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SEROGROUPS AND *Neisseria meningitidis PORA, FETA* AND *FHBP* GENE DISTRIBUTION IN VIETNAM FROM 2014 TO 2024

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ABSTRACT

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis,* is still a public health concern. There are 12 *N. meningitidis* serogroups, but serogroups A, B, C, Y and W are responsible for nearly all cases of IMD. This study aimed to investigate the serogroups, subgenotypes, FetA variants and FHbp antigenic profiles of *N. meningitidis* strains isolated in Vietnam from 2014 to 2024. Serogrouping of *N. meningitidis* by multiplex PCR assay and the genetic profiles, including the *porA, fetA,* and *fHbp* genes were analyzed using Sanger sequencing and bioinformatic methods. Of the 186 strain isolates, the most common serogroup was B (161 isolates, 86.6%), followed by serogroup C (n = 5, 2.7%) and the remaining rates were non-serogroupable. PorA was identified in this study, including 13 VR1, 21 VR2 and 6 VR3 families. The most common VR1 families were P1.22-25 (n = 36, 23.2%) followed by P1.7-2 (n = 31, 20.0%), P1.22 (n = 24, 15.5%), and P1.22-11 (n = 19, 12.3%). The most frequent VR2 variants were 14 (n = 60, 38.7%), 15-25 (n = 20, 12.9%). The three most common profiles of the *porA* gene were P1.22-25,14,36 (n = 24, 15.5%) and P1.22,14,36 (n = 22, 14.1%), and P1.22-11,15-25,36 (n = 19, 12.2%). For *FetA VR* types, the most common allele variant was F1-7, accounting for 40% (60/150), followed by F5-1 with 20% (30/150), and F3-16 with 12.7% (19/150). The FHbp variant group 2/subfamily A was most common (n = 142; 97.3%), followed by group 1/subfamily B (n = 4; 2.7%). Five variants of subfamily A were found. The A22 variant had the highest prevalence (n = 52; 19.7%). On the basis of genotypic data, poor matching was shown between the PorA type in the VA-MENGOC-BC vaccine as well as the PorA and FHbp components of the 4CMenB

vaccine to PorA and FHbp of meningococcal serogroup B strains isolated in Vietnam from 2014 to 2024.

Keywords: Factor H binding protein, genosubtype, invasive meningococcal disease, outer membrane protein porin A, serogroup.

INTRODUCTION

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis,* is still a public health concern that is associated with high morbidity and mortality (Nadel & Ninis, 2018). The meningococci are often present in the oral cavity and nasopharynx, with asymptomatic carriage. The prevalence varies by age and geographical areas (Read, 2019). When the *N. meningitidis* escapes the mucosal barrier and replicates within the blood, IMD often occurs and progresses rapidly, with a case fatality rate of about 8–15% even with suitable and timely antibiotic treatment (Batista *et al.*, 2017). There are about half a million cases of IMD worldwide each year, with incidence varying across geographical regions and age, but the highest rate occurs in infants and young children as well as adolescents (Parikh *et al.*, 2020). Based on the chemical structure of its polysaccharide capsule, *N. meningitidis* is separated into 12 serogroups: A, B, C, E, H, I, K, L, X, W, Y and Z. Five of these serogroups (A, B, C, W and Y) cause almost all IMD cases, with great variation in serogroup by geographic areas as well as over time (Purmohamad *et al.*, 2019). Recently, serogroup X has caused local outbreaks of meningococcal disease in Africa (Parikh *et al.*, 2020). Several molecular typing methods have been developed to study the molecular epidemiology of *N. meningitidis*. According to the recommendations of the European Centre for Disease Prevention and Control (ECDC), along with monitoring serogroups and testing antibiotic susceptibility, it is necessary to monitor molecular markers such as the variable regions of genes *porA* and *fetA*, the gene encoding the factor H binding protein - fHbp, and typing by multilocus sequence typing (MLST) (ECDC, 2012).

