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Meningococcal Antigen Typing System (MATS)-Based *Neisseria meningitidis* Serogroup B Coverage Prediction for the MenB-4C Vaccine in the United States

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ABSTRACT *Neisseria meningitidis* is the most common cause of bacterial meningitis in children and young adults worldwide. A 4-component vaccine against *N. meningitidis* serogroup B (MenB) disease (MenB-4C [Bexsero]; GSK) combining factor H binding protein (fHBP), neisserial heparin binding protein (NHBA), neisserial adhesin A (NadA), and PorA-containing outer membrane vesicles was recently approved for use in the United States and other countries worldwide. Because the public health impact of MenB-4C in the United States is unclear, we used the meningococcal antigen typing system (MATS) to assess the strain coverage in a panel of strains representative of serogroup B (NmB) disease in the United States. MATS data correlate with killing in the human complement serum bactericidal assay (hSBA) and predict the susceptibility of NmB strains to killing in the hSBA, the accepted correlate of protection for MenB-4C vaccine. A panel of 442 NmB United States clinical isolates (collected in 2000 to 2008) whose data were down weighted with respect to the Oregon outbreak was selected from the Active Bacterial Core Surveillance (ABCs; CDC, Atlanta, GA) laboratory. MATS results examined to determine strain coverage were linked to multilocus sequence typing and antigen sequence data. MATS predicted that 91% (95% confidence interval [CI_{95}], 72% to 96%) of the NmB strains causing disease in the United States would be covered by the MenB-4C vaccine, with the estimated coverage ranging from 88% to 97% by year with no detectable temporal trend. More than half of the covered strains could be targeted by two or more antigens. NHBA conferred coverage to 83% (CI_{95}, 45% to 93%) of the strains, followed by factor H-binding protein (fHbp), which conferred coverage to 53% (CI_{95}, 46% to 57%); PorA, which conferred coverage to 5.9%; and NadA, which conferred coverage to 2.5% (CI_{95}, 1.1% to 5.2%). Two major clonal complexes (CC32 and CC41/44) had 99% strain coverage. The most frequent MATS phenotypes (39%) were fHbp and NHBA double positives. MATS predicts over 90% MenB-4C strain coverage in the United States, and the prediction is stable in time and consistent among bacterial genotypes.

IMPORTANCE The meningococcal antigen typing system (MATS) is an enzyme-linked immunosorbent assay (ELISA)-based system that assesses the levels of expression and immune reactivity of the three recombinant MenB-4C antigens and, in conjunction with PorA variable 2 (VR2) sequencing, provides an estimate of the susceptibility of NmB isolates to killing by MenB-4C-induced antibodies. MATS assays or similar antigen phenotype analyses assume importance under conditions in which analyses of vaccine coverage predictions are not feasible with existing strategies, including large efficacy trials or functional antibody screening of an exhaustive strain panel. MATS screening of a panel of NmB U.S. isolates (n = 442) predicts high MenB-4C vaccine coverage in the United States.

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Meningococcal Antigen Typing System (MATS) predicts high MenB-4C vaccine coverage in US

KEYWORDS MATS, MenB-4C, NHBA, NadA, *Neisseria meningitidis*, PorA, SBA, fHbp

Neisseria meningitidis, responsible for a global annual disease burden of ~50,000 deaths and as many long-term disabilities, is currently the most common cause of bacterial meningitis in children and young adults (1, 2). In the United States, serogroup B (NmB) strains cause approximately 30% of disease in all age groups, including adolescents, and >60% of cases in infants aged <1 year. Licensed conjugate vaccines have been successful in reducing the circulation and disease burden of serogroups A, C, W, and Y (3). However, serogroup B has presented unique challenges in vaccine development due to the similarity between the NmB capsular polysaccharide and a human neural cell adhesion molecule, which creates the risk of generating an autoimmune reaction (4, 5). The reverse vaccinology approach, starting from the genomic information determined for the bacterium, has enabled the identification of noncapsular protein surface antigens that could help prevent invasive meningococcal disease (IMD) caused by any capsular serogroup (6, 7). These included neisserial adhesin A (NadA), neisserial heparin-binding antigen (NHBA), and factor H-binding protein (fHbp), which, in combination with New Zealand strain outer membrane vesicles (NZ OMV) harboring the PorA P1.4 antigen, constitute the *N. meningitidis* serogroup B 4-component (MenB-4C) vaccine (Bexsero; GSK). MenB-4C was the first broad-coverage MenB vaccine based on recombinant proteins (8) and is the only one approved (in January 2013) for use in individuals 2 months of age and above by the European Medicines Agency (8). In January 2015, the U.S. Food and Drug Administration approved this vaccine for use in persons 10 to 25 years of age. Subsequently, the U.S. Advisory Committee on Immunization Practices (ACIP) recommended the use of MenB vaccines for all persons ≥10 years of age in certain high-risk groups and suggested its use for adolescents 16 to 23 years of age (9, 10).

