



Genetic Features of a Representative Panel of 110 Meningococcal B Isolates to Assess the Efficacy of Meningococcal B Vaccines

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ABSTRACT Predictions of vaccine efficacy against *Neisseria meningitidis* serogroup B (NmB) disease are hindered by antigenic variability, limiting the representativeness of individual NmB isolates. A qualitative human serum bactericidal assay using endogenous complements of individual subjects (enc-hSBA) enables large panels of NmB isolates to be tested. A 110-isolate panel was randomly selected from 442 invasive NmB isolates from United States cases reported to the Centers for Disease Control (CDC) from 2000 to 2008. Typing analyses confirmed the 110-isolate panel is representative of the 442 isolates. The genetic features of the 110-isolate panel were compared against over 4,200 invasive NmB isolates collected from 2000 to 2018 in the United States, Australia, Canada, and nine European countries. Clonal complexes in the 110-isolate panel are also present in each geographical region; cumulative percentages show that these account for around 81% of the clonal complexes found in NmB isolates in other panels. For the antigens (fHbp, NHBA, PorA1.4, NadA) included in the currently licensed meningococcal serogroup B (MenB) vaccines, specifically considering the presence of at least one antigen with a matched genotype, the 110-isolate panel represents approximately 89% of the NmB isolates circulating worldwide, ranging from 87% for the European isolates to 95% and 97% for NmB isolates in the United States and Australia, respectively. The 110-isolate panel includes the most prevalent clonal complexes and genetic variants of MenB vaccine antigens found in a multinational collection of invasive NmB isolates. This panel is useful for assessing the efficacy of MenB vaccines in clinical trials worldwide.

IMPORTANCE *Neisseria meningitidis* serogroup B (NmB) is a major cause of invasive meningococcal disease (IMD). Predicting the effectiveness of vaccines against NmB is difficult because NmB is an uncommon disease and because antigens targeted by meningococcal serogroup B (MenB) vaccines have highly variable genetic features and expression levels. Therefore, a large number of NmB isolates from different regions would need to be tested to comprehensively assess vaccine effectiveness. We examined a panel of 110 isolates obtained from NmB IMD cases in the United States and compared the genetic features of this panel with those of panels from different countries around the world. We found the 110-isolate panel included the most common clonal complexes and genetic variants of MenB vaccine antigens that exist in the global collections of invasive NmB isolates. This confirms the value of the NmB 110-isolate panel in understanding the effectiveness of MenB vaccines in clinical trials worldwide.

KEYWORDS meningococcal disease, 4CMenB, MenB-FHbp, vaccine effectiveness, serogroup B, bacterial isolates, genetics, efficacy, clinical trial

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Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis* (meningococcus), has a rapid onset and is associated with poor outcomes, even with prompt treatment (1, 2).

The incidence of IMD is low, under one case per 100,000 population in most countries (1), which hinders the measurement of meningococcal vaccine efficacy in clinical trials (3).

The assessment of meningococcal vaccine efficacy in the field may be accelerated by model-based approaches calibrated with existing epidemiological data to demonstrate the real effectiveness and impact of meningococcal vaccines (4, 5). The immunogenicity of meningococcal vaccines can be measured via a serum bactericidal assay using human complement (hSBA) against isolates specific for each vaccine antigen (6). However, for *N. meningitidis* serogroup B (NmB), each antigen displays genetic variability and different genotype combinations, and antigen expression levels can vary greatly, limiting the representativeness of individual indicator strains. Accurate predictions of efficacy or effectiveness elicited by multicomponent meningococcal serogroup B (MenB) vaccines therefore requires the testing of a large panel of NmB isolates that represent the major genetic profiles of disease-causing isolates worldwide. This is addressed with a new approach, which consists of an hSBA that uses an endogenous complement present in each subject's serum (enc-hSBA) (7, 8). In contrast to the classical hSBA method, enc-hSBA offers a qualitative rather than quantitative assessment of the killing activity against each strain tested. However, this potential limitation is largely offset by its important advantages, including the possibility to test large panels of NmB isolates and the ability to account for intersubject variability in terms of the capability to elicit complement-mediated bactericidal killing of NmB isolates and for any synergistic effect of multiple vaccine antigens against circulating isolates with heterogeneous genetic features. Overall, the use of enc-hSBA on a broad strain panel can be considered the best proxy for predicting the effectiveness of a MenB vaccine in a clinical trial setting.

Two licensed MenB vaccines, MenB-FHbp (Trumenba, Pfizer) and 4CMenB (Bexsero, GSK), are currently available globally. MenB-FHbp is composed of two factor H-binding protein (fHbp) subvariants belonging to subfamily A (variant 3.45) and B (variant 1.55) (9). 4CMenB is a four-component MenB vaccine that includes three recombinant protein antigens, *Neisseria* adhesin A (NadA) peptide 3.8, neisserial heparin-binding antigen (NHBA) peptide 2, and fHbp subvariant 1.1, plus detergent-extracted outer membrane vesicles obtained from a New Zealand outbreak isolate, which contain porin A (PorA) protein with serosubtype P1.4 as the main vaccine antigen (10, 11). MenB-FHbp coverage has been demonstrated using 14 NmB strains that represent the breadth of the meningococcal fHbp diversity of approximately 80% circulating NmB strains (12). Strain coverage by 4CMenB has been predicted using the Meningococcal Antigen Typing System (MATS) and genetic MATS (gMATS) (13–15). In clinical trials of 4CMenB and MenABCWY vaccines, isolate coverage and serological correlates of clinical efficacy (breadth of coverage) will be assessed by enc-hSBA by using a panel of 110 invasive NmB isolates that were randomly selected from 442 isolates collected from 2000 to 2008 in 10 jurisdictions across the United States, participating in the Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance (ABCs) (16, 17). The 110-isolate panel has been characterized by multilocus sequence typing (MLST), NmB antigen sequence typing, and MATS (16).

