# The genomic epidemiology of *Neisseria meningitidis* carriage from a randomised controlled trial of 4CMenB vaccination in an asymptomatic adolescent population

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## Summary

**Background** Oropharyngeal carriage of *Neisseria meningitidis* is frequent during adolescence, representing a major source of invasive meningococcal disease. This study examined the impact of a serogroup B vaccination (*Bexsero*, GSK 4CMenB) programme on adolescent *N. meningitidis* carriage using genomic data.

**Methods** A total 34,489 oropharyngeal samples were collected as part of a state-wide cluster randomised-controlled trial in South Australia during 2017 and 2018 (NCT03089086). Samples were screened for the presence of *N. meningitidis* DNA by *porA* PCR prior to culture. Whole genome sequencing was performed on all 1772 *N. meningitidis* culture isolates and their genomes were analysed.

**Findings** Unencapsulated meningococci were predominant at baseline (36.3% of isolates), followed by MenB (31.0%), and MenY (20.5%). Most MenB were ST-6058 from hyperinvasive cc41/44, or ST-32 and ST-2870 from cc32. For MenY, ST-23 and ST-1655 from cc23 were prevalent. Meningococcal carriage was mostly unchanged due to the vaccination programme; however, a significant reduction in ST-53 capsule-null meningococci prevalence was observed in 2018 compared to 2017 (OR = 0.52; 95% CI: 0.30–0.87, p = 0.0106). This effect was larger in the vaccinated compared to the control group (OR = 0.37; 95% CI: 0.12–0.98, p = 0.0368).

**Interpretation** While deployment of the 4CMenB vaccination did not alter the carriage of hyperinvasive MenB in the vaccinated population, it altered the carriage of other *N. meningitidis* sequence types following the vaccination program. Our findings suggest 4CMenB vaccination is unlikely to reduce transmission of hyperinvasive *N. meningitidis* strains and therefore ongoing targeted vaccination is likely a more effective public health intervention.

**Funding** This work was funded by GlaxoSmithKline Biologicals SA.

*Keywords: Neisseria meningitidis*; Vaccination; Whole genome sequencing; Asymptomatic meningococcal carriage

## Introduction

*N. meningitidis* is commonly carried as part of the oropharyngeal microbiota and can cause fatal disseminated infections including meningitis and septicaemia. In Australia, incidence of invasive meningococcal disease (IMD) is highest in infants from age 0–4 years old, with a second peak in late adolescence and early adulthood.[1,2]

*N. meningitidis* is typically classified by serogroup (e.g. A, B, C, W, X and Y) using immunological analysis

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## Research in context

**Evidence before this study**

*Neisseria meningitidis* (*N. meningitidis*) is commonly carried asymptomatically in the oropharynx, especially by adolescents and young adults. This acts as a significant reservoir for new transmission events resulting in invasive meningococcal disease (IMD). Therefore, understanding how vaccination alters carriage of *N. meningitidis* has significance for vaccine implementation strategies and public health interventions. Before undertaking this study, we considered evidence from other studies regarding their effect on *N. meningitidis* carriage, these were found by using search terms such as "*N. meningitidis*" "*meningococcal*" "*carriage*" "*vaccine*" "*vaccination*" "*4CMenB*" in the Pubmed database. Only high quality, peer reviewed research articles, or registered clinical trials were included in our assessment of the evidence. Key evidence that preceded our study included a longitudinal analysis of a prior vaccination program conducted in Australia introduced in 2003 (MenCCV) targeting *N. meningitidis*. This data demonstrated that the introduction of the MenCCV vaccine reduced serogroup C (MenC) related IMD, resulting in serogroup B (MenB) becoming the dominant disease-causing serogroup (data summarised in Archer et al., 2017).

In response to ongoing disease incidence caused by MenB, the protein-based vaccine 4CMenB was developed. Rather than targeting the capsule this vaccine targets sub-capsular antigens (fHbp, NadA, and NHBA) and outer membrane vesicles containing PorA (reviewed in Serruto et al., 2012). Likewise, we considered evidence from previous phase 3 randomised controlled trial (randomised controlled trial) of meningococcal quadrivalent glycoconjugate (MenACWY-CRM) or 4CMenB vaccination conducted in England in 2010 on participants aged 18–24 years. This study utilised serogrouping and PCR-based detection of capsule type and demonstrated MenACWY-CRM specifically reduced carriage of vaccine-covered serogroups whereas 4CMenB had a broad effect, unrelated to serogroup or capsule type, on *N. meningitidis* carriage (data presented in Read et al., 2014). Our current study follows directly from the B-part of it randomised controlled trial study conducted in 2017 and 2018. B-part of it had the primary objective to estimate the difference in overall carriage prevalence of disease causing genogroups of *N. meningitidis* (e.g. A, B, C, W, X, Y) detected by PCR after 12-months in school aged adolescents who received two doses of 4CMenB (*Bexsero*, GSK), compared with unvaccinated students. This analysis found that MenB prevalence was unchanged after 12-months, however there was a shift in non-disease-causing strains (study presented in Marshall et al., 2018 and data presented in Marshall et al., 2020).