Efficacy studies to demonstrate the benefit of meningococcal vaccines are not feasible due to the relatively low incidence of the disease. Efficacy of glycoconjugate vaccines against serogroups A, C, Y, and W was estimated using the serum bactericidal antibody assay with rabbit (rSBA) or human complement (hSBA) (11, 12) accepted as a surrogate of protection in the clinical evaluation of meningococcal vaccines (13, 14).

Extrapolating this approach to NmB poses significant challenges. The meningococcal genome's plasticity facilitates adaptation of surface structures to changing environments through a variety of genetic mechanisms (15–17) and generates a high variability of sequence and level of surface expression for protein antigens, affecting strain susceptibility to vaccine-induced antibodies. To predict the effectiveness of a MenB vaccine, a large panel of bacterial isolates representative of meningococcal disease in the United States would need to be tested in hSBA versus a significant number of subjects, thus requiring large volumes of serum and human complement. This would be a challenging undertaking, especially in infant populations.

To overcome these limitations, the meningococcal antigen typing system (MATS) was developed and standardized across public health laboratories worldwide (18, 19). The MATS combines a sandwich enzyme-linked immunosorbent assay (ELISA) to measure the immunologic cross-reactivity and quantity of antigen expression for three MenB-4C protein antigens (fHbp, NadA, and NHBA) with genetic typing of the PorA variable 2 (VR2) region to determine the immune recognition potential for the OMV component. MATS typing for each antigen correlates with killing in the hSBA and predicts the susceptibility of NmB strains to killing in the hSBA, i.e., the strain coverage of the MenB-4C vaccine (18). MATS has been shown to be a conservative predictor of strain coverage by the MenB-4C vaccine in infants and adolescents (20). In addition, MATS coverage was shown to correlate with high rates of individual seroprotection (21).

MATS has been used to predict MenB-4C strain coverage in multiple countries, including Canada and countries in Europe (22–25), using cross-sectional panels of strain isolated from meningococcal disease cases in the respective countries during one or

