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Distribution of *Neisseria meningitidis* serogroup b (NmB) vaccine antigens in meningococcal disease causing isolates in the United States during 2009–2014, prior to NmB vaccine licensure

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SUMMARY

Objectives: Two *Neisseria meningitidis* serogroup B (NmB) vaccines are licensed in the United States. To estimate their potential coverage, we examined the vaccine antigen diversity among meningococcal isolates prior to vaccine licensure.

Methods: NmB vaccine antigen genes of invasive isolates collected in the U.S. from 2009 to 2014 were characterized by Sanger or whole-genome sequencing.

Results: During 2009–2014, the predominant antigen types have remained similar to those reported in 2000–2008 for NmB and 2006–2008 for NmC, NmY, with the emergence of a few new types. FHbp of subfamily B or variant 1 (B/v1) remained prevalent among NmB whereas FHbp of subfamily A or variant 2 and 3 (A/v2-3) were more prevalent among non-NmB. FHbp peptide 1 (B24/1.1) remains the most prevalent type in NmB. Full-length NadA peptide was detected in 26% of isolates, primarily in NmB and NmW. The greatest diversity of NhbA peptides was detected among NmB, with p0005 as the most prevalent type.

Conclusions: The prevalence and diversity of the NmB vaccine antigens have remained stable with common antigen types persisting over time. The data collected prior to NmB vaccine licensure provide the baseline to understand the potential impact of NmB vaccines on antigen diversity and strain coverage.

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Introduction

Neisseria meningiditis (Nm) is a commensal bacterium that can cause severe infections which are collectively known as meningococcal disease. Effective vaccines are important for disease control and prevention.[1] During 2009–2014, 4109 cases of meningococcal disease were reported in the United States, with serogroups B, C and Y accounting for the majority of cases.[2,3] Quadrivalent meningococcal polysaccharide conjugate vaccine (MenACWY) was recommended for adolescents aged 11–18 years in the United States in 2005 to protect against disease caused by Nm serogroups A, C, W and Y.[4] In 2015, 81.3% of adolescents aged 13–17 years had been vaccinated with ≥1 dose of MenACWY.[5]

Developing vaccines against *Neisseria meningitidis* serogroup B (NmB) was challenging as NmB capsular polysaccharide is immunologically similar to human antigens and poorly immunogenic.[6] A few outer membrane vesicle (OMV) vaccines have been proven effective in the control of NmB epidemics in different countries.[7,8] Studies later revealed that the functional immunogenic response to OMV vaccines stems primarily from a highly variable region of the outer membrane protein porin A (PorA).[9,10] Because of the diversity in PorA amino acid sequence in this region of the protein, immunological protection using OMV vaccines is generally limited to disease isolates that express the same PorA type as the vaccine strain and not suitable for broad protection against NmB isolates that have a different PorA type. Recently, the U.S. Food and Drug Administration approved two NmB vaccines for use in the United States.[11,12] MenB-FHbp (Trumenba®), licensed in the U.S. in

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Table 1
Meningococcal isolates collected from ABCs 2009–2014 and Expanded Surveillance Sites 2013–2014 in the United States.

Serogroup	ABCs 2009–2014	Expanded Surveillance Sites 2013–2014
Conjugated Vaccine Serogroups		
A	0 (0%)	0 (0%)
C	125 (29.0%)	101 (25.2%)
W	38 (8.8%)	41 (10.2%)
Y	152 (35.3%)	75 (18.7%)
Subtotal	315 (73.1%)	217 (54.1%)
B	100* (23.2%)	149 (37.2%)
Others**	16 (3.7%)	35 (8.7%)
Total	431*	401

* 155 serogroup B isolates were collected and characterized from ABCs 2009–2014. The number of serogroup B isolates was 100 after correction for disease prevalence in Oregon. The total number of isolates included in ABCs 2009–2014 was 486 before the correction for Oregon.

** "Others" include 15 nongroupable and one NmZ isolate from ABCs 2009–2014, and 34 nongroupable and one NmZ isolate from Expanded Surveillance Sites 2013–2014.

2014, is composed of recombinant lipidated factor H binding protein (FHbp). Based on amino acid sequence similarity, FHbp peptides can be grouped into two distinct subfamilies (A and B) or three variants (v1, v2, v3). A representative peptide from each subfamily is included in MenB-FHbp.[13] MenB-4C (Bexsero®), approved in the U.S. in 2015, includes three recombinant antigens, a non-lipidated FHbp peptide from subfamily B (v1), neisserial adhesin A (NadA), and neisserial heparin binding antigen (NhbA), formulated with OMVs from an epidemic NmB strain from New Zealand.[14] Because NmB vaccine antigens are also present in non-B meningococcal serogroups, the NmB vaccines may provide immunological cross-protection for non-B meningococcal disease.[15–17]

Continuous monitoring of the genetic diversity of NmB vaccine antigens is important to estimate the potential coverage of the two NmB vaccines. In this study, we determine the prevalence and distribution of FHbp, NadA, and NhbA protein antigen types among Nm isolates collected from the Active Bacterial Core surveillance (ABCs) network during 2009–2014 in the United States, prior to the licensure of the NmB vaccines. Characterization of additional isolates collected from Expanded Surveillance Sites in 2013–2014 is also included to identify other vaccine antigen types in circulating meningococcal isolates that were not captured in the ABCs network.