**Added value of this study**

These previous studies investigating the effect of 4CMenB on *N. meningitidis* carriage were limited to assessing serogroup and/or capsule type. In our study we apply whole genome sequencing analysis to the B-part of it study cohort, allowing for in-depth sub-capsular sequence typing (multi-locus sequence typing and clonal complex assignment) and vaccine antigen profiling (*Bexsero* antigen sequence typing) in this critical reservoir population. Here we demonstrate that capsule-null sequence type-53 carriage was significantly reduced in 4CMenB vaccinated individuals whereas other sequence types, including those most associated with disease in Australia, were not significantly altered. Our data also provides a snapshot of the *N. meningitidis* sequence types carried by this population over a one-year time frame.

**Implications of all the available evidence**

Collectively these studies highlight the complex effect of the 4CMenB vaccine on *N meningitidis* carriage. Our findings, in combination with work by others suggest 4CMenB vaccination is unlikely to reduce carriage of hypervirulent MenB *N. meningitidis* strains. Additionally, we found evidence supporting that 4CMenB vaccination does alter carriage of specific un-encapsulated meningococcus types not commonly associated with disease. Collectively these data imply that targeted vaccination in high-risk groups is a more effective public health intervention.

of capsule antigens, or into related genogroups by PCR detection of individual genes amongst their capsule biosynthesis regions. More recently, whole genome sequencing (WGS) has streamlined genogrouping by enabling the evaluation of the complete capsule biosynthesis region, including of non-typical meningococci such as capsule-null (*cnl*), non-groupable (NG), and rare genogroups such as E and Z. The incidence of IMD and the predominant causative genogroups vary by geographic region.[3] However, most incidence of disease is linked to encapsulated rather than *cnl* or NG strains.

Efforts to lower rates of IMD by reducing *N. meningitidis* carriage through mass vaccination programs have been extremely successful. Since the introduction of the meningococcal C (MenC) conjugate vaccination (MenCCV) to the National Immunization Programme in 2003, the incidence of IMD caused by MenC infection and the incidence of MenC carriage in Australia has been significantly reduced, suggesting substantial vaccine efficacy and herd protection has been achieved.[1] However, vaccination programmes that elicit associated herd protection can alter strain predominance and disease epidemiology, caused by underlying changes in carriage.[4] Despite the success of the MenCCV vaccine, genogroup B (MenB) remains the principal cause of IMD in infants and adolescents in Australia.[2,5]

Unlike MenC, the polysaccharide capsule of MenB is composed of polysialic acid repeating units that are antigenically similar to human neuronal cells, precluding the development of conjugate vaccines.[6] Therefore, the more recently licensed MenB vaccine *Bexsero*, GSK, 4CMenB (licenced in 2013) is a protein-based rather than capsular polysaccharide vaccine containing three outer-membrane protein antigens: fHbp, NHBA, NadA, and outer membrane vesicles (OMVs) containing the PorA antigen.[7]

In addition to defining genogroups, WGS enables high resolution typing based on analyses of multiple loci simultaneously. For example, *Bexsero* Antigen Sequence Typing (BAST) defines the allelic profiles of the major 4CMenB antigens, which are then used to estimate vaccine coverage by comparison with the outcomes of experimental studies. Additionally, WGS also permits classification of *N. meningitidis* into sequence types (ST) using the *Neisseria* multi-locus sequence typing (MLST) scheme consisting of seven core genes (*abcC, adk, aroE, fumC, gdh, pdhC, pgm*). These sequence types can then be further grouped into their related clonal complexes (cc).[8] Additionally, WGS also enables typing by core-genome MLST (cgMLST) based on a scheme of more than 1000 genes.[9] These sub-capsular classification schemes provide higher resolution typing and improve tracing of related isolates in epidemiological studies or during outbreaks compared to genogrouping alone.

Although multiple studies demonstrate the efficacy of 4CMenB vaccination, preventing MenB associated IMD (reviewed in[10]), less is known regarding how it impacts on carriage of *N. meningitidis* in the wider community. To address this, the effect of the 4CMenB vaccination on the carriage of *N. meningitidis* in adolescents was evaluated using the genomic data from a cluster randomised controlled trial (randomised controlled trial), known as the *B Part of It* study.[11,12]

## Methods

### Study population
Participants consisting of students aged from 15 to 19 throughout South Australia were enrolled in the B Part of It cluster randomised controlled trial, conducted between 2017 and 2018. A total of 34,489 years 10, 11 and 12 students were enrolled at baseline as described previously.[11] This cohort consisted of 16,127 students allocated to the control (mean 16 years [SD, 1.3]), and 18,362 allocated to the vaccinated group (mean 16 years [SD, 1.0]). One-year post intervention, follow-up samples were collected. Students were ineligible if they: had received 4CMenB previously; had previously had an anaphylactic reaction to any component of the vaccine; or were known to be pregnant. WCHN Human Research Ethics Committee approved the B Part of It trial. For full details of the randomised controlled trial protocol and participant demographics please refer to previously published work.[11,12]

### N. meningitidis isolation and whole genome sequencing
Oropharyngeal swabs that were *porA* positive from PCR screening (sensitivity 96.05%, specificity 91.58%[13]) were used to inoculate *Neisseria*-selective agar as previously published.[14] Plates were incubated for 48 h and then examined for the presence of *Neisseria* like colonies that were identified using MALDI ToF analysis. For subsequent DNA extraction, pure isolates were cultured overnight at 37 °C on selective agar, a single colony was placed in external lysis buffer (Roche), and total DNA was extracted using the QIASymphony SP with QIASymphony DSP Virus/Pathogen kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. DNA concentration was quantified using Quant-IT dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, USA).

