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Meningococcal carriage in adolescents in the United Kingdom to inform timing of an adolescent vaccination strategy

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KEYWORDS
Neisseria meningitidis;
Serogroup;
Carriage;
Factor H binding protein;
Adolescents

Summary *Objectives:* Recent development of serogroup B meningococcal (MenB) vaccines highlights the importance of pharyngeal carriage data, particularly in adolescents and young adults, to inform implementation strategies. We describe current UK carriage prevalence in this high risk population and compare methods of carriage detection.
Methods: In this multisite study, pharyngeal swabs were collected on 3–4 occasions over 6–12 months, from 1040 school and university students, aged 10–25 years. Meningococcal carriage was detected by standard culture combined with seroagglutination or PCR of cultured isolates, or by direct PCR from swab. The factor H binding protein (fHBP) variants present in meningococcal isolates were determined.
Results: Meningococcal serogroups B and Y were most common, with carriage up to 6.5% and 5.5% respectively, increasing throughout adolescence. Identification by seroagglutination was often unreliable, and the sensitivity of direct PCR detection was 66% compared to culture combined with PCR. Of MenB isolates, 89.1% had subfamily A variants of fHBP. The acquisition rate of MenB carriage was estimated at 2.8 per 1000 person-months.
Conclusions: If vaccination is to precede the adolescent rise in MenB carriage, these data suggest it should take place in early adolescence. Studies assessing vaccine impact should use molecular methods to detect carriage.
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Introduction

Neisseria meningitidis remains a leading cause of meningitis and septicaemia globally. There are 700–1200 cases of invasive disease annually in the UK with a case fatality rate of 5–15%.[1,2] Invasive disease is almost exclusively due to 6 capsular serogroups (A, B, C, W, X or Y) and effective capsule-based glycoconjugate vaccines are currently available for the prevention of A, C, W and Y infection.[3] Serogroup B meningococcus (MenB) now accounts for 80–90% of invasive disease in the UK,[2,4] 65% across Europe,[5] and 30% in North America.[6] The MenB capsule has a structure identical to human polysialic acid and is therefore poorly immunogenic, which has historically hampered vaccine development. Recently, a MenB vaccine based on four subcapsular antigens (4CMenB, 'Bexsero', Novartis, Italy) has been licensed in Europe while a vaccine composed of two lipidated factor H binding proteins (bivalent rLP2086, 'Trumenba', Pfizer, USA) has been licensed in the USA.

The human pharynx is the only effective reservoir for *N. meningitidis*, where it is carried as a commensal. Carriage prevalence is age-related with carriage rates of up to 20–50% being reported in late adolescence.[7] Carriage is also associated with social risk factors, including visiting night clubs, kissing, smoking, and living in closed communities such as military or university residences.[8–10] The relationship between carriage and disease is complex. Most disease is caused by a limited number of 'hyperinvasive' strains, although these are commonly carried harmlessly, and pharyngeal isolates are often non-pathogenic strains.[11] Nonetheless, carriage data remain invaluable for predicting disease epidemiology, and a vaccine which reduces carriage of invasive strains is likely to confer a herd immunity effect, as has been seen with meningococcal serogroup C (MenC) conjugate vaccines.[12]

The aim of this study was to address the relative paucity of recent information about carriage in UK adolescents, by sequentially collecting posterior pharyngeal swabs from students at secondary school (typically attended from 11 to 18 years of age in the UK) and first year of university. As both MenB vaccines include a recombinant factor H binding protein (fHBP) component - a meningococcal virulence factor which aids evasion of innate immunity - the study characterised the fHBP variant type of all meningococci isolated to add to epidemiological data on current strains circulating in the UK. In considering the likely need to assess the impact of vaccination on carriage in future studies, the study also explored laboratory methodologies for detecting carriage.

Methods

Study design and population

A longitudinal epidemiological study was performed to collect pharyngeal swabs from secondary school and first year university students, aged 10–25 years, during a 6–12 month period. Participants were enrolled and first swabbed between March–May 2011, across 13 secondary schools and 5 universities from 4 recruiting sites in the UK (Oxford, London, Bristol and Southampton). The study was approved by National Research Ethics Service Committee South Central (Oxford B: 10/H0605/80). Written informed consent was obtained from participants over the age of 18 and from their parent(s) or legal guardian (s) (with the child's assent) below this age. Approximately equal numbers were to be recruited in each of 5 age cohorts: 10–12, 13–14, 15–16, 17–18 and 19–25 years old.

The second study visit occurred two months after enrolment (during May–July 2011), at which time any additional participants required were enrolled to fulfil the target sample size. The third visit of the main study period occurred 6 months after initial enrolment period (September–October 2011). Students who were in their penultimate school year at enrolment (year 12), were invited for an additional visit 12 months after enrolment (February–March

2012), to address low original recruitment in year 13. Pharyngeal samples were collected at each visit.

Participants completed a questionnaire concerning risk factors for carriage such as smoking, kissing and numbers in household. The participant's history of any recent antibiotic usage and meningococcal vaccination was also sought from the participants or their parents and participants excluded if they had ever received an investigational vaccination against MenB infection. The primary objective was to estimate the prevalence of MenB throat carriage in secondary school and first year university students, as determined by seroagglutination of cultured isolates. Secondary and exploratory endpoints included estimation of the prevalence of non-B serogroups; comparison with prevalence when measured by 2 other microbiological methods, namely PCR of cultured isolates and direct PCR from swab transport medium; fHBP variant characterisation of the isolates collected; and estimation of carriage acquisition rates from the longitudinal data.

Sampling and bacterial identification

Two pharyngeal samples were collected simultaneously through the mouth using two cotton swabs held together, by gently rubbing the tonsils (or tonsillar fossae) and the posterior pharynx. One swab was plated immediately onto Thayer–Martin improved medium, and incubated within 3 h at the local laboratory, at 37 °C in 5% carbon dioxide. Culture plates were examined at 24, 48 and 72 h for suspected colonies of *N. meningitidis*; single representative colonies were subcultured onto Columbia Blood Agar then gram-stained and tested for oxidase-positivity. Identity as *N. meningitidis* was confirmed by commercial biochemical tests (API-NH, bioMerieux).