Materials and methods

Strain collection

Two sets of meningococcal isolates were included in this study. The first set of isolates was collected through the (ABCs) network, a population- and laboratory-based surveillance system supported by the Centers for Disease Control and Prevention (CDC) as part of its Emerging Infections Program network (http://www.cdc.gov/abcs/index.html). Participating sites during 2009–2014 included California (the three-county Bay area), Colorado (the five-county Denver area), Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York (the seven-county Rochester area and eight-county Albany area), Oregon, and Tennessee (20 urban counties). In 2014, the total population under surveillance was approximately 43.5 million, or 13.6% of the U.S. population. Among the 536 meningococcal disease cases reported through the ABCs during 2009–2014, Nm isolates from 486 cases (315 serogroups A, C, W, and Y, 155 NmB, 15 nongroupable, and one serogroup Z) were available and included in this study (Table 1 and S1).

The second set of isolates were collected during 2013–2014 from Expanded Surveillance Sites including 21 states and one large city (Alaska, Alabama, Arkansas, Arizona, California (non-ABCs), Florida, Indiana, Louisiana, Maine, Massachusetts, Michigan, Ohio, Oklahoma, Pennsylvania, Rhode Island, Tennessee (non-ABCs), Texas, Utah, Virginia, Washington, Wisconsin, and New York City). The proportion of isolates submitted from each state varied, ranging from 27% to 100% of the reported cases. A total of 401 Nm isolates (217 serogroups A, C, W, and Y, 149 NmB, 34 nongroupable, and one serogroup Z) from Expanded Surveillance Sites were characterized (Table 1 and S1).

Slide agglutination serogrouping and real-time polymerase chain reaction (rt-PCR) were performed at CDC to confirm the Nm species and serogroup designations.[18]

Molecular characterization of the U.S. meningococcal isolates

Sanger sequencing was performed as described previously with minor modifications.[19–21] For whole genome sequencing, genomic DNA was extracted with either the ArchivePure™ DNA purification kit (5prime, Gaithersburg, MD) or Gentra Puregene yeast/bacteria DNA extraction kit (Qiagen, Germantown, MD), followed by library preparation using NEBNext Ultra DNA library preparation kits for Illumina (New England BioLabs, Ipswich, MA). The libraries were then sequenced on the platforms of Illumina HiSeq 2500 with 100 bp or 250 bp paired-end kits or MiSeq with 250 bp paired-end kits (Illumina, San Diego, CA) at CDC or Pfizer Vaccine Research. Raw sequencing reads that met the high-quality criteria were trimmed and assembled.[22,23] Contigs with > 5x coverage and 200 bp in length were then BLAST searched for open reading frames corresponding to the NmB vaccine antigen genes *fHbp*, *nadA*, and *nhbA*, using the PubMLST website (https://pubmlst.org/). Each unique FHbp amino acid sequence was assigned a PubMLST peptide identifier (ID); the FHbp peptides identified among isolates from ABCs and Expanded Surveillance Sites were grouped into subfamilies A and B, corresponding to variant 1 (v1) and variant 2 or 3 (v2/3), respectively (Table S2). Similarly, each unique NadA amino acid sequence belonging to one of the NadA variants (NadA-1, NadA-2/3, or NadA-4/5) was assigned a PubMLST peptide ID (e.g., NadA-1.2 refers to NadA peptide 2 from variant 1) and each unique NhbA amino acid sequence was also given a unique identifier (e.g., p0002 is NhbA peptide 2).[24–27] A listing of FHbp, NadA, and NhbA peptides identified in this study is shown in Tables S2-4.

Data analysis

For phylogenetic analysis of NmB vaccine antigens, the multiple sequence alignment of deduced full-length protein sequences was generated by ClustalW with CLC Genomics Workbench 7. The networks were created by SplitsTree, v 4.0[28] with default parameters.

The prevalence and distribution of vaccine antigens was assessed using isolates obtained through the ABCs from 2009 to 2014. Oregon has reported higher rates of NmB and overall meningococcal disease that was first identified in 1993.[29] During 2009–2014, the incidence of NmB disease was 0.22 cases per 100,000 population in Oregon, compared with 0.05 cases per 100,000 population in the other ABCs sites. Therefore, NmB isolates from Oregon were weighted to compensate for the higher rates of NmB disease to accurately estimate the vaccine antigen distribution among serogroup B isolates in the United States. The weight was generated based on the proportion of the ABCs population contributed by Oregon (10%). A weight of 0.10 was applied to isolates collected from Oregon, and a weight of 0.90 (1– 0.10) was applied to isolates collected from other ABCs sites.

Table 2
The distribution of vaccine antigens among meningococcal isolates collected from ABCs 2009–2014 in the United States.

Serogroup	Number of Isolates	Intact FHbp ORF[+](%)	FHbp A/v2-3 (%)	FHbp B/v1 (%)	Intact NadA ORF[+](%)	Intact NhbA ORF[+](%)
NmB	100[*]	100	44.0	56.0	40.0	99.0[#]
NmC	125	85.6[**]	46.4	39.2	24.8	100
NmW	38	100	100	0	50.0	100
NmY	152	100	96.1	3.9	1.3	100

[*] The number of NmB isolates was 155 before correction for Oregon and 100 after correction.
[**] The remaining 14.4% ($n = 18$) of NmC isolates code for a truncated FHbp.
[#] One NmB isolate coded for an *nhbA* allele with a frameshift resulting in a truncated peptide.
[+] ORF stands for open reading frame.

