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Changes in Serogroup and Genotype Prevalence Among Carried Meningococci in the United Kingdom During Vaccine Implementation

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Background. Herd immunity is important in the effectiveness of conjugate polysaccharide vaccines against encapsulated bacteria. A large multicenter study investigated the effect of meningococcal serogroup C conjugate vaccine introduction on the meningococcal population.

Methods. Carried meningococci in individuals aged 15–19 years attending education establishments were investigated before and for 2 years after vaccine introduction. Isolates were characterized by multilocus sequence typing, serogroup, and capsular region genotype and changes in phenotypes and genotypes assessed.

Results. A total of 8462 meningococci were isolated from 47 765 participants (17.7%). Serogroup prevalence was similar over the 3 years, except for decreases of 80% for serogroup C and 40% for serogroup 29E. Clonal complexes were associated with particular serogroups and their relative proportions fluctuated, with 12 statistically significant changes (6 up, 6 down). The reduction of ST-11 complex serogroup C meningococci was probably due to vaccine introduction. Reasons for a decrease in serogroup 29E ST-254 meningococci (from 1.8% to 0.7%) and an increase in serogroup B ST-213 complex meningococci (from 6.7% to 10.6%) were less clear.

Conclusions. Natural fluctuations in carried meningococcal genotypes and phenotypes can be affected by the use of conjugate vaccines, and not all of these changes are anticipatable in advance of vaccine introduction.

The incorporation of protein-conjugate polysaccharide vaccines into immunization schedules has been an important advance in the control of invasive bacterial disease, especially meningitis. In 1992, Haemophilus influenzae serotype b conjugate (Hib) vaccine was the first such vaccine introduced in the United Kingdom (UK), followed by vaccines against Neisseria meningitidis, the meningococcus, and Streptococcus pneumoniae [1, 2]. In the case of meningococcal disease, still an important cause of morbidity and mortality worldwide [3], the deployment of meningococcal serogroup C conjugate (MCC) vaccines in the UK in 1999 [4] significantly reduced the incidence of serogroup C meningococcal disease, providing impetus for the use of
similar vaccines against other serogroups [5] (eg, the Meningitis Vaccine Project serogroup A conjugate vaccine for sub-Saharan Africa [6]).

In common with H. influenzae and S. pneumoniae, the meningococcus is an accidental pathogen, normally colonizing the human nasopharynx asymptomatically and rarely causing invasive disease [7, 8], and the ability of the conjugate polysaccharide vaccines to generate herd immunity is important in controlling the diseases they cause [2]. This is a consequence of the vaccines inducing immunity in individuals across the population that is not only long-lasting but also effective at eliminating the carriage of capsulate bacteria, thereby inhibiting their transmission. During the introduction of the MCC vaccines in the UK, the herd immunity effect was particularly strong [9, 10] for 2 reasons. First, the vaccination campaign targeted all individuals <18 years old [11] and later extended to those <25 years old, effectively immunizing the teenage age group among which carriage was highest [11, 12]. Second, the ST-11 complex-serogroup C meningococcal strain responsible for most serogroup C disease in the UK at that time was particularly susceptible to the effects of the vaccine, probably due to its high rate of capsule expression [9]. This had the beneficial effect of ensuring that the vaccine was effective, despite the suboptimal immunization schedule initially used for infants (immunization at 2, 3, and 4 months of age with no booster at 1 year of age) [13], because the outbreak strain was removed from asymptomatic transmission [9].

Although populations of carried meningococci are genetically and antigenically diverse, most disease is caused by a limited number of genotype and serogroup combinations [8]. Of the 13 recognized meningococcal serogroups, 5 (A, B, C, Y, and W135) are responsible for the majority of disease worldwide, with meningococci that elaborate sialic acid–based capsules (serogroups B, C, Y, and W135) causing most disease in Europe, North and South America, and Australasia [3]. The capsule is encoded at the cps genome region [14], which may be occupied by 1 of a number of possible gene clusters or a ~115-bp intergenic region, the capsule null locus (cnl) [15]. Which of the sialic acid–based capsules is synthesized depends on a single gene within the cps which is referred to by various nomenclatures, with the genes associated with the serogroups named as follows: serogroup B, siaDB or synD; serogroup C, siaDC or synE; serogroup W-135, siaDW or synF; and serogroup Y, siaDY or synG [16–18]. The siaD nomenclature is used here to maintain consistency with previous reports from this study [9].