The second swab was placed directly in transport medium (Digene Sample Transport Medium, Qiagen), and frozen for later PCR analysis.

The serogroup of cultured meningococcal isolates was assigned by 2 methods:

i) **Seroagglutination**. At local site laboratories, seroagglutination of several emulsified colonies was performed by slide agglutination with commercial antisera based on whole cell preparations for serogroups A, B, C, D, E, W, X, Y and Z (Difco™, BD Diagnostic Systems, Sparks, Maryland). Valid results were indicated by clumping with only one antiserum and lack of clumping in buffer alone (autoagglutination).
ii) **Isolate PCR**. Purified isolates were frozen on cryovial beads (Microbank, Pro-lab) at −70 °C. Frozen isolates were recovered, inspected for purity, then a 10 μL loopful of bacteria was solubilized in 1 ml 1X Phosphate Buffered Saline and diluted to provide a $1-5 \times 10^5$ cfu/mL sample for PCR analysis in Digene Sample Transport Medium. Real-time PCR (RT-PCR) assays for detecting serogroup specific capsule genes was conducted at PPD (Pharmaceutical Development LLC, Wayne, PA).

A third method of carriage detection and serogroup identification, assessed for MenB only given its importance for contemporary vaccine studies, was **direct swab PCR**. In this approach, DNA extraction and RT-PCR analysis was performed on 0.25 ml aliquots of transport medium from the second pharyngeal swab, bypassing the step of organism culture and isolation.

Real time PCR assay

RT-PCR assays were designed to both detect and serogroup *N. meningitidis*. Detection was achieved by amplification of the genes for surface protein porin A (*porA*, present in almost all strains of *N. meningitidis*) and *ctrA* (common to all meningococcal capsule operons regardless of the serogroup). Concurrently, genetic serogroup was assigned by a set of RT-PCR assays which relied on intrinsic differences in synthesis pathways between capsule type, and/or genetic diversity in sequence for shared genes between serogroups, for example the serogroup-specific sialic acid biosynthesis gene *siaD* was used to detect MenB (Rojas, E. et al. manuscript in preparation). The primers used were based on published sequence data, and all assays were qualified in accordance with guidelines published by the International Conference on Harmonisation[13,14] and United States Food and Drug Administration,[15] by Rojas et al. (manuscript in preparation). For further details, see Supplementary appendix 1.

Factor H binding protein sequence analysis

Determination of the fHBP variant type for all isolates was performed by nucleic acid sequencing using methods described previously.[16]

Statistical methods

A sample size of 1000–1200 subjects was chosen on the basis of confidence interval estimates. A conservative estimate for MenB prevalence of 2% would result in 95% CI of 1.2% and 3.1% with sample size 1000, and 1.3%–3.0% with sample size 1200, using the Clopper-Pearson exact method.[17] The expected size of these confidence intervals was considered small enough to provide meaningful estimates of prevalence.

This study was a descriptive epidemiological study; endpoints are estimates without formal hypothesis testing. For prevalence estimates, the numerator was the total number of positive results across all visits, and the denominator was the total number of times participants were tested across all visits. Exact 2-sided 95% confidence intervals (Clopper-Pearson) were calculated. For demographic analysis the intention to treat population was used, defined as all enrolled subjects. For longitudinal analysis, the number who had supplied at least one sample was used. The proportions of subjects with changes in meningococcal carriage were calculated with exact 95% confidence intervals using the F distribution.

Acquisition rate was the number of new meningococcal carriage cases (acquisitions) occurring during the study, divided by the total person-time of all initially negative subjects. Due to anticipated small numbers, data from all age groups were pooled. Any acquisition was assumed to have occurred at the timepoint halfway between assessments.

Results

The study enrolled 1040 participants in total, including 59 during the second recruitment period at the time of visit 2, although not all participants attended all visits (Fig. 1). The composition of the participants by age is shown in Table 1. Most participants were Caucasian (76%), and 55.8% were female. Antibiotic use in the preceding 2 weeks was low, between 2.2 and 3.6% of participants at visits 1—4.

Overall B and Y were the most prevalent serogroups detected. Carriage rates by age cohort are shown in Fig. 2 across all visits. Prevalence at each visit is plotted in Supplementary Fig. 4. Carriage of any meningococcus ranged from 3.9% in the youngest to 26.5% in the oldest cohort by seroagglutination method, or from 4.1% to 24.5% by isolate PCR.

Carriage prevalence of MenB

Prevalence of pharyngeal MenB carriage was similar in age cohorts 10—12 and 13—14, with a maximum prevalence of 1.5% by any method (Fig. 2A). Carriage was higher in the older age cohorts, with highest prevalence in the 19—25 age cohort: 3.6%, 6.5% and 6.9% by seroagglutination, isolate PCR and direct swab PCR, respectively.

Carriage prevalence of other meningococci

Carriage of serogroup Y meningococcus (MenY) reached similar prevalence to MenB (Fig. 2C), whereas carriage of MenC was extremely low, particularly when determined by isolate PCR (isolated on only 3 occasions in the entire study). Serogroups X and W were each detected in no more than 2.0% of any age cohort at any visit, by either