A weighted stratified analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC). A Cochran-Armitage test was conducted to test for a linear trend in change of FHbp subfamily/variant proportions, and 95% confidence intervals were calculated for the proportions of FHbp subfamily/variant in various age groups per serogroup. A Chi-squared goodness of fit test was used to test for a change in distribution in FHbp peptides among NmB isolates. The change in serogroup distribution was tested for individual differences between the same periods using Fisher's exact test.

Results

Diversity and prevalence of FHbp among the ABCs 2009–2014 isolates

The *fHbp* gene (intact or partial open reading frame) was present in all isolates included in this study. An intact FHbp open reading frame (ORF) was detected in 100% of NmB, NmW, and NmY isolates, and 85.6% of NmC isolates collected from ABCs in 2009–2014 (Table 2). Truncated FHbp, resulting from a frameshift mutation, was detected in 18 NmC isolates. Seventeen of these isolates coded for *fHbp* allele 669, previously identified among NmC isolates.[16,19] One isolate harbored *fHbp* allele 960, differing from *fHbp* allele 669 by a single nucleotide at position 278, but resulting in a truncated protein, with the same length as the one encoded by allele 669. The FHbp subfamily/variant distribution among NmB, NmC, NmW, and NmY is shown in Table 2. FHbp subfamily B/variant 1 (B/v1) peptides were predominant among NmB isolates, whereas subfamily A/variant 2/3 (A/v2-3) was more prevalent among the non-NmB serogroups. NmB isolates from Oregon had a higher proportion of FHbp B/v1 (70%) compared to the NmB isolates collected from the other ABCs sites (55.2%).

The distribution of full-length and truncated FHbp peptides among each serogroup is shown in Fig. 1A. A total of 64 unique full-length FHbp peptides were identified among NmB, NmC, NmW, and NmY isolates from ABCs in 2009–2014. With some of FHbp peptides being detected in multiple serogroups, a larger number of FHbp peptides was identified among NmB ($n = 38$) and NmC ($n = 31$) isolates than NmW ($n = 7$) and NmY ($n = 12$). Four (FHBp peptide 1 (B24/1.1), 19 (A22/2.19), 16 (A19/2.16, 21 (A07/2.21)) of the 38 FHbp peptides identified among NmB were detected in more than five isolates (weighted for Oregon), comprising 34% and 28% of NmB isolates carrying B/v1 and A/v2-3 FHbp peptides, respectively. FHbp peptide 1 (B24/1.1) was identified as the most prevalent peptide among NmB isolates (34%), followed by FHbp peptide 19 (A22/2.19; 15%). Five of the 31 FHbp peptides identified among NmC were detected in more than five isolates, comprising 39.2% and 8.8% of NmC isolates carrying A/v2-3 and B/v1 peptides, respectively; with FHbp peptide 25 (A15/2.25) being the most prevalent peptide among NmC isolates (20%). Thirty-two unique FHbp peptides (1 in both NmB and NmC, 13 in NmB and 18 in NmC) were identified for the first time among ABCs in 2009–2014, not previously detected among NmB isolates from ABCs 2000–2008 or NmC isolates from ABCs 2006–2008.[19] Despite the new FHbp peptides were identified, the most prevalent FHbp peptides remain the same. Twelve unique FHbp peptides were identified in NmY isolates from ABCs 2009–2014, with 78.9% of isolates containing FHbp peptide 25 (A15/2.25). Only 7 unique FHbp peptides were detected among NmW isolates, with 86.8% of isolates containing either FHbp peptide 22 (A10/2.22) or 16 (A19/2.16).

The pairwise amino acid sequence identity within FHbp A/v2-3 and B/v1 was at least 82% and 84%, respectively. This is illustrated in the FHbp phylogenetic tree in Fig. 2A.

Diversity and prevalence of NadA among the ABCs 2009–2014 isolates

Overall, only 22% of NmB, NmC, NmW, and NmY isolates (weighted for Oregon) from the ABCs 2009–2014 collection harbored a full-length NadA open reading frame (ORF). Of these, full-length NadA ORFs were detected in 50.0% of NmW, 40.0% of NmB, 24.8% of NmC, and 1.3% of NmY isolates (Table 2). NadA-1 was primarily detected among NmB isolates, while NadA-2/3 was mainly associated with non-NmB isolates (Fig 1B). Of the remaining *nadA* negative isolates, *nadA* was completely deleted in most of the isolates, while eleven isolates (10 NmB and 1 NmC) contained a truncated ORF resulting either from a frameshift mutation or IS element.

Nine unique full-length NadA peptides were identified among the 92 isolates (weighted for Oregon), 3 in NadA-1 variant (NadA-1.1, 1.100, 1.148) and 6 in NadA-2/3 (NadA-2/3.2, 2/3.3, 2/3.6, 2/3.91, 2/3.121, 2/3.147). The distribution of NadA peptides by serogroup is illustrated in Fig. 1B. All NmB isolates ($n = 40$, weighted for Oregon) contained a member of NadA-1 variant, with NadA-1.1 being the most common peptide (97.5%). NadA-2/3.6 was identified in all 19 of the NmW isolates. NadA-2/3.3 was the most prevalent peptide detected in NmC isolates (48.4%). Only two of the 152 NmY isolates contained NadA (NadA-2/3.6 and NadA-2/3.121).