Different serogroups and the cnl tend to be associated with different genotypes, identified as clonal complexes by multilocus sequence typing (MLST) [8, 19]. This includes the hyperinvasive lineages, a subset of the many genotypes present in carried meningococcal populations, which are responsible for most invasive disease [8]. These associations are not absolute, however [20], which leads to the possibility that immunization against asymptomatic carriage may generate a selective pressure for the emergence of vaccine escape variants [17], which is especially pertinent given the high rates of horizontal genetic exchange seen in Neisseria [21]. Such variants arise from time to time in meningococci, including changes between serogroups C and W-135 [17, 22, 23], but changes in response to vaccination campaigns have yet to emerge as a major public health threat.

Studies of the dynamics of carried meningococcal populations and the effects of immunization on them play an increasingly important part in the development of meningococcal vaccines and assessing their effectiveness [24]. Here, changes in predominant serogroups and genotypes of meningococci during the course of MCC vaccine introduction in the UK are described, and while the effect of the vaccine introduction on the targeted serogroup has been reported elsewhere [9, 10, 12], to our knowledge changes in the meningococcal population as a whole are described here for the first time.

METHODS

Study Design and Samples

The study design has been described elsewhere [9, 10, 12]. Briefly, meningococcal carriage was surveyed in 15–19-year-old students attending full-time education for 3 consecutive years in 7 centers throughout the UK, immediately before and 2 years after the introduction of MCC vaccines (1999, 2000, and 2001). Bacteria were isolated from nasopharyngeal swab samples. Each participant completed a questionnaire on risk factors for carriage; formal records of the numbers of individuals approached were not kept, but inspection of the school rolls indicated participation rates were usually in >50% of those approached. The demographic profile of those participating students matched that of their schools.

Isolate Characterization

Isolates were phenotyped for species and serogroup using microbiological methods performed by the Health Protection Agency (then Public Health Laboratory Service) Meningococcal Reference Unit, for isolates from England and Wales, or the Scottish Pneumococcal and Meningococcal Reference Laboratory (now the Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory) for isolates from Scotland. A heat-killed cell suspension was prepared, as described elsewhere [9], the multilocus sequence type was determined, and the presence of siaD alleles and the cnl within the cps region were detected by amplification with polymerase chain reaction (PCR) for each isolate. The allele classes of the siaD gene, siaDB (synD), siaDC (synE), siaDW (synF), and siaDY (synG), were identified by sequencing of PCR products. Isolate characteristics and questionnaire data were stored in a study-specific database within the BIGSdb database platform [25].
**Assignment of Sequence Types to Clonal Complexes**

Assignment of sequence types (STs) was performed automatically by the BIGSdb software using a reference set of central genotypes held within the database and approved by the *Neisseria* MLST Website management committee (http://pubmlst.org/neisseria/info/). STs that were not assigned to a clonal complex were examined by a combination of phylogenetic reconstruction with the neighbor joining algorithm using concatenated housekeeping gene sequences, split decomposition analysis [26], and eBURST analysis [27] of the same data to identify putative central genotypes of novel clonal complexes. These were compared with data in the PubMLST Web site [28], and where appropriate, new central genotypes were proposed to, and subsequently approved by, the management committee.

**Data Manipulation and Analysis**

Statistical analyses were performed using Intercooled Stata software for Windows (version 10; Stata). Proportions were compared using a χ² test or Fisher exact test as appropriate. Data are presented for the 3 years of the study. The following comparisons over time were made: 1999 and 2000, 2000 and 2001, and 1999 and 2001. There was a small degree of overlap between individuals who were sampled on successive years, but for this analysis each year was treated independently. Rate ratios and confidence intervals were calculated comparing 2001 with 1999 to summarize change over time and are tabulated together with the default (Woolf method) confidence intervals.

**RESULTS**

**Sample Collection and Exclusion Criteria**

From 1999 through 2000, 48,309 individuals were sampled across the 7 study centers (Bangor, Cardiff, Glasgow, Nottingham, Oxford, Plymouth, and Stockport). Of these, 47,765 (98.9%) met the inclusion criteria of completing a questionnaire demonstrating that they were in the correct age range (15–19 years of age when sampled). A total of 9,233 bacteria were isolated, of which 8,462 gave complete MLST profiles characteristic of meningococci, a carriage rate of 17.7%. Carriage increased slightly over the 3 years: it was 16.6% in 1999 (2,306 isolates from 13,901 individuals), 17.6% in 2000 (2,873 isolates from 16,295 individuals), and 18.7% in 2001 (3,283 isolates from 17,569) [9, 10]. The remaining 771 isolates (8.4% of isolates) comprised bacteria that were phenotypically characterized as other species (*Neisseria lactamica*, 324 isolates; *Moraxella catarrhalis*, 107 isolates; other organisms or uncultivable at the reference laboratory, 210 isolates) and 130 isolates (1.4%) that were phenotypically classified as meningococci but that had MLST profiles consistent with *N. lactamica*. The data set differed slightly from that of the 8,599 isolates reported elsewhere [9] as a consequence of using a MLST-based, rather than phenotypic, approach to speciation and by the exclusion of subjects on the basis of age calculated from the questionnaire and sample dates.

