Title: 'B Part of It' School Leaver study: a repeat cross-sectional study to assess the impact of increasing coverage with meningococcal B (4CMenB) vaccine on carriage of *Neisseria meningitidis*

Running title: Impact of 4CMenB vaccine on carriage

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ABSTRACT

Background:

Recombinant protein-based vaccines targeting serogroup B meningococci protect against invasive disease, but impacts on carriage are uncertain. This study assessed carriage prevalence of disease-associated meningococci from 2018-2020, as the proportion of vaccinated adolescents increased following introduction of a school-based 4CMenB immunisation program.

Methods:

Eligible participants who completed high school (age 17-25) in South Australia in the previous year had an oropharyngeal swab taken and completed a risk factor questionnaire. Disease-associated meningococci (genogroups A, B, C, W, X, Y) were detected by meningococcal and genogroup-specific polymerase chain reaction.

Results:

The final analysis included 4104 participants in 2018, 2690 in 2019, and 1338 in 2020. The proportion vaccinated with 4CMenB increased from 43% in 2018, to 78% in 2019, and 76% in 2020. Carriage prevalence of disease-associated meningococci in 2018 was 225/4104 (5.5%). There was little difference between the carriage prevalence in 2019 (134/2690, 5.0%, adjusted odds ratio [aOR] 0.82, 95% CI 0.64-1.05) and 2020 (68/1338, 5.1% aOR 0.82, 95% CI 0.57-1.17) compared to 2018.

Conclusions:

Increased 4CMenB uptake in adolescents was not associated with a decline in carriage of disease-associated meningococci. 4CMenB immunisation programs should focus on direct (individual) protection for groups at greatest risk of disease.

Registered at ClinicalTrials.gov:NCT03419533

INTRODUCTION

Invasive meningococcal disease (IMD), caused by the bacterium *Neisseria meningitidis* results in substantial morbidity and mortality worldwide.[1, 2] To develop IMD, *N. meningitidis* is first acquired, mainly through respiratory droplets from close contacts, resulting in oropharyngeal colonisation.[3] Rarely, colonisation is followed by invasive disease.

N. meningitidis is classified according to either the composition of its polysaccharide capsule (serogroup), or detection of capsular biosynthesis genes (genogroup).[2, 4] Meningococcal bacteria typically need to be encapsulated to cause invasive disease, with six groups responsible for the majority of disease globally over the last two decades (A, B, C, W, X, and Y), of which serogroups A and X rarely causes disease outside Africa.[1] However, unencapsulated meningococci are capable of causing severe disease in immunocompromised people, including those with hereditary complement deficiencies, or receiving eculizumab.[5] Potentially invasive meningococci may be non-capsulated in the nasopharynx and then switch on their capsule when they invade the bloodstream, usually shortly after acquisition.[6] Reducing the carriage prevalence of disease-associated *N. meningitidis* genogroups, especially in adolescents who have the highest meningococcal carriage rates in high-income countries, can prevent transmission and provide indirect population (herd) protection to other vulnerable and unvaccinated groups.[1, 7]

Meningococcal vaccines induce serum bactericidal antibodies that directly protect individuals against IMD.[8] Capsular polysaccharide-conjugate vaccines induce serogroup-specific salivary IgG and IgA antibodies,[9] which likely contribute to reduced pharyngeal carriage in vaccinated adolescents.[9, 10] As recombinant 'meningococcal B' vaccines were developed with novel technologies, using predominantly protein antigens, similar effects to those observed with meningococcal polysaccharide-conjugate vaccines are not guaranteed. The match between the outer membrane protein and antigens contained in the 4CMenB vaccine to those contained in group B and non-B *N. meningitidis* isolates remains an essential factor in the ability of the vaccine to

protect against IMD. There is no compelling evidence that 4CMenB vaccine reduces acquisition of pharyngeal carriage of group B meningococci, the serogroup for which the vaccine was licenced.[11-13] The impact of longer-term changes in meningococcal carriage following a population-based adolescent 4CMenB program are currently unknown.

In South Australia, 91% of high schools participated in a cluster RCT, with half receiving the 4CMenB vaccine (2 doses at least 1 month apart) in 2017 and the other half in 2018.[11] A state-funded 4CMenB immunisation program for South Australian children commenced in October 2018, and for adolescents 15 years of age in February 2019, with one year of catch up for 16-20-year-olds.[14] MenACWY-TT vaccine was introduced on the National Immunisation schedule in 2019 for 14-16 year olds. This study's primary objective was to establish if the high school 4CMenB immunisation program was associated with decreased carriage prevalence of disease-associated *N. meningitidis* among school leavers, as the proportion vaccinated increased over three years.

