5-8	CSF
1	B		15565	0.5/I	0.002/S	<0.5/S	0.094/R	NA	F504L, A510V, I515V, H541N, I566V (*penA939)*	WT	T91I (*gyrA 380)*	7/16-83	F5-2	STI; MR
1	B	Not assigned (linked to	13863	0.25/I	0.002/S	<0.5/S	0.19/R	<2/S	F504L, A510V, I515V, H541N, I566V (*penA587*)	WT	T91I (*gyrA 380)*	22/14	F5-1	MR
1	B	ST-5571)	5571	0.38/I	0.125/NS	<0.5/S	0.19/R	<2/S	F504L, A510V, I515V, H541N, I566V (*penA940)*	WT	T91I (*gyrA 380)*	22/14	F5-1	MR
1	B		13837									22/14	F5-1	Blood
1	B		13863									NA	NA	Blood
1	B		13863									22/14	F5-1	Specimen
1	C	Singleton	11004	<0.06/S	0.002/S	<0.5/S	<0.03/S	<2/S	NA	WT	WT	7-2/13-20	NA	
1	Y	ST-23	23	<0.06/S	0.002/S	<0.5/S	<0.03/S	NA	WT	WT	WT	5-2/10-1	F5-12	STI isolate
1	B	ST-162										NA	F5-9	CSF; §
1	B	Singleton	11338									18-1/34	F1-5	CSF

The minimal inhibitory concentration (in mg/l) breakpoints were as:
PEN: ≤0.06 mg/l (S: susceptible); 0.125-1 mg/l (I: Intermediate); >1 mg/l (R: Resistant).
CRO: ≤0.12 mg/l (S).
RIF: ≤1 mg/l (S); >1 mg/l (R).
CIP: ≤0.03 mg/l (S); ≥0.06 mg/l (R).
CHL: ≤2 mg/l (S) >2 mg/l (R).
NA: not accessed; WT: wild type; NS: non-susceptible; MR: multiple-resistant; §: 6/7 household genes were completely sequenced.

Table 2
Genotype of carriage isolates in Southern Vietnam from 2012 – 2024. The resistance profiles were from the previous study in 2016, which was performed with disk diffusion method, except CIP.

# of isolates	Serogroup	CC	ST	CRO	RIF	CIP	CHL	*rpoB*	*gyrA*	PorA_VR1/VR2	FetA	Comments
1	NG	ST-1136	1136	S	S	S	S	WT	WT	18-4/25-61	F4-1	
1	B		13859	S	S	≤ 0.03/S	S	WT	WT	18-4/25	F4-1	
6	NG		13859	S	S	≤ 0.03/S	S	WT	WT	18-4/25	F4-1	
5	B	Not assigned (linked to	1576	S	S	≤ 0.03/S	R	WT	WT	7-2/16-83	F5-2	
2	NG	ST-1576)	1576	na	na	na	na	WT	WT	12-24/13	F4-6	
7	NG		1576	na	na	S	R	WT	WT	12-24/13-1	F4-6	
1	B		13860	S	S	≤ 0.03/S	R	na	NA	19/15	F4-6	
15	B		13860	S	S	0.125/R	R	WT	T91I (*gyrA8)*	19/15	F4-6	
1	B		15344	S	S	0.125/R	R	WT	T91I (*gyrA8)*	19/15	F5-8	
1	NG	ST-175	175	na	na	na	S	WT	WT	18-1/3	F5-99	
1	B	ST-32	33	S	S	na	S	na	NA	20/15-35	F1-32	
1	B	ST-41/44	44	S	S	S	S	na	NA	na	na	
1	C		44	S	S	S	S	WT	WT	7-2/13-1	F1-7	
1	C		2973	S	S	S	S	WT	WT	7-2/16-83	F1-7	
1	C		14741	S	S	≤ 0.03/S	S	WT	WT	18-4/16-83	F1-7	
1	C		15217	S	S	S	S	WT	WT	7-2/13-21	F1-7	
1	C		15346	S	S	0.125/R	S	WT	D95N (*gyrA 296)*	7-2/13-19	F1-7	
1	NG		15346	na	na	na	S	WT	D95N (*gyrA 296)*	7-2/13-2	F1-7	
1	NG		15346	na	na	na	na	WT	D95N (*gyrA 296)*	7-2/13-1	F1-7	
1	NG		15346	na	na	S	S	WT	NA	7-6/13	F1-7	
1	NG		15346	na	na	na	na	WT	NA	7-2/13	F1-7	
6	C	ST-4821	15342	S	S	≤ 0.03/S	S	WT	WT	5-1/2-2	F5-8	
3	C		15343	S	S	na	S	WT	T91I (*gyrA8)*	5-1/2-2	F5-8	
1	B	Not assigned (linked to	5571	S	S	S	S	WT	WT	22/14	F5-1	
1	B	ST-5571)	13863	na	na	na	na	na	na	22/14	F5-1	
1	B	Not assigned	13857	S	S	S	S	na	na	7-2/16-83	F5-1	

