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Original article

Meningococcal carriage in Dutch adolescents and young adults; a cross-sectional and longitudinal cohort study

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ABSTRACT

Objectives: Current information on rates and dynamics of meningococcal carriage is essential for public health policy. This study aimed to determine meningococcal carriage prevalence, its risk factors and duration in the Netherlands, where meningococcal C vaccine coverage is >90%. Several methods to identify serogroups of meningococcal carriage isolates among adolescent and young adults were compared.
Methods: Oropharyngeal swabs were collected from 1715 participants 13–23 years of age in 2013–2014; 300 were prospectively followed over 8 months. Cultured isolates were characterized by Ouchterlony, real-time (rt-) PCR or whole-genome sequencing (WGS). Direct swabs were assessed by rt-PCR. Questionnaires on environmental factors and behaviour were also obtained.
Results: A meningococcal isolate was identified in 270/1715 (16%) participants by culture. Of MenB isolates identified by whole genome sequencing, 37/72 (51%) were correctly serogrouped by Ouchterlony, 46/51 (90%) by rt-PCR of cultured isolates, and 39/51 (76%) by rt-PCR directly on swabs. A sharp increase in carriage was observed before the age of 15 years. The age-related association disappeared after correction for smoking, level of education, frequent attendance to crowded social venues, kissing in the previous week and alcohol consumption. Three participants carried the same strain identified at three consecutive visits in an 8-month period. In these isolates, progressively acquired mutations were observed.
Conclusions: Whole genome sequencing of culture isolates was the most sensitive method for serogroup identification. Based upon results of this study and risk of meningococcal disease, an adolescent meningococcal vaccination might include children before the age of 15 years to confer individual protection and potentially to establish herd protection. **M.B. van Ravenhorst, Clin Microbiol Infect 2017;23:573.e1–573.e7**

Introduction

Neisseria meningitidis is a common colonizer of the upper respiratory tract in asymptomatic carriers, but may occasionally cause invasive meningococcal disease (IMD) [1]. Of the 12 *N. meningitidis* serogroups, six (A, B, C, X, W and Y) cause the majority of IMD worldwide. Whereas the overall carriage prevalence in the population is estimated to be ~10%, the incidence rate of IMD per 100 000 population varies between 0.5 in North America up to 1000 in epidemic settings [2,3]. Although IMD incidence is highest in infants under 5 years of age, meningococcal carriage prevalence is low at this early age. Carriage increases during childhood and peaks at around 24% among young adults and declines to around 8% in older adults [4].

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Experience with serogroup C (MenC) polysaccharide conjugate vaccines demonstrated herd protection through reduced colonization and transmission of MenC after vaccination of children and adolescents [2,5]. In the Netherlands, a single MenC conjugate vaccine was offered to all children aged 1–18 years in 2002. Routine vaccination at 14 months was subsequently introduced [6]. The vaccine coverage of the population was estimated at 94% in 2002 [7]. At present, meningococcal serogroup B (MenB) is the predominant cause of IMD in Europe [8,9]. Only recently, MenB vaccines have been made available through the licensure of two protein-based multicomponent vaccines: bivalent rLP2086, Trumenba® (Pfizer, Philadelphia, PA, USA) and 4CMenB, Bexsero® (GlaxoSmithKline Biologicals SA, Rixensart, Belgium) [10,11]. A recent study among university students showed a reduction in overall carriage after a second dose of 4CMenB, but no impact was demonstrated for MenB carriage [12]. Currently, European carriage data are mostly based on studies from the UK and France [13]; however, carriage characteristics change over time and may differ by country.

In this study, we compared different methods to identify meningococcal serogroups by culture and Ouchterlony, real-time (rt-) PCR or whole-genome sequencing (WGS) of meningococcal carriage isolates among Dutch adolescents and young adults. Furthermore, we determined the prevalence of meningococcal carriage, its risk factors and duration.