METHODS

Study Design and oversight

This investigator-led repeat cross-sectional study was conducted in the state of South Australia, which has a population of approximately 1.7 million people.[15] Each year there are approximately 14,500 adolescents that enrol in their final year (12/13) of high school.[16] Nationally, approximately 60% of school leavers enrol in higher education (university or vocational training) after leaving school.[17] Recruitment was conducted from 1st February to 27th August 2018, from 1st February to 10th December 2019, and 24th February to 11th September 2020.

Participants

Participants were eligible if they had completed high school in South Australia in the 12 months preceding enrolment and were between 17-25 years old.[18] In 2018, school leavers were recruited through mail-outs and text messages sent to previous RCT participants, tertiary education stalls at all three universities in South Australia and social media advertising.[18] In 2019 and 2020, a mail-out

to all 18 year-olds across South Australia from the Medicare database (Australia's universal health insurance scheme) was also conducted.[19]

Objectives

The primary objective was to detect any differences in carriage prevalence of disease-associated genogroups of *N. meningitidis* (genogroups A, B, C, W, X, or Y) in South Australian school leavers between 2018 (year 12 in 2017), 2019 (year 12 in 2018), and 2020 (year 12 in 2019). Disease-associated genogroups were chosen as the antigens in the vaccine may provide broad protection against non-B *N. meningitidis* carriage. Secondary objectives included estimating the difference in any *N. meningitidis* carriage, as well as for individual genogroups, between 2018, 2019, and 2020. Other secondary objectives included estimating the difference in the prevalence of *N. meningitidis* carriage in 4CMenB vaccinated school leavers compared to unvaccinated school leavers across 2018-2020 combined, and ascertainment of risk factors for carriage.[18] An exploratory objective was to investigate whether the effect of 4CMenB vaccination on carriage prevalence differed between 2018, 2019 and 2020.

Study process

Following informed consent, an oropharyngeal swab was taken from each participant and a risk factor questionnaire was completed. The questionnaire collected information from participants about smoking history, household size, recent antibiotic use, use of mouth wash, intimate kissing, socialising in pubs and clubs, and alcohol use.[18] Remoteness Area and Index of Relative Socio-economic Disadvantage (IRSD) were derived using the participant's postcode.[20]

Oropharyngeal swabs were placed in 2mL skim milk-tryptone-glucose-glycerol (STGG) medium and transported to the state-wide public pathology service, SA Pathology. All swabs underwent polymerase chain reaction (PCR) screening for the presence of specific meningococcal DNA (*porA* gene), before being frozen at -80°C. Those who had *porA* detected were further analysed to determine the genogroup (A, B, C, E, W, X, or Y), as described previously.[11, 18, 21] Direct plating

was not used due to its feasibility.[21] 'Disease-associated' carriage in this paper is defined as having A, B, C, W, X, or Y *N. meningitidis* genogroups detected. 'Any' carriage includes every sample that has meningococcal DNA (*porA* detected), and 'non-groupable' carriage is defined as failure to detect genogroup A, B, C, W, X, or Y, in those with *porA* detected. Whole-genome sequencing was performed on *N. meningitidis* isolates, and the multi-locus sequence type (MLST) was performed on the PubMLST website as described previously.[21, 22]

Participants were eligible to enrol once and were reimbursed with a $40 voucher for their time and travel costs. For those inadvertently enrolled more than once, only their first swab result and questionnaire responses were included. 4CMenB vaccination was confirmed for participants through either the high school cluster RCT study database or the Australian Immunisation Register (AIR). Participants were defined as vaccinated if they had received two 4CMenB doses at least a month apart, and the second dose had been received at least 28 days prior to their throat swab. MenACWY vaccination status was also confirmed for study participants from the AIR.