Vaccination is considered one of the most effective measures to control epidemics caused by *meningitidis*. Vaccines based on capsular polysaccharide protein conjugates are used to protect against meningococcal serogroups A, C, W, and Y disease but not against strains of serogroup B (NmB) (McCarthy *et al.*, 2018). The NmB capsular polysaccharide is similar to molecules present on human neuronal cells, so it has poor functional immunogenicity and is not a suitable vaccine antigen (Finne *et al.*, 1983). To overcome this challenge, Outer Membrane Vesicles (OMV) have been used to develop vaccines against meningococcal serogroup B disease, such as VA-MENGOC-BC, MenBvac, and MeNZB. Vaccines have been shown to be effective in the control of NmB epidemics in different countries (Petousis-Harris, 2018). Because of the high diversity in PorA protein, protective immunity of OMV vaccines was limited to disease isolates that have the same PorA type as the vaccine strain. Therefore, OMV vaccines were not suitable for broad protection against heterogeneous PorA type NmB (Holst *et al.*, 2009).

For the development of a broad vaccine against NmB, protein vaccine candidates have been introduced. By the technique of

reverse vaccinology, three surface-exposed proteins of NmB, including Neisseria adhesion A (NadA), factor H binding protein (FHbp) and Neisserial Heparin Binding Antigen (NHBA) were selected as promising antigens (McNeil *et al.*, 2013). At present, 4CMenB and rLP2086 are two cross-protective vaccines against NmB. Vaccines have been licensed and approved in several countries worldwide. The 4CMenB vaccine contains Toneatto three major protein antigens FHbp (variant 1.1), Neisserial Heparin Binding Antigen (NHBA, peptide 2), and Neisseria adhesion A (NadA, variant 3.1), plus the outer membrane vesicle (OMV, P1.7-2,4); rLP2086 contains two variants of FHbp (variants 1.55 and 3.45). Each vaccine was limited by the numbers and variants of the protein (Toneatto *et al.*, 2017). Consequently, they cannot provide universal protection against diverse NmB across different regions. Surveillance of vaccine antigens, especially fHbp, is important to estimate the potential coverage of those NmB vaccines. Therefore, we conducted this study to describe the serogroups and distribution of meningococcal PorA, FetA and FHbp types in Vietnam from 2014 to 2024 and provide information to predict vaccine coverage across NmB strains in Vietnam.

MATERIALS AND METHODS

Selection of *N. meningitidis* isolates

We collected 186 *N. meningitidis* strains, including 35 strains that were isolated from 2014 to 2018 and 151 strains isolated from 2019 to 2024. Bacteria were identified by Gram stain and using biochemical tests: oxidase reaction and API® NH cards (bioMérieux, France). Pure isolates were stored at -80°C in brain heart broth medium with 15% glycerol. This study has been considered and approved by the Joint Vietnam-Russia Tropical Science and Technology Research Center (Approval ref: 1047/CN-HĐĐĐ).

Polymerase chain reaction

Specific primers for *N. meningitidis* serogrouping were based on those described previously (Table 1) (Taha, 2000).

Table 1. Sequences of primers for *N. meningitidis* serogrouping.

Target gene for identification	Sequences (5'-3')	Size (bp)
orf-2 (A)	CGCAATAGGTGTATATATTCTTCC	400
	CGTAATAGTTTCGTATGCCTTCTT	
siaD (B)	GGATCATTTCAGTGTTTTCCACCA	450
	GCATGCTGGAGGAATAAGCATTAA	
siaD (C)	TCAAATGAGTTTGCGAATAGAAGGT	250
	CA ATCACGATTTGCCCAATTGAC	
siaD (W135)	CAGAAAGTGAGGGATTTCCATA	120
	CACAACCATTTTCATTATAGTTACTGT	
siaD (Y)	CTCAAAGCGAAGGCTTTGGTTA	120
	CTGAAGCGTTTTCATTATAATTGCTAA	

For determination of serogroups (A, B, C, Y, and W135), a multiplex PCR assay was performed with specific primers of the *siaD* gene (serogroups B, C, Y, and W135) and the *orf-2* gene (serogroup A).