Here, the genetic features of the 110-isolate panel are described in terms of clonal complexes and the distribution of individual MenB antigen genotypes relative to the global genetic diversity of NmB isolate sequences in the PubMLST database (18). These characteristics are also compared with the genetic features of a global collection of over 4,200 invasive NmB isolates from the United States, Australia, Canada, and nine European countries. MLST and antigen typing are also used to compare the 110-isolate panel with the original ABCs panel of 442 isolates. The main aim of these analyses was to confirm the suitability of the 110-isolate panel for assessing, by enc-hSBA testing, the effectiveness of MenB vaccines in clinical trials.

RESULTS

Phylogenetics. The phylogenetic distribution of the 110-isolate NmB panel, relative to around 6,700 MLST profiles of NmB isolates downloaded from PubMLST, was reconstructed in a minimum spanning tree created in PHYLOViZ, using the goeBURST algorithm (Fig. 1).

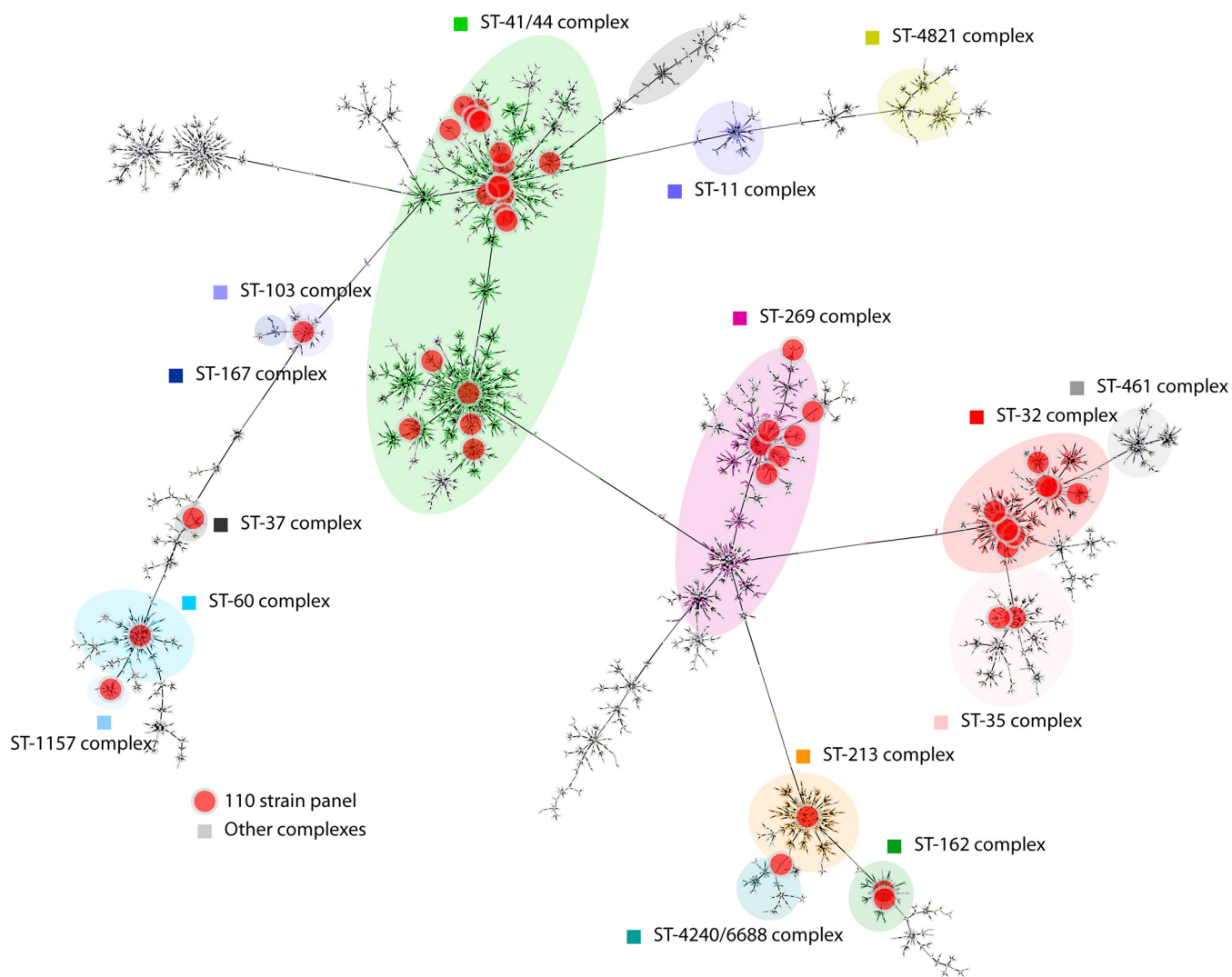


FIG 1 Phylogenetic distribution of the 110-isolate NmB panel in relation to around 6,700 multilocus sequence typing (MLST) profiles of NmB isolates downloaded from PubMLST, as reconstructed in a minimum spanning tree created in PHYLOVIZ using the goeBURST algorithm. The large red dots represent the 110-isolate panel STs, the small colored dots represent the major clonal complexes, and the small gray dots represent singlets or other minor clonal complexes. NmB, *Neisseria meningitidis* serogroup B; ST, sequence typing.