Sequencing libraries were prepared from DNA extracts using the Illumina Nextera XT Library Preparation kit (Illumina Inc., CA, USA) with slight modifications. Half the recommended volume was used for tagmentation reagents, amplification reagents and input DNA. Library clean-up was performed using AxyPrep MAG PCR Clean up kit (Corning Inc., NY, USA), and libraries were pooled manually, and sequenced on a NextSeq 550 platform with NextSeq 500/550 Mid-Output kit v2.5 (300 cycles) (Illumina Inc.).

### Genomic and statistical analyses
*De novo* assemblies were generated using paired end raw reads using the shovill pipeline (v1.0.4) with SPAdes (v3.13.1). Assembled contigs less than 1000 bp were removed, and those remaining were submitted to the PubMLST *Neisseria* database for storage and annotation.[8] Core-genome multi locus sequence typing of *N. meningitidis* using a scheme of 1422 loci (cgMLST v2) was assigned for all isolates through the PubMLST automated typing pipeline. The resultant distance matrix was visualised as a minimum spanning tree using GrapeTree, MSTreeV2 method.[15] All isolates included in this analysis had greater than 93% coverage of cgMLST loci (min = 93.67, median = 99.79%, mean = 99.72%). The vaccine coverage scheme is available in the PubMLST database (https://pubmlst.org/neisseria), including *Bexsero* Antigen Sequence Typing (BAST) was applied to estimate Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR).[16]

Associations between categorical data were made using Fischer's Exact test. Shapiro–Wilk normality tests were used to determine the distribution of the data and the appropriate non-parametric tests were selected based on these results. Differences in numerical data between two-populations were investigated using the Mann–Whitney U-test. Multiple group comparisons were performed by Kruskal–Wallis and Dunn's multiple comparisons. Confidence intervals were computed using normal approximation in a Bernoulli trial process.

All statistical analyses were performed using STATA15 or Rstudio.

### Role of the funding source
GSK Clinical Research and Development Board reviewed the protocol, but the funder had no role in the study management or data analysis.

## Results
### Isolate collection
In 2017, 34,459 baseline oropharyngeal swab samples were collected from adolescents in South Australia, excluding 30 participant samples that were rejected due to insufficient storage medium volume (Table 1). Of these 1222 were positive for *porA* at screening, and *N. meningitidis* isolates were obtained from 904 of the positive swabs (73% cultured). In 2018, 9644 participants were lost to follow-up and 24,815 oropharyngeal swab samples were collected. Of these 24,815 samples, 1264 were *porA*-positive at screening, and 868 isolates were obtained, giving a total of 1772 isolates for WGS analysis (71% of all positive samples). The proportion of isolates cultured from the *porA*-positive oropharyngeal swabs was consistent with prior method validation studies for throat swabs.[17] No cases of IMD were recorded in participants during the study period, for details of the study safety outcomes please refer to previously published work.[11,12]

### Overall population structure of adolescent meningococcal carriage in South Australia at study baseline
Based on the WGS derived genogroups of *N. meningitidis* culture isolates at baseline (Fig. 1a), genetically unencapsulated meningococci were the most abundant (n = 328, 36.3%; 95% CI: 33.2–39.5%). These consisted of either capsule-null (*cnl*) or non-groupable (NG) isolates. The *cnl* isolates were defined by detection of the capsule-null locus, and NG isolates were defined by detection of sequences that are associated with loss of capsule expression[18] but were genetically related to encapsulated isolates found in the population.[19] Of the remaining isolates, MenB was the most prevalent genogroup at baseline (n = 280, 31.0%; 95% CI: 28.0–34.1%). The next most common was genogroup Y (MenY: n = 185, 20.5%; 95% CI: 17.9–23.3%), followed by W (MenW: n = 46; 5.1%; 95% CI: 3.8–6.8%). Genogroups C, E, L, X, Z and
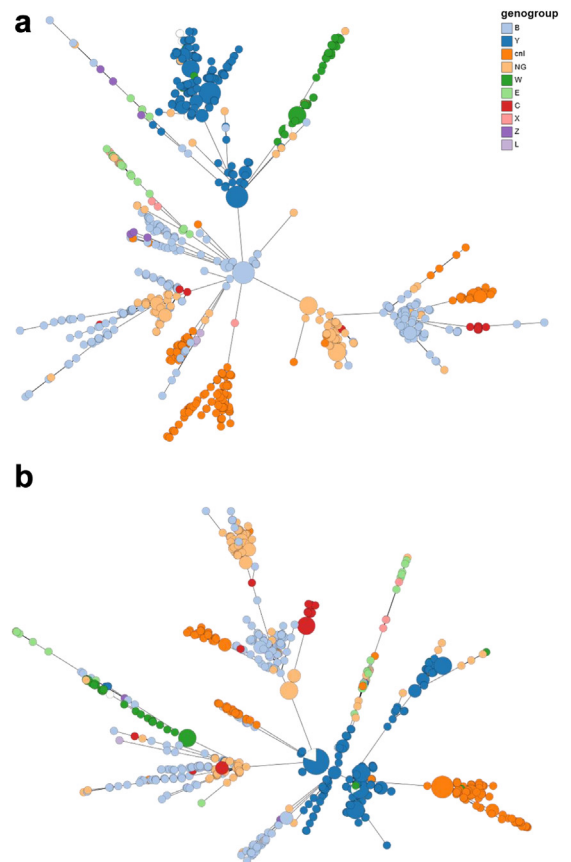
*Fig. 1:* Phylogeny of *Neisseria meningitidis* carried by South Australian adolescent sample population prior to vaccination in year 2017 (a) and one year after vaccination in 2018 (b). Tree structure was built using the GrapeTree package based on the cgMLST v2 *Neisseria* core gene MLST scheme (PubMLST) and nodes were coloured based on genogroup, white node represents isolates with indeterminate genogroup.