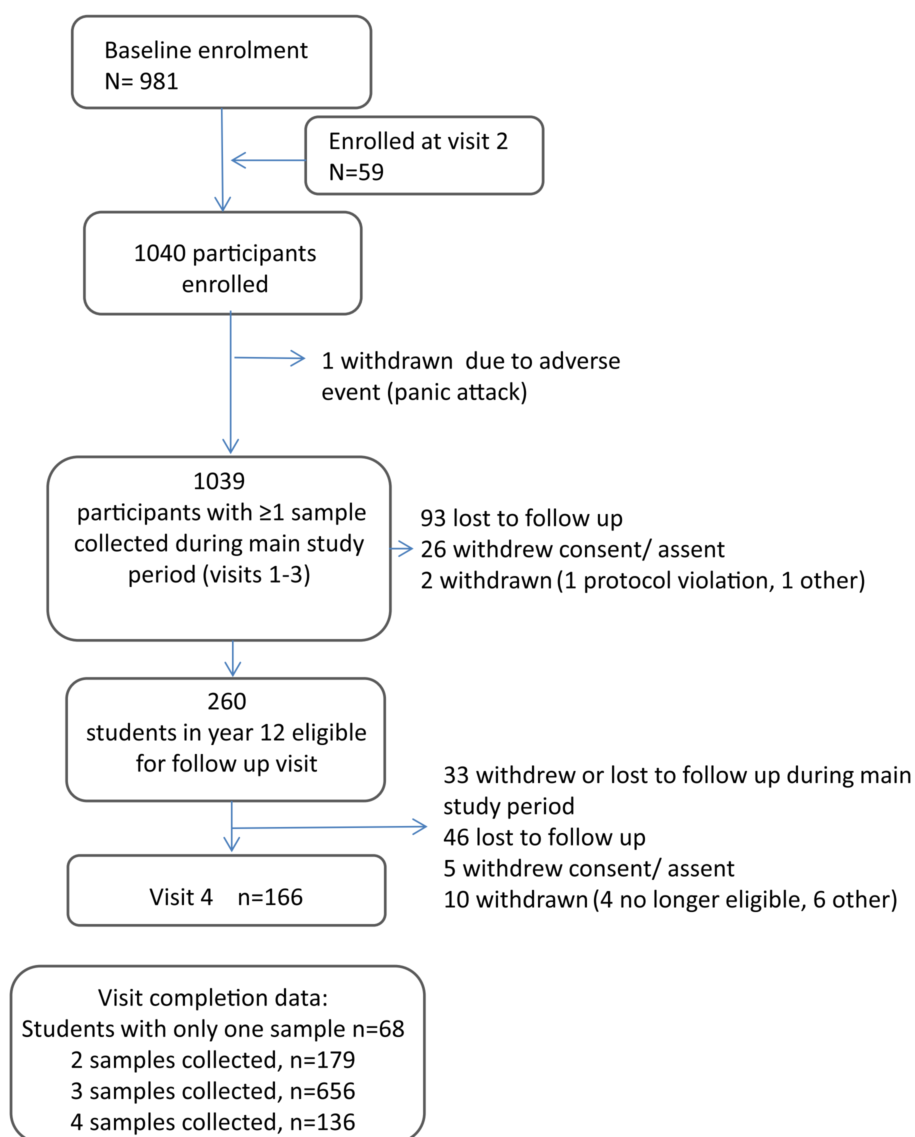


Figure 1 Summary of enrolment and disposition of study participants. All students enrolled in their penultimate year of secondary school (year 12) were invited to participate in follow-up sample collection period four, 12 months after enrolment.

Table 1 Number of participants who submitted an pharyngeal sample during each period of sampling, by age at enrolment.

Visit number	Period of collection	Number of participants swabbed	Age at enrolment	Male n (%)	All
Visit 1	March—May 2011	977	10—12 years	110 (55%)	200
			13—14 years	90 (54%)	169
			15—16 years	87 (48%)	182
			17—18 years	85 (34%)	254
			19—25 years	54 (31%)	172
Visit 2	May—July 2011	877 (including 59 newly enrolled)	10—12 years	104 (55%)	189
			13—14 years	103 (52%)	197
			15—16 years	85 (48%)	176
			17—18 years	72 (35%)	205
			19—25 years	32 (29%)	110
Visit 3	Sept—Oct 2011 (new academic year)	918	10—12 years	109 (55%)	197
			13—14 years	104 (53%)	198
			15—16 years	85 (48%)	176
			17—18 years	70 (34%)	207
			19—25 years	43 (31%)	140
Visit 4	Feb—March 2012	166	15—16 years	18 (33%)	54
			17—18 years	40 (36%)	112

The study enrolled 1040 students from secondary school and first year of university across 4 study sites, although not all participants attended all visits. Numbers recruited from Oxford (273 participants), Southampton (257), London (243), and Bristol (267) were similar.

seroagglutination or isolate PCR (data not shown), and no genetically-confirmed serogroup A (MenA) was detected.

Comparison of microbiological methods

Fig. 2 demonstrates substantial variation in detection of *N. meningitidis* by different microbiological methods, with carriage prevalence by seroagglutination generally lower than by isolate PCR or direct swab PCR. MenC however was detected more frequently by seroagglutination than by isolate PCR; the lack of genetic confirmation renders these probable false positive agglutination reactions.

As an illustration, the Venn diagram Fig. 3 shows the discrepancies for MenB detection by the three methods used. Of the 92 samples assigned MenB by isolate PCR, 59 (69%) were positive by direct swab PCR and only 44 (48%) by seroagglutination. Seven samples were positive for MenB by seroagglutination only, and were found to be false positives: 5 of these could not be confirmed as *N. meningitidis* in the central laboratory and 2 were MenY by PCR. Twenty-five samples were positive by direct swab PCR only. In 19 of these no *N. meningitidis* was isolated at the site laboratory or confirmed at the central laboratory, and 6 grew a non-MenB serogroup by isolate PCR. Over the 2899 sample pairs, the sensitivity and specificity of direct swab PCR relative to isolate PCR were 0.66 and 0.99 respectively.

The detection of non-MenB serogroups also demonstrated discrepancies between the two methods compared for these serogroups (seroagglutination and isolate PCR) (Table 2). Seroagglutination frequently assigned a serogroup which did not correspond with the PCR genogroup.