Statistical analysis

A sample size of 4,096 school leavers per year was estimated to provide 80% power to detect a clinically relevant 20% relative reduction in disease-associated carriage prevalence from 2018 to 2019 (two-tailed alpha=0.05), assuming a carriage prevalence of 8% in 2018.[23]

Analyses were undertaken according to a pre-specified statistical analysis plan. Logistic regression was used to compare annual carriage rates, as well as between vaccinated and unvaccinated school leavers, with effects described as odds ratios with 95% confidence intervals. For comparisons of carriage over time, global p-values from Wald tests were also reported to quantify evidence for a difference in carriage across any of the three years. Both unadjusted and adjusted analyses were performed, with adjustments made for risk factors for carriage of disease-associated meningococci identified in the original 'B Part of It' RCT: current cold or sore throat, smoking cigarettes, smoking water pipes, days out at a pub/club in last week (1 or more), people kissed (1 or more), and

ethnicity.[11] In addition, alcohol consumption in the previous month and previous MenACWY vaccination were included in adjusted models. No adjustments were made in analyses involving genogroups detected less frequently (C, W, X, or E). Due to differences in recruitment methods in 2020 and 2019 compared to 2018, additional analyses were conducted with adjustment for swab timing, age (years), socio-economic disadvantage, and geographical remoteness. Both adjusted models are presented in the results. In a post hoc analysis, the effect of 4CMenB vaccination on carriage was estimated separately for 2018, 2019, and 2020 by including an interaction term between vaccination status and year in the logistic models. Logistic regression was also used to quantify the effect of months (continuous variable) since 4CMenB vaccination on carriage among those vaccinated and identify risk factors for meningococcal carriage. All analyses were performed using Stata v.15 (StataCorp, Tx).[24]

Ethics approval

The study was approved by the Women's and Children's Health Network Human Research Ethics Committee (WCHN HREC). The protocol has been published and the study prospectively registered at ClinicalTrials.gov: NCT03419533.[18, 25]

RESULTS

After excluding duplicate swabs (n=220) and swabs with incomplete results (n=13), 4104, 2690, and 1338 swabs were available for analysis in 2018, 2019 and 2020, respectively. The mean age across all cohorts was 18.5 years, with 92% of the 2018 group participating in the 'B Part of It' RCT, compared to 79% in 2019, and 74% in 2020. Participants who had received two 4CMenB doses at the time of the swab increased from 43% in 2018 to 78% in 2019 and 76% in 2020. Less than 2% of participants in 2018 had been vaccinated longer than 12 months, compared to 51% in 2019, and 75% in 2020. Other baseline characteristics are described in Table 1, and baseline characteristics by vaccination status are described in Supplementary Table S1.

Carriage in school leavers in 2020 and 2019 compared to 2018

The primary outcome of carriage prevalence of disease-associated meningococci was 225/4104 (5.5%) in 2018, compared to 134/2690 (5.0%) in 2019, and 68/1338 (5.1%) in 2020. There was little evidence for an overall difference in disease-associated carriage between years (global p=0.25), tables 2 & 3.

Carriage prevalence was similar in 2019 and 2020 compared to 2018 for 'any' carriage (8.4%, and 7.9%, vs 9.6%; global p=0.29), genogroups B (2.4% and 3.2% vs 2.1%; global p=0.81), Y (2.1% and 2.3% vs 2.6%; global p=0.28), and non-groupable meningococci (3.5% and 2.8% vs 4.1%; global p=0.87). Small numbers of detections of genogroups C, W and X precluded adjusted analyses of these outcomes. There was some evidence for a change in carriage of genogroup W over time (global p=0.04), while no significant effects were detected for genogroups C (global p=0.62), or X (global p=0.12).

Carriage in 4CMenB-vaccinated versus unvaccinated participants

Across 2018-2020, there was no significant difference in disease-associated meningococci carried among participants who received two doses of 4CMenB vaccine 252/4904 (5.1%), compared to unvaccinated participants (175/3228 [5.4%]; aOR 0.84, 95% CI 0.68-1.04; p=0.11).

After adjustment for potentially confounding factors, overall (any) meningococcal carriage in vaccinated participants, 421/4904 (8.6%), was significantly lower than unvaccinated participants (305/3228 [9.5%]; aOR 0.83, 95% CI 0.70-0.98; p=0.03). No other significant differences were observed in the adjusted analyses for individual groups B, Y, or non-groupable *N. meningitidis*. In the unadjusted analysis, there was a reduction in genogroup W carriage in participants vaccinated with 4CMenB (unadjusted OR 0.42, 95% CI 0.20-0.91; p=0.03), table 4.

Exploratory post-hoc analyses showed some evidence to suggest that the effect of 4CMenB vaccination on disease-associated *N. meningitidis* differed between years in the pre-specified adjusted analysis (p-value for interaction=0.02), as shown in Supplementary Table S2. In 2019, a

reduction in disease-associated carriage was observed among vaccinated vs unvaccinated participants (aOR 0.53, 95% CI 0.36-0.79; p=0.002), while in 2018 and 2020, carriage rates did not vary markedly by vaccination status. The interaction effect did not persist after additional adjustment for swab timing, age, socioeconomic disadvantage and geographical remoteness (p-value for interaction=0.14).

