

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

Ana Belén Ibarz-Pavón,¹ Jenny MacLennan,¹ Nicholas J. Andrews,³ Stephen J. Gray,² Rachel Urwin,^{1,a} Stuart C. Clarke,⁴ A. Mark Walker,^{5,a} Meirion R. Evans,⁶ J. Simon Kroll,⁷ Keith R. Neal,⁸ Dlawer Ala'Aldeen,⁹ Derrick W. Crook,¹⁰ Kathryn Cann,^{10,a} Sarah Harrison,¹¹ Richard Cunningham,¹² David Baxter,¹³ Edward Kaczmarek,² Noel D. McCarthy,¹ Keith A. Jolley,¹ J. Claire Cameron,¹⁴ James M. Stuart,¹⁵ and Martin C. J. Maiden¹

¹Department of Zoology, University of Oxford; ²Meningococcal Reference Unit, Health Protection Agency, Manchester Medical Microbiology Partnership, Manchester Royal Infirmary; ³Health Protection Agency Centre for Infections, London; ⁴Division of Infection, Inflammation and Immunity, University of Southampton, School of Medicine, Southampton National Institute for Health Research Biomedical Research Unit in Respiratory Medicine, and Health Protection Agency, Southampton; ⁵University of Wales, Bangor, Gwynedd; ⁶Department of Primary Care and Public Health, Cardiff University; ⁷Imperial College School of Medicine, Norfolk Place, London; ⁸University of Nottingham, Epidemiology and Public Health, Community Health Sciences, Queen's Medical Centre; ⁹Division of Microbiology, School of Molecular Medicine, University Hospital, Nottingham; ¹⁰Nuffield Department of Clinical and Laboratory Sciences, John Radcliffe Hospital, Headley Way, University of Oxford; ¹¹Torbay Care Trust, Torquay; ¹²Derriford Hospital, Plymouth; ¹³Division of Epidemiology and Health Sciences, Medical School, The University of Manchester; ¹⁴Health Protection Scotland, Clifton House, Clifton Place, Glasgow; and ¹⁵School of Social and Community Medicine, University of Bristol, United Kingdom

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.

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^aPresent affiliations: Department of Biology, The Pennsylvania State University, Mueller Laboratory, University Park (R. U.); Tyn Lon, Pentraeth, Isle of Anglesey, United Kingdom (A. M. W.); Department of Microbiology, Stoke Mandeville Hospital, Aylesbury, Bucks, United Kingdom (K. C.).

Correspondence: Martin C. J. Maiden, PhD, Department of Zoology, University of Oxford, South Parks Rd, Oxford, OX1 3PS, United Kingdom (martin.maiden@zoo.ox.ac.uk).

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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 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, *siaD_B* or *synD*; serogroup C, *siaD_C* or *synE*; serogroup W-135, *siaD_W* or *synF*; and serogroup Y, *siaD_Y* 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, *siaD_B* (*synD*), *siaD_C* (*synE*), *siaD_W* (*synF*), and *siaD_Y* (*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 χ^2 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 9233 bacteria were isolated, of which 8462 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 (2306 isolates from 13 901 individuals), 17.6% in 2000 (2873 isolates from 16 295 individuals), and 18.7% in 2001 (3283 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 8599 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 8429 isolates (99.6%), with 3738 isolates (44%) grouped to 7 serogroups: B, C, 29E, W₁₃₅, X, Y, and Z. There were 4691 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 2050 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, 4290 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; 1163 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 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

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

Serogroup	No. (%) of isolates				Rate ratio, 2001 to 1999 (95% confidence interval)	<i>P</i>
	Year					
	1999 (<i>n</i> = 2306)	2000 (<i>n</i> = 2873)	2001 (<i>n</i> = 3283)	Total (<i>n</i> = 8462)		
B	564 (24.5)	681 (23.7)	805 (24.5)	2050 (24.2)	0.99 (.91–1.09)	.89
C	62 (2.7)	24 (0.8)	16 (0.5)	102 (1.2)	0.18 (.10–.31)	<.001
W ₁₃₅	155 (6.7)	234 (8.1)	251 (7.6)	640 (7.6)	1.13 (.93–1.37)	.22
X	27 (1.2)	31 (1.1)	44 (1.3)	102 (1.2)	1.13 (.70–1.83)	.61
Y	134 (5.8)	172 (6.0)	186 (5.7)	492 (5.8)	0.97 (.78–1.20)	.75
Z	10 (0.4)	10 (0.3)	16 (0.5)	36 (0.4)	1.11 (.51–2.45)	.79
Z/29E	3 (0.1)	7 (0.2)	5 (0.2)	15 (0.2)	1.16 (.28–4.85)	.84
29E	111 (4.8)	98 (3.4)	92 (2.8)	301 (3.6)	0.58 (.44–.76)	<.001
Not groupable	1215 (52.7)	1613 (56.1)	1863 (56.7)	4691 (55.4)
No result	25 (1.1)	3 (0.1)	5 (0.2)	33 (0.4)