**Serogroups**

Serogroup data were obtained for 8,429 isolates (99.6%), with 3,738 isolates (44%) grouped to 7 serogroups: B, C, 29E, W135, X, Y, and Z. There were 4,691 isolates (55.4%) that were not groupable by the reagent panel used, and no data were available for 33 isolates (0.4%). A single result was obtained for most isolates, with 15 (0.2%) assigned as serogroup Z/29E. Over the 3 years, serogroup B organisms were the most common, accounting for 2,050 isolates (24.2%), and serogroup Z the least common, with 36 isolates (0.4%). The proportion of serogroup C meningococci decreased highly significantly over the study period, from 2.7% to 0.5% of isolates (rate ratio for 2001 to 1999, 0.18; P < .001), as reported previously [9, 10], and the proportion of serogroup 29E isolates decreased from 4.85% to 2.8% (rate ratio for 2001 to 1999, 0.58; P < .001). The proportion of serogroup W135 isolates increased from 6.7% to 7.6%, but this was statistically insignificant. There were only marginal changes in the carriage proportion of other serogroups, none statistically significant (Table 1).

**Clonal Complexes**

Five new clonal complexes were defined during this study: ST-178 complex, ST-213 complex, ST-282 complex, ST-1117 complex, and ST-1136 complex. Including these designations, 4,290 isolates (88%) were assigned to 34 clonal complexes, and 572 isolates were not assigned. Carriage rates for clonal complexes ranged from 13.7% (ST-41/44 complex; 1,163 isolates) to a single isolate (ST-37, ST-106, and ST-376 complexes) over the whole study (Table 2). A number of temporal changes were observed in the rates of carriage of clonal complexes: the proportion of 6 decreased (the ST-11, ST-254, ST-865, ST-162, ST-212, and ST-41/44 complexes), with rate ratios for 2001 to 1999 ranging from 0.20 (95% confidence interval [CI], .10–.37 [ST-11 complex]) to 0.86 (95% CI, .75–.98 [ST-41/44 complex]), and the proportion of 6 increased (ST-213, ST-53, ST-461, ST-174, ST-269, and ST-1157 complexes), with rate ratios for 2001 to 1999 ranging from 0.18 (95% CI, .10–.37 [ST-11 complex]) to 0.86 (95% CI, .75–.98 [ST-41/44 complex]), and the proportion of 6 increased (ST-213, ST-53, ST-461, ST-174, ST-269, and ST-1157 complexes), with rate ratios for 2001 to 1999 ranging from 1.26 (95% CI, 1.06–1.50 [ST-53 complex]) to 3.98 (95% CI, 1.17–13.57 [ST-174 complex]). The remaining clonal complexes showed no significant change over the 3 years (14 clonal complexes) or were present in insufficient numbers for changes to be assessed (8 clonal complexes). The number of unassigned meningococci decreased from 9.3% in 1999 to 6.8% in 2001. The statistically strongest effects (P < .001) were the decreases in prevalence of the ST-11 complex (rate ratio for 2001 to 1999, 0.20) and ST-254 complex (rate ratio for 2001 to 1999, 0.38) and the increase in the proportion of the ST-213 complex (rate ratio for 2001 to 1999, 1.60). Most of the changes observed were consistent over the 3 years, with the exceptions of a significant increase in the ST-1117 complex.
in 2000 relative to 1999 and a significant decrease in the ST-60 complex in the same year (Table 2).