The pairwise amino acid sequence identity among peptides within NadA-1 and NadA-2/3 was at least 76% and 72%, respectively. Two peptides, NadA-1.100 and NadA-2/3.91, are less conserved (Fig. 2B) and each was identified just once among the ABCs isolates. The sequence identity within variants NadA-1 and NadA-2/3 increases to 98% and 97%, respectively when NadA-1.100 and NadA-2/3.91 are excluded from the comparison (Fig. 2B).

As shown in Table 3, full-length NadA peptides were more frequently detected in NmB isolates harboring a member of FHbp B/v1 (62.5%) than FHbp A/v2-3 peptides (11.4%). The percentage of NmC isolates that harbor full-length NadA peptides is similar among isolates containing FHbp A/v2-3 (25.9%) and B/v1 (30.6%). All 15 NmC isolates containing FHbp A/v2-3 have NadA-2/3.3, whereas NmC isolates that harbored a member of FHbp B/v1 have various NadA peptides, including NadA-1.1 (6/15), NadA-2/3.2 (5/15), and NadA-2/3.147 (4/15). Only one of the 18 NmC isolates with truncated FHbp contained NadA (NadA-2/3.91). All NmW and

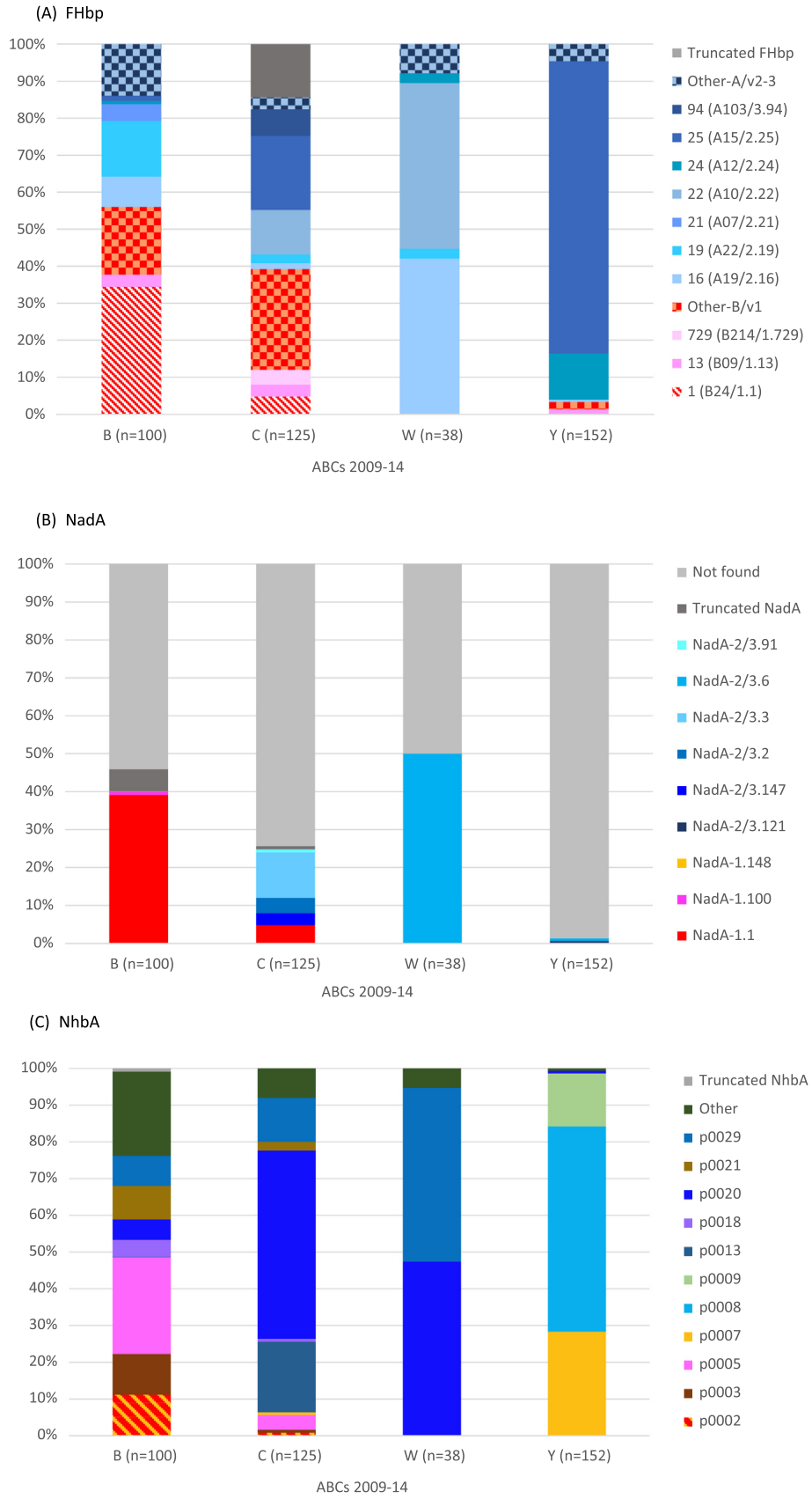

Fig. 1. Distribution of NmB vaccine antigen (A) FHbp, (B) NadA, and (C) NhbA among serogoups BCWY isolates from ABCs 2009–2014 in the United States. Peptides included in the 4CMenB were shaded in diagonal lines except for NadA antigen (NadA-2/3.8), which was absent in the collection. FHbp included in MenB-FHbp was not visually presented on the graph because it was detected in < 5 isolates (A05/3.45) or not represented (B01/1.55) in the collection. Peptides detected in < 5 isolates (in checker grid) were included in "Other-A/v2-3" and "Other-B/v1" for FHbp and "Other" for NhbA. NmB isolates were weighted for Oregon ($n = 100$). "Other-A/v2-3" includes 16 FHbp peptides in NmB, 4 FHbp peptides in NmC, 3 FHbp peptides in NmW, and 5 FHbp peptides in NmY. "Other-B/v1" includes 14 FHbp peptides in NmB, 19 FHbp peptides in NmC, and 3 FHbp peptides in NmY. "Other" NhbA includes 20 NhbA peptides in NmB, 8 NhbA peptides in NmC, 2 NhbA peptides in NmW, and 1 NhbA peptide in NmY.