There were no significant interaction effects between vaccination status and year for overall meningococcal carriage, genogroups B, W, Y, or non-groupable *N. meningitidis*.

Risk factors for carriage

Risk factors for carriage of disease-associated *N. meningitidis* among all participants included: attending a hotel or club in the previous week, intimately kissing at least one person in the previous week, drinking alcohol in the previous month, and not being in a relationship (Table 5). People identifying as of Asian ethnicity or living in a dwelling with more than one person per bedroom had a decreased risk of *N. meningitidis* carriage. Additional factors associated with carriage of any meningococci were receiving a swab between April to June (mid-spring to early winter) and living in an outer regional area of South Australia (Supplementary Table S3).

Time elapsed since vaccination

Among those who received two 4CMenB doses (n=4904), no significant effects of time elapsed since vaccination on carriage were detected for either any (aOR 1.00; 95% CI, 0.99 to 1.01; p=0.63), or disease-associated (aOR 1.01; 95% CI, 0.99 to 1.02; p=0.25) *N. meningitidis* carriage.

Meningococcal culture and whole-genome sequencing

Culturing of PorA PCR positive samples yielded 500 isolates (69% recovery: 2018, 288/393 [73%]; 2019, 148/227 [65%]; 2020, 64/106 [60%]). Whole-genome sequencing of these identified 133 genogroup B isolates with three predominant clonal complexes (ccs): cc41/44 (52 isolates, 39%); cc32 (50, 38%); cc213 (15, 11%). The MeNDeVAR index, indicating likely cross-reactivity to Bexsero

induced antibody responses, was available for 127 isolates (95%). Although there was some evidence of a decline in cc32 and cc41/44 isolates over time, the number of isolates was too small for any definite conclusions. It was notable that few of the cc32 isolates had exact antigen matches to Bexsero (Figure 1a-c).

DISCUSSION

This cross-sectional study found that 4CMenB vaccination was not associated with changes in carriage of disease-associated *N. meningitidis* in adolescents over time as vaccine coverage increased. Despite the proportion of school leavers who had completed a two-dose 4CMenB schedule in high school increasing by 33-35% in 2020 and 2019, compared to 2018, this was not associated with a decrease in carriage of *N.meningitidis*. This finding is consistent with current evidence that suggests recombinant 4CMenB vaccine does not significantly reduce pharyngeal carriage of disease-associated meningococci, including group B meningococci.[26]

Secondary analyses identified a significant reduction in the overall meningococcal carriage in 4CMenB vaccinated compared to unvaccinated adolescents. Carriage of non-groupable meningococci and, to a lesser extent disease-associated meningococci, contributed to the overall reduction in vaccinated compared to unvaccinated school leavers. However, this study is not powered to detect small differences in either individual genogroups or non-groupable carriage. Post hoc analysis of the 'B Part of It' RCT found a 29% reduction in carriage of non-groupable meningococci in vaccinated compared to unvaccinated high school students.[11] This was despite no significant reduction in overall carriage.[11] A similar effect on overall carriage was reported in secondary aggregated analysis from an RCT of 4CMenB vaccinated versus Japanese encephalitis-vaccinated University students from the UK.[13] It is not well understood how conjugate meningococcal vaccines reduce carriage. Mucosal antibodies, specifically IgG and IgA produced following vaccination, are thought to be the primary mechanism of reducing carriage acquisition and increasing clearance, although other immune mechanisms are likely to be important.[9, 27, 28] It

may be that for pharyngeal carriage of encapsulated invasive meningococcal strains, mucosal antibodies following recombinant vaccines are less able to bind to subcapsular protein antigens compared to polysaccharide specific antibodies after conjugate vaccines.

A decrease in the carriage of group W was observed in secondary analyses comparing carriage of individual genogroups in 2019 relative to 2018. A recent interrupted time series analysis conducted before and after introducing a childhood 4CMenB program in the UK estimated that 4CMenB directly prevented 98 (95% CI 34 to 201) group W IMD cases over four years in under five-year-olds.[29] Laboratory studies using the human complement serum bactericidal antibody assay (hSBA) have also found that 4CMenB-induced antibodies provided some cross-protection against meningococcal group C, W, Y, and X strains.[30-33] Due to the small numbers of school leavers with carriage of individual genogroups, especially W and X, these results should be interpreted with caution. Ideally, vaccine effectiveness against carriage would also be assessed against *N. meningitidis* strains that possess vaccine-related surface protein antigens, rather than genogroups alone. As the 4CMenB and MenACWY population coverage increase with the ongoing infant and adolescent programs in South Australia, it will be important to continue to monitor impacts on carriage.

