Changes in the Population Structure of Invasive *Neisseria meningitidis* in the United States After Quadrivalent Meningococcal Conjugate Vaccine Licensure

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**Background.** Meningococcal conjugate vaccines against serogroups A, C, W, and Y (MenACWY) are recommended for routine use in adolescents aged 11–18 years. The impact of these vaccines on the meningococcal population structure in the United States have yet to be evaluated.

**Methods.** Meningococcal isolates recovered during 2006–2010 (ie, after introduction of MenACWY) collected through Active Bacterial Core surveillance (ABCs) were characterized; serogroup distribution and molecular features of these isolates were compared to previously published data on ABCs isolates recovered from 2000 to 2005 (ie, before introduction of MenACWY). *P* values were generated using \( \chi^2 \) statistics and exact methods.

**Results.** There was a significant change (\( P < .05 \)) in serogroup distribution among all age groups between the 2 periods. A small proportion of isolates showed evidence of capsular switching in both periods. Between the 2 periods, significant changes were observed in the distribution of porin A, ferric enterobactin transport, and strain genotypes among vaccine and nonvaccine serogroups.

**Conclusions.** The population structure of US meningococcal isolates is dynamic; some changes occurred over time, but the basic structure remained. Vaccine-induced serogroup replacement was not observed, although a small proportion of isolates had undergone capsule switching, possibly driven by non–vaccine-mediated selection. Changes in the distribution of molecular features are likely due to horizontal gene transfer and changes in serogroup distribution.

**Keywords.** meningococcal disease; molecular epidemiology; vaccine impact.

Meningococcal disease remains a significant public health concern because of its high morbidity and mortality rate worldwide. In the United States, the incidence of meningococcal disease is relatively low, and most cases occur sporadically. The majority of the US cases are caused by serogroups B, C, or Y [1]. Two quadrivalent meningococcal conjugate vaccines (MenACWY) are licensed in the United States to protect against disease caused by serogroups C and Y, along with serogroups A and W. MenACWY is recommended for routine use in adolescents aged 11–18 years [2]. Since its introduction, coverage with MenACWY has been steadily increasing among children aged 13–17 years, from 11.7% in 2006 to 62.7% in 2010. The state-specific coverage with MenACWY ranged from 26% to 89.5% in 2010 [3]. A new vaccine against serogroup B disease was licensed on 29 October 2014 in the United States; others are still under clinical trial or development [4].

The *Neisseria meningitidis* population is antigenically and genetically diverse. The diversity results mostly from frequent horizontal gene transfer events and can change spatially and temporally [5]; it may also result
from vaccine-induced immune selection, which possibly provides an additional driving force for the diversity of surface-exposed antigens [6, 7]. The dynamic feature of the meningococcal population highlights the importance of monitoring potential changes in antigenic and genetic structures of this organism after the introduction of the new quadrivalent vaccine. Of biggest concern is the possibility that the incidence of infections caused by nonvaccine serogroups could increase following vaccine licensure, which was observed after introduction of pneumococcal conjugate vaccines in the United States [8]. An increase in the incidence of serogroup W meningococcal disease has recently been reported in Burkina Faso after the implementation of MenAfriVac, a conjugate serogroup A meningococcal vaccine [9]. An increase in the proportion of serogroup Y in relation to the other serogroup that cause invasive meningococcal disease was also observed in a few European countries [10–12].

Several approaches are commonly used for measuring meningococcal genetic and antigenic diversity. Multilocus sequence typing (MLST) is a DNA sequencing–based method that detects neutral genetic variations that do not have an apparent effect on phenotypes and has been widely used to determine strain sequence type (ST) and clonal complex (CC). This typing method has linked invasive meningococcal strains to certain CCs, also known as hyperinvasive lineages, regardless of the geographic locations [6, 7, 13–15]. The surface antigens porin A (PorA) and ferric enterobactin transport (FetA) are major components of some outer membrane vehicle–based vaccines. PorA and FetA sequence typing methods detect antigenic variations that occur within the surface-exposed variable regions of these proteins. A typing scheme combining the above molecular methods has been demonstrated to be useful for assessment of vaccine impact on meningococcal genetic and antigenic structure [16].