The commercialized DNA purification kit (Biobasic, Canada) was used to extract DNA from *N. meningitidis* following the manufacturer's protocol. A total 50 µl volume of components for PCR contained 19.5 µl H_2O, 25 µl of PCR Master Mix (2X), 1.25 µl of primer F/R (10 pM) and 3 µl of DNA template. The thermal cycle of the reaction: 94ºC in 2 min, followed by 35 cycles of 94ºC in 20 sec, 64ºC in 20 sec, and 72ºC in 60 sec, and finally at 72ºC in 10 min. The serogroups were determined by electrophoresis on a 2% agarose gel, based on specific DNA bands.

Molecular characterization of *N. meningitidis*

PorA, fetA and *fHbp* genes were amplified using previously described primers. Primers for PCR *porA*, *fetA* and *fHbp* genes were listed in Table 2. One hundred sixty one (161) NmB strains were selected for analysis in this study.

Table 2. Specific primers for PCR *porA*, *fetA* and *fHbp* genes.

Target gene	Sequences (5'-3')	Size (bp)	References
porA	AAACTTACCGCCCTCGTA TTAGAATTTGTGGCGCAAACCGAC	1100	Jelfs *et al.*, 2000
fetA	CGGCGCAAGCGTATTCGG CGCGCCCAATTCGTAACCGTG	1189	Castillo *et al.*, 2011
fHbp	GTCCGAACGGTAAATTATYGTG CTATTCTGVGTATGACTAG	895	Castillo *et al.*, 2011

The PCR reaction amplified *porA*, *fetA* and *fHbp* genes under the same components (except for primers). A total 50 µl column contained 19.5 µl of H_2O, 25 µl of PCR Master Mix (2X), 1.25 µl of primer F/R (10 pM) and 3 µl of DNA template. The thermal cycle for *porA* amplification: 94ºC in 5 min, 35 cycles of 94ºC in 1 min, 60ºC in 1 min, and 72ºC in 1 min 30 sec, finally at 72ºC in 4 min. For *fHbp* amplification, cycling conditions: 95ºC in 5 min, 40 cycles of 94ºC in 1 min, 55ºC in 1 min, and 72ºC in 2 min 30 sec, finally at 72ºC in 7 min. Cycling conditions for *fHbp* amplification: 94ºC in 5 min, 35 cycles of 95ºC in 15 sec, 50ºC in 15 sec, and 72ºC in 1 min 30 sec, finally at 72ºC in 5 min. The PCR products were purified with the EZ-10 Spin Column Kit (Biobasic, Canada) and subsequently subjected to Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit on the ABI prism 3100 Sequencer (Applied Biosystems, USA).

Bioinformatic analysis

The DNA sequence of *N. meningitidis* strains was examined in the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and was aligned by CLUSTAL_X. The variable loop of the VR1, VR2, and VR3 variable regions of *porA*, allele *fetA*, and *fHbp*

were classified based on the PubMLST database (https://pubmlst.org/organisms/neisseria-spp).

RESULTS

Source and serogroup typing of *N. meningitidis* strains

A total of 186 *N. meningitidis* strains in our study, including 29 strains (15.5%), were cultured from cerebrospinal fluid, blood or throat samples of patients, and 157 isolates (84.6%) were cultured from nasopharyngeal swabs of healthy adolescents. A multiplex PCR assay was used to identify meningococcal serogroups. The results showed that the 29 (100%) disease isolates were NmB. Of the 157 carrier isolates, the most common serogroups were B (n = 132, 84%) and C (n = 5, 3.2%); 20 isolates (12.7%) were non-serotypeable, all of which were isolated from the carrier group (Supplementary Figures S1 and S2).