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% *siaD_B*), ST-35 complex (42% and 67%, respectively), ST-41/44 complex (62% and 88%, respectively), ST-162 complex (79% and 95%, respectively), ST-213 complex (70% and 92%, 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% *siaD_C*), 31 ST-35 complex group C isolates (3% serogroup C and 11% *siaD_C*), and 20 group C ST-269 complex isolates (0.5% serogroup C, 5.5% *siaD_C*). The ST-11 complex (58% serogroup C, 89% *siaD_C*) and ST-212 complex (4 % serogroup C, 66% *siaD_C*) were mostly group C, with the ST-22 complex being mostly group W₁₃₅ (54% serogroup W₁₃₅, 85% *siaD_W*). 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 *cnl* (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 *siaD_B* 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.

Table 2. Meningococcal Clonal Complexes in the United Kingdom: Trends in Proportions of Carried Isolates, 1999–2001

Clonal complex (multilocus sequence typing)	No. (%) of isolates				Rate ratio, 2001 to 1999 (95% confidence interval)	<i>P</i>
	Year					
	1999 (<i>n</i> = 2306)	2000 (<i>n</i> = 2873)	2001 (<i>n</i> = 3283)	Total (<i>n</i> = 8,462)		
Decrease over 3 years (<i>P</i> ≤ .05)						
ST-11	43 (1.9)	26 (0.9)	12 (0.4)	81 (1.0)	0.20 (.10–.37)	<.001
ST-865	20 (0.9)	18 (0.6)	10 (0.3)	48 (0.6)	0.35 (.16–.75)	.005
ST-254	42 (1.8)	32 (1.1)	23 (0.7)	97 (1.1)	0.38 (.23–.64)	<.001
ST-162	28 (1.2)	29 (1.0)	19 (0.6)	76 (0.9)	0.48 (.27–.85)	.01
ST-212	29 (1.3)	24 (0.8)	21 (0.6)	74 (0.9)	0.51 (.29–.89)	.02
ST-41/44	349 (15.1)	389 (13.5)	425 (12.9)	1163 (13.7)	0.86 (.75–.98)	.02
Increase over 3 years (<i>P</i> ≤ .05)						
ST-53	191 (8.3)	264 (9.2)	342 (10.4)	797 (9.4)	1.26 (1.06–1.50)	.008
ST-1157	106 (4.6)	152 (5.3)	191 (5.8)	449 (5.3)	1.27 (1.00–1.60)	.05
ST-269	88 (3.8)	114 (4.0)	165 (5.0)	367 (4.3)	1.32 (1.02–1.70)	.03
ST-213	153 (6.6)	305 (10.6)	348 (10.6)	806 (9.5)	1.60 (1.33–1.92)	<.001
ST-461	6 (0.3)	12 (0.4)	26 (0.8)	44 (0.5)	3.04 (1.25–7.38)	.01
ST-174	3 (0.1)	15 (0.5)	17 (0.5)	35 (0.4)	3.98 (1.17–13.57)	.02
No change over 3 years (<i>P</i> > .05)						
ST-22	272 (11.8)	407 (14.2)	430 (13.1)	1109 (13.1)	1.11 (.96–1.28)	.15
ST-1117	35 (1.5)	85 (3.0)	66 (2.0)	186 (2.2)	1.32 (.88–1.99)	.17
ST-198	87 (3.8)	109 (3.8)	148 (4.5)	344 (4.1)	1.19 (.92–1.55)	.18
ST-23	91 (3.9)	106 (3.7)	154 (4.7)	351 (4.1)	1.19 (.92–1.53)	.18
ST-167	121 (5.2)	155 (5.4)	147 (4.5)	423 (5.0)	0.85 (.67–1.08)	.19
ST-92	9 (0.4)	10 (0.3)	7 (0.2)	26 (0.3)	0.55 (.20–1.46)	.23
ST-178	32 (1.4)	32 (1.1)	34 (1.0)	98 (1.2)	0.75 (.46–1.21)	.23
ST-750	44 (1.9)	49 (1.7)	49 (1.5)	142 (1.7)	0.78 (.52–1.17)	.23
ST-32	54 (2.3)	49 (1.7)	66 (2.0)	169 (2.0)	0.86 (.60–1.23)	.40
ST-1136	21 (0.9)	32 (1.1)	24 (0.7)	77 (0.9)	0.80 (.45–1.44)	.46
ST-103	37 (1.6)	46 (1.6)	61 (1.9)	144 (1.7)	1.16 (.77–1.74)	.48
ST-35	71 (3.1)	92 (3.2)	112 (3.4)	275 (3.2)	1.11 (.83–1.48)	.49
ST-282	16 (0.7)	17 (0.6)	24 (0.7)	57 (0.7)	1.05 (.56–1.98)	.87
ST-60	124 (5.4)	115 (4.0)	174 (5.3)	413 (4.9)	0.99 (.79–1.23)	.90
Low-prevalence complexes						
ST-8	7 (0.3)	1 (0.0)	4 (0.1)	12 (0.1)		
ST-18	5 (0.2)	2 (0.1)	3 (0.1)	10 (0.1)		
ST-364	3 (0.1)	2 (0.1)	3 (0.1)	8 (0.1)		
ST-116	1 (0.0)	3 (0.1)	0 (0.0)	4 (0.0)		
ST-334	2 (0.1)	0 (0.0)	0 (0.0)	2 (0.0)		
ST-106	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)		
ST-376	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)		
ST-37	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)		
Unassigned isolates	215 (9.3)	181 (6.3)	176 (5.4)	572 (6.8)		