Out of the 46 clonal complexes described in the PubMLST database, 11 clonal complexes are represented in the 110-isolate panel. These 11 clonal complexes correspond to the most relevant and hypervirulent NmB complexes (19), and 83.2% of the NmB isolates present in the PubMLST database belong to these 11 complexes (excluding singlets, i.e., profiles not assigned to a clonal complex). The positioning of isolates belonging to the 110-isolate panel is widespread within the clonal complex clusters, indicating the inclusion of the central (founder) sequence types for eight important clonal complexes (ST-41/44, ST-32, ST-269, ST-213, ST-162, ST-35, ST-60, and ST-1157) and the coverage of many satellite clonal complexes and substructures in each clonal complex (Fig. 1) (Fig. S1).

Reconstructions created from a phylogenetic network analysis of 1,332 fHbp peptides and 648 NHBA peptides downloaded from PubMLST were superimposed with the peptides present in the 110-isolate panel (Fig. 2 and 3). This shows that variability in fHbp and NHBA peptides in the 110-isolate panel is comparable to the global range of fHbp and NHBA peptides. Moreover, the fHbp and NHBA average protein sequence identity was measured within the repertoire of protein sequences present in the 110-isolate panel and within the remaining group of sequences in the PubMLST database. These within distances were then compared to the average identity between the pairs of sequences belonging to each of the two groups or

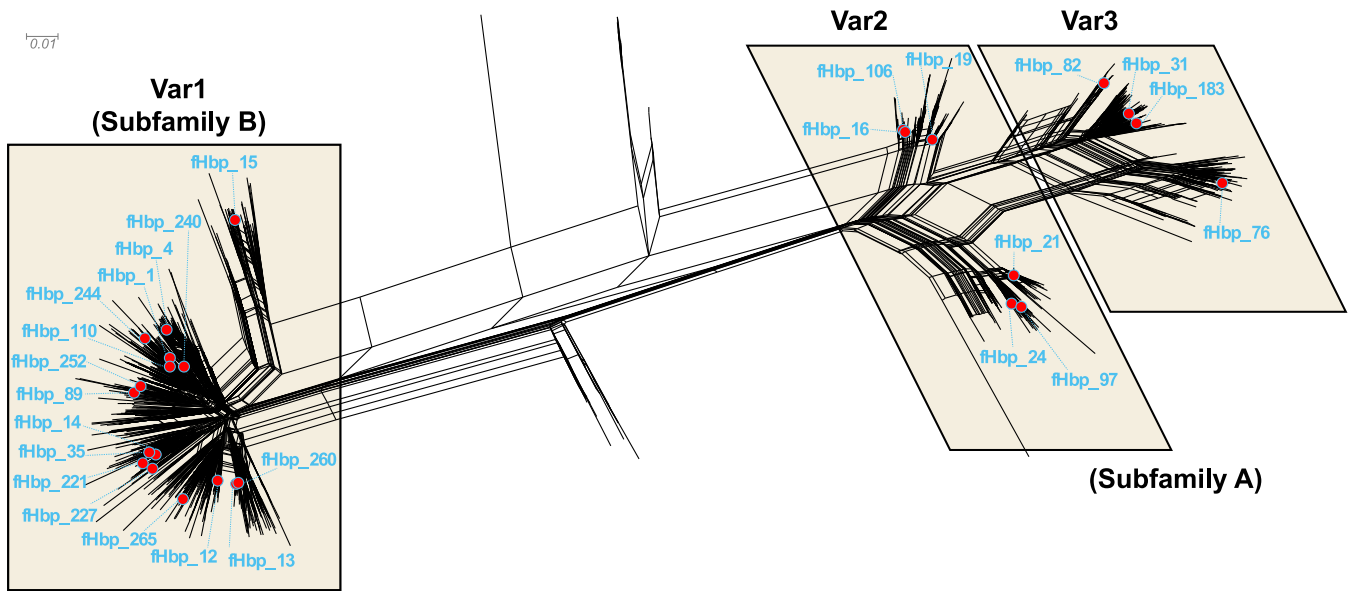


FIG 2 Phylogenetic network analysis of 1,332 factor H-binding protein (fHbp) peptides downloaded from PubMLST, showing (with red dots) the peptides present in the 110-isolate panel.

to the overall average protein identity, considering the totality of all molecules. For fHbp, the average (\pm standard error) peptide sequence identity present in the 110-isolate panel was $81.1\% \pm 1.3\%$, and the average identity of the remaining fHbp peptides was $80.5\% \pm 1.3\%$. These percentages were compared to the average identity of peptides between the two groups, which was $81.0\% \pm 1.3\%$, and to the overall identity (considering the entire set of sequences), which was $80.5\% \pm 1.3\%$. For NHBA, the average identity of the molecules pres-