Genotyping porA results

One hundred fifty-five (155) *porA* sequences were obtained from 161 NmB strains and most of the sequences could be identified by the PubMLST database (https://pubmlst.org/organisms/neisseria-spp/pora) (Table 3). We found 31 different combinations of three families, VR1, VR2 and VR3 in this study, including 13 VR1 families, 21 VR2 and 6 VR3 families (Table 3). The most common VR1 family was P1.22-25 (n = 36, 23.2%), followed by P1.7-2 (n = 31, 20.0%), P1.22 (n = 24, 15.5%), P1.22-11 (n = 19, 12.3%) and P1.12-14 (n = 18, 11.6%). For the VR2 family, the most prevalent variants were 14 (n = 60, 38.7%), 15-25 (n = 20; 12.9%) and 13-20 (n = 18, 11.6%) and some strains could not be determined based on the existing database. VR2 family 4, a component of 4CMenB, accounted for only 5.1% (n = 8). We observed that 78.7% of the analyzed strains carried one of two VR3 family 36 (n = 82, 52.9%) and VR3 family 35-1 (n = 40, 25.8%). The three most common profiles were P1.22-25,14,36 (n = 24, 15.5%), P1.22,14,36 (n = 22, 14.1%), and P1.22-11,15-25,36 (n = 19, 12.2%). These three profiles represent 41.9% of the strains analyzed.

Distribution of FetA

One hundred fifty (150) *fetA* sequences were obtained from 161 NmB strains. We observed the presence of 17 FetA allele variants, grouped into five families (F1, F2, F3, F4, and F5). The most common genotype was F1-7, with 60/150 strains, accounting for 40%, followed by F5-1 with 30/150 (20%), F3-16 with 19/150 (12.7%), F4-6 with 17/150 (11.3%), and F3-3 (8/150, 5.3%). Other alleles were less than 3% (Figure 1).

Diversity and distribution of FHbp

FHbp could be divided into three variant families, variants 1, 2, and 3 or two subfamilies A and B. Subfamily A included variants 2 and 3, and subfamily B corresponded to variant 1 (McNeil *et al.*, 2013). In this study, 146 *fHbp* sequences were obtained from 161 NmB isolates.

FHbp variant group 2 (subfamily A) was most common (n = 142, 97.3%), followed by group 1 (subfamily B) (n = 4, 2.7%). Five variants of subfamily A were found. The A22 variant was the most frequent (n = 52, 35.6%), followed by A24 (n = 41, 25.3%), A32 (n = 18, 12.3%), A20 (n = 11, 7.5%), and A07 (n = 10, 6.8%) (Table 4). There were 2.7% of the strains that showed FHbp variant 1, like the FHbp variant in the 4CmenB vaccine.

Table 3. Distribution of VR1, VR2, and VR3 meningococcal PorA.

No. of isolates (n = 155)	Genosubtype		
	VR1	**VR2**	**VR3**
23	22	14	36
2	22	9	35-1
1	5	2	36-2
18	12-14	13-20	35-1
2	18	25	35-1
1	18	ND	38-1
6	19	15	36
1	19	25	38-1
2	19	15-23	ND
2	20	2	36-2
2	21-2	28	36-2
1	21-2	ND	36-2
19	22-11	15-25	36
24	22-25	14	36
10	22-25	14	ND
2	22-25	ND	ND
1	5-1	10-12	36-2
1	5-2	10-1	36-2
1	7	16-103	35
3	7-2	13-9	35-1
3	7-2	14	ND
1	7-2	13-1	8
5	7-2	13	35-1
4	7-2	13-1	35-1
1	7-2	13-15	35-1
1	7-2	13-18	35-1
4	7-2	13-2	35-1
8	7-2	4	36
1	7-2	4-18	36
1	ND	15-25	36
3	ND	ND	38-1
1	5	2	36-2

ND: Non-determined.