### Association of Serogroup and cps Genotype With Clonal Complexes

For the 26 clonal complexes with >25 isolates, predominant serogroup and cps genotypes were evident (Table 3). Fifteen clonal complexes were associated with the sialic acid–containing serogroups, 8 of which were mostly group B: ST-32 complex (46% serogroup B, 86% siaDB), ST-35 complex (42% and 67%, respectively), ST-162 complex (79% and 95%, respectively), ST-41/44 complex (62% and 88%, respectively), ST-162 complex (79% and 95%, respectively), ST-213 complex (79% and 95%, respectively), ST-269 (50% and 74%, respectively), ST-282 complex (79% and 98%, respectively), and ST-461 complex (73% and 82%, respectively). Most of these clonal complexes rarely exhibited other serogroups, but there were 63 group C ST-41/44 complex isolates (1.5% serogroup C, 5.4% siaDC), 31 ST-35 complex group C isolates (3% serogroup C and 11% siaDC), and 20 group C ST-269 complex isolates (0.5% serogroup C, 5.5% siaDC). The ST-11 complex (58% serogroup C, 89% siaDC) and ST-212 complex (4% serogroup C, 66% siaDC) were mostly group C, with the ST-22 complex being mostly group W135 (54% serogroup W135, 85% siaDw). Group Y was associated with 4 clonal complexes (ST-167 complex, ST-23 complex, ST-174 complex, and ST-92 complex); 1 complex was serogroup X (ST-103 complex) and 1 was serogroup 29E (ST-60 complex). Four clonal complexes that contained nongroupable isolates were associated with the cntl (the ST-53, ST-198, ST-1117, and ST-1136 complexes). Three clonal complexes (ST-178, ST-254, and ST-1157 complexes) were not serogroupable and gave no result with the cps genotyping employed, indicating that they likely possessed capsules and capsular regions not included in the serogroup and genotyping panel (Table 3).

### Capsule Expression

For meningococci with a siaD gene, there was variation in capsule expression by both serogroup and clonal complex. Expression rates of serogroup B capsule were 60%–100% for most of the 11 clonal complexes with siaDB alleles, with the exceptions of the ST-32 complex, with 76 (52%) of 146 isolates expressing capsule, and the ST-865 complex, with 3 (13.5%) of 24 isolates expressing capsule. There was statistically significant variation (P < .002; Fisher exact test) in the rates of capsule expression of the 5 clonal complexes associated with serogroup C, from 2 (10%) of 20 isolates for the ST-269 complex to 46 (64%) of 72 isolates for the ST-11 complex. One clonal complex (ST-22 complex) was associated with serogroup W135 and 576 (61%) of these 945 isolates expressed this capsule. Rates of expression of the serogroup Y capsule ranged from 56% (ST-167 complex) to 28% (ST-22 complex) (Table 4).

### DISCUSSION

Herd immunity effects have made a major contribution to the success of many vaccines, and they have been of particular importance in vaccination with conjugate polysaccharide vaccines against encapsulated bacteria [2]; indeed, for these pathogens, the cost-effectiveness of immunization has been substantially enhanced by this phenomenon [29]. For accidental pathogens such as the meningococcus, inducing herd immunity against a subset of the antigenic repertoire of the organism potentially alters its niche, imposing selection pressure against those variants that express the vaccine antigens during carriage [21]. Given the complexity of the pharyngeal surface and its microbiota [30], it is difficult to anticipate the likely effects of this environmental change on the bacterial population, as the elimination of a variant is likely to affect the prevalence of other members of the microbiota.

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**Table 1. Carried Meningococcal Serogroups in the United Kingdom, 1999–2001**

<table>
<thead>
<tr>
<th>Year</th>
<th>Total (n = 8462)</th>
<th>Rate ratio, 2001 to 1999 (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>(n = 2306)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>(n = 2873)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>(n = 3283)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>564 (24.5)</td>
<td>681 (23.7)</td>
<td>.99 (.91–1.09)</td>
</tr>
<tr>
<td>C</td>
<td>62 (2.7)</td>
<td>24 (0.8)</td>
<td>0.18 (.10–.31)</td>
</tr>
<tr>
<td>W135</td>
<td>155 (6.7)</td>
<td>234 (8.1)</td>
<td>1.13 (.93–1.37)</td>
</tr>
<tr>
<td>X</td>
<td>27 (1.2)</td>
<td>31 (1.1)</td>
<td>1.13 (.70–1.83)</td>
</tr>
<tr>
<td>Y</td>
<td>134 (5.8)</td>
<td>172 (6.0)</td>
<td>0.97 (.78–1.20)</td>
</tr>
<tr>
<td>Z</td>
<td>10 (0.4)</td>
<td>10 (0.3)</td>
<td>1.11 (.51–2.45)</td>
</tr>
<tr>
<td>Z/29E</td>
<td>3 (0.1)</td>
<td>7 (0.2)</td>
<td>1.16 (.28–4.85)</td>
</tr>
<tr>
<td>29E</td>
<td>111 (4.8)</td>
<td>98 (3.4)</td>
<td>0.58 (.44–.76)</td>
</tr>
<tr>
<td>Not groupable</td>
<td>1215 (52.7)</td>
<td>1613 (56.1)</td>
<td></td>
</tr>
<tr>
<td>No result</td>
<td>25 (1.1)</td>
<td>3 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>
Despite widespread horizontal genetic exchange among meningococci, which can randomize genetic variation [31, 32], meningococcal populations are dominated by clonal complexes, which persist over time and during geographic spread [8, 33], 49 of which had been identified at the time of writing [8]. Isolates from cases of disease mostly belong to one of the dozen or so hyperinvasive lineages [3], with many more genotypes observed in asymptomatic carriage than from invasive disease [8, 20, 34]. Hyperinvasive meningococci are more likely to be identified than those that are carried yet rarely cause disease and it is likely that there are more noninvasive clonal complexes than are currently recognized. For example, the 572 meningococci unassigned to a clonal complex likely represented members of as yet undefined clonal complexes that were present at low