Materials and methods

Design and participants

The study was conducted in the Netherlands between January 2013 and March 2014. Healthy participants aged 13–23 years were recruited randomly and solely based on age from 15 educational institutions. Exclusion criteria included previous MenB vaccination, antibiotic use during the month before enrolment, or participation in any other clinical trial with an investigational drug. Written informed consent was obtained from both parents/guardians of participants aged <18 years and from all participants. Among all participants, all participants in the last year of secondary school who were included in the year 2013 were enrolled in the longitudinal study cohort and had study visits three times within 8 months after enrolment, twice before and once after starting tertiary school or universities. The study was conducted in compliance with ethical principles originating from the Helsinki Declaration, within the guidelines of Good Clinical Practice and the study was registered at the Dutch Trial register (www.trialregister.nl; NTR3785).

Clinical procedures

Trained research nurses visited schools and universities to collect swabs and demographic data, and provided questionnaires on environmental factors and behaviour based on previously identified risk factors [14–16]. Two oropharyngeal swabs, each of the tonsils/the tonsillar fossa and the posterior pharynx, were simultaneously collected to compare different methods for identification of meningococcal serogroups. One was inoculated on a Thayer–Martin agar and incubated at 37°C with 5% CO_2 immediately after swabbing and the second ('Direct swab', which was only collected at baseline from participants who were included in the year 2013) was placed directly into storage transport media (STM, DIGENE, Biomérieux, Marcy l'Etoile, France), and was kept at 4°C on study location. Within 5 h of collection, the samples arrived at the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM, Amsterdam, the Netherlands) for microbiological processing.

Bacterial identification

Meningococcal isolates were isolated at the NRLBM as previously described [17]. Up to a maximum of five colonies of isolates identified as *N. meningitidis* were frozen in Microbank tubes and stored at −80°C. Direct swabs were also stored at this temperature. Direct swabs and frozen colonies were shipped on dry ice to Pfizer Vaccine Research and Development for further identification by rt-PCR (both swabs) and WGS (cultured swabs only) (Pearl River, NY, USA).

Phenotyping cultured isolates. Serogrouping of cultured isolates was performed by means of microprecipitation in a modified Ouchterlony assay for serogroups A, B, C, E, W, X, Y and Z as previously described [18].

Real-time PCR. This was performed on cultured isolates in the subset of participants from whom two swabs were collected as previously described [19]. Isolates negative for group-specific assays but positive for *por*A or *ctr*A were defined as non-groupable meningococcal isolates (NG). Direct rt-PCR assays were performed on swab transport medium (without culture) of the second swab to detect *por*A, *ctr*A and genetic targets diagnostic for MenB [19].

WGS of cultured isolates and sequence analysis. All meningococcal isolates were analysed by WGS as previously described [20], and genogroup was identified using capsule locus genes [21]. Isolates without the capsule locus but positive for *por*A were identified as NG meningococcal isolates. Phylogenomic analysis using core genome sequences was conducted in Harvest Suite [22]. Genomes of carriage isolates were compared and maximum likelihood phylogenetic trees were built through Parsnp, a fast core-genome multi-aligner. Then the alignment and trees were visualized in Gingr and iTOL [23].

Statistical analyses

This study was a descriptive epidemiological *N. meningitidis* carriage study. On the basis of confidence interval (CI) estimates, an overall sample size of 1700 participants was chosen. Using the Clopper–Pearson exact method, a conservative estimate for MenB carriage prevalence of 2% would result in a 95% CI between 1.4% and 2.8% within a sample size of 1700 individuals [24]. Continuous variables were presented as means with standard deviation (SD), and categorical variables as numbers and percentages. Differences in proportion were tested with the Fisher's exact test. Statistical tests were two-sided and a p-value <0.05 was considered statistically significant. Data were analysed using SPSS statistics 22 (IBM, Armonk, NY, USA). Serogroup-specific meningococcal carriage was defined by WGS of cultured isolates. For laboratory methods comparison analysis, WGS of cultured isolates was used as reference. Possible risk factors for meningococcal carriage were determined using results of the first visit based on previous publications [14,15,25]. The association of age and carriage was evaluated with logistic regression analysis. We estimated the univariate association of age with carriage as well as an estimate corrected for all identified confounders. A confounder was defined as a variable that had a significant association with both age and carriage, and that altered the correlation coefficient of age by at least 10% if added to the model. A multivariate logistic regression model that included all evaluated risk factors for carriage simultaneously was also developed. Model stability was evaluated by performing stepwise backward model selection based on likelihood ratio testing with a p-in of 0.05 and p-out of 0.1. Acquisition rate was defined by the number of new carriers for the 8-month study period divided by

the total person-time of all initially negative participants. Acquisition was assumed to have occurred at the time-point halfway between assessments.