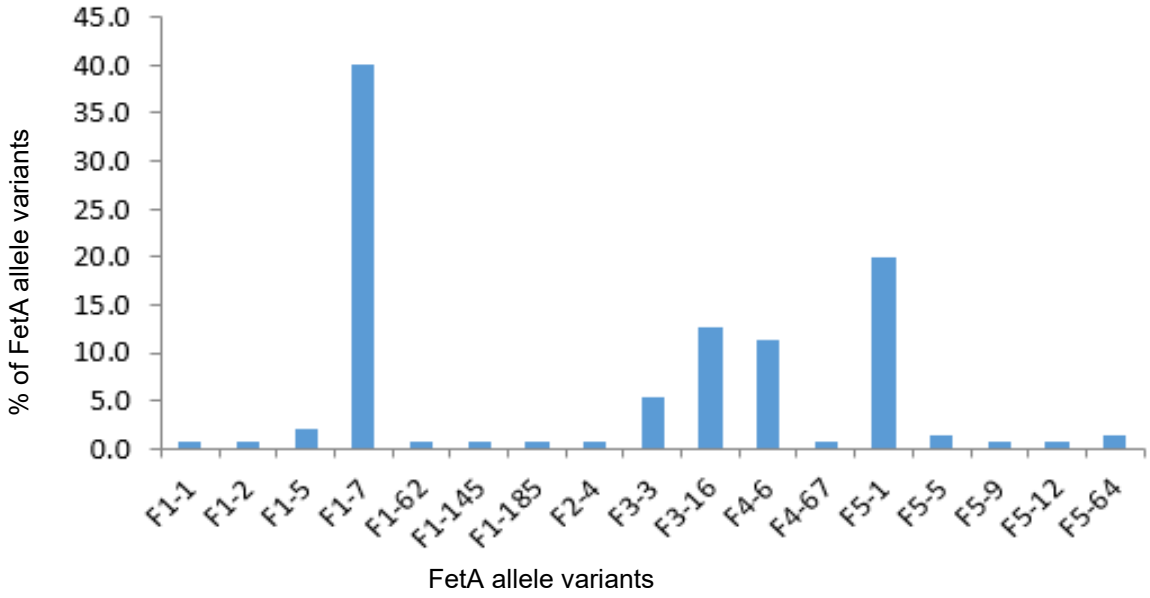

Figure 1. Distribution of *fetA* allele variants.

Table 4. Distribution of VR1, VR2, and VR3 meningococcal PorA.

No. of isolates (n = 146)	Novartis variant group	Sub family	Module group	Modular variable segment allele				
				A	B	C	D	E
3	1	B	ND	A1.1	B1.1	C1.22	D1.9	E1.26
18	2	A32	III	A1.1	B1.1	C2.1	D2.1	E2.1
10	2	A07	III	A1.1	B1.1	C2.1	D2.1	E2.3
41	2	A24	VI	A1.1	B1.1	C2.12	D1.1	E2.4
4	2	A	ND	A1.1	B1.1	C2.2	D1.1	E2.1
52	2	A22	VI	A1.1	B1.1	C2.2	D1.1	E2.1
11	2	A20	VI	A1.1	B1.1	C2.4	D1.1	E2.9
6	2	A	ND	A1.19	B2.1	C2.2	D1.1	E2.2
1	1	B	ND	ND	B1.1	ND	D1.2	E1.53

Based on amino acid sequence similarity and antigenic cross-reactivity, FHbp variants have been subdivided into two subfamilies, three variant groups. fHbp also can be classified into modular groups based on different combinations of five variable segments. Isolates in the variant 2 group included modular groups III and VI. Modular group VI has the majority among variant 2 isolates (n = 104; 71.2%) and modular group III accounted for 19.2% (n = 28). Four isolates with variant 1 (subfamily B) did not determine the modular group.