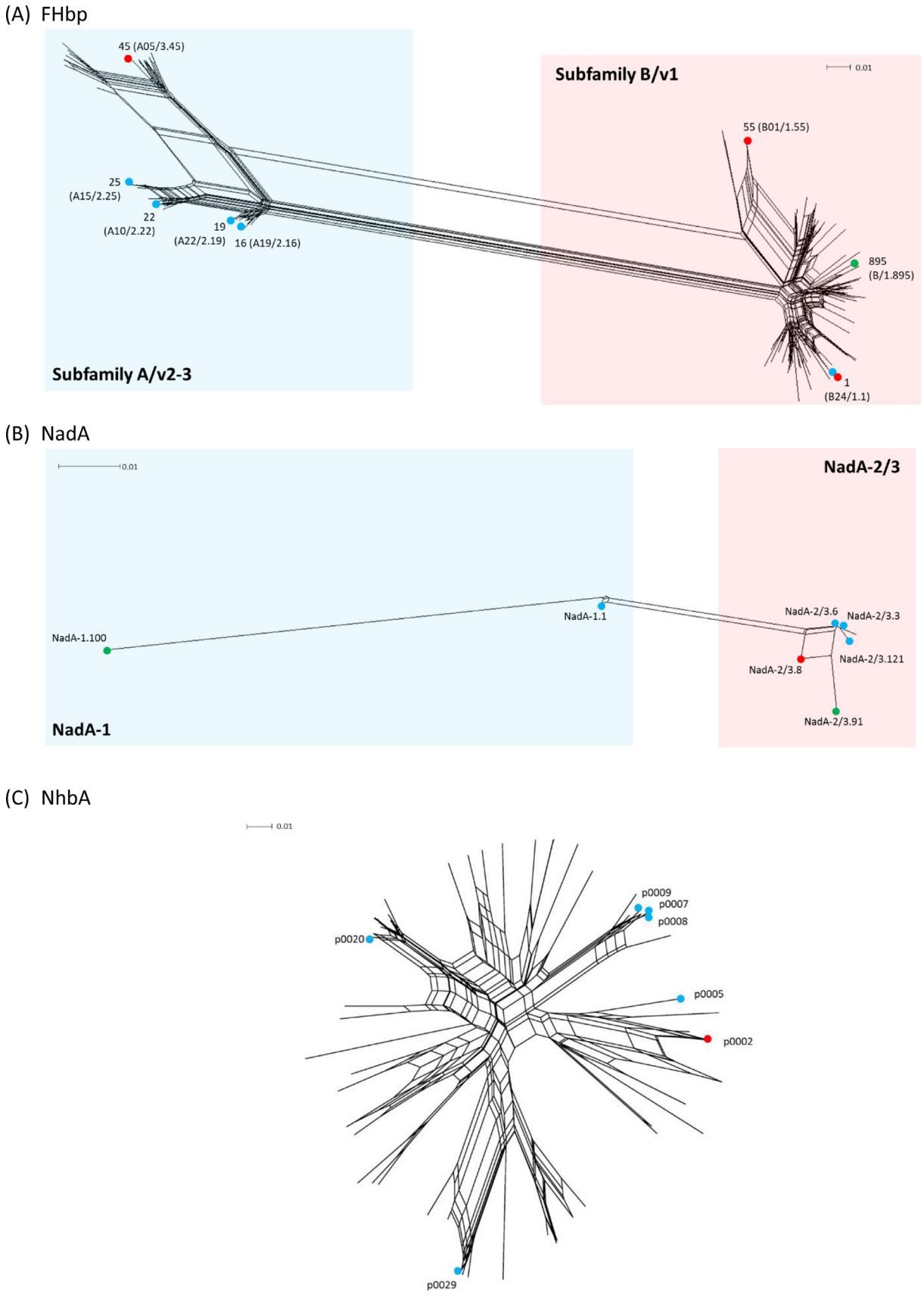

Fig. 2. Phylogenetic analysis of (A) FHbp, (B) NadA, and (C) NhbA peptides detected in ABCs and Expanded Surveillance Sites. Protein variants corresponding to antigens in MenB-FHbp and 4CMenB NmB vaccines are shown in red circles; the predominant peptides detected in NmB, NmC, NmW, and NmY were shown in blue, green, orange, and grey circles, respectively. FHbp peptide 895 (B259/1.895) identified from Expanded Surveillance Sites and NadA peptides with low sequence similarity compared to others were showed in purple circles.

Table 3
Frequency of intact NadA ORFs among meningococcal isolates from ABCs 2009–2014 harboring FHbp A/v2-3 or B/v-1 peptides.