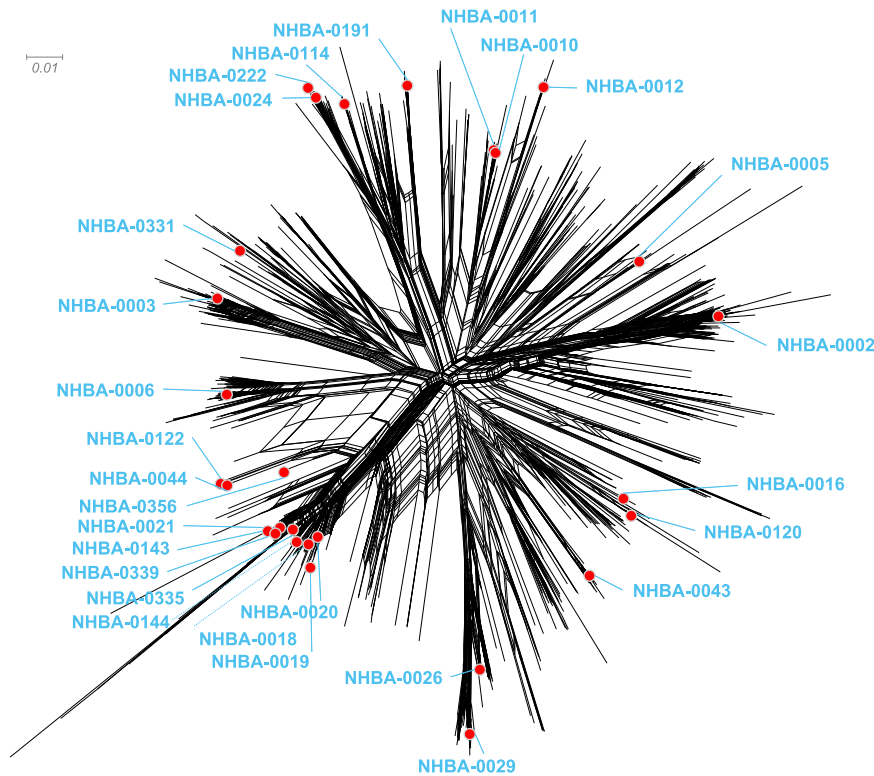


FIG 3 Phylogenetic network analysis of 648 neisserial heparin-binding antigen (NHBA) peptides downloaded from PubMLST, showing (with red dots) the peptides present in the 110-isolate panel.

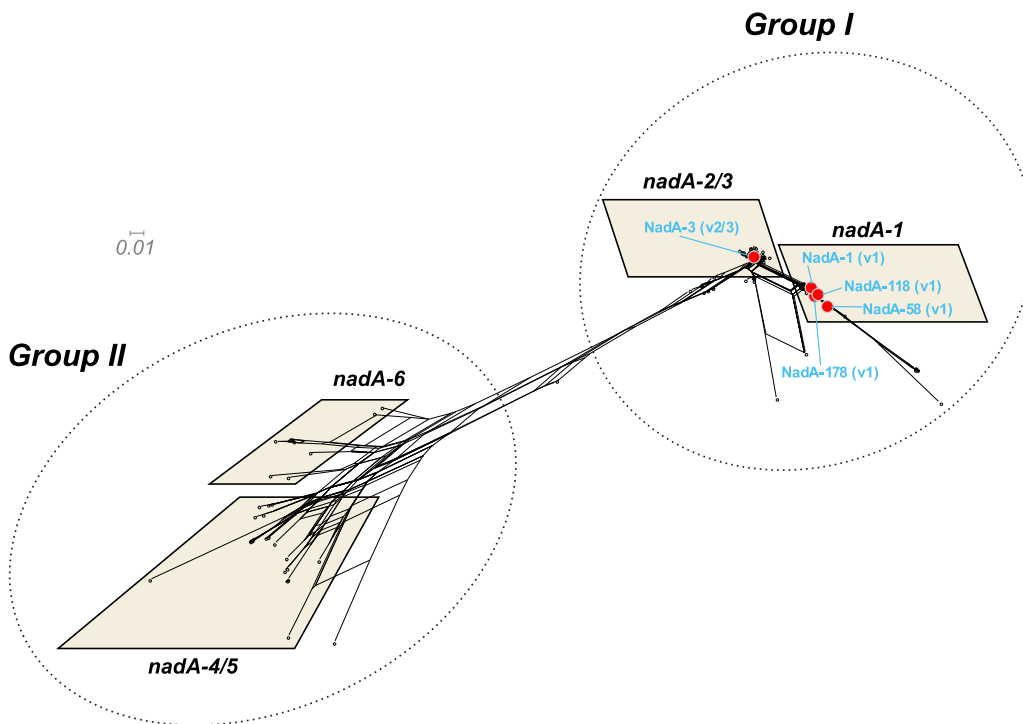


FIG 4 Phylogenetic network analysis of 130 *Neisseria* adhesin A (NadA) peptides downloaded from PubMLST, showing (with red dots) the peptides present in the 110-isolate panel.

ent in the 110-isolate panel was $86.4\% \pm 1.0\%$, and the average identity of the remaining NHBA molecules was $87.9\% \pm 1.0\%$. In comparison, the average identity of molecules between the two groups was $86.9\% \pm 1.1\%$, and overall, the average identity was $86.4\% \pm 0.9\%$. Both comparisons confirm an unbiased diversity of the fHbp and NHBA peptides represented in the 110-isolate panel in comparison to the global molecular diversity of these antigens. The situation is slightly different for the NadA molecular variability (Fig. 4). The peptide sequence identity present in the 110-isolate panel was $97.7\% \pm 0.5\%$, indicating similar sequences in this panel, while the average identity of the remaining NadA peptides was $81.0\% \pm 1.2\%$. The average identity of peptides between the two groups was $85.9\% \pm 0.9\%$ and overall, the average identity was $81.3\% \pm 1.0\%$.