DISCUSSION

IMD remains a significant global public health problem worldwide. IMDs caused by MnB accounted for a high percentage in many regions. In the United States and Canada, NmB was predominant, with a case fatality rate of 10.9% and 7.8%, respectively (Asturias *et al.*, 2022). NmB was the major cause of IMD and responsible for more than 50% of IMD cases in Europe from 2008 to 2017 (Parikh *et al.*, 2020). NmB was predominant in Singapore and in the Philippines (Marshall, 2021). In Vietnam, data from the meningococcal surveillance system in Southern Vietnam showed that the incidence rate annually averaged 0.02 per 100,000 inhabitants per year during 2012 - 2021 (Phan *et al.,* 2024). The incidence rates (per 100,000 population) in the soldier military, ranging from 0.22 to 2.67 between 2018 and 2021. Most of the cases were caused by NmB (94%) (Van *et al.*, 2021). Among 109 isolates from Southern Vietnam during the period 1980s - 2019, serogroup B predominated with 94% compared to C with 6% (Phan *et al.*, 2020). Similarly, NmB predominated among serogroups at 56% (*n* = 34), followed by serogroup C at 21% (n = 13), and the remainder were non-serotypeable (Hoan *et al.*, 2014). In this study, 161/186 (86.6%) isolates belong to serogroup B. Together with published data, NmB is circulating at a high rate in Vietnam and caused most cases of invasive disease.

The high genetic diversity found among the PorA regions VR1 and VR2 of *N. meningitidis*. The Australian Meningococcal Surveillance Programme Annual Report, 2023, showed the predominant types were P1.7-2,4 (32%; 26/82), P1.7,16-26 (16%; 13/82) and P1.22,14 (9%; 7/82) (Lahra *et al.*, 2024). NmB strain P1.19,15: F4-28: ST-34 accounted for 64.1% of isolates in Lithuania from 2009 to 2019 (Sereikaite *et al.*, 2023). The predominant type of combination VR1 and VR2 PorA in NmB in Morocco from 2011 to 2016 was P1.19,15 (Razki *et al.*, 2018). Previous PorA typing results of serogroup B isolates in Southern Vietnam between 2012 and 2021 showed the predominant types of P1.19,15 (Phan *et al.,* 2024). Overall, P1.22-25,14, P1.22,14, and P1.22-11,15-25 were the predominant subtypes in our study. VR2 family 4, a component of 4CMenB, accounted for only 5.1% in strains. Results indicated low coverage of the PorA antigen component in the 4CMenB vaccine to NmB isolates in Vietnam.

We found 17 FetA allele variants, of which F1-7, F5-1, and F3-16 were the most common, accounting for 72.7% (109/150) of the total strains. Similarly, a study in Brazil (2016–2018) observed 17 FetA allele variants, with F5-1 being one of the most common variants (de Lemos *et al.*, 2020). Another study reported that the FetA allele variant F5-1 was also frequently found in Western Australia between 2000 and 2011 (Boan *et al.*, 2014). The FetA variants F4-6 and F1-5 were the most popular in serogroup B isolates in Southern Vietnam (Phan *et al.*,

2020). The FetA allele F5-1 is prevalent in many regions, thus, we suggest that it may be another potential candidate for vaccine design.

For NmB disease, the molecular epidemiology of FHbp has been the focus of numerous studies. The main import from the results of these studies is that FHbp subfamily distribution can vary geographically. The recognition of FHbp as an important virulence factor and its attributes as a vaccine antigen contributed to the development of two recombinant MenB vaccines, which are either directed to FHbp as the sole target or contain FHbp as one of the antigenic components. MenB-FHbp includes two variants of FHbp, one from subfamily A (variant A05) and the second from subfamily B (variant B01). In contrast, 4CMenB contains nonlipidated FHbp from subfamily B (variant B24). In this study, we found the highest percentage of meningococcal strains carrying the FHbp variant group 2 (subfamily A) (97.3%), FHbp variant group 1 (subfamily B) was only 2.7%.