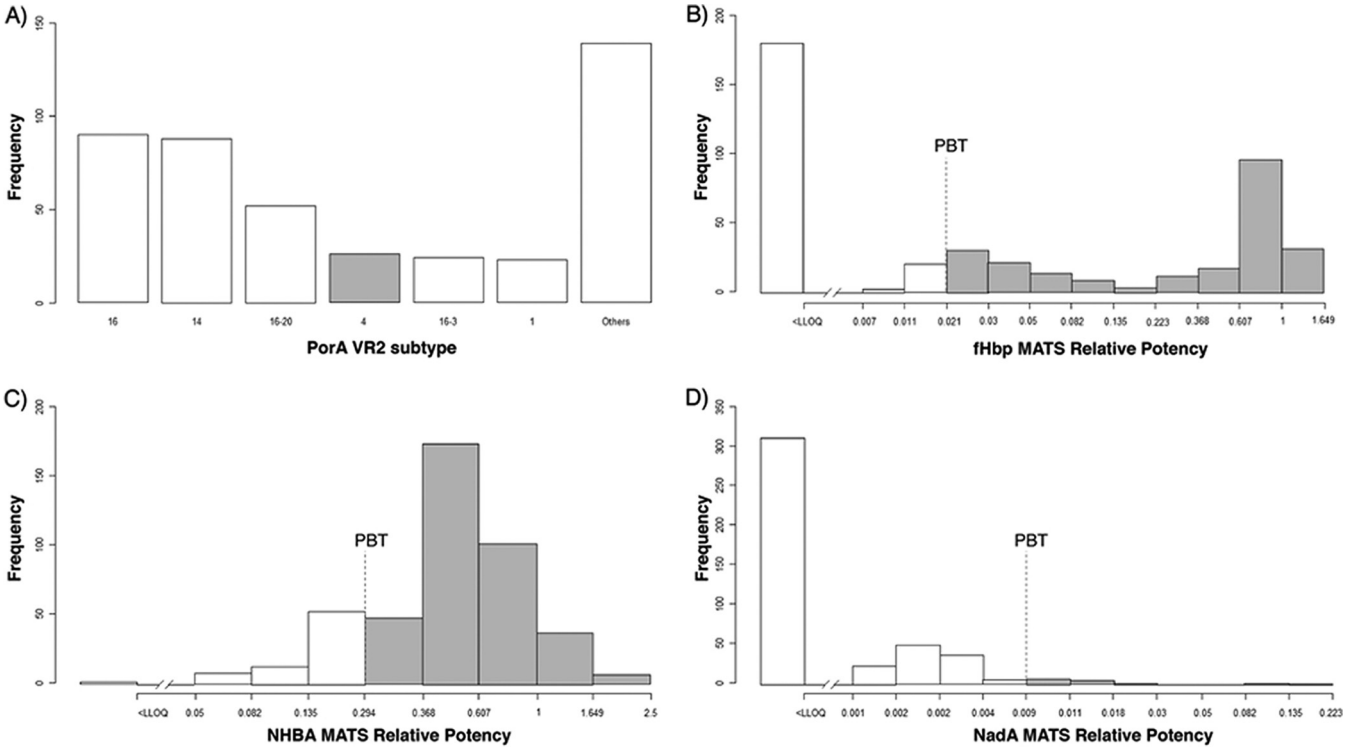

FIG 1 MenB-4C antigen frequency distribution in NmB U.S. isolates ($n = 442$). (A) Prevalence of PorA VR2 subtypes. Most of the NmB isolates belonged to the PorA 16 VR2 subtype (20.4%), followed by the PorA 14 VR2 subtype (19.9%). PorA VR2 subtype 4 is expressed in 5.9% of the isolates. (B) Frequency distribution of fHbp MATS relative potencies (RPs). Among the U.S. NmB isolates tested, 262/442 (59%) expressed fHbp above the MATS lower limit of quantitation (LLOQ) (gray bars). (C) Frequency distribution of NHBA MATS relative potencies (RPs) among the U.S. NmB isolates ($n = 442$). A total of 440 of the NmB isolates tested expressed NHBA above the MATS LLOQ (gray bars). (D) Frequency distribution of NadA MATS relative potencies (RPs). Among the U.S. NmB isolates tested, only 132 of 442 (30%) expressed NadA above the MATS LLOQ (gray bars).

two epidemiological years. So far, however, the MenB-4C strain coverage has not been estimated in the United States; neither has the longitudinal stability of such predictions been examined.

In this study, we evaluated a longitudinal panel of 442 U.S. NmB disease isolates (collected in 2000 to 2008) that were selected by the Active Bacterial Core Surveillance (ABCs; CDC) laboratory that are representative of serogroup B meningococcal disease in the United States (26). MATS analysis was performed on each NmB isolate, and the results were correlated with antigen genotyping data to determine MenB-4C strain coverage in the United States and to analyze its longitudinal trends from 2000 to 2008.

RESULTS

PorA VR2 subtype 4, covered by the OMV component of the MenB-4C vaccine (18), was identified in 23 strains (5.3%), while PorA subtypes 16 and 14 (each approximately 20%) predominated in 2000 to 2008 (Fig. 1A).

Among the NmB U.S. isolates tested, the specific genotype of fHbp protein included in MenB-4C, variant 1 and peptide 1, was identified in 144 of 442 strains (33%). Among the fHbp variant 1 results, the most common peptides were peptides 1, 13, and 4 (33%, 7.2%, and 5.0%, respectively) (see Table 2). The most common factor H-binding protein variant 2 peptides were peptides 19, 24, and 16 (11%, 7.2%, and 5.0%, respectively); all other variant 2 and 3 peptides were identified in less than 5% of isolates (see Table 2). MATS detected quantifiable fHbp expression in 262/442 (59%) isolates (Fig. 1B). The NmB isolates with nonquantifiable MATS relative potencies (RP) for fHbp harbored fHbp variants 2 and 3, which were not included in the MenB-4C vaccine. NHBA expression was detected in 440 (95%) NmB isolates with a quantifiable MATS RP. NHBA peptides 5, 20, 10, 29, 3, and 21 were predominant in the U.S. NmB isolates (see Table 3). The specific genotype of NHBA included in the MenB-4C vaccine is peptide 2. This specific