Comparisons with the ABCs panel and other global panels. A comparison of the genetic features of the 110-isolate panel with the original 442-isolate ABCs panel shows close similarities in terms of distribution of clonal complexes and MenB antigens fHbp, NHBA, and PorA variable region 2 (VR2) matched with peptide 4, as well as *nadA* gene presence (Fig. S2; Table S1). For most isolates, the potential for NadA peptide expression could not be determined due to the absence of the *nadA* gene, the presence of frameshift mutations, or the presence of insertion elements (Table S1). Isolate coverage prediction by gMATS was also similar: 85% (upper limit [UL], lower limit [LL]: 76, 95) for the original 442-isolate ABCs panel (15) and 85% (UL, LL: 74, 97) for the 110-isolate panel. These comparisons confirm that the 110-isolate panel reflects the diversity seen in the original 442-isolate panel.

The wider relevance of the 110-isolate panel was assessed by comparing its genetic features (clonal complexes and distribution of individual MenB antigen genotypes) against invasive NmB isolate panels from different geographical regions (Table S2): the United States (ABCs panel of 442 isolates plus a panel of 306 isolates collected in 2015–2017 by the Enhanced Meningococcal Disease Surveillance [EMDS]), Australia (collected from 2007 to 2011), Canada (2006 to 2009 and 2010 to 2014), and nine European countries combined (various collection years between 2007 and 2018; see Table S2). The comparison shows some differences in the relative frequencies of specific clonal complexes, such as a lower

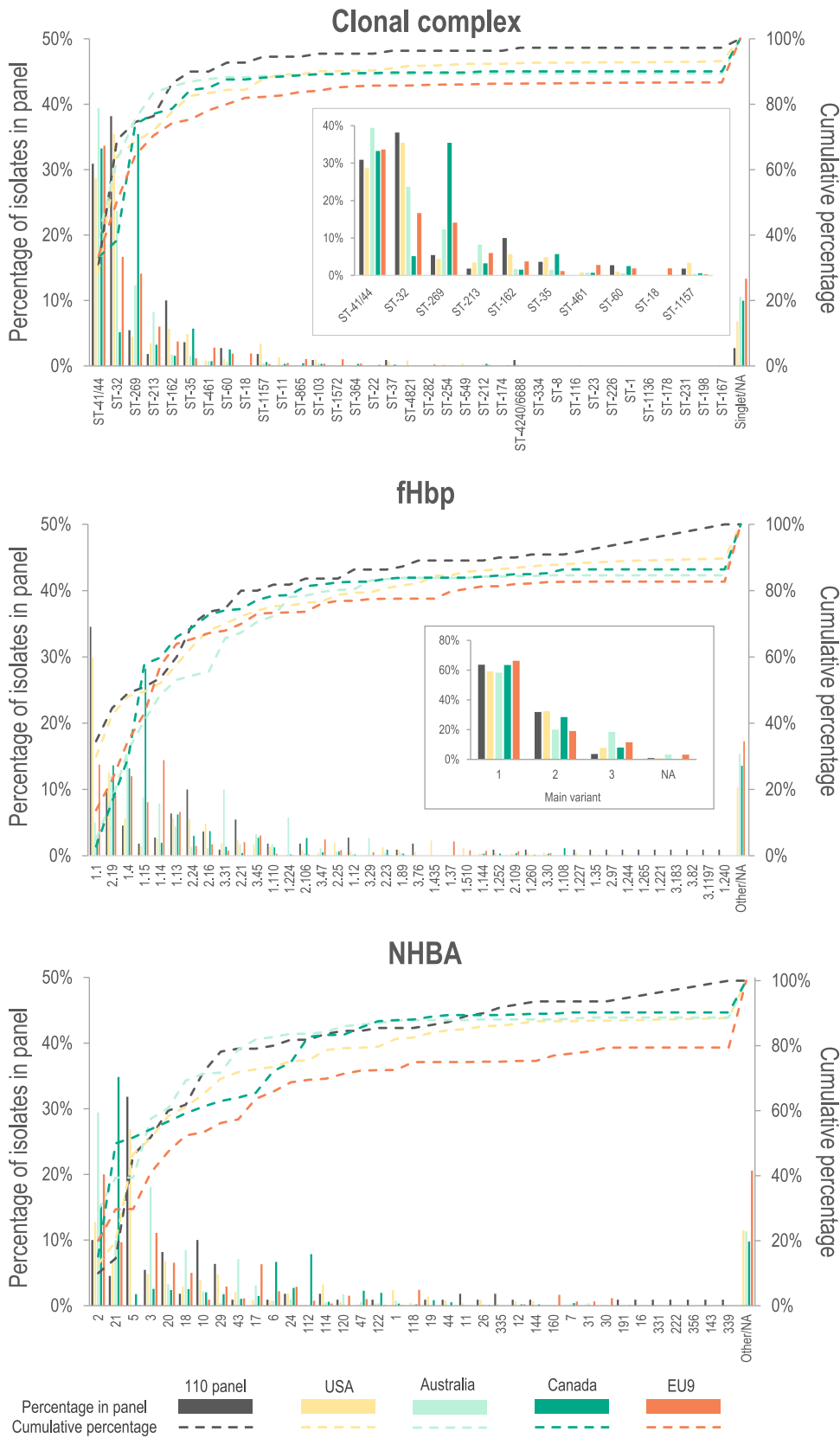


FIG 5 Clonal complex, fHbp peptides, and NHBA peptides distribution in NmB invasive disease cases reported in the United States, Australia, Canada, and nine European countries in comparison to the 110-isolate panel. The inset graphs show the 10 most frequent clonal complexes and the fHbp variant 1, 2, and 3 distributions. EU9: Finland, France, Germany, Greece, Italy, Norway, Poland, Spain, UK; fHbp, factor H-binding protein; NmB, *Neisseria meningitidis* serogroup B; NA, not available; NHBA, neisserial heparin-binding antigen.