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 3. Predominant Serogroups and *cps* Genotypes of the 26 Most Prevalent Carried Meningococcal Clonal Complexes Isolated in the United Kingdom, 1999–2001

Serogroup ^a	<i>cps</i> Genotype ^b	Clonal complex or complexes
B	<i>siaD_B</i>	ST-32, ST-35, ST-41/44, ST-162, ST-213, ST-269, ST-282, ST-461
C	<i>siaD_C</i>	ST-11, ST-212
Y	<i>siaD_Y</i>	ST-23, ST-92, ST-167, ST-174
W ₁₃₅	<i>siaD_W</i>	ST-22
29E	No result	ST-60, ST-254
X	No result	ST-750
Z	No result	ST-103
Not groupable	<i>cnl</i>	ST-53, ST-198, ST-1117, ST-1136
Not groupable	No result	ST-178, ST-254, ST-1157

^a 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*.

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 ≤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 associated 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 meningococci. 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 meningococci [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

Table 4. Expression of Serogroup by Different *siaD* Associated Clonal Complexes

Group, <i>siaD</i> :cc combination ^a	Serogroup positive	Genotype positive	Percentage of isolates expressing serogroup
B			
<i>siaD_B</i> :ST-18	10	10	100.0
<i>siaD_B</i> :ST-461	31	36	86.1
<i>siaD_B</i> :ST-162	58	72	80.6
<i>siaD_B</i> :ST-282	45	56	80.4
<i>siaD_B</i> :ST-213	542	740	73.2
<i>siaD_B</i> :ST-41/44	714	1023	69.8
<i>siaD_B</i> :ST-1157	9	13	69.2
<i>siaD_B</i> :ST-269	178	272	65.4
<i>siaD_B</i> :ST-35	115	192	59.9
<i>siaD_B</i> :ST-32	76	146	52.1
<i>siaD_B</i> :ST-865	3	24	12.5
C			
<i>siaD_C</i> :ST-11	46	72	63.9
<i>siaD_C</i> :ST-8	6	10	60.0
<i>siaD_C</i> :ST-41/44	15	63	23.8
<i>siaD_C</i> :ST-213	2	15	13.3
<i>siaD_C</i> :ST-269	2	20	10.0
W₁₃₅			
<i>siaD_W</i> :ST-22	576	945	61.0
Y			
<i>siaD_Y</i> :ST-174	21	27	77.8
<i>siaD_Y</i> :ST-167	190	339	56.1
<i>siaD_Y</i> :ST-92	12	23	52.2
<i>siaD_Y</i> :ST-23	128	319	40.1
<i>siaD_Y</i> :ST-22	19	67	28.4

^a *siaD_B* is equivalent to *synD*, *siaD_C* to *synE*, *siaD_W* to *synF*, and *siaD_Y* to *synG*.