In a post hoc analysis, 4CMenB vaccination was found to have a greater effect on carriage of disease-associated meningococci in 2019 compared to 2018 in the pre-specified adjusted model. This effect was not replicated in 2020, albeit in a smaller sample, and there was no evidence that the effect of 4CMenB changed over time according to a more extensive adjusted model. It is possible that the expression of vaccine proteins change following a large adolescent program, which could lead to small fluctuating variations in the vaccine impact on carriage. In Australia, this has been previously demonstrated to occur in a natural cycle of approximately 2 years in the absence of a vaccine program and relates to the travel-related introduction of strains, their ability to spread in the local community and the proportion of the community that is naturally immune.[34, 35] This post-hoc result should be interpreted with caution as almost all unvaccinated participants in 2018 were participants in the RCT, with the proportion decreasing in 2019, and 2020, suggesting differences

between vaccinated and unvaccinated cohorts in different years. Due to these differences, additional characteristics were added in the adjusted model, including swab timing, age, socio-economic disadvantage, and remoteness. In this model, the interaction between year and vaccination was no longer significant, suggesting confounding was present in the pre-specified adjusted model.

One of the largest studies investigating risks factors associated with meningococcal carriage to date found that active and passive smoking, intimate kissing, and attending pubs and clubs were independently associated with *N. meningitidis* carriage in the UK.[36] Findings in more recent carriage studies conducted in the Americas and Europe have also found that going out,[12, 37-40] cigarette smoking,[12, 37-39] and kissing[39, 40] are associated with higher meningococcal carriage. Only one study identified drinking alcohol as an independent risk factor.[39] In this cross-sectional study, the low proportion of participants reported smoking cigarettes and e-cigarettes (<3%) or a water pipe (<5%) may explain a lack of observed association with carriage. Living in a residence where the number of persons per bedroom was greater than 1 but not more than 2 was associated with reduced carriage, compared to living in a residence with one person per bedroom or less. The WHO Housing and Health guidelines note that whether a household is “crowded” depends on many factors, not just the number of persons per bedroom.[41] Despite adjusting for several other risk factors for carriage, this marginal reduction in risk may be due to residual/unmeasured confounding.

The other observed risk factors were similar to those previously reported, including among adolescents in the South Australian RCT.[11] Drinking alcohol in the previous month was identified as an independent risk factor for carriage and is potentially modifiable, although already targeted by harm minimisation campaigns in Australia for other diseases.

Strengths and limitations

One of the study's main strengths is that both 4CMenB and MenACWY vaccinations were validated using the RCT database and the Australian Immunisation Register, respectively. Very few participants

in this study were vaccinated with MenACWY, providing the opportunity to observe the effects of 4CMenB vaccine on carriage with little potential for confounding due to receipt of other meningococcal vaccines. The majority of the staff conducting the swabbing had 2-3 years' experience, received specific training, and were also involved in the cluster RCT, ensuring consistent technique.

Limitations include the lower than anticipated recruitment in 2019 and 2020. Many of those enrolled as school leavers in 2018 were previous RCT participants who were swabbed when they returned to receive their 4CMenB vaccination (RCT control group). The 2019 and 2020 cohorts were only invited to have a swab taken, making recruitment more challenging. The recruitment period was extended in 2019 and 2020, compared to 2018, to increase enrolment. In 2020, recruitment commenced in university orientation week as planned in February, but restrictions due to COVID-19 meant that swabbing could not recommence until the second half of the year. The smaller than expected samples in 2019 and 2020, and lower observed carriage prevalence than anticipated, reduced the study's ability to detect small differences in carriage between years. Lower rates of smoking and other risk factors for carriage are likely to be the primary reason for lower than anticipated carriage prevalence. This was reflected in the UK, where the reduced prevalence of behaviours associated with carriage has contributed to reduced carriage over 15 years.[42] Extending the recruitment period and the different recruitment methods used in 2020 and 2019, compared to 2018, may have also increased the risk of bias and confounding. To minimise the risk of bias due to confounding, we adjusted for known pre-specified risk factors for carriage. Due to differences in recruitment methods from 2019, additional analyses were conducted to adjust for the timing of the swab, participant's age, socio-economic disadvantage, and remoteness. However, some residual unmeasured confounding may still be present.