Results

Participants' characteristics

At baseline, 1727 participants were assessed for eligibility and 1715 (99%) were enrolled (Fig. 1). Median age at inclusion was 16.9 years (±SD 2.0) and 1056/1727 (62%) were female. Of all participants, 1310/1715 (76%) were recruited from secondary school and 405/1715 (24%) from tertiary school/University. The numbers of enrolled participants were 906/1715 (53%) and 809/1715 (47%) for the years 2013 and 2014, respectively. At the first visit, a meningococcal isolate was identified by culture of oropharyngeal swabs in 270 (16%) of 1715 participants.

Characterization of meningococcal serogroups of cultured isolates at the first visit

Results of WGS were available for 267/270 (99%) cultured isolates (Table 1). The most prevalent serogroups identified by WGS were MenB, with 72/267 (27%) isolates, followed by MenX (38/267; 14%) and MenY (34/267; 13%). Of isolates identified by WGS, 82/267(31%) isolates did not harbour the capsule locus in the genome sequence, and were therefore identified as capsule null.

Ouchterlony was available for 268/270 (99%) cultured isolates (Table 1). MenB was the most common serogroup identified by the modified Ouchterlony with 37/268 (14%) isolates, followed by MenY (20/268; 7%) and MenE (17/268; 6%). Of isolates determined by Ouchterlony, 180/268 (67%) isolates were identified as Non-Groupable (NG).

Real-time PCR was performed on a subset of 177/270 (66%) cultured isolates (Table 2). Of these cultured isolates, 48/177 (27%), 19/177 (10%) and 16/177 (9%) were identified by rt-PCR as MenB, MenX and MenE, respectively. Identified by rt-PCR, 25/177 (14%) isolates were identified as NG (i.e. negative for all eight capsular types). The rt-PCR was positive for more than one group-specific assay in 37/177 (21%) isolates and a serogroup was therefore not assigned.

MenB identified by direct rt-PCR at the first visit

A direct swab for rt-PCR was collected per protocol from 906/1715 (53%) participants. Direct rt-PCR targeting MenB was available for 902/906 (99.5%) swabs. MenB was detected by direct rt-PCR in 49/902 (5%) swabs.

Comparison of conventional diagnostic methods for detecting carriage serogroup

Of isolates with a MenB capsule locus identified by WGS of cultured isolates, 37/72 (51%) were correctly serogrouped by Ouchterlony, and 34/72 (47%) were NG (Table 1). Of isolates with genogroup X or Y, Ouchterlony resulted in the corresponding phenotype in 3/38 (8%) and 13/34 (59%), respectively. Of isolates identified by WGS of cultured isolates, only 5/267 (2%) harboured the MenC polysaccharide capsule gene; none of these were detected by Ouchterlony.

Of cultured isolates assessed by rt-PCR, 144/177 (64%) were classified in accordance with the WGS assignment (Table 2). Though both WGS and rt-PCR of cultured isolates identified a MenB carriage prevalence of 27%, the detection of MenB by the two methods showed discrepancies, e.g. MenB assignments by rt-PCR that were not confirmed by WGS and vice-versa (see Supplementary material, Fig. S1). The sensitivity of rt-PCR to identify MenB among cultured isolates was 90% compared with WGS as reference. Of isolates that did not harbour the capsule locus identified by WGS, 25/52 (48%) were NG by rt-PCR (Table 2). In one isolate rt-PCR assigned a serogroup (MenW) that did not correspond with the WGS result (MenX).

Direct rt-PCR had a sensitivity of 76% (39/51) when compared with WGS of cultured isolates derived from the partner swab (see Supplementary material, Fig. S1). Of isolates identified by WGS, 12/51 (24%) MenB isolates were not confirmed by direct rt-PCR. In addition, direct rt-PCR was positive for MenB in ten additional samples that were not confirmed by WGS of cultured isolates. Of these samples, 9/10 (90%) samples were negative for meningococci by culture and 1/10 (10%) isolates lacked the capsule locus by WGS.