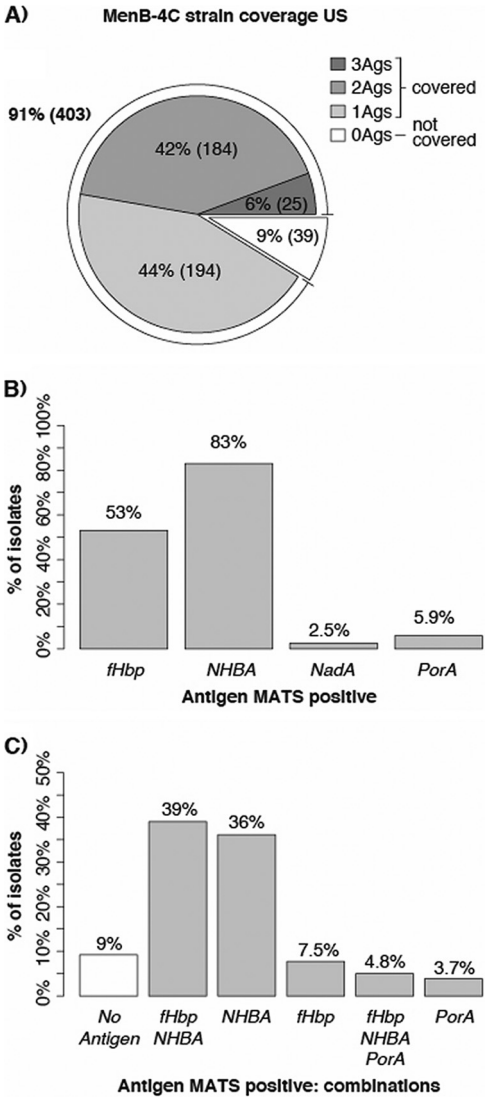

FIG 2 MenB-4C vaccine strain coverage potential among the U.S. NmB isolates ($n = 442$). (A) Among the U.S. NmB isolates tested, 194/442 (44%) expressed one antigen (1Ags), 184/442 (42%) two antigens (2Ags), and 25/442 (5.7%) three antigens (3Ags) at levels over the PBT thresholds for the respective antigens. Overall, MATS estimates 91% coverage of the 4CMenB vaccine among U.S. NmB isolates. 0Ags, no antigens. (B) Contribution of individual antigens to 4CMenB coverage of the U.S. NmB isolates ($n = 442$). NHBA conferred maximum coverage potentials, with 83% of NHBA-positive strains exhibiting PBT values over the threshold, followed by fHbp (53%), PorA (5.9%), and NadA (2.5%). (C) Frequency of MATS phenotypes among the U.S. NmB isolates. The most frequent combination was represented by MATS double positives for fHbp and NHBA antigens (39%) followed by MATS single positives for NHBA antigen (36%). The MATS-negative phenotype accounted for 9%.

genotype was identified in only 42 of 442 (9.5%) isolates, but MATS demonstrated the immune reactivity of anti-NHBA antibodies with the majority ($n = 440$) of test isolates, irrespective of the NHBA genotype (Fig. 1C).

The *nadA* gene was harbored by 170/442 (39%) strains, and 78% of these isolates (132/170) expressed NadA with quantifiable MATS RP (Fig. 1D).

Figure 2 summarizes the results of the MenB-4C strain coverage analysis performed. Results indicated that 91% (95% confidence interval [CI_{95}], 72% to 96%) of the NmB U.S. isolates are predicted to be covered by the MenB-4C vaccine. While 5.7% (25/442) of the isolates had three antigens covered (RP > positive bacterial threshold [PBT] and/or PorA = P1.4), 42% (184/442) and 44% (194/442) had two antigens and one antigen covered, respectively (Fig. 2A). NHBA alone or in combination with another antigen(s)

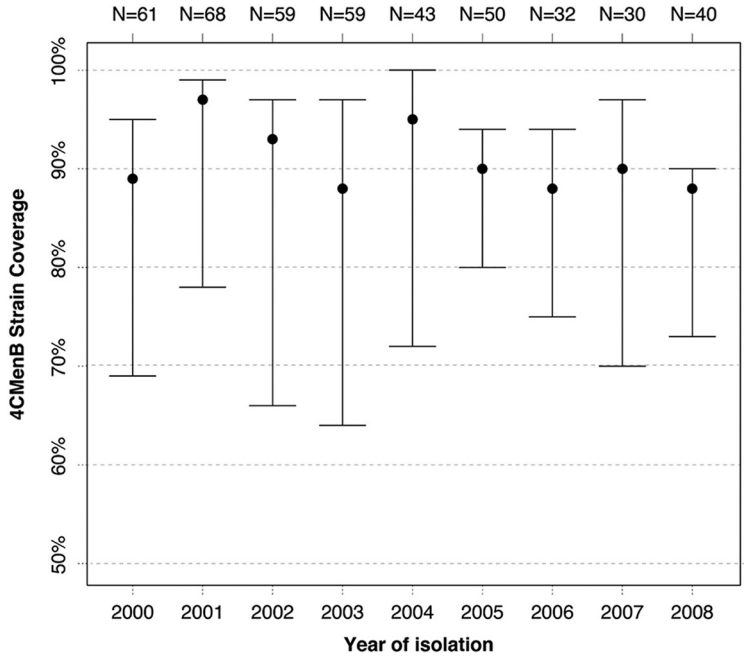

FIG 3 MATS coverage of NmB strains isolated in the United States during 2000 to 2008 by at least 1 antigen (95% CI) by year. Comparisons of point estimates of predicted strain coverage across groups did not identify any statistically significant difference ($P = 0.567$).