TABLE 1 Relative percentages of isolates harboring porin A variable region 2 (PorA VR2) matched with peptide 4 and *Neisseria* adhesin A (*nadA*) gene presence in *Neisseria meningitidis* serogroup B (NmB) invasive disease cases reported in United States, Australia, Canada, and nine European countries in comparison to 110-isolate panel

Antigens	No. (percentage) of isolates in panel				
	110-isolate panel	USA (748 isolates) ^a	Australia (520 isolates)	Canada (407 isolates) ^b	EU9 (2,601 isolates) ^c
PorA VR2					
Peptide 4	8 (7.3%)	46 (6.1%)	105 (20.2%)	61 (15.0%)	449 (17.3%)
Others	102 (92.7%)	700 (93.6%)	415 (79.8%)	346 (85.0%)	2,144 (82.4%)
NA	0	2 (0.3%)	0	0	8 (0.3%)
<i>nadA</i> gene					
Present	42 (38.2%)	266 (35.6%)	162 (31.2%)	33 (8.1%)	544 (20.9%)
Absent	63 (57.3%)	425 (56.8%)	343 (66.0%)	365 (89.7%)	1,713 (65.9%)
FS/IS/NA	5 (4.5%)	57 (7.6%)	15 (2.9%)	9 (2.2%)	344 (13.2%)

^aData for two epidemiological panels combined. Averages of the relative percentages of the two panels remain the same for PorA VR2. For the *nadA* gene, 35.2% (present), 56.1% (absent), and 8.8% (FS/IS/NA).

^bData for two epidemiological panels combined. Averages of the relative percentages of the two panels are for PorA VR2 14.7% (peptide 4) and 85.3% (others). For the *nadA* gene, 7.9% (present), 90.2% (absent), and 1.9% (FS/IS/NA).

^cData for 12 epidemiological panels combined. Averages of the relative percentage of the panels are for PorA VR2 18.7% (peptide 4), 81.2% (others), and 0.1% (NA). For the *nadA* gene, 21.4% (present), 66.3% (absent), and 12.3% (FS/IS/NA). EU9: Finland, France, Germany, Greece, Italy, Norway, Poland, Spain, UK; FS/IS/NA, frameshift mutation/presence of DNA insertion within *nadA* gene/not available.

frequency of ST-32 and higher frequency of ST-269 in Canada than in other panels (Fig. 5). However, cumulative distribution percentages suggest comparable prevalent clonal complexes across the different isolate collections, with major clonal complexes in the 110-isolate panel (excluding singlets) also being present in the other geographical areas. These represent 89% (95% confidence interval [CI]: 86% to 92%) of the isolates for the United States, 88% (86% to 91%) for Australia, 88% (83% to 93%) for Canada, and 78% (70% to 86%) for Europe, with an average of 81% (74% to 88%) of isolates in the other panels, overall.

The proportions of individual fHbp, NHBA, and PorA variable region 1 and 2 (VR1 and VR2) peptides vary among the different isolate panels, but the cumulative distributions of the variants show close similarities (Fig. 5; Fig. S3). The fHbp peptides found in the 110-isolate panel represent averages of 79% (95% CI: 75% to 83%), 70% (66% to 74%), 79% (74% to 85%), and 71% (63% to 79%) of isolates collected in the United States, Australia, Canada, and Europe, respectively, while the NHBA peptides in the 110-isolate panel represent on average, respectively, 84% (81% to 88%), 83% (80% to 87%), 78% (72% to 84%), and 65% (56% to 74%) of the isolates. For the PorA VR1 and VR2 peptides, these percentages are 78% (95% CI: 73% to 82%) for the United States, 53% (49% to 58%) for Australia, 68% (62% to 75%) for Canada, and 60% (52% to 68%) for Europe (excluding Italy, which lacked PorA VR1 data). The frequencies of the 10 most common peptides (by average decreasing order of distribution) for each of these MenB antigens are also comparable between the 110-isolate panel and other panels, with prevalence ranging from 67% (Australia) to 74% (Canada) for fHbp, from 64% (Europe) to 82% (Australia) for NHBA, and from 45% (Europe) to 60% (Canada) for PorA VR1 and VR2.

PorA VR2 with peptide 4 (the variant contained in the 4CMenB vaccine) was present in 7% of isolates in the 110-isolate panel, similar to the proportion in the United States collection (6%), with the highest proportion being observed in the Australia panel (20%) (Table 1). The *nadA* gene was detected in 38% of isolates in the 110-isolate panel, while the distribution of *nadA*-positive NmB isolates in the other isolate panels ranged from 8% (Canada) to 36% (United States) (Table 1).

For each geographical panel, we also investigated the percentage of isolates showing a match with the repertoire of antigen genotypes present in the 110-isolate panel, taking into consideration the presence of at least one antigen included in the currently licensed MenB vaccines (fHbp and NHBA peptides, PorA VR2 with peptide 4, and *nadA* gene presence). In this analysis, the percentage of isolates represented by the 110-isolate panel was 87% (95% CI: 82% to 93%) for Europe, 90% (86% to 94%) for Canada, 95% (93%

to 97%) for the United States, and 97% (96% to 99%) for Australia. On average, the 110-isolate panel represents approximately 89% (95% CI: 84% to 94%) of the NmB isolates circulating worldwide.