<table>
<thead>
<tr>
<th>Clonal complex (multilocus sequence typing)</th>
<th>1999 (n = 2306)</th>
<th>2000 (n = 2873)</th>
<th>2001 (n = 3283)</th>
<th>Total (n = 8,462)</th>
<th>Rate ratio, 2001 to 1999 (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decrease over 3 years (P ≤ .05)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-11</td>
<td>43 (1.9)</td>
<td>26 (0.9)</td>
<td>12 (0.4)</td>
<td>81 (1.0)</td>
<td>0.20 (1.10–37)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ST-865</td>
<td>20 (0.9)</td>
<td>18 (0.6)</td>
<td>10 (0.3)</td>
<td>48 (0.6)</td>
<td>0.35 (1.16–75)</td>
<td>.005</td>
</tr>
<tr>
<td>ST-254</td>
<td>42 (1.8)</td>
<td>32 (1.1)</td>
<td>23 (0.7)</td>
<td>97 (1.1)</td>
<td>0.38 (1.23–64)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ST-162</td>
<td>28 (1.2)</td>
<td>29 (1.0)</td>
<td>19 (0.6)</td>
<td>76 (0.9)</td>
<td>0.48 (1.27–85)</td>
<td>.01</td>
</tr>
<tr>
<td>ST-212</td>
<td>29 (1.3)</td>
<td>24 (0.8)</td>
<td>21 (0.6)</td>
<td>74 (0.9)</td>
<td>0.51 (1.29–89)</td>
<td>.02</td>
</tr>
<tr>
<td>ST-41/44</td>
<td>349 (15.1)</td>
<td>389 (13.5)</td>
<td>425 (12.9)</td>
<td>1163 (13.7)</td>
<td>0.86 (1.75–98)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Increase over 3 years (P ≤ .05)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-53</td>
<td>191 (8.3)</td>
<td>264 (9.2)</td>
<td>342 (10.4)</td>
<td>797 (9.4)</td>
<td>1.26 (1.06–1.50)</td>
<td>.008</td>
</tr>
<tr>
<td>ST-1157</td>
<td>106 (4.6)</td>
<td>152 (5.3)</td>
<td>191 (5.8)</td>
<td>449 (5.3)</td>
<td>1.27 (1.00–1.60)</td>
<td>.05</td>
</tr>
<tr>
<td>ST-269</td>
<td>88 (3.8)</td>
<td>114 (4.0)</td>
<td>165 (5.0)</td>
<td>367 (4.3)</td>
<td>1.32 (1.02–1.70)</td>
<td>.03</td>
</tr>
<tr>
<td>ST-213</td>
<td>153 (6.6)</td>
<td>305 (10.6)</td>
<td>348 (10.6)</td>
<td>806 (9.5)</td>
<td>1.60 (1.33–1.92)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ST-461</td>
<td>6 (0.3)</td>
<td>12 (0.4)</td>
<td>26 (0.8)</td>
<td>44 (0.5)</td>
<td>3.04 (1.25–7.38)</td>
<td>.01</td>
</tr>
<tr>
<td>ST-174</td>
<td>3 (0.1)</td>
<td>15 (0.5)</td>
<td>17 (0.5)</td>
<td>35 (0.4)</td>
<td>3.98 (1.17–13.57)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>No change over 3 years (P &gt; .05)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-22</td>
<td>272 (11.8)</td>
<td>407 (14.2)</td>
<td>430 (13.1)</td>
<td>1109 (13.1)</td>
<td>1.11 (1.96–1.28)</td>
<td>.15</td>
</tr>
<tr>
<td>ST-1117</td>
<td>35 (1.5)</td>
<td>85 (3.0)</td>
<td>66 (2.0)</td>
<td>186 (2.2)</td>
<td>1.32 (0.88–1.99)</td>
<td>.17</td>
</tr>
<tr>
<td>ST-198</td>
<td>87 (3.8)</td>
<td>109 (3.8)</td>
<td>148 (4.5)</td>
<td>344 (4.1)</td>
<td>1.19 (0.92–1.55)</td>
<td>.18</td>