Age, carriage and risk factors for carriage at the first visit

The relationship between age and carriage was non-linear (Fig. 2a, and see Supplementary material, Table S1). Overall, *N. meningitidis* carriage increased rapidly in early adolescence, from 15/318 (4.7%) 13–14 years of age to 113/501 (22.6%) at 17–18 years (OR 5.88, 95% CI 3.66–10.29, p <0.001). MenB carriage prevalence was highest in the 21–23-year age cohort; 5.3%–7.9% depending on the diagnostic test (Fig. 2b). Smoking, level of education, the average estimated number of times per week going to crowded social venues, kissing in the last week and drinking alcohol were

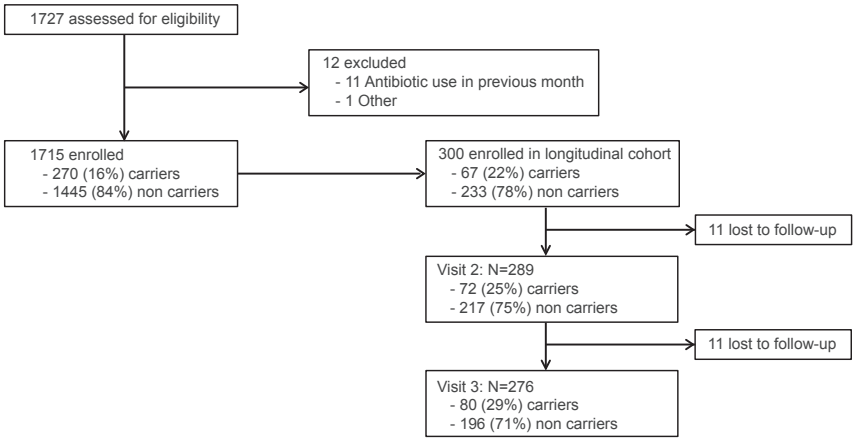

Fig. 1. Flow-chart for inclusion and follow-up. Carriers were identified by culture of oropharyngeal swabs. Note. Oropharyngeal swabs and questionnaires of participants in the longitudinal study cohort were collected at enrolment and 3 months and 8 months after enrolment.

Table 1
Serogroup distribution of isolates detected by whole-genome sequencing and Ouchterlony of cultured isolates at the first visit ($n = 270$)

Ouchterlony, *n*	Isolate WGS, *n*										Total
	B	C	E	W	X	Y	Z	Capsule null	Negative	ND	
B	**37**	0	0	0	0	0	0	0	0	0	37
C	0	**0**	0	0	0	0	0	0	0	0	0
E	0	0	**16**	0	0	0	0	1	0	0	17
W	0	0	0	**8**	0	1	0	0	0	0	9
X	0	0	0	0	**3**	0	0	0	0	0	3
Y	0	0	0	0	0	**20**	0	0	0	0	20
Z	0	0	0	0	0	0	**2**	0	0	0	2
NG	34	5	3	4	35	13	2	**80**	1	3	180
ND	1	0	0	0	0	0	0	1	0	0	2
Total	72	5	19	12	38	34	4	82	1	3	270

Abbreviations: n = number of isolates; ND, not done; NG, non-groupable; Negative, WGS negative for *porA* and *ctrA*; WGS, whole genome sequencing.
Numbers in bold were concordant between the two assays.

Table 2
Serogroup distribution of isolates according to isolate real-time PCR and whole genome sequencing of cultured isolates at the first visit ($n = 177$)

Isolate rt-PCR[a]	Isolate WGS									Total
	B	C	E	W	X	Y	Z	Capsule null	ND	
B	**46**	0	0	0	0	0	0	2	0	48
C	0	**2**	0	0	0	0	0	1	0	3
E	0	0	**10**	0	0	0	0	6	0	16
W	0	0	0	**10**	1	0	0	2	0	13
X	0	0	0	0	**10**	0	0	7	2	19
Y	0	0	0	0	0	**7**	0	2	1	10
Z	0	0	0	0	0	0	**4**	2	0	6
NG[b]	0	0	0	0	0	0	0	**25**	0	25
Unassigned[c]	5	1	2	1	14	9	0	5	0	37
Total	51	3	12	11	25	16	4	52	3	177