had RP values that were greater than the PBT values determined for 83% (CI_{95}, 45% to 93%) of the NmB isolates followed by fHbp with 53% (CI_{95}, 46% to 57%). The proportions of MATS-predicted coverage for PorA and NadA were 5.9% and 2.5% (CI_{95}, 1.1% to 5.2%), respectively (Fig. 2B). In the case of NadA, the low contribution may have been due to the growth conditions in use for MATS testing, which repress *nadA* expression (27, 28). Having four antigens and two possible states (MATS positive or negative) each, $2^4=16$ antigen combinations or MATS phenotypes can be observed. As shown in Fig. 2C, only 5 of the 16 phenotypes were observed with a frequency of >1%, accounting overall for 96% of the panel. The most frequent MATS phenotype was the double-positive phenotype for fHbp and NHBA antigens, followed by NHBA positive, negative for all antigens, fHbp positive, and triple positive for fHbp, NHBA, and PorA.

Data corresponding to predicted strain coverage by year of strain isolation in the United States from 2000 to 2008 are shown in Fig. 3 (see also Table S1 in the supplemental material). Point estimates of predicted strain coverage were similar across different years, and the 95% CIs largely overlapped. Lower levels of strain coverage were seen in the isolates collected in 2006 and 2008 (88%) and 2000 (89%); higher levels of strain coverage were seen in 2001 (97%) and 2004 (95%). A 2-sided chi-square test was used to test the differences across years in the predicted MATS coverage and in the upper and lower limit of the 95% CIs. No statistical significant difference across years was observed (P value = 0.567, P value = 0.457, and P value = 0.654, respectively). A linear regression model was used to test the strain coverage stability over time, and no statistically significant trend was observed either (P value = 0.222; Table S2), indicating high and consistently stable strain coverage in the United States over almost a decade.

Data corresponding to the predicted strain coverage classified by clonal complex (CC) are shown in Table 1. Two major CCs accounting for more than half of the circulating strains (CC32 and CC41/44) had predicted strain coverage of 99% (CI_{95}, 98% to 100% and 73% to 100%, respectively). The two other CCs with frequencies of >5%, CC162 and CC35, also had high levels of strain coverage (91% and 85%, respectively) but with broader confidence intervals (CI_{95}, 53% to 100% and 29% to 92%, respec-

TABLE 1 Distribution of clonal complexes in the panel of 442 NmB strains

Clonal complex	Frequency (n = 442)	% of total	Predicted % coverage (95% CI) by all antigens
CC32	162	37	99 (98–100)
CC41/44	123	28	99 (73–100)
CC162	43	9.7	91 (53–100)
CC35	24	5.4	85 (29–92)
CC269	19	4.3	68 (32–89)
CC60	9	2.0	89 (67–100)
CC213	6	1.4	50 (33–50)
Unassigned[a]	25	5.7	64 (32–76)
Other (n = 14)	31	7.0	68 (55–84)

[a]Unassigned, the sequence type (ST) of strains in this collection has been identified, but the ST belongs to no clonal complex (CC).

tively), suggesting antigenic variability. Minor CCs had high strain coverage (between 50% and 89%), too.

Tables 2 and 3 report predicted strain coverage by genotype of the fHbp and NHBA antigens, respectively. Within fHbp variant 1 (60% of the panel), fHbp covered >90% of all major peptides corresponding to identification numbers (IDs) in the PubMLST *Neisseria* sequence typing database with the exception of ID 13 (fHbp coverage, 24% [CI_{95}, 0% to 76%]; 6% of the isolates) and 81% of the minor IDs, for an overall 89% coverage by fHbp. Within fHbp variants 2 and 3 (40% of the panel), fHbp provided almost no coverage, and yet each antigenic variant was significantly (50% to 100%) covered by other MenB-4C antigens, for an overall 83% coverage by non-fHbp MenB-4C antigens. Within the eight major NHBA peptide IDs (IDs 5, 20, 2, 10, 29, 3, 21, and 1, accounting for three-quarters of the circulating strains), NHBA provided consistently high (84% to 100%) coverage. Coverage by NHBA for minor peptide IDs was also high (69% [CI_{95}, 41% to 84%]).

Due to the low number of isolates with levels of NadA MATS relative potency above the positive bacterial threshold (PBT; see Materials and Methods), the relationship between strain coverage and antigen genotype was not investigated for this antigen.