Serogroup	Number of Isolates	% of FHbp A/v2-3 isolates with an intact NadA ORF	% of FHbp B/v1 isolates with an intact NadA ORF
NmB	100*	11.4 (5/44*)	62.5 (35/56*)
NmC	107**	25.9 (15/58)	30.6 (15/49)
NmW	38	50 (19/38)	0 (0/0)
NmY	152	1.4 (2/146)	0 (0/6)

* NmB isolates were weighted for Oregon.
** Only NmC isolates with full-length FHbp ORFs were included.

Table 4
NmB vaccine antigens among meningococcal isolates collected from Expanded Surveillance Sites 2013–2014 in the United States.

Serogroup	Number of Isolates	Number of FHbp peptide types*		Number of NadA peptide types*		Number of NhbA peptide types*	
		Shared	Unique	Shared	Unique	Shared	Unique
NmB	149	20	15	1	1	19	17
NmC	101	15**	9**	4	2	8	2
NmW	41	5	2	2	0	3	0
NmY	75	6	5	0	1	3	4

* Peptides identified from isolates collected from both the ABCs 2009–2014 and Expanded Surveillance Sites 2013–2014 were described as "shared", whereas those only identified in isolates from the Expanded Surveillance Sites were termed "unique".
** Examples of truncated FHbp identified from NmC isolates ($n = 25$) were excluded; these include 23, 1, and 1 isolates coding for *fhbp* alleles 669, 1124, and 1125 as defined by PubMLST, respectively. Only full-length FHbp peptides were included for determining these counts.

NmY isolates with full-length NadA peptides contained one of the FHbp A/v2-3 peptides (Table 3).

Diversity and prevalence of NhbA among the ABCs 2009–2014 isolates

All ABCs 2009–2014 isolates harbored an intact *nhbA* allele except for one NmB isolate having a frameshift mutation that is predicted to code for a truncated protein (Table 2). Overall, a total of 38 NhbA peptides were identified in the NmB, NmC, NmW, and NmY isolates. Pairwise amino acid sequence identity was at least 65% among the NhbA peptides (Fig. 2C). The greatest number of unique NhbA peptides ($n = 29$) was observed among NmB isolates (Fig. 1C). NhbA peptides p0005 (26%) and p0002 (11%) predominate among NmB isolates. NhbA p0005 was detected more frequently in NmB isolates collected in Oregon (56%) than from other ABCs sites (25%). A total of 20 low-frequency (defined as being detected in fewer than 5 isolates) NhbA peptides were found in 23% of the NmB isolates. Of the 17 NhbA peptides identified in NmC isolates, peptide p0020 was the most prevalent (51.2%). A small number of unique NhbA peptides were identified in NmW and NmY isolates (Fig. 1C), with 94.7% of NmW isolates containing NhbA p0020 or p0029, and 98.6% of NmY isolates containing NhbA p0007, p0008, or p0009. All eighteen NmC isolates with a truncated FHbp contained NhbA p0020 and only one had a full length NadA peptide (NadA-2/3.91). The NmB isolate with the NhbA frameshift mutation contained FHbp peptide 1 (B24/1.1) and NadA-1.1.

Emerging antigen types from expanded surveillance sites

Additional vaccine antigen types were identified among NmB, NmC, NmW, and NmY isolates ($n = 366$) collected from the Expanded Surveillance Sites (Table 1). Of the 29 FHbp peptides that were not identified in the ABCs 2009–2014 collection, fifteen were detected in NmB, 9 in NmC, 2 in NmW, and 5 in NmY isolates (Table 4). FHbp peptide 276 (B153/1.276) was detected in 5 NmB isolates and FHbp peptide 895 (B259/1.895) in 20 NmC isolates. Each of the remaining 27 peptides unique to Expanded Surveillance Sites collection was detected in < 5 isolates. While most of these emerging FHbp peptides were only detected in a specific serogroup, FHbp peptide 89 (B36/1.89) was detected in three sergoroups (1 NmB, 2 NmC, and 3 NmW isolates). In addition, two new *fhbp* alleles 1124 and 1125, each encoding a truncated FHbp, were identified in NmC isolates; the truncated FHbp was predicted to be the same length as those detected among NmC isolates from the ABCs. Unique NadA peptides identified among isolates collected from Expanded Surveillance Sites included NadA-1.150 in NmB, NadA-2/3.127 in NmC, and NadA-2/3.8 in NmC and NmY. A total of 23 unique NhbA peptides were identified in isolates collected from non-ABC sites: 17 in NmB, 2 in NmC and 4 in NmY (Table 4). Aside from NhbA p0150 (identified in one NmB and one NmY isolate), other new NhbA peptides were restricted to a single serogroup and found in fewer than 5 isolates.

The antigen peptides identified among isolates from the Expanded Surveillance Sites showed high amino acid sequence identity to those identified from ABCs sites. Analysis of all peptides from ABCs and Expanded Surveillance Sites illustrated that pairwise amino acid sequence identity was at least 82% within FHbp A/v2-3, 84% within FHbp B/v1, 75% within NadA-1, 70% within NadA-2/3, and 65% within NhbA peptides. The phylogenetic relationships of each FHbp, NadA and NhbA peptides identified in this study are displayed in Fig. 2.

Epidemiological characteristics of FHbp

The FHbp subfamily/variant distribution among ABCs 2009–2014 isolates by patient age and serogroup is described in Table 5 (with weighting for Oregon in NmB isolates). As noted in Hoiseth et al.,[30] genes coding for FHbp B/v1 were primarily detected in NmB isolates collected from individuals 1–64 years old, while FHbp A/v2-3 peptides were more frequently detected in isolates collected from infants <1 year old and adults over 65 (Table 5). The majority of NmC isolates have FHbp A/v2-3 peptides, particularly among isolates collected from infants and children <10 years old and adults over 65 years old.