Abbreviations: *n*, number of isolates; ND, not done; NG, non-groupable; WGS, whole genome sequencing.
Numbers in bold were concordant between the two assays.
[a] Real-time PCR was only performed on cultured isolates from a subset of participants of whom a second oropharyngeal swab was collected.
[b] Non-groupable was defined as positive porA and/or ctrA, but negative for group-specific assays.
[c] Real-time PCR positive for multiple group-specific assays.

identified as confounders of the association between age and meningococcal carriage. The significant association between age group and meningococcal carriage was lost when corrected for these confounders. After correcting for all other variables in the Supplementary material (Table S1), education level, the average estimated number of times per week going out, kissing in the past week, drinking alcohol and smoking 10–20 cigarettes per day were statistically independent predictors of carriage. Model stability, using backward stepwise model selection, resulted in the predictors of carriage.

Duration of carrier state and acquisition

Of all participants, 300/1715 (17%) in the last year of secondary school at baseline were included in the longitudinal cohort (Fig. 1). Median age at inclusion was 17.5 years (±SD 0.9) and 163/300 (54%) were female. Of the participants in the longitudinal cohort, 67/300 (22%) carriers were identified at enrolment by culture, and 72/289 (25%) and 80/276 (29%) after 3 months and 8 months, respectively. Of all participants who completed the whole study period, 158/276 (57%) did not carry a meningococcal isolate by culture at any of the three sampling visits (see Supplementary material, Fig. S2). A meningococcal isolate at all three sampling visits was identified in 21/276 (5%) participants. The same genogroup was identified by WGS at all three sampling visits in 12/21 (57%) participants. MenB (5/12; 42%) and MenW (3/12; 25%) were most frequently identified in the cases of persistent carriage.

The acquisition rate of colonization by meningococci irrespective of the serogroup was 45.4 per 1000 person-months during the whole study period. That of MenB was 9.4 per 1000 person-months.

Detailed analysis of carriage isolates

The WGS of 419 meningococcal carriage isolates collected during all three visits were compared to evaluate genetic diversity of isolates between individuals and isolate persistence within an individual (see Supplementary material, Fig. S3). Isolates from the same genogroup and clonal complex (CC) generally tended to cluster together but a large diversity of isolates that were not clustered by region or age group was observed. However, in several instances we identified isolates within a cluster that had a different genogroup compared with the neighbouring isolates in the cluster (see Supplementary material, Fig. S3). In addition, isolates without a capsule locus (NG) located in a serogroup-restricted cluster have been noted indicating loss of the capsule locus by deletion.

There were 5/276 (2%) cases of persistent carriage of MenB in the longitudinal cohort (Fig. 3a). Of these participants, 2/5 (40%) acquired a replacement strain during a follow-up visit or were colonized by a mixture of strains (participants D and E). The isolates from the other three cases of persistent MenB carriage were, in each case, nearest neighbours on the WGS phylogenetic tree (participants A, B and C; Fig. 3a) and identical with respect to genogroup, CC and other fine typing markers. Examination of the WGS data revealed a progressive accumulation of single nucleotide polymorphisms and/or recombination events across the genome over the 8-month surveillance period from each of these participants (Fig. 3b).

Discussion

Meningococcal disease originates from the oropharyngeal niche through contact with a carrier. In the present study to evaluate *N. meningitidis* carriage and acquisition we found that WGS of cultured isolates was able to provide a precise picture of both genogroup and isolate identity. Specifically, we were able to closely track isolates within the population and could assess that there was a large diversity of isolates that were not clustered by region or age.