Conclusion

There was no evidence from this study to suggest that vaccination coverage up to 78% with 4CMenB results in a reduction of disease-associated *N. meningitidis* carriage in older teenagers. This finding is consistent with earlier studies and reaffirms that 4CMenB immunisation programs should focus on direct (individual) protection for groups at greatest risk of meningococcal disease.

Declaration of interests:

HSM is an investigator on vaccine trials sponsored by Industry (the GSK group of companies, Novavax, Pfizer). HM's and MMc's institution receives funding for investigator-led studies from Industry (Pfizer, the GSK group of companies). HM and MMc receive no personal payments from Industry. RB performs contract research on behalf of Public Health England for the GSK group of companies, Pfizer and Sanofi Pasteur. The Immunisation Department where MR and SL are employed has provided vaccine manufacturers with post-marketing surveillance reports on pneumococcal and meningococcal infection which the companies are required to submit to the UK Licensing authority in compliance with their Risk Management Strategy. A cost recovery charge is made for these reports. PR is an investigator on vaccine trials sponsored by Industry (the GSK group of companies, Novavax, Pfizer). PR's institution receives funding for investigator-led studies from Industry (Pfizer, the GSK group of companies, CSL). PR has been a member of scientific vaccine advisory boards for Industry (Pfizer, the GSK group of companies, Sanofi) but has not received any personal payments from Industry. Between May 2015 and May 2019 AF was President of the European Society for Paediatric Infectious Diseases which, during this period, received sponsorship from GSK for its annual congress. AF does advisory work related to vaccines for the UK government, the World Health Organisation and several companies developing vaccines. He also leads clinical trials of vaccines funded by the UK government, charities and vaccine manufacturers. AF receives no personal remuneration or benefits in kind for any of this work apart from his salary via the University of Bristol from the Higher Education Funding Council and the NHS. AF is a member of the UK Department of Health's Joint Committee on Vaccination, Chair of the WHO European Technical

Advisory Group of Experts in which capacity he attends SAGE.. JW was employed by the GSK group of companies throughout the course of this work. She no longer works at GSK and is now an independent consultant. KV is anemployee of the GSK group of companies and holds shares in the GSK group of companies as part of his employee remuneration.

Contributions:

All authors were involved in the design of the study. MMc participated in the data collection, entry and cleaning. The analysis plan was written by MMc, HM, TS, JB and conducted by JB and MMc, with all authors involved in the interpretation. MMc wrote the draft manuscript. All authors reviewed and approved the final version for publication.

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REFERENCES

1. Borrow R, Alarcon P, Carlos J, et al. The Global Meningococcal Initiative: global epidemiology, the impact of vaccines on meningococcal disease and the importance of herd protection. Expert Rev Vaccines **2017**; 16:313-28.

2. Chang Q, Tzeng YL, Stephens DS. Meningococcal disease: changes in epidemiology and prevention. Clin Epidemiol **2012**; 4:237-45.

3. DeVoe IW. The meningococcus and mechanisms of pathogenicity. Microbiol Rev **1982**; 46:162-90.

4. Rouphael NG, Stephens DS. *Neisseria meningitidis*: biology, microbiology, and epidemiology. Methods Mol Biol **2012**; 799:1-20.

5. McNamara LA, Potts CC, Blain A, et al. Invasive Meningococcal Disease due to Nongroupable *Neisseria meningitidis*-Active Bacterial Core Surveillance Sites, 2011-2016. Open Forum Infect Dis **2019**; 6:ofz190.

6. Jones DM, Borrow R, Fox AJ, Gray S, Cartwright KA, Poolman JT. The Lipooligosaccharide Immunotype as a Virulence Determinant in *Neisseria Meningitidis*. Microb Pathog **1992**; 13:219-24.

7. Maiden MC, Stuart JM, Group UKMC. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. Lancet **2002**; 359:1829-31.

8. Jones GR, Williams JN, Christodoulides M, Jolley K, Heckels JE. Lack of immunity in university students before an outbreak of serogroup C meningococcal infection. J Infect Dis **2000**; 181:1172-5.

9. Clark SA, Borrow R. Herd Protection against Meningococcal Disease through Vaccination. Microorganisms **2020**; 8.

10. Vianzon V, Illek B, Moe GR. Effect of Vaccine-Elicited Antibodies on Colonization of *Neisseria meningitidis* Serogroup B and C Strains in a Human Bronchial Epithelial Cell Culture Model. Clin Vaccine Immunol **2017**; 24:e00188-17.