DISCUSSION

Molecular epidemiology is a component of bacterial surveillance programs that is important for understanding the incidence and diversity of the *Neisseria meningitidis*

TABLE 2 Distribution of most frequent factor H-binding protein variant and peptide identities in the panel of 442 NmB strains

Variant	fHbp peptide ID[a]	Frequency (n = 442)	% of total	Predicted coverage (95% CI) by fHbp	Predicted coverage (95% CI) by all antigens
1	1[b]	144	33	99 (99–99)	99 (99–99)
	13	25	5.7	24 (0–76)	68 (52–92)
	4	22	5.0	100 (91–100)	100 (100–100)
	14	17	3.8	94 (47–100)	100 (94–100)
	110	8	1.8	100 (100–100)	100 (100–100)
	12	6	1.4	100 (17–100)	100 (67–100)
	Other IDs	42	9.5	81 (57–86)	100 (74–100)
Total for variant 1		264	60	89 (77–95)	97 (89–99)
2	19	48	11	0	94 (56–98)
	24	32	7.2	0	91 (41–100)
	16	22	5.0	0	82 (27–86)
	21	13	2.9	0	92 (62–100)
	25	11	2.5	9.1 (0–9.1)	55 (36–64)
3	31	7	1.6	0	86 (0–86)
	30	5	1.1	0	80 (60–100)
	76	5	1.1	0	100 (100–100)
2/3	Other IDs	35	7.9	0	66 (43–83)
Total for variants 2 and 3		178	40	0.5 (0–0.5)	83 (46–91)

[a]ID, identification number in PubMLST *Neisseria* sequence typing database. fHbp, factor H-binding protein.
[b]Subvariant included in MenB-4C multicomponent vaccine.

TABLE 3 Distribution of the most frequent neisserial heparin-binding antigen peptides in the panel of 442 strains

NHBA peptide ID[a]	Frequency (n = 442)	% of total	Predicted coverage (95% CI) by NHBA	Predicted coverage (95% CI) by all antigens
5	127	29%	87% (31%–100%)	100% (98%–100%)
20	43	9.7%	98% (53%–100%)	98% (63%–100%)
2[b]	42	9.5%	100% (93%–100%)	100% (100%–100%)
10	29	6.6%	97% (55%–100%)	97% (55%–100%)
29	26	5.9%	92% (31%–100%)	96% (42%–100%)
3	26	5.9%	100% (88%–100%)	100% (96%–100%)
21	25	5.7%	84% (40%–92%)	88% (40%–96%)
1	18	4.1%	100% (100%–100%)	100% (100%–100%)
Other (n = 47)	106	24%	50% (21%–71%)	69% (41%–84%)

[a]ID, identification number in PubMLST *Neisseria* sequence typing database.
[b]Peptide included in the MenB-4C multicomponent vaccine.

isolates collected in the United States. In order to predict the possible vaccine coverage potential, it is critical to assess the expression of vaccine antigens in the representative *N. meningitidis* isolates. A major limitation of the molecular approach to determine the potential for strain coverage by a vaccine is that it has not been possible thus far to obtain information about the expression levels of the protein encoded in the bacteria from genetic data. Expression of the protein is necessary if the bacteria are to be targeted by protective antibodies directed against the antigen of interest. Studies to characterize the geographic and temporal distribution of the vaccine antigens among the members of the *N. meningitidis* population in the United States are important to understand the disease dynamics and strategies for deployment of MenB vaccines. Considering the importance of antigen diversity and level of expression in predicting MenB-4C vaccine coverage, MATS was used to assess the expression and cross-reactivity to vaccine-induced antibodies of MenB-4C antigens among the isolates selected in this study as representative of NmB meningococcal disease in the United States.

MATS predicted MenB-4C vaccine coverage of over 90% of circulating NmB strains in the United States. For the first time globally, strain coverage was estimated for a significant period of time and was found to be consistent over a 9-year time period (2000 to 2008). Among the MenB-4C vaccine antigens, NHBA alone was shown to contribute to strain coverage in over 80% of the isolates in a consistent manner across antigen genotypes. In contrast, fHbp coverage potential (53%) was restricted to the variant 1 antigen genotypes, as the NmB isolates harboring fHbp variants 2 and 3 were MATS negative for this antigen. Nonetheless, 83% of the fHbp variant 2/3 strains were covered by other MenB-4C antigens, suggesting the importance of the multiple vaccine components. MATS represents a conservative predictor of strain coverage in relation to hSBA as it does not take into account synergy of combinations of antigens or the contribution of other components present in the outer membrane vesicles (20, 29). Also, MATS does not factor in the interactions between antigens present at levels below the PBT. This is particularly relevant for MenB-4C vaccine as it contains multiple components that may provide a greater synergistic effect even when the individual components are present at levels below the bactericidal threshold (30).

Genetic diversity among the endemic NmB isolates is well documented (24). This diversity is also seen in virulence genes that are vaccine targets. Possible changes in the expression profiles of these genes in response to genotypic variations cannot be ruled out. A change in the virulence factor's structure might impact its immune reactivity, jeopardizing the positive outcome of vaccination. Evidence for this can be drawn from the MATS data obtained in this study with reference to fHbp, whose immune reactivity was restricted by the variant class of this gene that corresponds to vaccine antigen. Only those strains expressing variant 1 and its subclasses had expressed immune susceptibility to the vaccine. In contrast, NHBA, despite the molecular variations in the gene that encode the target peptides, was found to show immune reactivity to antibodies induced by the major and minor peptide IDs.