The overall meningococcal carriage prevalence of 16% observed in this study was comparable to carriage rates reported for UK populations in this age cohort [4,13]. MenB was the most common serogroup identified by WGS with 72/267 (27%) cultured isolates followed by MenX (38/267; 14%) and MenY (34/267; 13%). MenB is

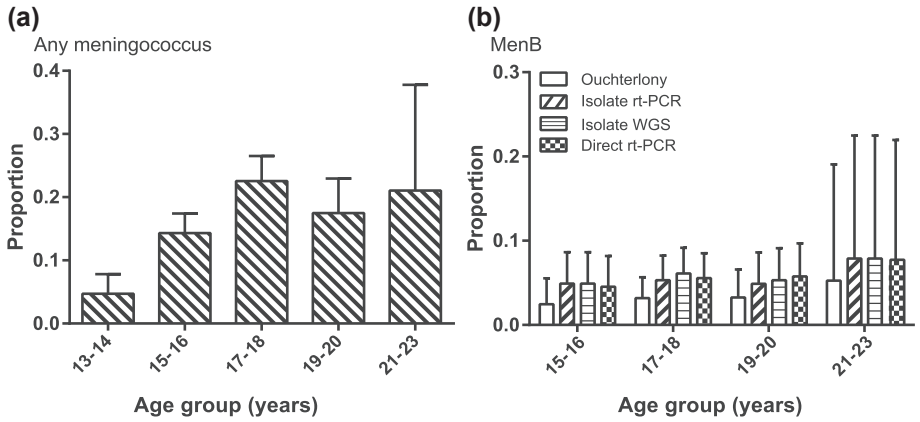

Fig. 2. Meningococcal carriage prevalence by age cohort at the first visit identified by culture of oropharyngeal swabs. (a) Overall meningococcal carriage identified by culture of oropharyngeal swabs from all 1715 participants at the first visit. (b) Meningococcal serogroup B carriage prevalence at the first visit identified by each detection method for a subset of participants of whom a second swab was collected; Ouchterlony, rt-PCR and WGS of cultured isolates and direct rt-PCR of the second swab. Note. Error bars indicate 95% CI.

also the most common isolate found in disease isolates [9]. MenY disease has traditionally been rare in Europe; however, the incidence has been rising in the Netherlands and at the time of the study it was the second most common serogroup causing disease in adolescents and young adults [26]. We identified six isolates with the MenC loci, but expression of capsule was not detected. The low prevalence may reflect high uptake and use of MenC conjugate vaccines in the Netherlands since 2002 [7]. However, low rates of MenC carriage were also detected in the UK before vaccination when MenC disease rates were as high as 0.2 per 10 000 population suggesting that MenC carriage might be transient and therefore difficult to detect [27,28].

It has been demonstrated that when conjugate vaccines targeting MenC and MenA were implemented on a large scale, the transmission of these meningococci was prevented, leading to herd protection [5,16]. To monitor the impact on carriage after vaccine implementation, accurate diagnostic approaches are required. We found that WGS was the most sensitive method for serogroup identification compared with Ouchterlony and rt-PCR. The discrepancies between the two techniques can be explained by the fact that rt-PCR interrogates a small portion of one gene essential for capsule synthesis; whereas, WGS provides a view of the entire *cps* operon. Direct rt-PCR has potential logistical advantages over traditional culture. However, direct-PCR was less sensitive to identify MenB compared with WGS, probably because of the lack of culture enrichment [29].

The impact of protein-based vaccines, such as the MenB vaccines, on meningococcal carriage and transmission needs to be established. Therefore, it is important to determine meningococcal carriage rates by age, specifically in which age group carriage is initially detected, so that participants can be vaccinated before the period of maximum risk for acquisition. In a small study with 4CMenB of young adults in the UK, impact on MenB carriage was not observed [12]. In the current study, we demonstrate a notable increase in carriage between the ages 13–14 and 15–16 years. For future studies evaluating the impact on carriage acquisition by vaccination, the age group of 13–14 years would be the preferred target group. The increase in carriage in our study was strongly linked to life style rather than age. Also observed in a UK study, it was found that social behaviour, rather than age or sex, was associated with higher frequency of meningococcal carriage among teenagers [14]. Since life style changes over time and between countries, information regarding the risk factors for carriage can aid in Public Health decisions about target ages for vaccination campaigns. In addition, it is important to understand carriage dynamics in the specific trial population before a vaccine interventional study is initiated.