11. Marshall HS, McMillan M, Koehler AP, et al. Meningococcal B Vaccine and Meningococcal Carriage in Adolescents in Australia. N Engl J Med **2020**; 382:318-27.

12. McNamara LA, Thomas JD, MacNeil J, et al. Meningococcal Carriage Following a Vaccination Campaign With MenB-4C and MenB-FHbp in Response to a University Serogroup B Meningococcal Disease Outbreak-Oregon, 2015-2016. J Infect Dis **2017**; 216:1130-40.

13. Read RC, Baxter D, Chadwick DR, 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**; 384:2123-31.

14. Meningococcal B Immunisation Program. Available at: *https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/conditions/immunisation/immunisation+programs/meningococcal+b+immunisation+program*. Accessed 5th April 2021.

15. 3101.0 - Australian Demographic Statistics, Mar 2019. Available at: *https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Mar%202019?OpenDocument*. Accessed 11th December 2019.

16. Statistics and reports about schools, preschools, students and staff. Available at: *https://www.education.sa.gov.au/department/about-department/statistics-and-reports-about-sites-students-and-staff*. Accessed 31st March 2021.

17. Education and Work, Australia. Available at: *https://www.abs.gov.au/statistics/people/education/education-and-work-australia/latest-release*. Accessed 31st March 2021.

18. Marshall HS, McMillan M, Koehler A, et al. B Part of It School Leaver protocol: an observational repeat cross-sectional study to assess the impact of a meningococcal serogroup B (4CMenB) vaccine programme on carriage of *Neisseria meningitidis*. BMJ Open **2019**; 9:e027233.

19. Medicare. Available at: *https://www.health.gov.au/health-topics/medicare*. Accessed 8th December 2020.

20. 1270.0.55.005 - Australian Statistical Geography Standard (ASGS): Volume 5 - Remoteness Structure, July 2016 Available at:

https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1270.0.55.005July%202016?OpenDocument. Accessed 14th October 2020.

21. McMillan M, Walters L, Mark T, et al. B Part of It study: a longitudinal study to assess carriage of *Neisseria meningitidis* in first year university students in South Australia. Hum Vaccin Immunother **2019**; 15:987-94.

22. Jolley KA, Maiden MC. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics **2010**; 11:595.

23. Fitzpatrick PE, Salmon RL, Hunter PR, Roberts RJ, Palmer SR. Risk factors for carriage of *Neisseria meningitidis* during an outbreak in Wales. Emerg Infect Dis **2000**; 6:65-9.

24. Stata Statistical Software. 15 ed: College Station, TX: StataCorp LP.

25. Study to Assess Oropharyngeal Carriage of *N. Meningitidis* in South Australian School Leavers (B Part Of It). Available at: *https://clinicaltrials.gov/ct2/show/NCT03419533*. Accessed 2nd November 2020.

26. McMillan M, Chandrakumar A, Wang HLR, et al. Effectiveness of Meningococcal Vaccines at Reducing Invasive Meningococcal Disease and Pharyngeal *Neisseria meningitidis* Carriage: A Systematic Review and Meta-analysis. Clin Infect Dis **2021**; 73:e609-e19.

27. Zhang Q, Finn A. Mucosal immunology of vaccines against pathogenic nasopharyngeal bacteria. J Clin Pathol **2004**; 57:1015-21.

28. van Ravenhorst MB, den Hartog G, van der Klis FRM, van Rooijen DM, Sanders EAM, Berbers GAM. Induction of salivary antibody levels in Dutch adolescents after immunization with monovalent meningococcal serogroup C or quadrivalent meningococcal serogroup A, C, W and Y conjugate vaccine. PLoS One **2018**; 13:e0191261.

29. Ladhani SN, Campbell H, Andrews N, et al. First real world evidence of meningococcal group B vaccine, 4CMenB, protection against meningococcal group W disease; prospective enhanced national surveillance, England. Clin Infect Dis **2020**.

30. Biolchi A, De Angelis G, Moschioni M, et al. Multicomponent meningococcal serogroup B vaccination elicits cross-reactive immunity in infants against genetically diverse serogroup C, W and Y invasive disease isolates. Vaccine **2020**; 38:7542-50.

31. Hong E, Giuliani MM, Deghmane AE, et al. Could the multicomponent meningococcal serogroup B vaccine (4CMenB) control *Neisseria meningitidis* capsular group X outbreaks in Africa? Vaccine **2013**; 31:1113-6.