CIP: ≤ 0.03 mg/L (S); ≥ 0.06 mg/L (R).
NA: not accessed; WT: wild type

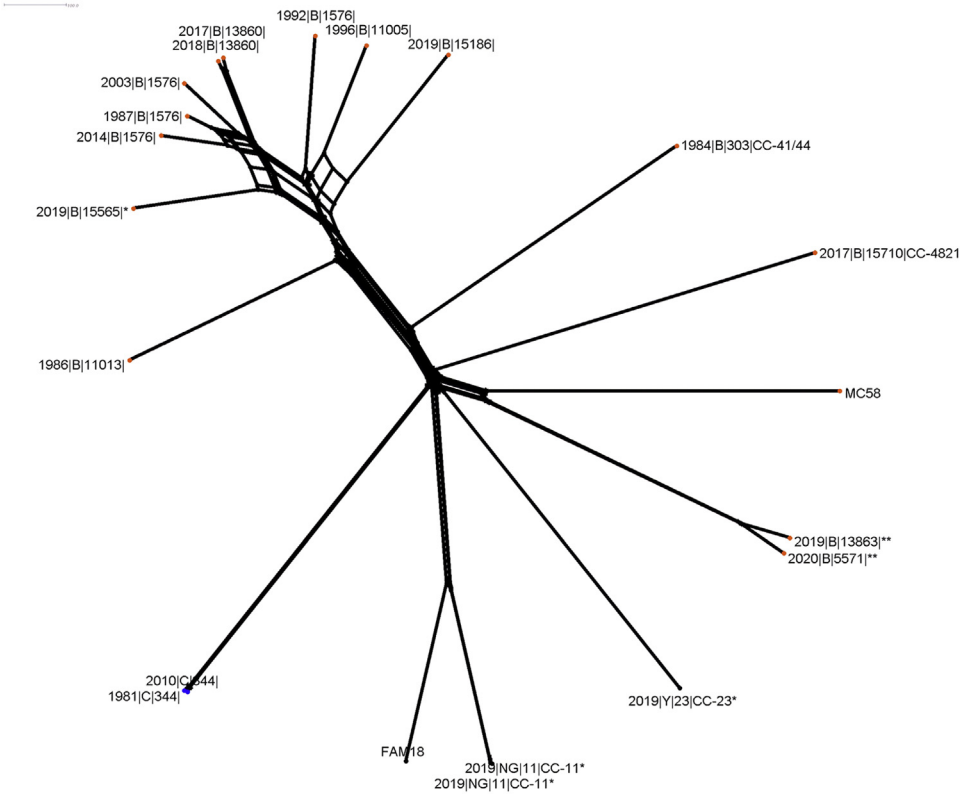

Figure 1. The network depicted a relationship between STs in Southern Vietnam, using the gene-by-gene approach on the PubMLST website. Orange circle depicted NmB ST-1576 lineage, orange square for other NmB ST, black for CC-11, blue for NmC, and black for references (FAM18 and MC58 strains). The labels were annotated according to order: the year of collection|serogroup|sequence type|clonal complex. * sexually transmitted diseases; ** non-susceptible CRO.