In approximately 6% (n = 22) of isolates, the isolate PCR assay was positive for multiple serogroup targets. To investigate this, the purity of these isolates was assessed post hoc by dilution and subculture to produce 10 daughter colonies which were genetically compared by porA genotyping. By this approach, some samples submitted by site laboratories were found to be mixed. Sequencing of *siaD* was performed on all MenB isolates to verify the RT-PCR results, and this revealed sequence diversity at the primer site which necessitated use of a second set of primers to enable detection of all MenB isolates.

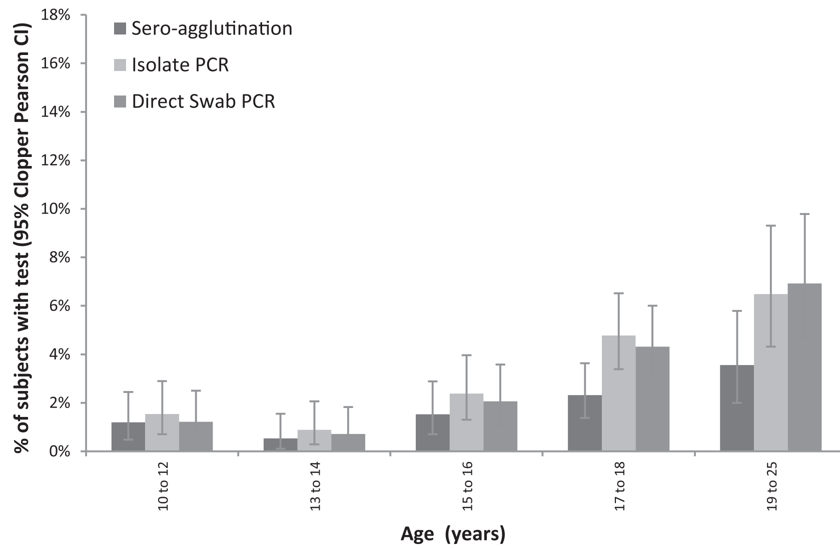
Characterisation of fHBP variants

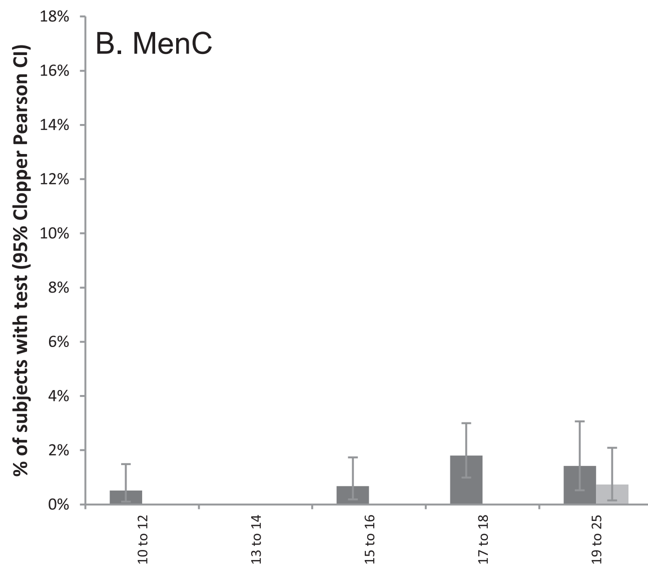
fHBP variants for all meningococcal isolates were analysed. Variants are divided in two immunologically distinct subfamilies, A and B.[16] Of isolates identified as serogroup B by PCR (n = 92), the majority (89.1%), belonged to subfamily A, and the most common sub-family variants were A05 (27.2%), A22 (27.2%) and A12 (15.2%), although some variation was noted between the four geographical study sites (Table 3).

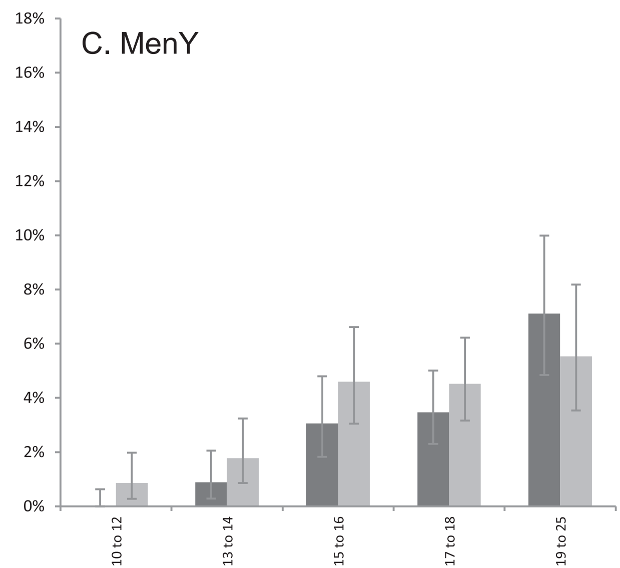
Changes in carriage within individuals

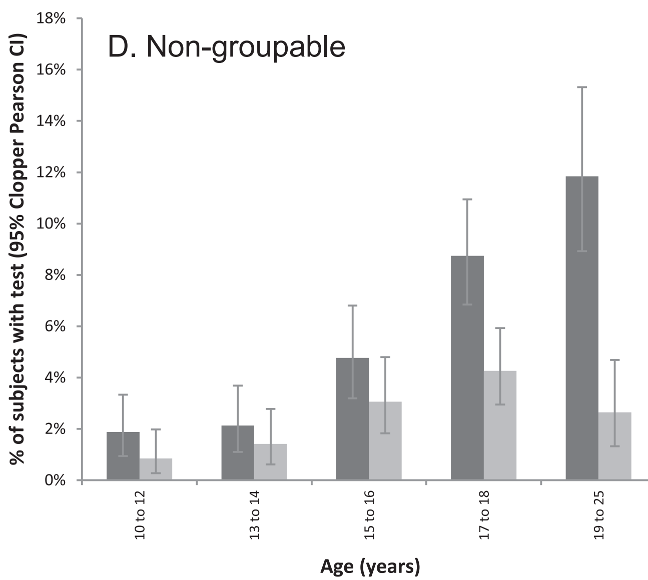
In view of the discrepant results mentioned earlier, analysis of carriage acquisition was based on isolate PCR data. Among participants initially negative for MenB carriage at enrolment, 1.9% (95% CI: 1.1, 2.9) became MenB carriers during the 6 month main study period. Of those who were initially MenB carriers, 48% (95% CI: 33, 63) became negative during the study period. This equates to an estimated acquisition rate of 2.8 per 1000 person-months by isolate PCR, pooling data from all age groups.