Up to now, there are 2 types of meningococcal B vaccines used in Vietnam. VA-MENGOC BC (Finlay Institute, Cuba) contains OMVs from the B4:P1.19,15: L3,7,9 strain against meningococcal serogroup B. VR1 and VR2 types of PorA in strains (P1.19,15) are identical to the VA-MENGOC BC vaccine, accounting for 3.9%. Indicating that the effectiveness of the vaccine against heterologous serogroup B strains in Vietnam should be evaluated in the population. The remaining vaccine is 4CMenB (Bexsero, GSK) with the four antigenic components described above. The distribution of the predominant FHbp variant 2 or subfamily A in NmB strains in Vietnam was different from variant in the 4CmenB vaccine. It is necessary to evaluate the compatibility of the 4 antigens (NadA, FHbp, NHBA and PorA) in the 4CMenB vaccine with the antigens of NmB strains in Vietnam, thereby predicting the protective ability of this vaccine.

CONCLUSION

Of the 186 strain isolates in Vietnam over the period 2014 - 2024, the most predominant serogroup was B (86.6%), followed by serogroup C (2.7%). The three most common profiles of the *porA* gene were P1.22-25,14,36, P1.22,14,36, and P1.22-11,15-25,36. For FetA VR types, the major allele variant was F1-7, accounting for 40%, followed by F5-1 with 20%. The FHbp variant group 2 was the most common, accounting for 97.3%, meaning a similar number of subfamily A. On the basis of genotypic data, poor matching was shown between the PorA type in the VA-MENGOC-BC vaccine as well as PorA and FHbp components of the 4CMenB vaccine to PorA and FHbp of meningococcal serogroup B strains isolated in Vietnam from 2014 to 2024.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPLEMENTARY FIGURES