indeterminate (genogroup not detected) together represented 7.2% of isolates (n = 65; 95% CI: 5.6–9.1%). We did not detect any genogroup A isolates in our carriage population. These results are consistent with the reported global distribution of genogroups associated with IMD[3] and with our previously reported PCR-based genogroup detection for this study cohort.[11]

MenB isolates exhibited diverse MLST-defined sequence types (ST) and clonal complexes (cc)

| | Baseline (2017) | Year 1 follow-up (2018) |
|---|---|---|
| Enrolled participants, n | 34,489 | 34,489 |
| Collected swabs, n | 34,459 | 24,815 |
| PorA PCR detected, n | 1222 | 1264 |
| Meningococcal culture, n (%) | 904 (74.0%) | 868 (68.6%) |

*Table 1:* Number and percent recovery of *Neisseria meningitidis* culture isolates obtained from *porA* positive samples at baseline and 1 year follow up.

(Supplementary Figure S1a and b, and Supplementary Table S1). The majority of MenB isolates were ST-6058 from the hyperinvasive cc41/44 (n = 41, 4.5%; 95% CI: 3.3–6.2%), ST-32 from cc32 (n = 40, 4.4%; 95% CI: 3.2–6.0%), or ST-2870 from cc32 (n = 30, 3.3%; 95% CI: 2.3–4.8%). Amongst MenY, cc23 was predominant, and the most prevalent sequence types from this complex were ST-23 (n = 79, 8.7%; 95% CI: 7.0–10.8%) and ST-1655 (n = 66, 7.3%; 95% CI: 5.7–9.2%). Those isolates that were *cnl* consisted mostly of ST-823 from cc198 (n = 60, 6.6%; 95% CI: 5.1–8.5%) and NG isolates were mostly ST-35 (n = 56; 6.2%; 95% CI: 4.8–8.0%) from cc35 and ST-6058 (n = 40; 4.4%; 95% CI: 3.2–6.0%) from cc41/44.

## Overall population structure of meningococcal carriage one year following intervention

One year following vaccination, WGS analysis indicated the overall genogroup composition of the *N. meningitidis* carriage isolate population had changed compared to baseline (Fig. 1b, and Supplementary Table S1). While unencapsulated (*cnl* and NG) *N. meningitidis* remained the most abundant (n = 318; 36.6%; 95% CI: 33.4–40.0%), and the prevalence of MenW remained stable (n = 37; 4.3%; 95% CI: 3.1–5.9%), MenY prevalence increased making these the most common isolates carried by South Australian adolescents in 2018 (n = 225; 25.9%; 95% CI: 23.1–29.0%). Conversely, MenB isolates were less prevalent (n = 224; 25.8%; 95% CI: 23.0–29.0%), and these differences were found to be significant by Fischer's Exact test (MenY: OR = 1.36; 95% CI: 1.08–1.71, p = 0.0068, MenB: OR = 0.78; 95% CI: 0.63–0.96, p = 0.0178).

Overall, the population structure of each genogroup categorised by sequence type and clonal complex was mostly unaltered one year after intervention (Supplementary Figure S1c and d, and Supplementary Table S1). ST-6058 from cc41/44 (n = 30; 3.5%; 95% CI: 2.4–5.0%), ST-32 from cc32 (n = 28; 3.2%; 95% CI: 2.2–4.7%) and ST-154 from cc41/44 (n = 19; 2.2%; 95% CI: 1.4–3.5%) were the most prevalent sequence types amongst the MenB. While ST-1655 from cc23 (n = 110; 12.7%; 95% CI: 10.6–15.1%), ST-23 from cc23 (n = 53; 6.1%; 95% CI: 4.6–8.0%) and ST-1624 from cc167 (n = 33; 3.8%; 95% CI: 2.7–5.4%) were the three most prevalent sequence types amongst the MenY. Likewise, *cnl* ST-823 from cc198 (n = 66; 7.6; 95% CI: 6.0–9.6%), ST-35 from cc35 (n = 77; 8.9%; 95% CI: 7.1–11.0%) and ST-6058 from cc41/44 (n = 36; 4.1%; 95% CI: 3.0–5.8%) non-groupable meningococci remained most prevalent.

Prevalence of specific sequence types differed at year one compared to baseline (Fig. 2). A significantly higher proportion of MenY ST-1655 from cc23 (OR = 1.84; 95% CI: 1.32–2.58, p = 0.0002), and non-groupable ST-35 from cc35 meningococci (OR = 1.47; 95% CI: 1.02–2.15, p = 0.0378) were detected in 2018 compared to 2017.