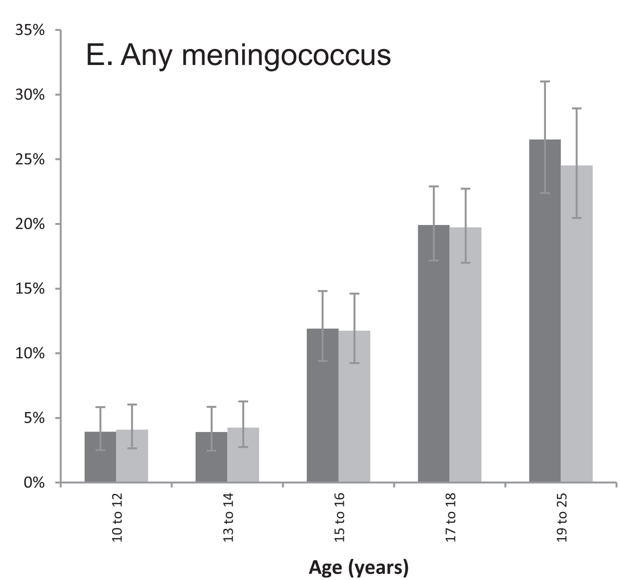
When individual participants are followed longitudinally across visits, 53/1039 (5.1%) were MenB carriers at any time, of whom 53% (28/53) were carriers at more than one visit and 45% (24/53) at two or more consecutive visits.


A. MenB
% of subjects with test (95% Clopper Pearson CI)
Sero-agglutination
Isolate PCR
Direct Swab PCR
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
10 to 12
13 to 14
15 to 16
17 to 18
19 to 25
Age (years)


B. MenC
% of subjects with test (95% Clopper Pearson CI)
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
10 to 12
13 to 14
15 to 16
17 to 18
19 to 25


C. MenY
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
10 to 12
13 to 14
15 to 16
17 to 18
19 to 25


D. Non-groupable
% of subjects with test (95% Clopper Pearson CI)
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
10 to 12
13 to 14
15 to 16
17 to 18
19 to 25
Age (years)


E. Any meningococcus
35%
30%
25%
20%
15%
10%
5%
0%
10 to 12
13 to 14
15 to 16
17 to 18
19 to 25
Age (years)

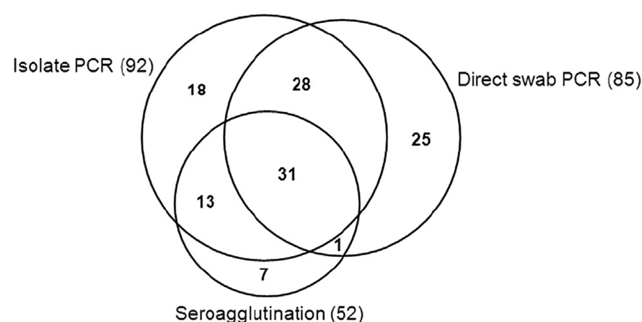


Figure 3 The number of MenB positive results across all pharyngeal samples collected, as detected by 3 laboratory methods: seroagglutination, isolate PCR, and direct swab PCR. Of 123 samples in which MenB was detected by any of the 3 methods used, the number positive by one or more methods is depicted[a].
[a] Two direct swab PCR samples had insufficient volume for testing (both were isolate PCR positive and seroagglutination negative).

Carriage of MenY was acquired and lost with similar frequency to MenB: 2.5% (95% CI:1.6, 3.6) became carriers during the study, and 51% (95% CI:35, 67) of initial carriers became negative.

Discussion

This is the largest contemporary study of meningococcal carriage through the second decade of life. We found MenB and MenY to be the most commonly carried serogroups, with prevalence reaching 6.5% for MenB and 5.5% for MenY in the 19—25 age cohort, by isolate PCR. Carriage rates in our 19—25 cohort are similar to those found in a recent vaccine study by Read et al. in which 3000 UK university students had baseline MenB prevalence of 9% and MenY prevalence of 7%, using a combined serogroup and genogroup assignment strategy.[18] In younger adolescents our study prevalence of any meningococcal carriage was lower than estimated by a European meta-analysis,[7] which predicted carriage of 7.7% in 10 year olds, rising to 23.7% in 19 year olds, but the authors comment that many studies included in that analysis may have been subject to recruitment bias.

The largest previous meningococcal carriage study in the UK took place around the introduction of a MenC vaccine into the UK childhood immunisation schedule in 1999, which was accompanied by a catch-up campaign for all aged below 18 years.[12,19,20] Carriage prevalence in 14,000 adolescents aged 15—19 years immediately pre-vaccination was 5.6% for serogroup B, 1.7% for serogroup Y, and 0.98% for serogroup C, using PCR methodology to assign serogroup. Two years later, carriage of serogroup C had dropped to 0.5% (and 0.09% by seroagglutination). Our data confirm that vaccination has been effective in maintaining very low carriage of serogroup C. This is likely to be due to a combination of persistent immunity among previously immunised individuals and herd immunity.[21] From knowledge of UK MenC vaccine coverage it is expected that 85% of participants would have received a single dose of MenC vaccine in 1999/2000.[22]