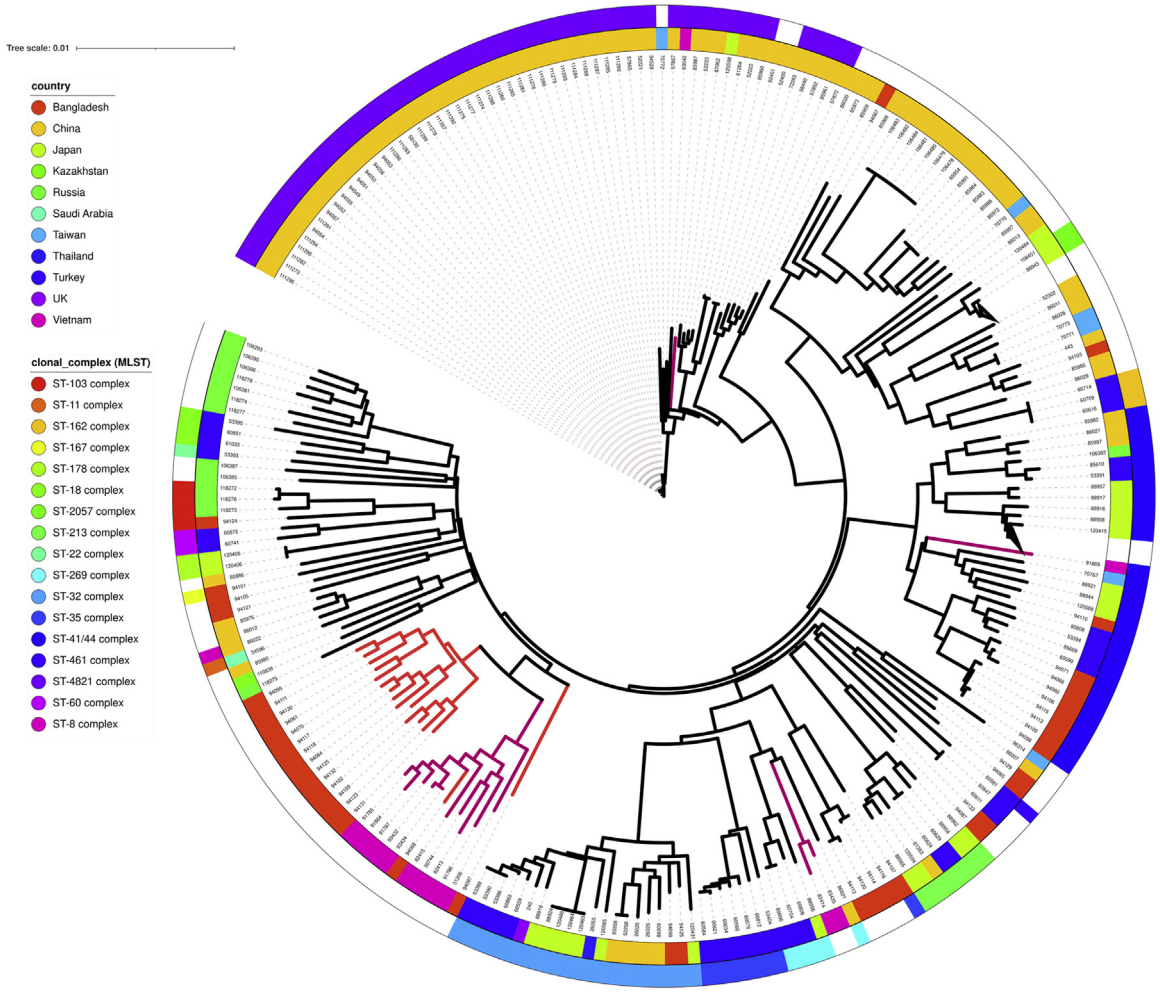

Figure 2. Determining the relationships between isolates in Vietnam and in other countries of Asia, building a neighbor-joining tree with cgMLST scheme of 1605 loci. The outer most circle illustrates the year of collection, the middle represents for clonal complexes and the inner depicts for countries. Green branch represents for isolates in Vietnam, orange for the rest.

prevalence of ST-1576 lineage which is strongly associated with CHL resistance. The CHL-resistant Nm was first detected in Southern Vietnam in 1987 [23], and our study confirmed that these were ST-1576.

In 2012, a cluster of 10 IMD cases detected among industrial workers in Ho Chi Minh City was due to NmB ST-1576, indicating the significance of this lineage in regional epidemiology.

The mechanism of CHL resistance is due to the action of chloramphenicol acetyltransferase (CAT enzyme) produced by *catP* gene. CAT attaches an acetyl group of Acetyl-CoA to CHL, preventing CHL from binding to bacterial ribosomes and thus inhibiting bacterial growth [23,24]. However, it remains unexplained why *catP* gene is exclusively present within ST-1576 lineage but has not been horizontally transferred to other serogroups or CC.

Regarding the emergence of ST-1576 lineage, we hypothesize that the CHL-resistant lineage may have emerged in the 1980s when CHL was the standard for treatment for IMD in Vietnam. This extended use may have allowed the selection of the resistance among Nm. This may be because Oberti and colleagues reported that no detection of CHL-resistant Nm was found in Southern Vietnam in the 1976-1979 epidemic [7]. Moreover, one isolate in this study, 13515, designed as B:P1.5,2:F1-5:ST-11013, may be the earliest ST-1576 lineage isolate known when being collected in 1986. Remarkably, this isolate was susceptible to CHL and did not harbor *catP* gene.