DISCUSSION

The immunogenicity of MenB vaccines can be measured by hSBA against isolates specific for each vaccine antigen. However, due to the genetic variability of each antigen and the different genotype combinations and because antigen expression can vary greatly, a large panel of isolates needs to be tested to enable accurate predictions of vaccine efficacy or effectiveness. The approach developed by GSK assesses the bactericidal activity of vaccine-induced antibodies using the endogenous complements of individual subjects (enc-hSBA), allowing for testing across large panels of epidemiologically relevant NmB isolates (8).

We characterized the genetic features of a 110-isolate NmB panel that was qualified for use in enc-hSBA testing and was derived from 442 NmB isolates collected from IMD cases in the United States from 2000 to 2008. The 110-isolate panel was randomly selected to ensure the representation of the most frequent MenB vaccine antigen genotypes that were likely to be important in the generation of bactericidal antibodies, as assessed by enc-hSBA. The 110-isolate panel was found to reflect the diversity seen in the original 442-isolate ABCs panel, which has undergone extensive characterization in terms of the prevalence and genetic diversity of the MenB vaccine antigens NadA, NHBA, fHbp, and PorA VR2 (15–17). Moreover, the genetic characteristics of the ABCs 2000 to 2008 panel appear to be stable over time, as a comparison with isolates collected by ABCs in 2009 to 2014 showed that the predominant MenB vaccine antigen types remained the same (20).

Although there are differences in the frequencies of specific clonal complexes, the variety of clonal complexes present in the 110-isolate panel account for around 81% of the NmB isolates from the other countries that were analyzed in this study. Similarly, although there are differences in the frequencies of particular peptides, the fHbp, NHBA, and PorA VR1 and VR2 peptides in the 110-isolate panel represent on average 73%, 70%, and 63%, respectively, of the isolates in the other geographical regions assessed in this study. For the antigens included in the currently licensed MenB vaccines (fHbp, NHBA, PorA with peptide 4, and NadA), specifically considering the presence of at least one MenB vaccine antigen, the 110-isolate panel represents approximately 89% of the NmB isolates circulating worldwide, ranging from 87% for the European isolates to 95% and 97% for the NmB isolates in the United States and Australia, respectively. These data suggest that the 110-isolate panel includes the most prevalent clonal complexes and genetic variants of MenB vaccine antigens found in NmB isolates globally. Moreover, reconstructions created from a phylogenetic network analysis and a molecular identity analysis show that the diversity in fHbp and NHBA peptides present in the 110-isolate panel is comparable to the global range of fHbp and NHBA peptides. Although the 110-isolate panel is intended to be used to measure MenB vaccine effectiveness in clinical trials and not to estimate NmB isolate coverage, our results suggest the stable representation of the major circulating strains, even when considering fluctuations in isolate evolution. This is in line with evidence generated from other studies, which show the persistence of the most frequent clonal complexes and antigen variants in circulating NmB isolates monitored over long periods of time (21–25).

There were limitations to this assessment. The collection years for the isolate panels ranged from 2000 to 2018 overall, with 9 of the 17 panels containing isolates collected between 2000 and 2010. The regular monitoring of isolates collected in more recent years is planned to assess possible changes in the epidemiology of circulating strains. New data from MATS testing of recent isolates collected in the United States (2015 to 2017) and in other countries (e.g., the United Kingdom, Canada, Italy, Spain, etc.) will supplement these analyses, and augment critical information from the evaluation of hyperendemic and outbreak strain genotypes. This information could be used to update the panel, if required, by excluding less frequent genotypes and by adding recently emerging genotypes, thus ensuring the representativeness of the panel in different geographical regions and over time. Also, the 110-isolate panel was obtained

via a process of random selection from the ABCs panel and from the identification of isolates suitable for laboratory growth and for use in the enc-hSBA. Consequently, certain uncommon or emerging clonal complexes (e.g., ST-11/CC11 and ST-461/CC461) are not present in the 110-isolate panel. Moreover, our analysis takes into account *nadA* gene presence/absence rather than the expression of specific NadA peptide variants because complete sequencing data were not available for all panels (15). Also, the *nadA* gene was present in only 8% to 36% of isolates in each panel. Finally, the isolates in the ABCs panel originated from specific areas (10 jurisdictions) under surveillance in the United States during the period from 2000 to 2008 (17), and this may not be nationally representative.

In conclusion, the 110-isolate panel is a genetically diverse panel of NmB isolates and represents the majority of genotypes reported globally in the *N. meningitidis* PubMLST database. Moreover, the panel includes the most prevalent clonal complexes and genetic variants of MenB vaccine antigens found in a collection of over 4,200 invasive NmB isolates reported by reference laboratories that monitor NmB disease cases in the United States, Australia, Canada, and Europe. The NmB 110-isolate panel was found to reflect the diversity seen in the original 442-isolate ABCs panel of IMD NmB cases collected in the United States from 2000 to 2008. The NmB 110-isolate panel can therefore be considered a key tool for assessing, by enc-hSBA testing, the effectiveness of MenB vaccines in clinical trials with the potential for worldwide application, provided that epidemiologic differences in the genotypes of NmB circulating isolates are well-represented in the 110-isolate panel. It will be important to constantly monitor the epidemiological evolution of circulating NmB strains to assess the relevance of the 110-isolate panel in the future.