The high prevalence of MenY is in keeping with recent literature, which has shown increasing MenY carriage over the last decade. Two studies in a UK university, 10 years apart, demonstrated a significant rise in MenY carriage in incoming students from 1.7% in 1997 to 2.9% in 2009.[8,23] Carriage in the equivalent age cohort (17—18 years) in our study was 4.5% by isolate PCR. This may reflect a further rise in MenY, or geographical variation; the latter should be countered by our study's multisite design. Over the same decade, MenY invasive disease has more than doubled in the UK,[2] although provisional data from 2013 suggests that it still accounts for only 10.4% of cases (14% in 15—19 year olds).[24] The peak incidence of MenY disease in the UK is among those over 65 years of age, in contrast to other serogroups, although a lesser peak in 15—19 year olds has been observed.[25] Of interest, the USA saw a dramatic rise in the proportion of invasive disease caused by serogroup Y in the 1990s, from 2% to the current rate of approximately 30%.[26] There is evidence that the proportion of cases due to MenY is also rising in other European countries,[27] although in the limited contemporary carriage data from across Europe, MenB is generally dominant.[28]

The distribution of meningococcal carriage across the age cohorts that were sampled shows a gradual rise in later adolescence, and accords with findings from a large meta-analysis of studies.[7] Any immunisation campaign designed to reduce pharyngeal carriage of MenB (or MenY), and thereby induce herd immunity, should aim to immunise prior to this rise, i.e. in the early teenage years or before, in order to prevent acquisitions and interrupt transmission.

There are limited data on whether new MenB vaccines, based on non-capsular antigens, reduce carriage,[29] yet the effectiveness and cost-benefit of a MenB vaccination programme may depend to a significant degree on this factor.[30] A trial that evaluated whether 4CMenB had an impact on carriage suggested its effect may be modest.[18] The reasons for this are uncertain, but vaccine trial participants mixed with an unvaccinated population and therefore the effect on carriage might be expected to be smaller than in the setting of an immunisation programme. In addition, the trial did not report how well the vaccine antigens matched the strains of meningococcus circulating in that population. It is not yet clear which subcapsular antigens might be

Figure 2 Prevalence estimates of meningococcal carriage by age cohort (at enrolment), of selected serogroups. **A.** Prevalence of MenB carriage as detected by 3 laboratory methods: seroagglutination of cultured isolates, RT-PCR of cultured isolates ('isolate PCR') and RT-PCR of swab transport medium ('direct swab PCR'). **B** Prevalence of MenC, **C** MenY, and **D** non-groupable[a] *N. meningidis* carriage and **E** carriage of any meningococcus as detected by seroagglutination, and by isolate PCR. Error bars indicate 95% confidence intervals.
[a] For the isolate PCR method, non-groupable was defined as a positive porA or ctrA result, but negative RT-PCR for all group-specific assays (ie A, B, C, E, X, W, Y and Z).

Table 2 Discrepancies between assignment of serogroup according to seroagglutination and RT-PCR of cultured isolates, for all swab cultures that were positive by either method.

Isolate PCR result	Seroagglutination result									
	A	B	C	D	E	W	X	Y	Z	Non-groupable
A	**0**	0	0	0	0	0	0	0	0	0
B	4	**44**	5	3	0	1	0	7	1	30
C	0	0	**0**	0	0	0	0	0	0	3
D[a]	—	—	—	—	—	—	—	—	—	—
E	1	0	5	0	**17**	1	1	4	1	31
W	0	0	1	1	1	**9**	0	1	0	7
X	0	1	2	0	0	0	**1**	1	0	5
Y	1	3	1	1	1	2	0	**61**	0	30
Z	0	0	0	1	1	0	1	0	**1**	10
Non-groupable[b]	1	1	12	2	2	3	2	6	1	**46**
No growth[c]	0	4	0	0	0	0	0	0	0	5
Negative[d]	0	1	1	0	0	0	0	0	0	2

Numbers in bold were concordant between the two assays.
[a] Serogroup D was only tested for by seroagglutination, not by isolate PCR.
[b] For the isolate PCR method, non-groupable was defined as a positive *porA* or *ctrA* result, but negative RT-PCR for all group-specific assays (ie groups A, B, C, E, X, W, Y and Z).
[c] No growth at central laboratory.
[d] Isolate negative for *porA* and *ctrA* at central laboratory i.e. not confirmed as *N meningitidis*.

responsible for any effect on pharyngeal carriage of MenB vaccines, but the proportion of strains potentially 'covered' would depend on the antigenic types expressed by carriage strains, and their similarity to vaccine antigens.[31]

Since fHBP is included in both 4CMenB (subfamily B type) and bivalent rLP2086 (both subfamilies A and B), we determined the fHBP variants of isolates to add to the available information on circulating strains of MenB in England.[16,32,33]

Table 3 Factor H binding protein variants among all serogroup B and non-serogroup B meningococcal carriage isolates that had successful sequencing of the fHBP gene, over entire study period.

Number of MenB isolates	Oxford	Southampton	London	Bristol	Total
	24	14	19	35	92
Subfamily A	23 (95.8%)	11 (78.6%)	14 (73.7%)	34 (97.1%)	82 (89.1%)
A05	4 (16.7%)	6 (42.9%)	0	15 (42.9%)	25 (27.2%)
A22	14 (58.3%)	2 (14.3%)	5 (26.3%)	4 (11.4%)	25 (27.2%)
A12	4 (16.7%)	2 (14.3%)	0	8 (22.9%)	14 (15.2%)
A19	0	0	4 (21.1%)	4 (11.4%)	8 (8.7%)
Subfamily B	1 (4.2%)	3 (21.4%)	5 (26.3%)	0	9 (9.8%)
B24	0	0	3 (15.8%)	0	3 (3.3%)
B03	0	2 (14.3%)	0	0	2 (2.2%)
B204	0	0	2 (10.5%)	0	2 (2.2%)
B98	1 (4.2%)	0	0	0	1 (1.1%)
Number of non-B isolates	86	47	40	107	280
Subfamily A	60 (69.8%)	26 (55.3%)	20 (50.0%)	65 (60.7%)	171 (61.1%)
A15	31 (36.0%)	10 (21.3%)	7 (17.5%)	30 (28.0%)	78 (27.9%)
A73	3 (3.5%)	5 (10.6%)	6 (15%)	8 (7.5%)	22 (7.9%)
A103	6 (7.0%)	5 (10.6%)	3 (7.5%)	3 (2.8%)	17 (6.1%)
A19	3 (3.5%)	2 (4.3%)	1 (2.5%)	9 (8.4%)	15 (5.4%)
Subfamily B	23 (26.7%)	17 (36.2%)	20 (50.0%)	39 (36/4%)	99 (35.4%)
B09	13 (15.1%)	14 (29.8%)	10 (25%)	27 (25.2%)	64 (22.9%)
B16	7 (8.1%)	2 (4.3%)	4 (10%)	4 (3.7%)	17 (6.1%)
B03	2 (2.3%)	1 (2.1%)	1 (2.5%)	1 (0.9%)	5 (1.8%)
B06	0	0	2 (5.0%)	2 (1.9%)	4 (1.4%)