Additionally, *cnl* ST-53 from cc53 (OR = 0.52; 95% CI: 0.30–0.87, p = 0.0106), MenB ST-2870 from cc32 (OR = 0.44; 95% CI: 0.21–0.88, p = 0.0134) and Men B ST-1097 from cc41/44 (OR = 0.19; 95% CI: 0.05–0.55, p = 0.0005) were all significantly less prevalent after one year. Changes to clonal complex abundance associated with these sequence types were less pronounced due to compensatory shifts in prevalence of the other sequence types within these complexes. For example, the change in overall abundance of non-groupable cc35 did not significantly increase (OR = 1.43; 95% CI: 0.99–2.07%, p = 0.0508). Likewise, MenB cc32 was not significantly reduced (OR = 0.76; 95% CI: 0.55–1.10, p = 0.0962). However, in-accord with sequence type, the abundance of MenY cc23 isolates increased significantly (OR = 1.31; 95% CI: 1.02–1.68; p = 0.0312). Likewise, *cnl* cc53 (OR = 0.56; 95% CI: 0.33–0.92%, p = 0.0187) and MenB cc41/44 (OR = 0.73; 95% CI: 5.53–1.00, p = 0.0430) were reduced in 2018. BAST analysis for MenB ST-1097 indicated the major vaccine antigens for these isolates were not covered by the *Bexsero* 4CMenB vaccine (Supplementary Table S2). There was insufficient experimental data to determine vaccine antigen reactivity for the other sequence types that had altered prevalence.

## The effect of 4CMenB vaccination on the meningococcal population at baseline and one year after intervention

Despite these changes to strain abundance one year post vaccination, it was unclear if they were related to vaccination or to extrinsic factors effecting *N. meningitidis* carriage in the wider population. Therefore, we assessed the effect of 4CMenB by directly comparing the vaccine and control groups *N. meningitidis* carriage isolates. Isolates were segregated by genogroup and sequence type, then their prevalence were analysed. There were no significant differences in overall prevalence of isolates between the control and vaccine group at baseline (Mann–Whitney; p = 0.2408). Likewise, there was no difference in the overall carriage prevalence at one-year post-intervention in either vaccine or control groups (Kruskal–Wallis; p = 0.3471, Dunn's multiple comparisons; Baseline-Control vs Year1-Control; p = 0.3862, Baseline-Vaccine vs Year1-Vaccine; p = 0.4501). MenY and MenB genogroup prevalence was equivalent between the vaccine and control groups (MenY: OR = 1.09; 95% CI: 0.79–1.49, p = 0.5881, MenB: OR = 1.27; 95% CI: 0.93–1.74, p = 0.1397).

In addition, the effect of immunisation with 4CMenB on carriage of specific sequence types were independently assessed (Fig. 2 and Supplementary Table S1). Isolates of MenY ST-1655 were by far the most prevalent in both groups one-year post-intervention (control: n = 54; 11.6%, 95% CI: 9.0–15.1%, vaccinated: n = 56; 13.8%, 95% CI: 10.6–17.6%) and were not significantly different between the treatment groups
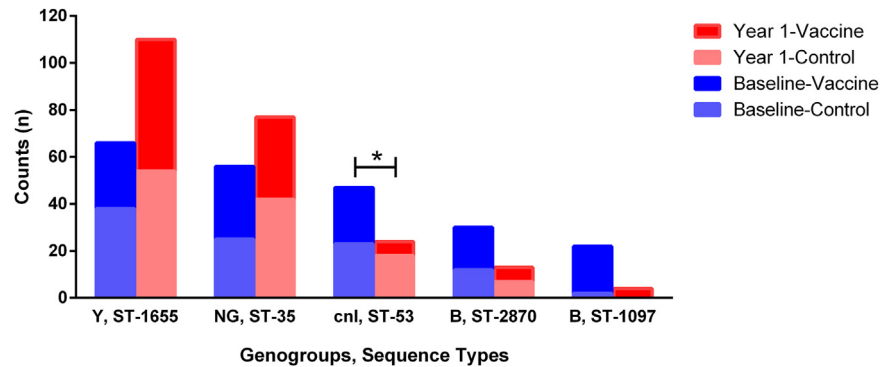
**Fig. 2:** *Neisseria meningitidis* carriage strain sequence types with prevalence that were significantly different at baseline (blue) compared to one year following the vaccination trial (red). Odds ratio analyses were then performed to compare the proportion of each strain in the vaccinated (bold) and control groups (pale), the proportion of *cnl* ST-53 was significantly reduced (p = 0.0368) in the vaccinated cohort one year following vaccination compared to the control group. All other strains were not significantly different between vaccine and control groups.

(OR = 1.20, 95% CI: 0.79–1.83, p = 0.4136) despite their increased overall prevalence in 2018.