In this evaluation, we demonstrated that WGS of cultured isolates provided data sufficient for genogroup assignment and isolate characterization. Clustering of the isolates by their genetic markers (e.g. CC) is useful to identify where capsule switching may have occurred within the study population. Capsule switching has relevance for disease outbreak surveillance as the virulent CC11 strain has been involved in capsule switching from capsule group C to group W through recombination of the entire cps cluster [30,31]. In this study, we have identified several isolates that appear to have capsule switching or capsule deletion events compared with closely related strains within the cluster, highlighting the genetic plasticity of the meningococcal bacteria and the ability of WGS to identify these types of genetic changes within a population. Longitudinal carriage studies are important for understanding the dynamics of transmission. Acquisition rate and duration of carriage are important variables for models to predict the epidemiological and economic impact of vaccination. One strength of our longitudinal study cohort is the high-attendance for sampling visits achieved in this particular age cohort. Our longitudinal cohort showed that carriage can persist for at least 8 months. A previous longitudinal carriage study observed a high turnover rate of meningococcal carriage among UK students and indicated that carriage of a particular meningococcal strain may not necessarily protect against colonization by homologous or heterologous strains [32]. WGS was also useful for describing carriage dynamics. We found long-term carriage of MenB and were able to identify whether the same strain was present at multiple visits or a new strain was acquired during the follow up. We found five cases of persistent carriage, of which three most likely carried the same strain retrieved in three consecutive visits over an 8-month period. Most interestingly, the isolates showed progressively acquired mutations or recombinations across the genome, indicating its potential relevance in long-term adaptation to the host. *Neisseria meningitidis* has long been recognized to have highly dynamic population structure due to frequent homologous recombination and horizontal gene transfer [33]. Yet very little is known about within-host evolution of *N. meningitidis*. Accumulation of mutations in the genome from these carriage isolates may potentially increase virulence. We believe that the present methodology allowed for the first time the investigation of meningococcal carriage with such high accuracy and this is highly relevant for better understanding of the dynamics of carriage and transmission within populations.