The 4 commonest variants from subfamily A and subfamily B are listed, with the corresponding number (and proportion, in brackets) of isolates accounted for at each study site.

Our MenB carriage isolates contained fHBP variants from subfamily A much more frequently than subfamily B (89.1% vs. 9.8% of isolates). The relative proportion of carriage isolates expressing variants from each subfamily has varied in the literature, and indeed varied between the four geographic sites in the current study. Isolates from cases of invasive disease overall carry fHBP subfamily B variants more frequently,[16,34] although in infants subfamily A strains appear relatively more important.[32] A vaccine using only subfamily B may have less impact on carriage of these strains.

Once vaccines are in use, further studies will be required to measure their impact on carriage, and our comparisons between laboratory methods have implications for study design. It is not surprising that we found seroagglutination, which was originally developed for typing invasive isolates, to be less useful for studies of the carriage state where isolates may express capsule weakly or be unencapsulated strains of low pathogenicity,[12] although it does allow comparison with historical data. Many 'non-groupable' isolates by seroagglutination (Fig. 2D) were successfully grouped by real-time PCR, indicative of low capsule expression. In addition, seroagglutination is subjective, and use of whole cells in the manufacture of commercial sera may result in nonspecific reactivity. During the course of this study, the inaccuracy of serogroup detection by agglutination became apparent (Table 2), and we suggest that molecular methods are preferred for grouping isolates from carriage studies.

We explored the use of PCR analysis of swab transport medium (direct swab PCR), as it would be logistically attractive to remove the 'bedside' inoculation and culture stages of carriage detection that are required for a fastidious bacterium. However, compared to isolate PCR, direct swab PCR had a sensitivity of 0.66 and would lead to substantial underestimation of carriage. Although some difference could be accounted for by sampling error, this is more likely to reflect enrichment of genetic material during growth.

This is the first study to have examined meningococcal throat carriage longitudinally across school age students in the UK. Carriage prevalence reflects both acquisition and duration of carriage, variables which are difficult to ascertain but important for modelling. Our study found a MenB acquisition rate of 2.8/1000 person-months. A single negative swab however can result from sampling error or failure of laboratory detection methods, so this may be an overestimation. Acquisition is very likely to be age-related, but the small number of MenB acquisitions in the study meant data had to be pooled across age groups. Approximately half of carriers had MenB detected at two or more consecutive visits in the 6 month study period, suggesting that carriage usually persists for several months (although we did not investigate whether these were the same or different strains). This is in keeping with a study of a university cohort[10] which found 45% of carriers (33 individuals) were persistent carriers over a 5–6 month period. Other studies have suggested carriage duration may be much longer, for example over 20 months when a statistical model was used to take into account the limited sensitivity of pharyngeal swabs, which may be only 60–80%.[35] Increasing the number of swabbing visits would be one way of improving the accuracy of data on carriage acquisition and duration, but in secondary school age children this is not very feasible. We experienced fairly high non-attendance for swabbing visits (Fig. 1), likely related to dislike of the procedure and pressure of school work and exams.

In conclusion, this study provides valuable current data on meningococcal carriage in UK adolescents, demonstrating a dominance of MenB and MenY, and low carriage of MenC. Carriage rates rise gradually throughout adolescence, suggesting that an immunisation programme designed to reduce acquisition of MenB or MenY would be best placed in the early teenage years or before. Studies assessing vaccine impact face the challenges and limitations of carriage detection methodologies, and we suggest PCR of cultured isolates is the preferred method for carriage detection at present.

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Conflict of interest statement

AJP, AF, MDS, PTH, SNF and SCC act as chief, principal or co-investigators for clinical trials conducted on behalf of their respective NHS Trusts and/or Universities, sponsored by vaccine manufacturers including Pfizer but receive no personal payments from them. AF, MDS, PTH, SNF and SCC have participated in advisory boards for vaccine manufacturers, but receive no personal payments for this work. MDS, SCC and SNF have received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS Trusts or Universities, or to independent charities. AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunisation (JCVI) but the views expressed in this manuscript do not necessarily reflect the views of DH or JCVI. NREK, ASA, HJ, KUJ, JE and LP are employees of Pfizer.