</tr>
<tr>
<td>ST-23</td>
<td>91 (3.9)</td>
<td>106 (3.7)</td>
<td>154 (4.7)</td>
<td>351 (4.1)</td>
<td>1.19 (0.92–1.53)</td>
<td>.18</td>
</tr>
<tr>
<td>ST-167</td>
<td>121 (5.2)</td>
<td>155 (5.4)</td>
<td>147 (4.5)</td>
<td>423 (5.0)</td>
<td>0.85 (0.67–1.08)</td>
<td>.19</td>
</tr>
<tr>
<td>ST-92</td>
<td>9 (0.4)</td>
<td>10 (0.3)</td>
<td>7 (0.2)</td>
<td>26 (0.3)</td>
<td>0.55 (1.20–4.67)</td>
<td>.23</td>
</tr>
<tr>
<td>ST-178</td>
<td>32 (1.4)</td>
<td>32 (1.1)</td>
<td>34 (1.0)</td>
<td>98 (1.2)</td>
<td>0.75 (1.46–2.12)</td>
<td>.23</td>
</tr>
<tr>
<td>ST-750</td>
<td>44 (1.9)</td>
<td>49 (1.7)</td>
<td>49 (1.5)</td>
<td>142 (1.7)</td>
<td>0.78 (1.52–1.17)</td>
<td>.23</td>
</tr>
<tr>
<td>ST-32</td>
<td>54 (2.3)</td>
<td>49 (1.7)</td>
<td>66 (2.0)</td>
<td>169 (2.0)</td>
<td>0.88 (1.60–1.23)</td>
<td>.40</td>
</tr>
<tr>
<td>ST-1136</td>
<td>21 (0.9)</td>
<td>32 (1.1)</td>
<td>24 (0.7)</td>
<td>77 (0.9)</td>
<td>0.80 (0.45–1.44)</td>
<td>.46</td>
</tr>
<tr>
<td>ST-103</td>
<td>37 (1.6)</td>
<td>46 (1.6)</td>
<td>61 (1.9)</td>
<td>144 (1.7)</td>
<td>1.16 (0.77–1.74)</td>
<td>.48</td>
</tr>
<tr>
<td>ST-35</td>
<td>71 (3.1)</td>
<td>92 (3.2)</td>
<td>112 (3.4)</td>
<td>275 (3.2)</td>
<td>1.11 (0.83–1.48)</td>
<td>.49</td>
</tr>
<tr>
<td>ST-282</td>
<td>16 (0.7)</td>
<td>17 (0.6)</td>
<td>24 (0.7)</td>
<td>57 (0.7)</td>
<td>1.05 (1.56–1.98)</td>
<td>.87</td>
</tr>
<tr>
<td>ST-60</td>
<td>124 (5.4)</td>
<td>115 (4.0)</td>
<td>174 (5.3)</td>
<td>413 (4.9)</td>
<td>0.99 (1.79–2.33)</td>
<td>.90</td>
</tr>
<tr>
<td><strong>Low-prevalence complexes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-8</td>
<td>7 (0.3)</td>
<td>1 (0.0)</td>
<td>4 (0.1)</td>
<td>12 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-18</td>
<td>5 (0.2)</td>
<td>2 (0.1)</td>
<td>3 (0.1)</td>
<td>10 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-364</td>
<td>3 (0.1)</td>
<td>2 (0.1)</td>
<td>3 (0.1)</td>
<td>8 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-116</td>
<td>1 (0.0)</td>
<td>3 (0.1)</td>
<td>0 (0.0)</td>
<td>4 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-334</td>
<td>2 (0.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-106</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.0)</td>
<td>1 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-376</td>
<td>1 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-37</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.0)</td>
<td>1 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unassigned isolates</td>
<td>215 (9.3)</td>
<td>181 (6.3)</td>
<td>176 (5.4)</td>
<td>572 (6.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
complexes were represented by 