Microbes are under constant evolutionary pressure posed by several factors, includ-

ing vaccines. Given the possibility of genotypic changes in a particular virulence gene in response to vaccine pressure, an ideal vaccine should have inherent fail-safe mechanisms. One such strategy is to create a multicomponent vaccine such as MenB-4C. The presence of multiple components in MenB-4C vaccine implies that coverage may be maintained for strains that express multiple antigens in the event of mutation or loss of expression of a single antigen. It was previously noted that the bactericidal activity is increased for strains expressing two or more of the vaccine antigens at levels higher than the positive bactericidal threshold (18).

Although this study was limited to the evaluation of NmB strains circulating up to 2009, the stability of the strain coverage prediction over the 9-year period investigated suggests that no major differences should be expected in the ensuing years. Also, all genetic lineages significantly represented in the country were covered at levels of >85%, suggesting that temporal oscillations in the frequency of clonal complexes would not affect significantly the MenB-4C strain coverage. Postimplementation surveillance capable of monitoring both genetic (antigens or full-genome sequencing) and phenotypic (MATS) variations will be the key to quick adaptation of the public health strategy to the potential response of the pathogen to vaccination (31), especially when joined with dynamic modeling techniques that quantify in nearly real time the effectiveness of immunization campaigns (32). Also, adopting the same tools and standards for bacterial typing implementation worldwide, earlier epidemiological evidence from one country could help in preventive adjustments of the implementation strategy in other areas.

In September 2015, the United Kingdom was the first country to introduce MenB-4C into the national infant immunization program, offering the vaccine to all infants born after 1 July 2015, with a 2-plus-1 schedule at 2, 4, and 12 months and with two small catch-up cohorts for infants born between 1 May and 30 June (3, 4, and 12 months and 4 and 12 months). A detailed multifaceted plan is in place for enhanced meningococcal disease surveillance in England that will provide invaluable data on the usefulness of MATS for monitoring vaccine impact, characterizing meningococci causing meningococcal disease in both vaccinated and unvaccinated cohorts and the impact on meningococcal disease in infants. The reduced infant schedule implemented from September 2015 in the United Kingdom national immunization program provided the first evidence of effectiveness of 83% for a two-dose vaccine against all NmB disease, equivalent to a vaccine effectiveness of 94% against the most highly predicted NmB disease-preventable strains (33). This vaccine effectiveness measured in the field exceeds significantly the MATS coverage predictions for the United Kingdom (67% in 2014/2015, representing the last year prior to mass vaccination). Considering the higher (91%) MATS strain coverage predictions in the United States, the effectiveness of MenB-4C vaccination in the United States could be potentially superior to the UK results, particularly considering the burden of disease in the infant population for which a direct link was established between MATS strain coverage and pooled hSBA titers and individual seroprotection (21). Recently, the FDA approved the use of MenB-4C before licensure to control several outbreaks in U.S. universities (34). The FDA subsequently licensed MenB-4C for the age group of 10 to 25 years, and the ACIP recommended its use for all persons of age 10 and older in high-risk groups (9). No cases of meningococcal disease caused by *N. meningitidis* serogroup B have been reported among vaccinated students, although the numbers were too low to allow statistically powered determinations of efficacy (34). A recent immunogenicity study conducted among U.S. college students during a NmB outbreak indicated that 87% to 100% of vaccinated students showed immunoreactivity to reference NmB strains compared to modest (66.1%) reactivity to the NmB outbreak strain (35). This difference in the levels of immune reactivity among the MenB-4C seropositives reconfirms our incomplete understanding of strain susceptibility to vaccine-induced bactericidal activity and the tendency of hSBA to underestimate the vaccine efficacy (36). Additional data regarding the breadth and duration of protection provided by meningococcal B vaccine will be key in the decision-making process pertaining to vaccine dosage and schedule.

This report has provided a detailed breakdown of molecular characterization and MATS results for U.S. invasive NmB strains, estimating a high level of strain coverage for the MenB-4C vaccine in the United States. Overall, the data provide a useful baseline for monitoring MenB-4C. However, due to anticipated changes in the distribution of clonal complexes and antigen genotypes over time, continuous detailed surveillance and monitoring of the antigen expression of circulating strains will be needed.

MATERIALS AND METHODS

Strain selection and classification. A representative panel of 442 U.S. NmB isolates collected by the Active Bacterial Core surveillance (ABCs; CDC) in 2000 to 2008 were tested in MATS. Among these U.S. isolates, representation of fHbp 1.1 was weighted to account for higher rates of serogroup B and overall meningococcal disease occurring in Oregon. Multilocus sequence types, clonal complexes, and antigen genotypes for these isolates had previously been determined (26). The U.S. isolates ($n = 442$) tested in this study included the major clonal complexes of endemic U.S. NmB strains. Clonal complexes CC32 (37%), CC41/44 (28%), and CC162 (9.7%) accounted for the majority of the strains (Table 1).