Previous presentation of the data

Part of this data was presented at the International Pathogenic Neisseria Conference 2012, Würzburg, Germany; poster P120.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2015.02.006

References

1. Gray SJ, Trotter CL, Ramsay ME, Guiver M, Fox AJ, Borrow R, 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**(Pt 7):887—96.
2. Ladhani SN, Flood JS, Ramsay ME, Campbell H, Gray SJ, Kaczmarski EB, et al. Invasive meningococcal disease in England and Wales: implications for the introduction of new vaccines. *Vaccine* 2012;**30**(24):3710—6.
3. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 2009;**27**(Suppl. 2):B51—63.
4. Public Health England. Invasive meningococcal infections (England and Wales), annual report for 2011/12. www.hpa.org.uk/hpr/archives/2013/hpr18-2213.pdf [cited 2013 24 September].
5. European Centre for Disease Prevention and Control. *Annual Epidemiological Report 2012. Reporting on 2010 surveillance data and 2011 epidemic intelligence data*. Stockholm: ECDC; 2013. p. 168—71.
6. Centers for Disease Control and Prevention. Summary of Notifiable diseases - 2010. *MMWR* 2012;**59**(53):43.
7. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**(12):853—61.
8. Neal KR, Nguyen-Van-Tam JS, Jeffrey N, Slack RC, Madeley RJ, Ait-Tahar K, et al. Changing carriage rate of Neisseria meningitidis among university students during the first week of term: cross sectional study. *BMJ* 2000;**320**(7238):846—9.
9. MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC, Roberts R, et al. Social behavior and meningococcal carriage in British teenagers. *Emerg Infect Dis* 2006;**12**(6):950—7.
10. Bidmos FA, Neal KR, Oldfield NJ, Turner DP, Ala'Aldeen DA, Bayliss CD. Persistence, replacement, and rapid clonal expansion of meningococcal carriage isolates in a 2008 university student cohort. *J Clin Microbiol* 2011;**49**(2):506—12.
11. Yazdankhah SP, Caugant DA. Neisseria meningitidis: an overview of the carriage state. *J Med Microbiol* 2004;**53**(Pt 9): 821—32.
12. Maiden MC, Ibarz-Pavón AB, Urwin R, Gray SJ, Andrews NJ, Clarke SC, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 2008;**197**(5):737—43.
13. ICH document Q2A. Guidance for industry:validation of analytical procedures. www.fda.gov/downloads/Drugs/Guidances/ucm073381.pdf; 1995.
14. ICH document Q2B. Guidance for industry: validation of analytical procedures. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073384.pdf; 1996.
15. FDA document. Guidance for industry: bioanalytical method validation. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf; 2001.
16. Murphy E, Andrew L, Lee KL, Dilts DA, Nunez L, Fink PS, et al. Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B Neisseria meningitidis. *J Infect Dis* 2009;**200**(3):379—89.
17. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934;**26**: 404—13.
18. Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. *Lancet* 2014;**384**(9960):2123—31.
19. Maiden MC, Stuart JM. UK Meningococcal Carriage Group. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet* 2002; **359**(9320):1829—31.
20. Ibarz-Pavón AB, Maclennan J, Andrews NJ, Gray SJ, Urwin R, Clarke SC, et al. Changes in serogroup and genotype prevalence among carried meningococci in the United Kingdom during vaccine implementation. *J Infect Dis* 2011;**204**(7): 1046—53.
21. Pollard AJ, Green C, Sadarangani M, Snape MD. Adolescents need a booster of serogroup C meningococcal vaccine to protect them and maintain population control of the disease. *Arch Dis Child* 2013;**98**(4):248—51.
22. 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**(Suppl. 1):S58—67.
23. Ala'aldeen DA, Oldfield NJ, Bidmos FA, Abouseada NM, Ahmed NW, Turner DP, et al. Carriage of meningococci by university students, United Kingdom. *Emerg Infect Dis* 2011;**17**(9): 1762—3.
24. Public Health England. Invasive menigococcal disease by calendar year, age group and capsular group. England & Wales, 1998 to 2013* (*provisional). Available at: www.gov.uk/government/publications/meningococcal-disease-laboratory-confirmed-cases-in-england-and-wales [4 June 2014].
25. Ladhani SN, Lucidarme J, Newbold LS, Gray SJ, Carr AD, Findlow J, et al. Invasive meningococcal capsular group Y disease, England and Wales, 2007-2009. *Emerg Infect Dis* 2012; **18**(1):63—70.
26. Harrison LH. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis* 2010;**50**(Suppl. 2):S37—44.
27. Bröker M, Jacobsson S, Kuusi M, Pace D, Simões MJ, Skoczynska A, et al. Meningococcal serogroup Y emergence in Europe: update 2011. *Hum Vaccin Immunother* 2012;**8**(12): 1907—11.
28. Soriano-Gabarró M, Wolter J, Hogea C, Vyse A. Carriage of Neisseria meningitidis in Europe: a review of studies undertaken in the region. *Expert Rev Anti Infect Ther* 2011;**9**(9): 761—74.
29. Dellicour S, Greenwood B. Systematic review: Impact of meningococcal vaccination on pharyngeal carriage of meningococci. *Trop Med Int Health* 2007;**12**(12):1409—21.
30. Christensen H, Hickman M, Edmunds WJ, Trotter CL. Introducing vaccination against serogroup B meningococcal disease: an economic and mathematical modelling study of potential impact. *Vaccine* 2013;**31**(23):2638—46.
31. Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, et al. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. *Lancet Infect Dis* 2013;**13**(5):416—25.
32. Hoiseth SK, Murphy E, Andrew L, Vogel U, Frosch M, Hellenbrand W, et al. A multi-country evaluation of Neisseria meningitidis serogroup B factor H-binding proteins and implications for vaccine coverage in different age groups. *Pediatr Infect Dis J* 2013;**32**(10):1096—101.
33. Ala'aldeen DA, Flint M, Oldfield NJ, Omer SA, McNeil LK, Jiang Q, et al. Human antibody responses to the meningococcal factor H binding protein (LP2086) during invasive disease, colonization and carriage. *Vaccine* 2010;**28**(48):7667—75.
34. Anderson AS, Hao L, Jiang Q, Harris SL, Jones TR, Perez JL, et al. Potential impact of the bivalent rLP2806 vaccine on Neisseria meningitidis carriage and invasive serogroup B. *Dis Hum Vaccin Immunother* 2012;**9**(3).
35. Trotter CL, Gay NJ. Analysis of longitudinal bacterial carriage studies accounting for sensitivity of swabbing: an application to Neisseria meningitidis. *Epidemiol Infect* 2003;**130**(2): 201—5.