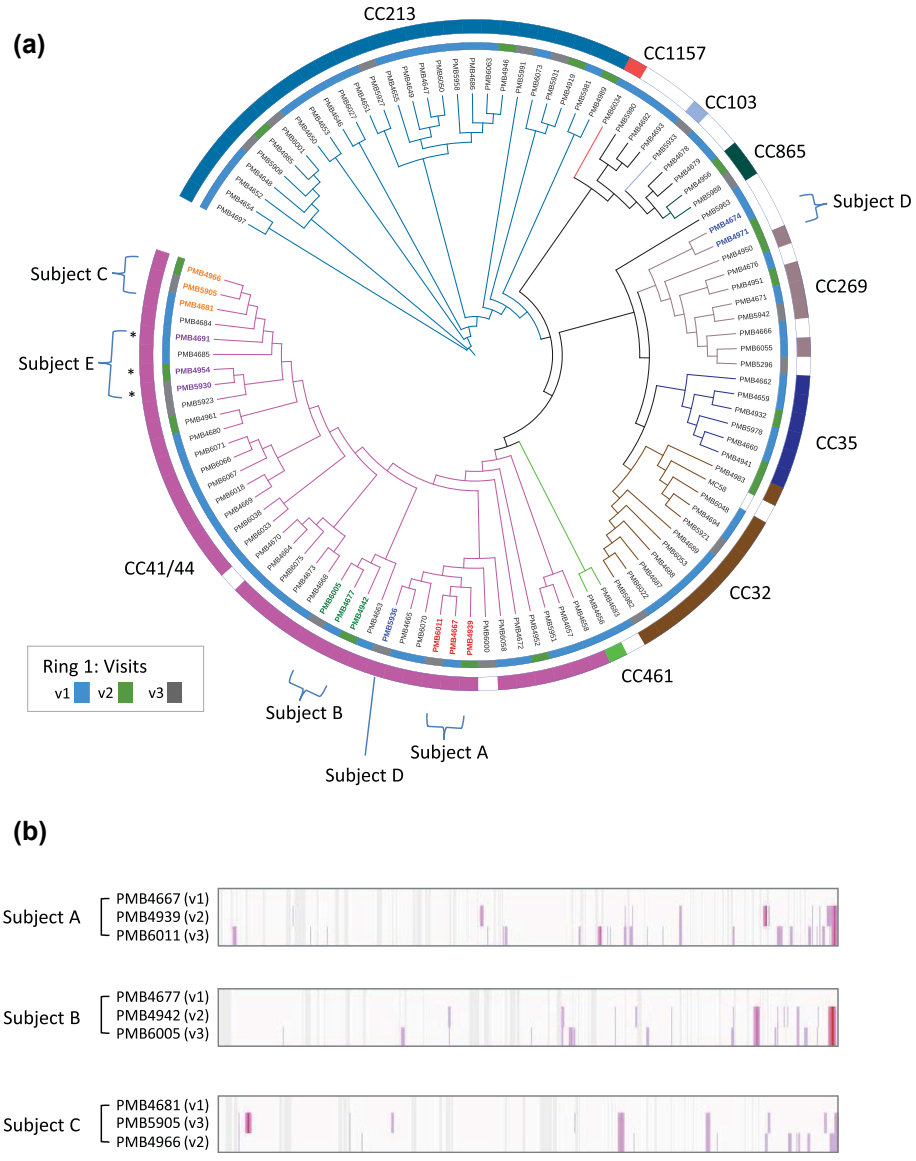

Fig. 3. Phylogeny of carriage isolates based on comparative genomic analysis. (a) Meningococcal serogroup B carriage ($n = 105$). (b) Distribution of single nucleotide polymorphisms (SNPs) in MenB isolates from three participants showing persistence of carriage across three longitudinal visits. The visits (ring 1) and clonal complexes (CCs) (ring 2) were annotated in colour strips along the circular tree. In ring 2, CCs were labelled accordingly, whereas white strip represents non-typeable (NT). Tree branches were coloured to match CCs (inferred from the majority isolates of the cluster). The isolate IDs of three persistent cases (carriage isolates collected from three longitudinal visits) were highlighted in different colours. Persistent carriage in three different participants is accompanied by a progressive accumulation of SNPs and/or recombination events. The WGS of isolates from visit 1 for each individual was used as a baseline or reference data set. Vertical light grey lines for each of the visit 1 WGS data sets represent sequences that are not part of the *N. meningitidis* core genome and are shared in WGS of carriage isolates from visits 2/3. In each case, WGS of isolates collected at visits 2 and 3 revealed an accumulation of SNPs and/or recombination events.

A limitation of the current study is that the vaccination history of the participants was not known. However, a single MenC-TT vaccination in 2002 can be assumed in all participants of the current study due to the high vaccine coverage of 94% during the catch-up campaign in 2002 [7].

In conclusion, data from the current study show that WGS of cultured isolates is recommended to monitor baseline *N. meningitidis* carriage and the impact on carriage after vaccine implementation. Results of this study can be used as a pre-implementation reference, showing that MenB, MenX and MenY were the most common serogroups identified by WGS of cultured isolates. Based upon the results of this study and the risk of IMD, adolescent meningococcal vaccination might include children before the age of 15 years because this is the age when increased carriage acquisition occurs related to behavioural changes. This study provides useful information for conducting a clinical trial to look at the effect of meningococcal vaccine (particularly MenB vaccine) on carriage.

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manuscript. This publication made use of the Neisseria Multi Locus Sequence Typing website (http://pubmlst.org/neisseria) developed by Keith Jolley and located at the University of Oxford [21]. The development of this database has been funded by the Wellcome Trust and European Union. Phenotyping of cultured isolates was performed at the NRLBM, Amsterdam, The Netherlands; rt-PCR was performed at PPD Vaccine and Biologics Laboratory, Wayne, PA, USA; WGS was performed at Pfizer Vaccine Research & Development.

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Transparency declaration

MBvR, MWB, VMDS and MAvH report no conflict of interest. AvdE declares to have received grants for pneumococcal epidemiology studies from Pfizer. EAMS declares to have received unrestricted research support from Pfizer, grant support for vaccine studies from Pfizer and GSK and fees paid to the institution for advisory boards or participation in independent data monitoring committees for Pfizer and GSK. ASA, JE, LH, KUJ, HJ, NK and LP were all employees of Pfizer Vaccine Research and Development at the time of the study implementation, conduct, and analysis and may hold stock in the company.

Note

Information of this manuscript was presented during the International Pathogenic Neisseria Conference 2016, Manchester, UK.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.02.008.

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