MATERIALS AND METHODS

Meningococcal B isolate panels. All of the isolate panels were of invasive disease NmB isolates recovered from the blood or cerebrospinal fluid of patients.

The 110-isolate panel was selected from a 442-isolate panel collected from the ABCs catchment areas during the time period from 2000 to 2008 in the United States (16, 17). A total of 140 NmB isolates were randomly selected from the ABCs panel to identify 110 isolates suitable for laboratory conditions (7). The 110 isolates were qualified for use in the enc-hSBA which, as described previously (7, 8), uses the endogenous complements present in the serum samples of each subject and is based on an assay described by Goldschneider et al. (26).

The panels of invasive NmB isolates collected in Australia, Canada, and nine European countries (Finland, France, Germany, Greece, Italy, Norway, Poland, Spain, and the UK) (22, 27–35) (Table S2) were described previously (15) with the addition of more recent isolate collections from Canada (2010 to 2014) (36) and the United Kingdom (2017 to 2018). These isolate panels can be considered to be representative of each country. For the United States, in addition to the ABCs panel of 442 isolates, a more recent panel of 306 isolates from the EMDS system, collected from 2015 to 2017 (<https://www.cdc.gov/meningococcal/surveillance/index.html>), was included in the analyses.

The whole-genome sequencing (WGS) of the entire collection of United States strains was conducted using Illumina platforms (HiSeq2500 or MiSeq; San Diego, CA). The Illumina reads were trimmed with cutadapt (37) to remove adaptor sequences and reads below the quality score of 28 (Q28) and 75 bp. *De novo* short-read assembly was carried out using SPAdes 3.7.0 (38) with the “careful” option.

Since the United States ABCs panel of 442 isolates was previously characterized by Sanger technology (17), the genotyping of MenB antigens and MLST results were compared to WGS results. In cases of incomplete information from the Illumina sequencing, the data were complemented with previously generated Sanger data. Antigens, MLST alleles, and corresponding peptide identification numbers (protein variants) were assigned from the sequences by using the PubMLST *Neisseria* MLST database (downloaded from February to August 2020; <https://pubmlst.org/neisseria/>).

Phylogenetic analysis. The clonal complex distribution of the 110-isolate panel was characterized using the phylogenetic relationships of the global MLST profiles of NmB isolates downloaded from February to August 2020 from PubMLST (<https://pubmlst.org/neisseria/>) by PHYLOVIZ (version 2.0) (39), using the goeBURST algorithm.

Phylogenetic reconstructions were made for fHbp, NHBA, and NadA by the analysis of protein peptide sequences downloaded from PubMLST. Sequence alignment was performed via MULTIPLE Sequence Comparison by Log-Expectation (MUSCLE version 3.8). The resulting distance matrices were computed with SplitsTree (version 4.14) (40), and the phylogeny was represented by NeighborNet method (41).

The antigen average molecular identity was measured by computing the number of identical amino acids per site and averaging over all sequence pairs within and between the compared groups. The standard error estimate of the average was obtained via a bootstrap procedure (50 replicates). All ambiguous positions were removed for each sequence pair (pairwise deletion option), and the analyses were conducted with MEGA X software (42).

Genetic features of the NmB 110-isolate panel and the comparison with global panels. The wider relevance of the 110-isolate panel was assessed by comparing its genetic features (clonal complexes

and distribution of individual MenB antigen genotypes), as characterized by MLST, against other global panels of invasive NmB isolates. For each single panel, the 95% CI of the point estimate frequency of genotypes matching with the 110-isolate panel was estimated, assuming a binomial distribution. Results from different panels were aggregated according to each country's geographical region, with the average frequency values calculated for each region, and the consequent CIs calculated by quadratically averaging single panel variances. Overall comparisons were made of the frequencies of individual MenB antigen genotypes in the 110-isolate panel and of the frequencies in panels from the United States (ABCs panel and EMDS panel combined), Australia, Canada, and nine European countries combined. Overall point estimates were computed as an average of the single panels' point estimates, and as CIs via quadratic average.

Antigen-specific predicted isolate coverage by gMATS was calculated as described previously (15).

Trademark statement. Bexsero is a trademark owned by or licensed to GSK. Trumenba is a trademark owned by Pfizer.

Data availability. GSK makes available the anonymized individual participant data and the associated documents from interventional clinical studies which evaluate medicines upon the approval of proposals submitted to www.clinicalstudydatarequest.com. To access data for other types of GSK sponsored research, study documents without patient-level data, and clinical studies not listed, please submit an inquiry via the website.

Genotyping data for most of the isolate panels analyzed in the present paper were published previously as supplementary information (15). Complete genotyping information for the USA EMDS panel (2015 to 2017), the ABCs panel (2000 to 2008), and the 110-isolate panel are provided as supplemental material (Table S3). Genomic data for the USA ABCs and EMDS panels are publicly available (National Center for Biotechnology Information, NCBI: accession numbers [PRJNA853880](https://www.ncbi.nlm.nih.gov/nuclink/PRJNA853880) and [PRJNA858835](https://www.ncbi.nlm.nih.gov/nuclink/PRJNA858835)).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, PDF file, 16.9 MB.

FIG S2, PDF file, 0.2 MB.

FIG S3, PDF file, 0.2 MB.

TABLE S1, PDF file, 0.2 MB.

TABLE S2, PDF file, 0.3 MB.

TABLE S3, XLSX file, 0.1 MB.

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