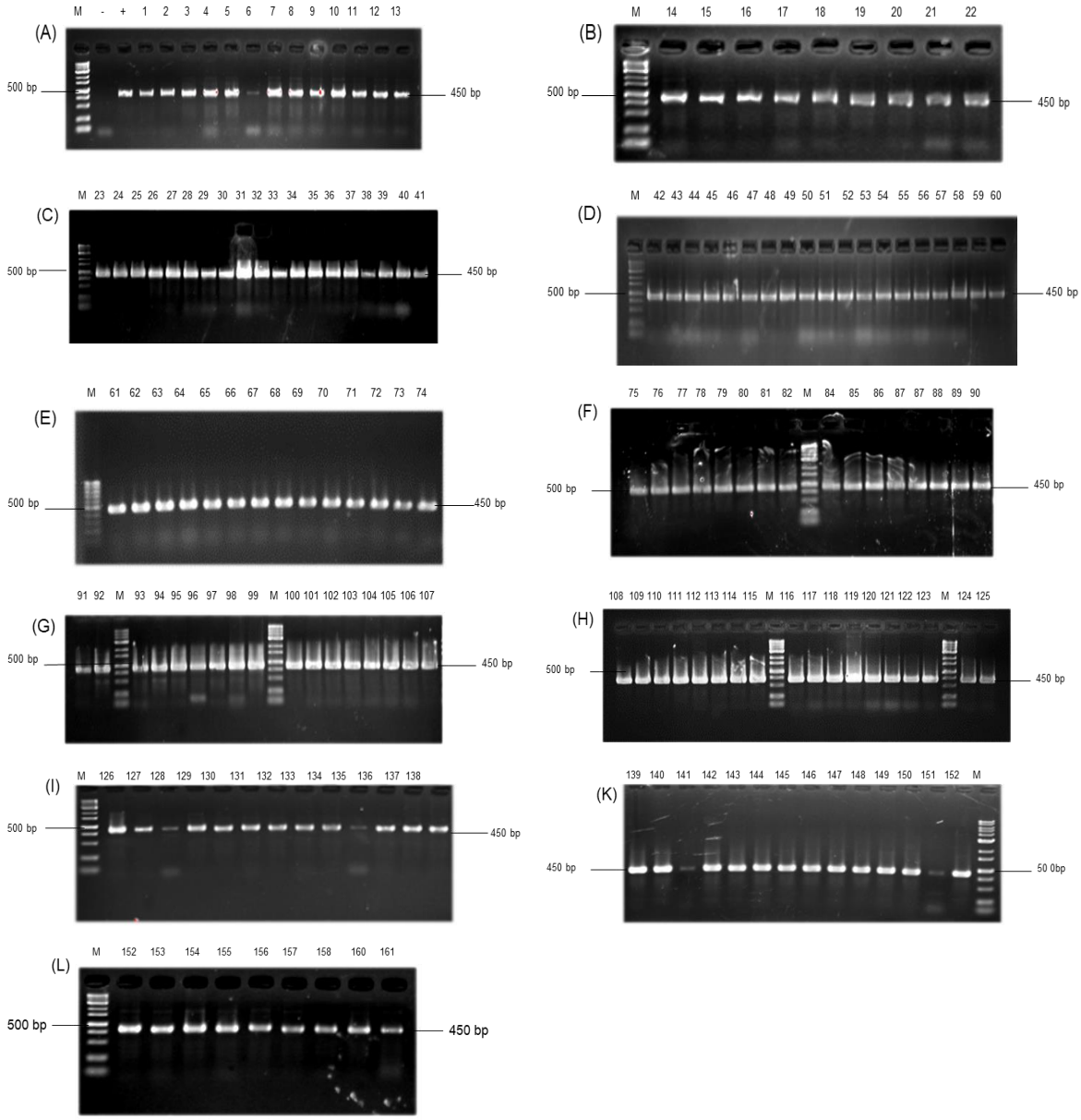

Figure S1. Multiplex PCR for detection of *N. meningitidis* serogroup B. (A–C) PCR amplification results of serogroup B from 29 clinical isolates (samples 1–29). (C–L) PCR results from 132 carrier isolates positive for serogroup B (samples 30–161).

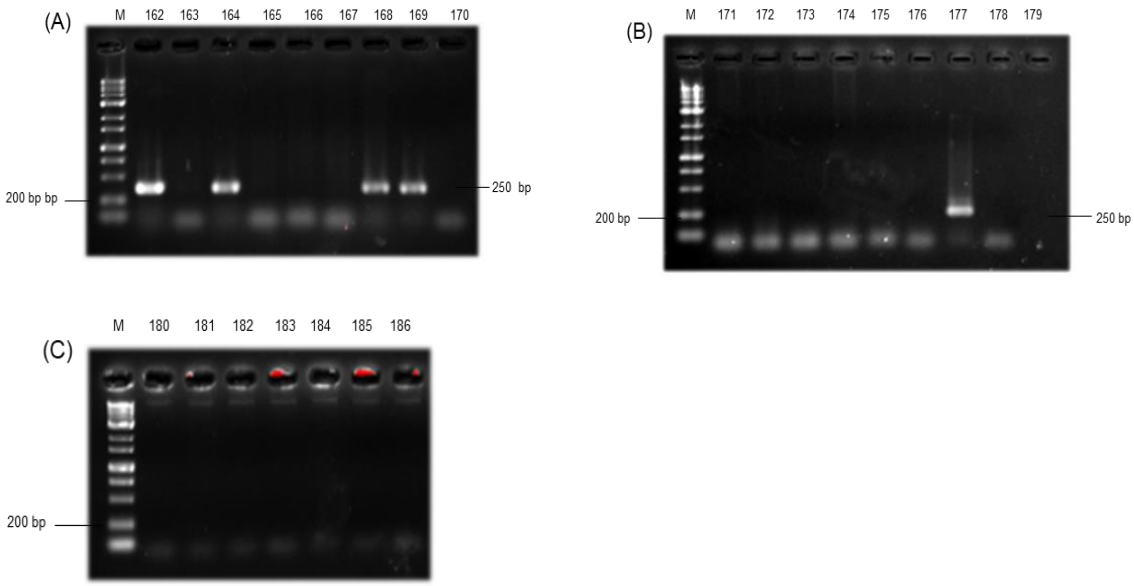

Figure S2. Multiplex PCR for detection of *N. meningitidis* serogroup C. **(A–C)** PCR amplification results of serogroup C from carrier isolates positive (samples: 162, 164, 168, 169 and 177), the remaining 20 strains were non-serogroupable.