To assess changes in the population structure of N. meningitidis in the United States, we used MLST and PorA/FetA typing methods to characterize meningococcal isolates collected from 2006 to 2010. Serogroup distribution, capsule switching, and molecular features of these isolates were compared to previously published data on isolates recovered from 2000 to 2005 [17].

### METHODS

#### Meningococcal Strain Collection

During 2006–2010, 678 cases were reported through the Active Bacterial Core surveillance (ABCs) network, and isolates were available from 639 cases. A total of 610 N. meningitidis isolates (379 vaccine-serogroup isolates and 231 non–vaccine-serogroup isolates), which are available at the Centers for Disease Control and Prevention (CDC) and represent 95.5% of all ABCs isolates (610 of 639), were characterized in this study and compared to 1175 ABCs isolates (624 vaccine-serogroup isolates and 551 non–vaccine-serogroup isolates) from 2000 to 2005 (Table 1) [17]. Vaccine serogroups included serogroups A, C, W, and Y; nonvaccine serogroups include serogroups B, X, E, and Z; nongroupable isolates; and unknown serogroups (possible H, I, K, or L). The ABCs network is a population- and laboratory-based surveillance system supported by the CDC as part of its Emerging Infections Program network (http://www.cdc.gov/abcs/index.html). During 2000–2010, the participating states included California (3 counties), Colorado (5 counties), Connecticut, Georgia, Maryland, Minnesota, New York (15 counties), Oregon, and Tennessee (11 counties). New Mexico joined the ABCs network in 2004; isolates before 2004 were not available from this state. In 2010, the total population under surveillance was approximately 40.6 million, or 13.6% of the US population.

Identification and serogrouping of N. meningitidis was performed at state public health laboratories, after which the isolates were sent to the CDC, where slide agglutination serogrouping and real-time polymerase chain reaction were performed to confirm the N. meningitidis serogroup [18].

#### Characterization of N. meningitidis Isolates

MLST loci, porA, and fetA were sequenced and analyzed using DNASTAR Lasergene 7 or a Web-based computational tool, Meningococcus Genome Informatics Platform [19]. ST and CC were determined using MLST as previously described on the PubMLST Web site (http://pubmlst.org/neisseria/) [20]. A minimum spanning tree was constructed to convey the clonal
structure of meningococcal isolates from 2006 to 2010, using BioNumerics, version 6.6. PorA and FetA types were determined by sequencing the 2 variable regions of porA (VR1 and VR2) and the variable region of fetA [21, 22] and then comparing the translated DNA sequence with the existing PorA and FetA types listed on the PubMLST Web site. Strain genotype in this study was defined as CC:PorA:FetA. A common molecular type was defined as a molecular type (CC, ST, PorA, FetA, PorA:FetA, or CC:PorA:FetA) that was present in ≥5 isolates. A capsule-switching event is defined as an isolate of a certain serogroup belonging to a CC that is commonly associated with a different serogroup.

Data Analysis
Data were analyzed using SAS, version 9.2 (SAS Institute, Cary, North Carolina). The changes (P value) in the molecular features of meningococcal isolates between the 2 periods were determined using χ² statistics and exact methods.

Oregon has reported higher rates of serogroup B and overall meningococcal disease because of hyperendemic serogroup B disease that was first reported in 1993 [23]. During 2001–2010, the incidence of serogroup B meningococcal disease was 0.7 cases per 100 000 population in Oregon, compared with 0.12 cases per 100 000 population in the other ABCs sites [1]. To better estimate the prevalence and distribution of the molecular types (ST, CC, PorA, FetA, PorA:FetA, and strain genotype) of serogroup B isolates circulating in the United States, analyses of serogroup B isolates were weighted to account for the increased incidence of serogroup B meningococcal disease in Oregon. The weight was generated on the basis of the proportion of serogroup B isolates were weighted to account for the increased incidence of serogroup B meningococcal disease in Oregon, and a weight of 0.90 (1.00) was assigned to isolates from Oregon, and a weight of 0.10 (0.10) was assigned to isolates from other ABCs sites (Table 1).