Genotypic classification of antigens. The classification of fHbp followed the scheme available on the PubMLST *Neisseria* sequence typing database (http://pubmlst.org/neisseria/), which separates peptide subvariants into three major variant classifications: variants 1, 2, and 3. Unique NHBA peptides and *nadA* variants were numbered as previously described (37, 38). PorA subtyping had been previously performed by PCR amplification and sequencing of the VR2 region of the gene (26). PorA variants had been assigned therein according to variable region 2 (VR2) sequences on the PubMLST *Neisseria* sequence typing database.

Meningococcal antigen typing system (MATS) ELISA. The MATS ELISA methodology (18), reagents, reference strains, and recombinant antigens were supplied by Novartis Vaccines and Diagnostics, Siena, Italy (now part of the GSK group of companies). Briefly, bacteria were cultured overnight on chocolate agar (BD Biosciences, San Jose, CA) and were suspended in Mueller-Hinton (MH) broth (BD Biosciences, San Jose, CA) to an optical density at 600 nm (OD_{600}) of 0.4. Empigen BB detergent (Sigma, St. Louis, MO) was added to the bacterial suspension to reach a final dilution of 1:11, and this suspension was incubated at 45°C for 1 h for bacterial inactivation. Twofold serial dilutions of bacterial extract in MH broth with Empigen were carried out in ELISA plates (Costar, Corning, NY) that had been precoated with rabbit polyclonal antibodies against fHbp, NHBA, or NadA. The plates were incubated for 1 h at 37°C and then washed with phosphate-buffered saline (PBS)–0.05% Tween. The plates were incubated for 1 h at 37°C with biotinylated antigen-specific rabbit polyclonal antibody to detect the primary antibody-bound antigens. After being washed, the plates were developed with streptavidin-horseradish peroxidase (HRP) (Jackson ImmunoResearch, West Grove, PA) and *o*-phenylenediamine dihydrochloride (OPD; Sigma). The reaction was stopped with 4N H_2SO_4 and read at 492 nm. A reference strain for each antigen (H44/76 for fHbp, NGH38 for NHBA, and 5/99 for NadA) was also included in the respective microtiter plates. Results were analyzed with StatLIA software (Brendan Technologies, Carlsbad, CA). The relative potency (RP) for each unknown strain was calculated by comparing the fit of the five-parameter logistic regression curves to 2-fold serial dilutions of extracts from the reference strain and the unknown strain. The reference strain for each antigen was assigned an arbitrary value of 100.

Estimation of NmB strain coverage. In a previous study, it was shown that the presence of at least one antigen with a relative potency (RP) value greater than the positive bactericidal threshold (PBT; 0.021 for fHbp, 0.294 for NHBA, and 0.009 for NadA) or the presence of PorA P1.4 correlated with bactericidal activity in the hSBA by pooled sera taken after immunization (18). Therefore, the MATS data could be used to predict strain coverage by the multicomponent MenB-4C vaccine. Strains that did not meet these criteria were deemed not covered. In this study, the predicted strain panel coverage was defined as the proportion of strains with a MATS relative potency value greater than the positive bactericidal threshold value for one or more antigens and/or with PorA P1.4. It should be noted that the PBT was set using pooled sera from infants and that strain coverage predictions do not account for variations in the individual seroresponses.

Statistical analysis. As described in the MATS interlaboratory standardization study (19), empirical estimates of the 95% CIs for the positive bactericidal thresholds were derived with a log-normal approximation based on the overall ranges of assay reproducibility (0.014 to 0.031 for fHbp, 0.169 to 0.511 for NHBA, and 0.004 to 0.019 for NadA). These values were used to define the 95% CIs of the strain coverage data. No CI was calculated for coverage by the PorA antigen, as the typing was performed genotypically (VR2 = P1.4).

All statistical evaluations were performed using R statistical software (http://www.r-project.org) version 2.13.1.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00261-17.

TABLE S1, PDF file, 0.1 MB.

TABLE S2, PDF file, 0.05 MB.

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M.S., G.B., L.S., and D.M. are currently employed by the GSK group of companies. G.R., E.K., and P.S. have performed research on behalf of the Centers for Disease Control and Prevention (Atlanta, GA, USA). G.C. was employed at the CDC at the time of the study.

G.R., M.S., D.M., and G.C. participated in the conception and design of the study. E.K., P.S., and G.B. participated in the acquisition of data. All of us participated in the analysis and interpretation of the data and in the drafting and revision of the manuscript.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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