The proportion of *cnl* ST-53 (OR = 0.37; 95% CI: 0.12–0.98, 0.0368) was less in the vaccinated compared to the control group at one year (Fig. 2), despite having equal prevalence at baseline (control n = 23, vaccinated n = 24; OR = 0.87; 95% CI: 0.46–1.64, p = 0.6551). This was consistent with an overall reduction in *cnl* cc53 at year one (OR = 0.37; 95% CI: 0.14–0.96, p = 0.0307). No significant differences in strain prevalence were found between the vaccine and control groups for all other sequence types (NG ST-35, MenB ST-2870, MenB ST-1097) or their related clonal complexes (Men Y cc23, NG cc35, MenB cc32, MenB cc41/44). BAST analysis suggested there was insufficient data for ST-53 *cnl* to determine *Bexsero* vaccine antigen reactivity (Supplementary Table S2).
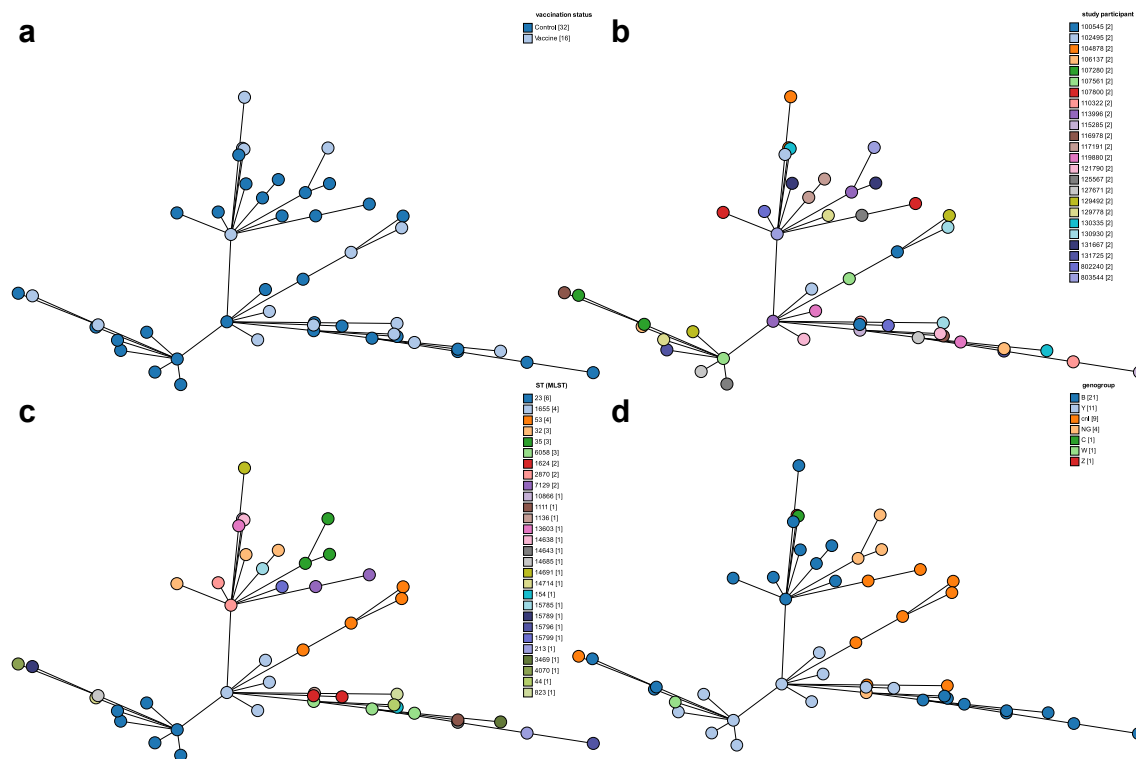
### Carriage in study participants with *N. meningitidis* isolates recovered in both sampling years

To assess the intra-host impact of 4CMenB vaccination, we analysed carriage isolate genomes recovered in both years from the same study participant (n = 64 vaccinated, n = 77 control). For 83% of participants (n = 117), 2017 and 2018 isolates were basically indistinguishable based on sequence type and cgMLST phylogenetic analysis (Fig. 3). All but one of these 117 participants had the same genogroup detected in both years (Supplementary Figure S2). For this individual, matched isolates were both ST-823 cc198, and were phylogenetically related by cgMLST, but had different genogroups (2017: *cnl*, 2018 MenW), possibly indicating capsule switching. The incidence of participants having the same or different isolate type(s) in both years was not altered by vaccination status (OR = 1.86; 95% CI: 0.68–5.34, p = 0.2610). For participants with different

isolate types recovered in both years (n = 24), sequence type and genogroup switching appeared to be random (Fig. 4). There were no significant differences in the isolate types that switched after one year comparing between the vaccine and control groups.
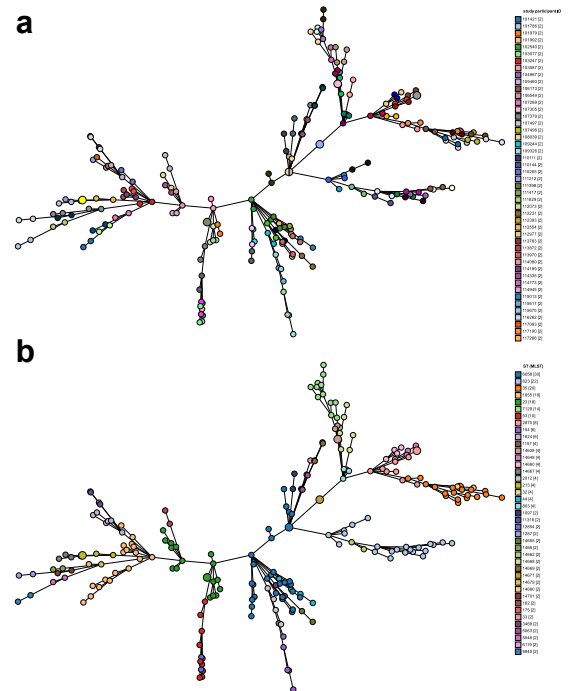


**Fig. 3:** Phylogeny of carriage isolates taken from study participants that had the same *Neisseria meningitidis* sequence type recovered in both sampling years. Tree structure was built using the GrapeTree package based on the cgMLST v2 scheme (PubMLST) and nodes were coloured based on study participant ID (a), or sequence type (b). Large nodes represent two overlayed isolates. Branch lengths are presented on a log scale.