RESULTS
Serogroup Distribution and Capsule Switching
Serogroups B, C, and Y were the most common cause of meningococcal disease in both periods. A significant change (P = .001) in serogroup distribution of the US meningococcal isolates was observed among all age group between the 2 study periods (Table 1) but not among adolescents. Among all age groups, the proportion of vaccine serogroups A, C, W, and Y increased from 65.3% in 2000–2005 to 71.3% in 2006–2010, while the proportion of nonvaccine serogroups decreased from 34.7% in 2000–2005 to 28.7% in 2006–2010. There was a significant change in the proportion of serogroups Y (P = .03) and B (P < .001) between 2000–2005 and 2006–2010 (Table 1). Among Oregon cases, the proportion involving serogroup B decreased from 67.6% in 2000–2005 to 47.4% in 2006–2010 (P < .001; data not shown).

The proportion of isolates recovered during 2000–2005 and 2006–2010 that had evidence of capsular switching was 5.5% (2005 and 2010, respectively. No association was

Genetic Diversity
The study isolates were highly diverse during both periods (Table 2). A total of 311 STs (231 in 2000–2005 vs 125 in 2006–2010), 27 assigned CCs (25 in 2000–2005 vs 21 in 2006–2010), 139 PorA types (107 in 2000–2005 vs 64 in 2006–2010), and 51 FetA types (45 in 2000–2005 vs 33 in 2006–2010) were identified. A total of 45 isolates belonged to an unassigned CC. A porA mutation (deletion or frameshift mutation) was detected in 12 isolates (7 in 2000–2005 and 5 in 2006–2010). A fetA deletion was detected in 10 isolates (7 in 2000–2005 and 3 in 2006–2010). No association was


<table>
<thead>
<tr>
<th>Serogroup(s), Genotype</th>
<th>2000–2005, No. (% of Isolates)a</th>
<th>2006–2010, No. (% of Isolates)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine serogroups A, C, W, and Y</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Clonal complex</td>
<td>8 (98.2)</td>
<td>7 (1.8)</td>
</tr>
<tr>
<td>Sequence type</td>
<td>12 (84.8)</td>
<td>74 (15.2)</td>
</tr>
<tr>
<td>FetA type</td>
<td>11 (95.5)</td>
<td>18 (4.5)</td>
</tr>
<tr>
<td>PorA type</td>
<td>11 (91.3)</td>
<td>37 (8.7)</td>
</tr>
<tr>
<td>Serogroup Bb</td>
<td>Clonal complex</td>
<td>7 (95.2)</td>
</tr>
<tr>
<td>Sequence type</td>
<td>14 (67.8)</td>
<td>32 (32.2)</td>
</tr>
<tr>
<td>FetA type</td>
<td>13 (94.8)</td>
<td>19 (5.2)</td>
</tr>
<tr>
<td>PorA type</td>
<td>16 (82.9)</td>
<td>62 (17.1)</td>
</tr>
<tr>
<td>Other nonvaccine serogroups</td>
<td>Clonal complex</td>
<td>2 (46.9)</td>
</tr>
<tr>
<td>Sequence type</td>
<td>2 (40.6)</td>
<td>17 (59.4)</td>
</tr>
<tr>
<td>FetA type</td>
<td>2 (53.1)</td>
<td>9 (46.9)</td>
</tr>
<tr>
<td>PorA type</td>
<td>2 (40.6)</td>
<td>13 (59.4)</td>
</tr>
</tbody>
</table>

Results for 2000–2005 were previously reported [17].

Abbreviations: FetA, ferric enterobactin transport; PorA, porin A.

a A total of 1175 and 610 isolates were collected during 2000–2005 and 2006–2010, respectively. The total number of genotypes detected and the proportion of these genotypes among US isolates were shown for both common types, which appeared in ≥5 isolates, and rare types, which appeared in <5 isolates.

b Results of proportions are presented with a weight of 0.10 for Oregon isolates and 0.90 for all other isolates.
observed between *porA* and *fetA* mutations. Neither *porA* nor *fetA* mutations were associated with a serogroup. Three isolates with a *porA* or *fetA* mutation belonged to an unassigned CC; the remaining isolates belonged to assigned CCs.