The derivatives of ST-1576 show a high degree of diversity, with 10 different ST and 15 distinct finetypes being identified among 28 pathogenic strains. This lineage can be found in various states: encapsulated and non-encapsulated forms, causing IMD, STI, or being carried asymptomatically. In this study, however, the carriage NmB ST-1576 isolates showed lower diversity, with only three ST and four finetypes. NmNG ST-1576, meanwhile, expressed a unique finetype: P1.7-2,16-83:F5-2. It is important to note that the carriage isolates were collected in specific population, military camps, in a short period, which may have limited the diversity among them.

Currently, only a few countries have reported the NmB ST-1576 in the PubMLST database, including one from Italy in 2002 and two in the United States in 1997 and 2008. The majority circulates from Northern to Southern Vietnam and other South East Asian countries, such as Thailand, Laos, and Cambodia [25–27]. Our results suggested that NmB ST-1576 poses a significant public health thread in Vietnam and neighboring countries. Hence, it is crucial to implement effective surveillance and control measures to prevent and control its spread.

Another remarkable finding was related to CC4821, which showed a single finetype, P1.5-1,2-2:F5-8. This finetype was first described in our study as it was not reported among other isolates within the ST-4821 complex. The three IMD isolates in Vietnam were intermediate to PEN due to bearing mutations of *penA939*, while being susceptible to CIP. However, we found three carriage isolates had a mutation of *gyrA8* at T91I which is responsible for CIP resistance. Additionally, one IMD isolate, typed as B:P1.5-1,2-2:F5-8:ST-4821, clustered into lineage L44.4, which is mostly distributed in China and India [28].

We also observed that almost 40% of IMD and 30% of carriage strains in Southern Vietnam expressing *porA* of P1.19,15 which is identical to the vaccine strain used in the MenB vaccine, VA-Mengoc BC (Finlay Institute, Cuba) [29]. Indicating that this MenB vaccine may be effective against NmB in Vietnam as ever seen in Cuba and other Latin American countries [4,30].

Of great concern was the emergence of resistance to antibiotics used for treatment and prophylaxis such as CRO, PEN, CHL, and CIP. We found seven IMD isolates were resistant/non-susceptible to at least two different antibiotics as mentioned above. Although Nm nonsusceptible to third-cephalosporin has been reported worldwide [6,10,31], it remains extremely rare. CRO is the first-frontline antibiotic for treatment IMD, while PEN and CHL are only used for certain cases [32]. The previous study showed that 50% (63 out of 126) carriage isolates were resistant to CIP between 2012 and 2014 [11]. When matching to the results of *gyrA* typing from this work, these isolates contained mutations in *gyrA8* and *gyrA296* at T91I and D95N, respectively. The suboptimal hygiene and crowded condition in military camps may enhance the risk of wider transmission of the CIP-resistant Nm strains. Moreover, a high frequency of CIP resistance was also detected among STI NmUC isolates in Southern Vietnam [33]. These suggested that CIP resistance may widely spread and become a problem in public health in Vietnam. Therefore, there is a need for surveillance of the circulating of CIP-resistant Nm.

One of the main limitations in this report was on the analysis which was limited to Southern Vietnam only, while it may exist regional variations in epidemiology, strain distribution, and antimicrobial resistance. Additionally, the absence of WGS for all isolates may limit the ability to broaden genomic understanding, which could offer insights into the dynamics of adaptation and transmission. Lastly, the lack of information on *penA* among carriage isolates limits the prevalence of PEN resistance in the community. This may give the potential difference in resistance profiles between IMD and carriage strains.

Conclusions

Meningococcal serogroup B predominantly circulated in Southern Vietnam and produced more than 90% of cases. NmB ST-1576 lineage strains contributed mainly to the IMD cases in Vietnam. This ST has existed in Vietnam since 1986, has highly diversity with many different genotypes, and is mainly distributed in Vietnam and neighbor countries. The emerging antibiotic-resistant strains raise a great concern in the community, especially resisting CRO and CIP which are currently used for treatment and chemoprophylaxis.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

This study has been considered and approved by the Institutional Review Boards of the Pasteur Institute in Ho Chi Minh City (approval Ref: 06/2023/CN-HDDD).

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Author contributions

TVP was responsible for sequencing work, analysis data and wrote the first draft with input from PDQ and MKT. VTTD and HNLT performed bacterial culture and antimicrobial susceptible tests. CMT and was involved into day-to-day testing management. MKT and NVT contribute to the study design and oversaw result interpretations. NVT and LCQ contributed study design and establishment the surveillance network. PDQ and LCQ analyzed and interpreted epidemiological information. MKT and NVT contribute to the study design and oversaw result interpretations. All authors contributed to refinement and approved the manuscript.

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