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 replacement in Spain [41], ST-11 complex meningococci expressing different serogroup variants of the ST-11 complex did not spread in the UK during or immediately after the vaccine introduction. Indeed, although serogroup W₁₃₅ 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 meningococci 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 obvious. No other serogroup changed significantly over this time period, although there were increases in serogroups W₁₃₅, 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,

was consistent with a vaccine effect. This is very unlikely to be due to serological cross-reactivity between the structurally distinct serogroup 29E (3-deoxy-D-manno-octulosonic) [43] and serogroup C (sialic acid) polysaccharides, although immunological 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 *siaD_C* 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 consequence 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 PubMLST 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, meningococcal clonal complexes are characterized by particular antigenic repertoires that persist for decades and during geographic 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 potential 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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References

1. Makwana N, Riordan FA. Bacterial meningitis: the impact of vaccination. *CNS Drugs* 2007; 21:355–66.
2. Trotter CL, McVernon J, Ramsay ME, et al. Optimising the use of conjugate vaccines to prevent disease caused by *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Vaccine* 2008; 26:4434–45.
3. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 2009; 27:B51–63.
4. Campbell H, Borrow R, Salisbury D, Miller E. Meningococcal C conjugate vaccine: the experience in England and Wales. *Vaccine* 2009; 27:B20–9.
5. Pace D, Pollard AJ, Messonier NE. Quadrivalent meningococcal conjugate vaccines. *Vaccine* 2009; 27:B30–41.
6. Okoko BJ, Idoko OT, Adegbola RA. Prospects and challenges with introduction of a mono-valent meningococcal conjugate vaccine in Africa. *Vaccine* 2009; 27:2023–9.
7. Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*. *Vaccine* 2009; 27(suppl 2):B71–7.
8. Caugant DA, Maiden MC. Meningococcal carriage and disease—population biology and evolution. *Vaccine* 2009; 27(suppl 2):B64–70.
9. Maiden MC, Ibarz-Pavon AB, Urwin R, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 2008; 197:737–43.
10. Maiden MC, Stuart JM, Group UMC. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet* 2002; 359:1829–31.
11. Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. *Vaccine* 2001; 20:S58–67.