**Fig. 4:** Phylogeny of carriage isolates taken from study participants that had different *Neisseria meningitidis* sequence types recovered in both sampling years. Tree structure was built using the GrapeTree package based on the cgMLST v2 scheme (PubMLST) and nodes were coloured based on vaccination status (a), study participant ID (b), sequence type (c), or genogroup (d). Branch lengths are presented on a log scale.

## Discussion

*N. meningitidis* is usually transmitted through social contact among healthy carriers.[20] Previous studies have shown carriage rates are highest in adolescents and that this population is the most significant reservoir for IMD causing meningococci.[20–22] Changes to the strains that commonly cause IMD, consistent with changes to their carriage prevalence, have occurred following introduction of other meningococcal vaccination programs.[5] However, the effect of protein based MenB vaccines on carriage are less well understood. Here we report findings from the largest genomic analysis conducted on *N. meningitidis* carriage to date studying the impact of introduction of the 4CMenB vaccine (*Bexsero*, GSK) in this critical age demographic. Our findings offer large scale insight into the genomic epidemiology of *N. meningitidis* carriage in an Australian context and provide supportive evidence for 4CMenB vaccination influencing carriage of specific *cnl* isolates.

Core genome MLST was used to assess the relatedness of *N. meningitidis* isolates at baseline and one year after intervention (Fig. 1). Genogroups B, Y, and W were most common amongst encapsulated meningococci, and have been associated with IMD in South Australia.[2] Notably, ST-6058 from the hyperinvasive cc41/44 were

well represented in the South Australian adolescent population, and this has been shown to be a major disease-causing strain in the neighbouring state, Victoria.[23] The isolation of ST-6058 meningococci with different capsule types (MenB and MenY), as well as no capsule (non-groupable), supports its ability to undergo capsule switching.[24] Such a capacity could enable these closely related virulent meningococcal clones to avoid vaccine-induced or natural protective immunity directed towards capsular antigens and potentially drive capsule replacement amongst carriage populations.

This analysis also showed that the majority of the unencapsulated isolates were genetically similar to the encapsulated meningococci. Specifically, cc32 and cc35 meningococci clustered together, despite some being genogroup B (MenB), and others being unencapsulated (*cnl* or non-groupable meningococci). This supports that these unencapsulated meningococci were likely genogroup B, Y, or W that became capsule deficient due to acquisition of loss-of-function mutations, or regulatory mutations within the promoter of capsule biosynthesis regions, as suggested by a previous study.[19] While *cnl* and non-groupable meningococcal associated IMD is rare, reporting of these cases has been increasing in recent years.[25,26] Most of these cases occur in those with

hereditary complement deficiencies or during treatment with the complement component inhibitor eculizumab.[26]

Capsule switching in meningococci, along with extended recombination events and horizontal gene transfer, has lent support to the hypothesis that multiple meningococcal variants may be carried simultaneously in asymptomatic carriers.[27,28] Given that a single colony was chosen for sequencing from the culture plates, this study could not consider strain heterogeneity within an individual; however, our data demonstrated genomic heterogeneity at the population level. Specifically, genogroup B isolates from our study were phylogenetically diverse despite having the same capsular biosynthesis genes. The diversity of MenB lineages observed in this study may be related to its endemicity within the Australian population, where time and other environmental factors allow the meningococci to diversify and co-evolve with their host.

Contrary to MenB, MenW and MenY were not associated with significant IMD in Australia until relatively recently.[1,29] Consistent with this, our data shows these emerging genogroups are phylogenetically distinct from other variants and have lower genetic diversity, consisting of one or two major clonal complexes. Notably, these strains from MenW and MenY included hyperinvasive meningococcal clonal complexes (MenW cc11, MenY cc23 and cc41/44).[30] Australian adolescents with these hyperinvasive MenW and MenY are potential carriers for transmission to immune-compromised patients and elderly populations that have recently been associated with IMD caused by these lineages.[1,31,32] The detection of hyperinvasive MenW and MenY in our carriage population is consistent with the Australian Meningococcal Surveillance Program reporting IMD associated with these lineages.[33]

In this study the carriage of meningococci from the South Australian adolescent population was screened at two occasions one year apart. A positive meningococcal PCR result was only detected in one of the two samples in the majority of the sampled population despite overall carriage rates remaining stable.[14] This suggests that most participants acquired circulating meningococcal clones from transmission events occurring amongst the wider population. For the small number of participants with isolates recovered in both sampling years (n = 141), the majority (83%) carried the same isolate type, strongly suggestive of persistent carriage over the duration of the study. Carriage in these participants was unaltered by 4CMenB vaccination irrespective of genogroup or sequence type. Therefore, this study supports the notion of a homeostatic meningococcal carriage population in South Australia. Further analysis into the longitudinal duration and demographics of individuals that support persistent carriage would be of interest in future studies.