The number of unique molecular types (STs, CCs, PorA, and FetA) among serogroup B was higher than among the other serogroups. Common molecular types that appeared in $\geq 5$ isolates accounted for majority of the isolates of vaccine serogroups and serogroup B (Table 2). For example, 12 common STs that were present in $\geq 5$ isolates accounted for 84.8% of the isolates of vaccine serogroups in 2000–2005, and 74 uncommon STs that were detected in $<5$ isolates were accounted for the remaining 15.2% of isolates of vaccine serogroups. However, the pattern among isolates of other nonvaccine serogroups was different; the uncommon molecular types that appeared in $<5$ isolates accounted for most of these isolates.

**Clonal Structure of Meningococcal Isolates**

The clonal structure of meningococcal isolates from 2006 to 2010 (Figure 1) was compared to the clonal structure of 2000–2005 isolates [17]. A total of 14 CCs were detected in $\geq 5$ isolates in at least 1 period, with 13 (CC11, CC22, CC23, CC32, CC35, CC41/44, CC60, CC103, CC162, CC167, CC174, CC213, and CC269) appearing in both periods and 1 (CC1157) appearing in 9 serogroup B isolates in 2006–2010 only (Figure 1). CC174, a common CC in 2006–2010 ($n = 6$), was detected in

![Figure 1](https://academic.oup.com/jid/article-abstract/211/12/1887/881910)

**Figure 1.** Clonal structure of US meningococcal isolates of vaccine serogroups (A) and serogroup B (B), 2006–2010. A minimum spanning tree, by multilocus sequence type (ST), was created using BioNumerics, version 6.6, for vaccine-serogroup isolates ($n = 379$) and serogroup B isolates ($n = 206$). The size of the circles is proportional to the number of isolates represented. Each clonal complex (CC) is represented by a different color. STs that do not belong to any CCs are in black circles.
only 1 isolate in 2000–2005; CC269, a common CC in 2000–2005 (n = 25), was detected in only 3 isolates from 2006–2010. Of the 13 CCs appearing with low frequency (in <5 isolates), 6 (CC37, CC254, CC198, CC461, CC349, and CC178) were present in both periods; CC865 appeared only in 2006–2010; the others (CC5, CC8, CC175, CC334, CC364, and CC4240/6688) appeared only in 2000–2005. The prevalent CCs among serogroup B in England, Wales, and Canada were CC269, CC41/44, and CC269, respectively [24–26]. Although the ST composition among each common CC showed some differences between the 2 periods, the prevalent STs within each common CC in 2006–2010 also predominated.
in 2000–2005 (Figure 1). New STs that appeared in 2006–2010 are single-locus variants of the central ST or a ST of the same CC that was detected in 2000–2005.

In both periods, serogroup B was mainly associated with CC32, CC41/44, CC269, CC162, CC213, CC60, and CC35; serogroup C, with CC11 and CC103; serogroup Y, with CC23 and CC32, CC41/44, CC269, CC162, CC213, CC60, and CC35; and serogroup W, with CC22. These prevalent CCs accounted for >83% of the isolates among each serogroup.

Distribution of PorA and FetA Types
A total of 27 PorA types and 18 FetA types were detected in ≥5 isolates in at least 1 period. Of these common types, 17 PorA and 13 FetA types appeared in both periods. The remaining types were detected in only 1 period (Figure 2A and 2B). A total of 305 unique PorA:FetA combinations were identified, with 220 combinations in 2000–2005 and 146 combinations in 2006–2010. Thirty-two PorA:FetA combinations were detected in ≥5 isolates in at least 1 period. Significant changes were observed in the distribution of various PorA types among B (P < .0001), C (P < .0001), Y (P = .0008), and other non–vaccine-serogroup (P = .001) isolates and the distribution of FetA types among B (P = .0005) and C (P = .0001) isolates between the 2 periods (Figure 2A and 2B and Table 3).

Particular PorA and FetA types were associated with specific serogroups (Figure 3A). P1.5-1,2-2:F5-8 and P1.5-1,10-1:F4-1 were strongly associated with serogroup Y, accounting for 82% of serogroup Y isolates. P1.7,16-2:F3-3 predominated among serogroup B isolates, accounting for 30% of the isolates.