The FHbp subfamily/variant distribution among ABCs 2009–2014 isolates by collection states and serogroup is described in Table S5. In spite of the overall prevalence of FHbp B/v1 peptides among NmB isolates (Table 2), genes coding for FHbp A/v2-3 peptides were detected in the majority of NmB isolates collected from the following states: Colorado (100%), Connecticut (66.7%), Georgia (57.1%), New Mexico (66.7%), and New York (75%).

Table 5
Distribution of isolates that code for full length ORFs by FHbp subfamily/variant in the ABCs 2009–2014 collection as a function of patient age and serogroup.

Serogroup (No. of Isolates)	Age group	Number of Isolates	% Isolates that code for FHbp A/v2-3 (95% CI)	% Isolates that code for FHbp B/v1 (95% CI)
NmB ($n=100$*)				
	<1	29*	72.4* (54.6–87.9)	27.6* (12.2–45.4)
	1–<10	18*	27.8* (6.49–47.1)	72.2* (52.9–92.5)
	10–25	21*	28.6* (7.4–45.2)	71.4* (54.8–92.6)
	26–64	27*	37.0* (19.8–56.5)	63.0* (43.5–80.2)
	65+	5*	60.0* (14.2–100)	40.0* (0–85.8)
NmC ($n=107$**)				
	<1	6	66.7 (54.2–87.6)	33.3 (12.4–45.8)
	1–<10	17	52.9 (27.8–77)	47.1 (23.0–72.2)
	10–25	31	48.4 (30.2–66.9)	51.6 (33.1–69.9)
	26–64	41	48.8 (32.9–64.9)	51.2 (35.1–67.1)
	65+	12	83.3 (51.6–97.9)	16.7 (2.1–48.4)
NmW ($n=38$)				
	<1	8	100 (63.1–100)	0 (0–36.9)
	1–<10	2	100 (15.8–100)	0 (0–84.2)
	10–25	2	100 (1.26–98.7)	0 (0–84.2)
	26–64	16	100 (79.4–100)	0 (0–20.6)
	65+	10	100 (69.2–100)	0 (0–30.6)
NmY ($n=152$)				
	<1	13	96.1 (75.3–100)	3.9 (0–24.7)
	1–<10	8	100 (63.1–100)	0 (0–36.9)
	10–25	27	100 (81.0–99.9)	0 (0.09–19.0)
	26–64	54	96.3 (90.1–100)	3.7 (0.05–9.89)
	65+	50	92.0 (80.1–97.8)	1.9 (2.22–19.2)

* NmB isolates were weighted for Oregon.
** Truncated FHbp identified from NmC isolates ($n=25$) was excluded from these totals.

Similarly, while FHbp A/v2-3 peptides were more frequent among all NmC isolates (Table 2), genes coding for FHbp B/v1 peptides were present in more than 62.5% of NmC isolates collected from Colorado (62.5%), Connecticut (75%), Georgia (80%), Maryland (80%), New Mexico (66.7%), and Tennessee (100%).

No significant linear trend for change in FHbp subfamily/variant distribution by year in ABCs 2009–2014 isolates was detected (Cochran–Armitage test for trend, $p=0.689$).

Discussion

Our data indicate that the predominant NmB vaccine antigen types remained the same among serogroup B, C, W, and Y isolates from ABCs 2009–2014 when compared with isolates from ABCs 2000–2008 (NmB) and ABCs 2006–2008 (NmC, NmW, NmY).[19] FHbp B/v1 peptides remained prevalent among NmB isolates in the two study periods (2009–14 vs 2000–08). A number of new FHbp peptides were identified among NmB with low frequency in the isolate collection of ABCs 2009–2014, whereas a number of peptides detected in 2000–08 were not present among the 2009–2014 isolates. No significant change ($p=0.214$) in the distribution of FHbp peptides among NmB isolates was observed when compared to the data collected from ABCs 2000–2008.[19] The pairwise amino acid sequence identity among FHbp B/v1 and A/v2-3 peptides remained the same in ABCs 2000–2008 and ABCs 2009–2014 (A/v2-3: 82% and B/v1: 84%). We were unable to analyze the relative distribution of NhbA and NadA peptides in NmB between the two study periods because only a subset of isolates from the ABCs 2000–2008 collection were characterized for these antigens. Truncated FHbp sequences were detected only in NmC isolates, as previously reported in the United States,[19] despite the frequent horizontal gene transfer among meningococcal isolates. One serogroup B isolate contains an *nhbA* allele with a frameshift mutation that encodes a truncated protein, a finding previously observed only among non-NmB isolates in the United States. The DNA allele (allele 862) of the truncated NhbA found in our study was different from the one identified from a NmB isolate in Spain (allele 457).[31] This observation underscores the needs to continuously monitor the genetic diversity of these vaccine antigens.