12. MacLennan J, Kafatos G, Neal K, et al. Social behavior and meningococcal carriage in British teenagers. *Emerg Infect Dis* **2006**; 12:950–7.
13. Auckland C, Gray S, Borrow R, et al. Clinical and immunologic risk factors for meningococcal C conjugate vaccine failure in the United Kingdom. *J Infect Dis* **2006**; 194:1745–52.
14. Frosch M, Weisgerber C, Meyer TF. Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B. *Proc Natl Acad Sci U S A* **1989**; 86:1669–73.
15. Claus H, Maiden MC, Maag R, Frosch M, Vogel U. Many carried meningococci lack the genes required for capsule synthesis and transport. *Microbiology* **2002**; 148:1813–9.
16. Claus H, Vogel U, Muhlenhoff M, Gerardy-Schahn R, Frosch M. Molecular divergence of the *sia* locus in different serogroups of *Neisseria meningitidis* expressing polysialic acid capsules. *Mol Gen Genet* **1997**; 257:28–34.
17. Swartley JS, Marfin AA, Edupuganti S, et al. Capsule switching of *Neisseria meningitidis*. *Proc Natl Acad Sci U S A* **1997**; 94:271–6.
18. Dolan-Livengood JM, Miller YK, Martin LE, Urwin R, Stephens DS. Genetic basis for nongroupable *Neisseria meningitidis*. *J Infect Dis* **2003**; 187:1616–28.
19. Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A* **1998**; 95:3140–5.
20. Yazdankhah SP, Kriz P, Tzanakaki G, et al. Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *J Clin Microbiol* **2004**; 42:5146–53.
21. Maiden MC, Spratt BG. Meningococcal conjugate vaccines: new opportunities and new challenges. *Lancet* **1999**; 354:615–6.
22. Cano R, Larrauri A, Mateo S, Alcalá B, Salcedo C, Vazquez JA. Impact of the meningococcal C conjugate vaccine in Spain: an epidemiological and microbiological decision. *Euro Surveill* **2004**; 9:11–5.
23. Vogel U, Claus H, Frosch M. Rapid serogroup switching in *Neisseria meningitidis*. *N Engl J Med* **2000**; 342:219–20.
24. Trotter CL, Maiden MC. Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. *Expert Rev Vaccines* **2009**; 8:851–61.
25. Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **2010**; 11:595.
26. Huson DH. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* **1998**; 14:68–73.
27. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* **2004**; 186:1518–30.
28. Jolley KA, Chan MS, Maiden MC. mlstdbNet—distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics* **2004**; 5:86.
29. Ortega-Sanchez IR, Meltzer MI, Shepard C, et al. Economics of an adolescent meningococcal conjugate vaccination catch-up campaign in the United States. *Clin Infect Dis* **2008**; 46:1–13.
30. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. *J Bacteriol* **2010**; 192:1422–31.
31. Holmes EC, Urwin R, Maiden MC. The influence of recombination on the population structure and evolution of the human pathogen *Neisseria meningitidis*. *Mol Biol Evol* **1999**; 16:741–9.
32. Jolley KA, Kalmusova J, Feil EJ, et al. Carried meningococci in the Czech Republic: a diverse recombining population. *J Clin Microbiol* **2000**; 38:4492–8.
33. Buckee CO, Jolley K, Recker M, et al. Role of selection in the emergence of lineages and the evolution of virulence in *Neisseria meningitidis*. *Proc Natl Acad Sci U S A* **2008**; 105:15082–7.
34. Claus H, Maiden MC, Wilson DJ, et al. Genetic analysis of meningococci carried by children and young adults. *J Infect Dis* **2005**; 191:1263–71.
35. Feavers IM, Gray SJ, Urwin R, et al. Multilocus sequence typing and antigen gene sequencing in the investigation of a meningococcal disease outbreak. *J Clin Microbiol* **1999**; 37:3883–7.
36. Gray SJ, Trotter CL, Ramsay ME, et al. Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit. *J Med Microbiol* **2006**; 55:887–96.
37. Russell JE, Urwin R, Gray SJ, Fox AJ, Feavers IM, Maiden MC. Molecular epidemiology of meningococcal disease in England and Wales 1975–1995, before the introduction of serogroup C conjugate vaccines. *Microbiology* **2008**; 154:1170–7.
38. Jones DM, Sutcliffe EM. Group A meningococcal disease in England associated with the Hajj. *J Infect* **1990**; 21:21–5.
39. Jelfs J, Munro R, Ashto FE, Caugant DA. Genetic characterization of a new variant within the ET-37 complex of *Neisseria meningitidis* associated with outbreaks in various parts of the world. *Epidemiol Infect* **2000**; 125:285–98.
40. Vogel U, Claus H, Frosch M, Caugant DA. Molecular basis for distinction of the ET-15 clone within the ET-37 complex of *Neisseria meningitidis*. *J Clin Microbiol* **2000**; 38:941–2.
41. Castilla J, Vazquez JA, Salcedo C, et al. B:2a:P1.5 meningococcal strains likely arisen from capsular switching event still spreading in Spain 10.1128/JCM.01495-08. *J Clin Microbiol* **2009**; 47:463–5.
42. Taha MK, Achtman M, Alonso JM, et al. Serogroup W135 meningococcal disease in Hajj pilgrims. *Lancet* **2000**; 356:2159.
43. Bhattacharjee AK, Jennings HJ, Kenny CP. Structural elucidation of 3-deoxy-D-manno-octulosonic acid containing meningococcal 29-E capsular polysaccharide antigen using C-13 nuclear magnetic-resonance. *Biochemistry* **1978**; 17:645–51.
44. Griffiss JM, Brandt BL. Immunological relationship between the capsular polysaccharides of *Neisseria meningitidis* serogroups Z and 29E. *J Gen Microbiol* **1983**; 129:447–52.
45. Ala'Aldeen DA, Neil K, English A, Hawkey P. Isolation of a serogroup 29E meningococcal strain carrying Group-C *siaD* capsular gene. *J Infect* **2002**; 44:56–7.
46. Lucidarme J, Comanducci M, Findlow J, et al. Characterisation of *fHbp*, *nhba* (gna2132), *nadA*, *porA* and sequence type in group B meningococcal case isolates collected in England and Wales during January 2008, and potential coverage of an investigational group B meningococcal vaccine. *Clin Vaccine Immunol* **2010**; 17:919–29.
47. Brehony C, Jolley KA, Maiden MC. Multilocus sequence typing for global surveillance of meningococcal disease. *FEMS Microbiol Rev* **2007**; 31:15–26.
48. Urwin R, Russell JE, Thompson EA, Holmes EC, Feavers IM, Maiden MC. Distribution of surface protein variants among hyperinvasive meningococci: implications for vaccine design. *Infect Immun* **2004**; 72:5955–62.
49. Jolley KA, Wilson DJ, Kriz P, McVean G, Maiden MC. The influence of mutation, recombination, population history, and selection on patterns of genetic diversity in *Neisseria meningitidis*. *Mol Biol Evol* **2005**; 22:562–9.
50. Jodar L, Feavers IM, Salisbury D, Granoff DM. Development of vaccines against meningococcal disease. *Lancet* **2002**; 359:1499–508.