Distribution of Strain Genotype
The isolates analyzed were represented by 344 unique-strain genotypes (CC:PorA:FetA), including 243 in 2000–2005 and 157 in 2006–2010. A total of 36 strain genotypes were identified in ≥5 isolates, including 26 in 2000–2005 and 15 in 2006–2010. CC11: P1.5-1,10-62:F3-6 was detected only in 2006–2010, and CC32: P1.7,16-2:F3-3 was detected only in 2000–2005. The common strain genotypes accounted for >80% of vaccine-serogroup isolates and 37%–56% of non–vaccine-serogroup isolates (Figure 3). Some association between serogroup and strain genotype was also observed. The distribution of strain genotypes (CC:PorA:FetA) was significantly different among the 2 periods (P < .001) among isolates of both vaccine serogroups and serogroup B. Thirteen common-strain genotypes were more frequently observed in one studied period than in the other (P < .05; Table 3). Strain genotypes that appeared with low frequency and in only 1 period are listed in Supplementary Table 1.

DISCUSSION
Vaccination remains one of the most successful and cost-effective public health interventions to eliminate vaccine-preventable infectious diseases. Pathogens have evolved mechanisms to escape host immunity through diversifying the population and altering antigenic features. The incidence of meningococcal disease in the United States has declined since 1996, even before the recommendation for MenACWY among adolescents. The overall annual incidence had decreased 64%, from 1.1 cases per 100 000 population in 1996 to 0.4 cases per 100 000 population in 2005, and continues to decline among all age groups since 2005 [1]. The decline occurs in both vaccine serogroups (C and Y) and serogroup B.

Serogroups B, C, and Y remained to be the most common serogroups before and after MenACWY introduction. However, there was a significant change in serogroup distribution among the US meningococcal strains, with an increase in the proportion of serogroup Y and decrease in the proportion of serogroup B from 2000–2005 to 2006–2010. Capsule switching occurred in both periods, which may contribute to the changes in serogroup distribution. Most of the switching events have occurred.
between serogroup B and C strains, with more switching from B to C than from C to B, indicating that MenACWY is less likely to be the driving force for these capsule-switching events. This is similar to the experience in the United Kingdom after meningococcal vaccination, where capsule-switching events were detected but not induced by vaccination [27].

The meningococcal isolates in this study showed a high level of genetic diversity, which is not surprising because most of them were from sporadic cases, and only a very small proportion of isolates were from known outbreaks or clusters. Between the 2 study periods, there are some significant changes in the distribution of each molecular type among different serogroups, including both vaccine and nonvaccine serogroups. Most of the common molecular types predominated in both periods, indicating the persistence of certain CCs and outer membrane antigen types over time. Some short-lived molecular types disappeared after the introduction of MenACWY, but new molecular types appeared with low frequency. The new STs either belong to an existing or unassigned CC. There is no evidence of emergence of new hyperinvasive clones in the United States. Consistent with previous observations by other research groups, some association between serogroup and CC was observed in this study [17, 24, 28].

One protein-based vaccine against serogroup B disease is currently available, and others are currently under clinical trial or development [4]. For these vaccines, understanding the genetic diversity of these protein immunogens is important to estimate vaccine coverage [25, 29]. Both PorA and FetA have been included in outer membrane vehicle-based vaccines to provide strain-specific protections against serogroup B meningococcal disease [4, 28, 30]. Given the high diversity of PorA and FetA among the isolates analyzed in this study, vaccines with PorA and FetA as primary antigens may not provide broad protection against US strains. Inclusion of multiple PorA and FetA variants may potentially improve coverage but would be technically changing.

In conclusion, the population structure of US meningococcal isolates is dynamic; some changes occurred over time, but the basic structure remained. Vaccine-induced serogroup replacement was not observed, although a small proportion of isolates had undergone capsule switching driven by horizontal gene transfer. Changes in the distribution of molecular types among different serogroups before and after vaccine introduction are likely due to horizontal gene transfer events and changes in serogroup distribution. With the further increase in MenACWY coverage (>70%) in 2011 and beyond, the impact of vaccine on the population structure of N. meningitidis in the United States should be reevaluated after sustained high MenACWY coverage among adolescents has been achieved.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary
data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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