Other vaccinations, such as the pneumococcal vaccine, have resulted in significant decrease in nasal carriage rates of vaccine serotypes and increased indirect immunity in the general population when provided to infants and young children.[4] This has resulted in replacement of pneumococcal carriage with non-vaccine types that also have the capacity to cause severe infections.[34] In our study, we did not observe evidence of strain replacement comparing between vaccine and control groups. We did observe a general reduction in MenB and an increase in MenY isolates amongst the carriage study samples one year after vaccination. Accordingly, we observed significant reductions in MenB ST-1097 and ST-2870 carriage, whereas MenY ST-1655 isolates had increased strain prevalence one year after vaccination. Since these effects were equivalent in both vaccine and control groups, it is unclear whether this was an indirect effect of vaccination or a result of extraneous factors controlling *N. meningitidis* carriage in the wider population. This increase in MenY prevalence may have implications for MenY associated disease, whether caused by indirect effect of vaccination or natural fluctuations in meningococcal populations. However, since the introduction of MenACWY vaccine into the Australian national immunisation program schedule for children in 2018 and then adolescents in 2019, the incidence of IMD has been declining in Australia, indicating effective protection against MenY and other covered serotypes.[33]

Even though vaccination is one of the most successful public health interventions, deployment of the 4CMenB vaccination did not reduce the prevalence of hyperinvasive meningococci within the vaccinated population. This was illustrated by the hyperinvasive strain MenB cc41/44, which was the most prevalent MenB lineage in the study cohort, being detected with equal frequency in control and vaccine groups at year one. This finding provides strong evidence that 4CMenB does not prevent transmission of hyperinvasive MenB strains in this population. These data are consistent with the previous study that reported 4CMenB vaccination did not effect carriage prevalence of disease-associated meningococcal strains in vaccinated university students one month after receiving a second dose.[35]

Despite this, the 4CMenB vaccination may have influenced carriage of unencapsulated meningococcal strains. At the one-year follow-up, *cnl* ST-53 from cc53 was significantly less prevalent in the vaccine group compared to the control group. This may indicate that transmission of these meningococci is lower within the vaccinated population. At the time of writing, there was insufficient experimental evidence to conclude cross reactivity of vaccine induced immunity for the BAST types amongst *cnl* ST-53 meningococci and is a cause for further investigation. Notably, BAST analysis estimates vaccine reactivity against the four major vaccine antigens: fHbp, NHBA, NadA, and PorA. The 4CMenB vaccine also includes OMVs that may promote broad-spectrum immunity unrelated to BAST profiles[7] and

studies have shown that 4CMenB vaccination may offer some cross-species protection against *Neisseria gonorrhoeae* infection.[36] This implies that immune responses to 4CMenB cannot be inferred solely by BAST profile. A possible explanation as to why only unencapsulated meningococci were affected by vaccination may be that these isolates are more antigenically exposed and less capable of immune escape. In support of this theory, a previous longitudinal meningococcal carriage study had shown a loss of carriage of unencapsulated meningococci was more common than encapsulated meningococci,[37] potentially reflecting enhanced immunogenicity towards unencapsulated strains. Also, the 4CMenB vaccine is a protein-based rather than capsule-based vaccine that may have a greater impact on unencapsulated strains. One way to confirm the effect of 4CMenB on the carriage reduction of *cnl* ST-53 meningococci would be to perform a controlled experimental study to assess serum reactivity from immunised and non-immunised individuals against this sequence type.

Despite not demonstrating a reduction in the prevalence of hyperinvasive variant carriage, this does not preclude 4CMenB vaccine efficacy for prevention of IMD. Although we did not observe strain replacement in this study, reduced carriage of non-disease-causing meningococci such as *cnl* ST-53 could result in recolonisation, potentially by disease-causing strains in the long-term. This potential for strain replacement in 4CMenB vaccinated individuals should be considered when monitoring rates of IMD in the future. To date, there are limited longitudinal studies on meningococcal carriage and effect of vaccination, suggesting prolonged surveillance may prove beneficial. Collectively our data implies deployment of 4CMenB vaccination is unlikely to reduce transmission and carriage of hyperinvasive strains of *N. meningitidis*, suggesting ongoing targeted vaccination in high-risk groups may be required to prevent IMD.

Our study had limitations. Analyses were limited to meningococcal colonisation that were detectable by *porA* PCR. Additionally, typing was performed from a single meningococcal isolate from each plate, and the effectiveness of the 4CMenB vaccination could not be estimated where participants harbour multiple strains of meningococci. Aside from participant loss to follow-up, there were a proportion of samples that screened positive by *porA* PCR but were unable to be cultured, and thus were excluded from this study. In addition, meningococcal carriage in the South Australian adolescent population were lower than anticipated, thus reducing the power of this study to measure effectiveness of the vaccination for all carriage variants. Despite these limitations, our study represents the largest longitudinal WGS data set on meningococcal carriage in adolescents to date and suggests carriage can persist and transmission is still plausible even with 4CMenB vaccination. Nevertheless, further epidemiological analysis and sampling covering a longer period should be conducted for the true assessment of the effectiveness of this vaccination programme in this population.

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