Supported by the observation that 8 previously described clonal 

precise association varies with time and place [20]. Although 
tend to be associated with a particular serogroup, although the 
disease-associated capsules with hyperinvasive lineages that 

prevalence in the UK at the time of sampling—a view that is 
supported by the observation that 8 previously described clonal 

complexes were represented by \( \leq 12 \) isolates in this study.

Almost all meningococci that cause disease elaborate one of 
the disease-associated capsules with hyperinvasive lineages that 
tend to be associated with a particular serogroup, although the 

precise association varies with time and place [20]. Although 

possession of a capsule is normally necessary for virulence, it is 

not in itself sufficient, because certain clonal complexes are as-

sociated with these capsules yet rarely or never invade [20]. Other 

clonal complexes are associated with the \( cnl \) genotype, and 

these meningococci can be considered nonvirulent, yet they can 

acquire capsules and cause disease. For example, the ST-53 

complex is typically \( cnl \) and thought to be noninvasive, but the 

first ST-53 meningococcus identified was a serogroup C clinical 

isolate from the UK [35]: only 2 of the 797 ST-53 complex 

meningococci isolated here possessed the \( siaD_C \) gene. Non-

disease serogroups are also associated with clonal complexes, 

showing that this phenomenon is not limited to invasive me-

ningococci. Consistent with disease patterns in the UK [36], no 

serogroup A meningococci were isolated: the reasons for the 

disappearance of this serogroup from transmission in the UK 
since the 1970s [37] despite reintroduction [38] remain to be 

explained, highlighting the dynamic nature of meningococcal 
carriage and therefore disease prevalence, which alters over time 

for reasons that remain poorly understood.

The UK’s implementation of the MCC vaccines was prompted 
by the increased incidence of serogroup C meningococcal 
disease, due to the global spread of serogroup C ST-11 me-

ningococci [39]. The strain responsible (C: P1.5,2: ST-11 (cc11)), 

identified as ET-15 by multilocus enzyme electrophoresis), was 

first identified in Canada in 1986 and spread widely in North 

America, Europe, Israel, and Australia during the 1990s [39, 40]. 

The MCC vaccines were very effective against this strain in the 

UK and other countries by herd immunity effects [9]; however, 

despite the potentially strong selective pressure imposed by 
vaccine introduction, and the report of possible serogroup re-

placement in Spain [41], ST-11 complex meningococci ex-

pressing different serogroup variants of the ST-11 complex did 

not spread in the UK during or immediately after the vaccine 

introduction. Indeed, although serogroup \( W_{135} \) ST-11 complex 

meningococci were introduced into the UK by Hajj pilgrims 

[42] and increased in the postvaccination period, they did not 

replace the serogroup C ST-11 clonal complex meningococci as 

a major cause of disease in the UK [36].

Although the 80% reduction in carriage of serogroup C me-

ningococci was attributable to the vaccine introduction, the 

reason for the 40% reduction in serogroup 29E carriage, with a 
similar level of statistical significance (Table 1), was less ob-

vious. No other serogroup changed significantly over this time 

period, although there were increases in serogroups \( W_{135}, X, Y, \) 

and \( Z \). Although a causal link between vaccine introduction 

and the reduction in serogroup 29E was not established, the 

year-on-year reduction, similar to that seen for serogroup C,

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>cps Genotype*</th>
<th>Clonal complex or complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>( siaD_C )</td>
<td>ST-11, ST-212</td>
</tr>
<tr>
<td>Y</td>
<td>( siaD_Y )</td>
<td>ST-23, ST-92, ST-167, ST-174</td>
</tr>
<tr>
<td>W(_{135})</td>
<td>( siaD_W )</td>
<td>ST-22</td>
</tr>
<tr>
<td>29E</td>
<td>No result</td>
<td>ST-60, ST-254</td>
</tr>
<tr>
<td>X</td>
<td>No result</td>
<td>ST-750</td>
</tr>
<tr>
<td>Z</td>
<td>No result</td>
<td>ST-103</td>
</tr>
<tr>
<td>Not groupable</td>
<td>( cnl )</td>
<td>ST-63, ST-198, ST-1117, ST-1136</td>
</tr>
<tr>
<td>Not groupable</td>
<td>No result</td>
<td>ST-178, ST-254, ST-1157</td>
</tr>
</tbody>
</table>

* Serogroup was detected immunochemically.

b The genotyping test detected only the \( siaD \) alleles or the capsule null 

locus (\( cnl \)). \( siaD_B \) is equivalent to \( synD, siaD_C \) to \( synE, siaD_W \) to \( synF, \) and \( siaD_Y \) to \( synG. \)