It was noted that the distribution of prevalent FHbp peptides varies by geographic region.[30,32] As described in Hoiseth et al., FHbp B/v1 peptides were identified in 57.1% of the U.S. NmB isolates (2000–2005), whereas the proportion of NmB harboring FHbp B/v1 ranged from 59.8% to 79% in the Czech Republic, France, Norway, Spain, Germany, and England (including Wales and Northern Ireland) during 2001–2006.[30] FHbp peptide 1 (B24/1.1) has consistently been the most common FHbp detected in U.S. NmB isolates since 2000 (data before 2000 is not available). FHbp peptides 4 (B16/1.4) and 15 (B44/1.15) were the most prevalent peptides reported in England, together accounting for 45.5% of NmB isolates in 2001–2006.[32] However, FHbp peptides 4 and 15 were only detected among 5.9% of NmB (weighted for Oregon) ABCs isolates during 2000–2014. Similar observations were also made with NhbA and NadA. A European study using isolates collected from 2007–2008 reported that the predominant NhbA peptides in NmB isolates was different from those in the United States. NhbA p0002, the antigen included in MenB-4C, was the most common peptide in the European study, while NhbA p0005 was the most common peptide identified in this study in the United States (Fig. 2C). NhbA p0002 was detected in 22%–31.7% of NmB isolates in France, England (and Wales), Germany, Italy, and Norway.[33] NhbA p0002 was detected in only 11.3% of ABCs NmB isolates in 2009–2014. While NadA-1.1 was the predominant peptide detected among NmB isolates in both Europe and the United States, the prevalence was notably different.[31,33] NadA-1.1 was found in 10% of NmB isolates collected from 2009–2010 in Spain,[31] but was detected in 39% of isolates in the current study (97.5% of NmB isolates that code for a full length protein).

While the ABCs strain collection provides population-based data on the distribution of the NmB vaccine antigens in the United States, analysis of invasive isolates from Expanded Surveillance Sites in 2013–14 provided insight into additional vaccine antigen

types in circulation. Because not all Expanded Surveillance Sites have provided a consistent proportion of meningococcal isolates, it is not feasible to assess the vaccine antigen prevalence and distribution. Enhanced Meningococcal Disease Surveillance was implemented in 2015 and includes 44 states and three large jurisdiction health departments. The isolates collected from Enhanced Meningococcal Disease Surveillance will allow for a more broad assessment of the distribution of NmB vaccine antigens in the United States.[34]

The genetic diversity of NmB vaccine antigens can impact NmB vaccine coverage. FHbp provides immunological cross-protection against disease isolates harboring sequence diverse FHbp peptides from the same subfamily, but only limited protection across subfamily.[35,36] All NmB isolates from ABCs 2009–2014 have the genetic potential to express FHbp, with 56% containing a member of FHbp B/v1 and 44% of A/v2-3. A larger percentage of NmB isolates containing FHbp B/v1 peptides have the potential to express full-length NadA peptides (62.5%). Among the NmB isolates that have an FHbp A/v2-3 peptide, only 11.4% have the potential to express full-length NadA peptides. All NmB isolates except for one from ABCs 2009–2014 contain NhbA, and the pairwise amino acid sequence identity among NhbA peptides identified is at least 70% to p0002 (antigen in MenB-4C). Rajam et al. showed the antibodies elicited from the antigen was able to cross-react with a variety of NhbA collected from NmB isolates in the U. S. (2000–2008);[37] 75% of NmB isolates from ABCs 2009–2014 carried one of these NhbA, which have potential to be cross-protected. However, the gene encoding PorA that was an OMV component of Bexsero® was only detected in 8 of 155 ABCs NmB isolates.

The genetic diversity of vaccine antigens is not enough to accurately predict the strain coverage. Additional factors that impact vaccine coverage include antigen accessibility to bactericidal antibodies and the level of antigen expression at the bacterial surface. The serum bactericidal assay using human complement (hSBA), a measure of vaccine elicited antibodies that initiate complement-mediated killing of invasive MenB isolates, is recognized as the surrogate of efficacy for meningococcal vaccines.[38] Recent studies using sera from human subjects vaccinated with MenB-FHbp have shown vaccine coverage for isolates harboring diverse FHbp peptides in the United States and Europe.[39–41] In addition, MenB-4C showed high NmB strain coverage using isolates collected in various countries.[33,41–50] However, testing of a panel of representative meningococcal isolates that express each of the hundreds of unique FHbp sequence variants using hSBA require large volumes of serum and human complement, which has limited the routine use of hSBA in assessing strain coverage. . To help predict the susceptibility of NmB isolates to vaccine immune sera, assays designed to measure the level of meningococcal antigen expression have been developed (MEASURE,[51] and MATS,[52]). Previous reports have shown that the level of FHbp surface expression can differ by as much as 15-fold, in some cases even when comparing strains that have the same FHbp peptide.[40,51,53–55] It should be noted that MATS does not evaluate the impact of OMV components (except PorA) on strain coverage. Evaluation of antigen expression among the NmB isolates included in this study is currently under investigation.

Epidemiological analysis has demonstrated that FHbp subfamily/variant distribution varies by geographic locations and age groups. A higher proportion of NmB isolates that carried FHbp A/v2-3 peptides was detected in infants <1 year old and adults over 65 years old; a similar distribution was observed in the previous study[19] as well as in data collected from Europe.[30] Since the two licensed NmB vaccines are formulated with different compositions, it is important to monitor vaccine antigen distribution and expression among different age groups. A recent study from the United Kingdom reported on the effectiveness of MenB-4C against serogroup B disease after two doses to infants.[56] While encouraging, evaluation of the long-term impact of vaccination on meningococcal serogroup B disease is warranted.

Declaration of Competing Interest

PL, LH, and ASA are current employees of Pfizer and may hold stock options.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.09.001.

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