Table 3. Predominant Serogroups and \( cps \) Genotypes of the 26 

Most Prevalent Carried Meningococcal Clonal Complexes 

Isolated in the United Kingdom, 1999–2001

Table 4. Expression of Serogroup by Different \( siaD \) Associated 

Clonal Complexes

<table>
<thead>
<tr>
<th>Group, ( siaD ) combination</th>
<th>Serogroup corresponding to ( genotypes )</th>
<th>Genotype positive</th>
<th>Percentage of isolates expressing serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>( siaD_B ) ST-18</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-261</td>
<td>31</td>
<td>86.1</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-162</td>
<td>58</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>( siaB_D ) ST-282</td>
<td>45</td>
<td>80.4</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-213</td>
<td>542</td>
<td>73.2</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-41/44</td>
<td>714</td>
<td>69.8</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-1157</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-269</td>
<td>178</td>
<td>65.4</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-35</td>
<td>115</td>
<td>59.9</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-32</td>
<td>76</td>
<td>52.1</td>
</tr>
<tr>
<td></td>
<td>( siaD_B ) ST-865</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>C</td>
<td>( siaD_C ) ST-11</td>
<td>46</td>
<td>63.9</td>
</tr>
<tr>
<td></td>
<td>( siaD_C ) ST-8</td>
<td>6</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>( siaD_C ) ST-41/44</td>
<td>15</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>( siaD_C ) ST-213</td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>( siaD_C ) ST-269</td>
<td>2</td>
<td>10.0</td>
</tr>
<tr>
<td>W(_{135})</td>
<td>( siaD_W ) ST-22</td>
<td>576</td>
<td>61.0</td>
</tr>
<tr>
<td>Y</td>
<td>( siaD_Y ) ST-174</td>
<td>21</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td>( siaD_Y ) ST-167</td>
<td>190</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>( siaD_Y ) ST-92</td>
<td>12</td>
<td>52.2</td>
</tr>
<tr>
<td></td>
<td>( siaD_Y ) ST-23</td>
<td>128</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td>( siaD_Y ) ST-22</td>
<td>19</td>
<td>28.4</td>
</tr>
</tbody>
</table>

* \( siaD_B \) is equivalent to \( synD, siaD_C \) to \( synE, siaD_W \) to \( synF, \) and \( siaD_Y \) to \( synG. \)
was consistent with a vaccine effect. This is very unlikely to be
due to serological cross-reactivity between the structurally dis-
tinct serogroup 29E (3-deoxy-D-manno-octulosonic) [43] and
serogroup C (sialic acid) polysaccharides, although immuno-
logical cross-reactivity of the chemically related 29E and Z
capsules is known [44] and was seen in this study. A serogroup
29E isolate with a siaDC capsule gene has been reported [45],
but none of the 29E isolates examined exhibited this unusual
genotype. Thus, the reduction in carriage of serogroup 29E
(mostly ST-254 complex) meningococci may be a conseque-
tce of a secondary interaction of meningococcal genotypes or an
unrelated change in carriage prevalence.

The other noteworthy change in the carried meningococcal
population over this time was the increase in the prevalence of
serogroup B ST-213 organisms. This clonal complex was absent
from 325 meningococcal isolates obtained from invasive disease
in England and Wales sampled from 1975 through 1995 [37], but
there was a sustained increase of this complex among disease
isolates in England and Wales after 1999 [46]. In the Impact
of Meningococcal Epidemiology and Population Biology on Public
Health in Europe (EU-MenNet) study, which analyzed >4000
representative disease isolates from throughout Europe from
2000 through 2002, the ST-213 clonal complex was present in the
UK (vaccine introduced in 1999), Ireland (2000), the Netherlands
(2002), and Denmark (vaccine not introduced), but not in other
European countries [47]. Furthermore, although the PubMedLST
database (http://pubmlst.org/neisseria) is not a representative
epidemiological sample, it is intriguing that none of the 266
members of the ST-213 complex that were deposited at the time
of writing predated 1999. These data are not conclusive, but the
increase in the invasive serogroup B ST-213 meningococci
may have been related to the vaccine introduction, although
the increase in incidence may also have been due to the natural
meningococcal population change: this increase was in the
context of a decrease in meningococcal disease rates in the
UK [46]. This example highlights the importance of ongoing
surveillance of both carried and disease-causing meningococci
in the absence of comprehensive vaccines.

Irrespective of their likelihood to cause invasive disease, me-
ningococcal clonal complexes are characterized by particular
antigenic repertoires that persist for decades and during geo-
graphic spread [48]. The reasons for this remain a matter of
debate, but neither neutral nor micro-epidemic evolutionary
processes can account for the structuring observed [49], whereas
models that incorporate selection for fitness during transmission
can explain these features of meningococcal populations [33]. In
the absence of universal meningococcal polysaccharide vaccines,
which are precluded by the reluctance to include the serogroup
B capsular antigen [50], these ideas have important implications
for vaccine design. The effect of the MCC vaccine in eliminating
ST-11 complex serogroup C meningococci illustrates the poten-
tial of an approach that targets particular transmission
phenotypes. In principle, vaccines based on rationally assembled
cocktails of antigens, which are effective against carriage, could be
used to target those invasive clonal complexes that are typically
associated with serogroup B capsules [48]. The results of the
present study, however, demonstrate that mass vaccination
campaigns affect population structure in ways that are not readily
anticipatable in advance of the intervention. Consequently, such
strategies require long-term surveillance of types that are found in
carriage and their association with invasive disease.

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