32. Fazio C, Biolchi A, Neri A, et al. Cross-reactivity of 4CMenB vaccine-induced antibodies against meningococci belonging to non-B serogroups in Italy. Hum Vaccin Immunother **2021**; 17:2225-31.

33. Ladhani SN, Giuliani MM, Biolchi A, et al. Effectiveness of Meningococcal B Vaccine against Endemic Hypervirulent *Neisseria meningitidis* W Strain, England. Emerg Infect Dis **2016**; 22:309-11.

34. Mowlaboccus S, Mullally CA, Richmond PC, et al. Differences in the population structure of *Neisseria meningitidis* in two Australian states: Victoria and Western Australia. PLoS One **2017**; 12:e0186839.

35. Mowlaboccus S, Perkins TT, Smith H, et al. Temporal Changes in BEXSERO(R) Antigen Sequence Type Associated with Genetic Lineages of *Neisseria meningitidis* over a 15-Year Period in Western Australia. PLoS One **2016**; 11:e0158315.

36. MacLennan J, Kafatos G, Neal K, et al. Social behavior and meningococcal carriage in British teenagers. Emerg Infect Dis **2006**; 12:950-7.

37. Breakwell L, Whaley M, Khan UI, et al. Meningococcal carriage among a university student population - United States, 2015. Vaccine **2018**; 36:29-35.

38. Soeters HM, Whaley M, Alexander-Scott N, et al. Meningococcal Carriage Evaluation in Response to a Serogroup B Meningococcal Disease Outbreak and Mass Vaccination Campaign at a College-Rhode Island, 2015-2016. Clin Infect Dis **2017**; 64:1115-22.

39. van Ravenhorst MB, Bijlsma MW, van Houten MA, et al. Meningococcal carriage in Dutch adolescents and young adults; a cross-sectional and longitudinal cohort study. Clin Microbiol Infect **2017**; 23:573 e1- e7.

40. Watle SV, Caugant DA, Tunheim G, et al. Meningococcal carriage in Norwegian teenagers: strain characterisation and assessment of risk factors. Epidemiol Infect **2020**; 148:e80.

41. Household crowding. WHO Housing and Health Guidelines. Geneva: World Health Organization, **2018**.

42. MacLennan JM, Rodrigues CMC, Bratcher HB, et al. Meningococcal carriage in periods of high and low invasive meningococcal disease incidence in the UK: comparison of UKMenCar1-4 cross-sectional survey results. Lancet Infect Dis **2021**; 21:677-87.

43. Rodrigues CMC, Jolley KA, Smith A, Cameron JC, Feavers IM, Maiden MCJ. Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) Index: a Rapid and Accessible Tool That Exploits Genomic Data in Public Health and Clinical Microbiology Applications. J Clin Microbiol **2020**; 59.

Table 1. Baseline characteristics for 2018, 2019, and 2020 school leavers

	2018 (N = 4107)	**2019 (N = 2695)**	**2020** (N = 1343)
Timing of swab			
Jan-March	2474 (60.2)	1582 (58.7)	741 (55.2)
April-June	1534 (37.4)	573 (21.3)	0 (0.0)
July-Sept	99 (2.4)	365 (13.5)	602 (44.8)
Oct-Dec	0 (0.0)	175 (6.5)	0 (0.0)
4CMenB vaccination			
Yes, <=12 months since dose 2	1708 (41.6)	728 (27.0)	13 (1.0)
Yes, >12 months since dose 2	70 (1.7)	1384 (51.4)	1011 (75.3)
Partial (1 dose only)	49 (1.2)	64 (2.4)	42 (3.1)
No	2280 (55.5)	519 (19.3)	277 (20.6)
MenACWY vaccination	55 (1.3)	75 (2.8)	278 (20.7)
Participation in the 'B Part of It' RCT	3760 (91.6)	2121 (78.7)	988 (73.6)
Age in years: mean (SD)	18.45 (0.7)	18.49 (0.5)	18.55 (0.60)
Gender (Female)	2400 (58.4)	1660 (61.6)	810 (60.3)
Smoking in the last week	97 (2.4)	79 (2.9)	32 (2.4)
Smoked e-Cigarette in the last week	55 (1.3)	106 (3.9)	18 (1.3)
Smoked water pipe in last month	192 (4.7)	127 (4.7)	30 (2.2)
Drank alcohol in the last month	2846 (69.3)	1866 (69.2)	937 (69.8)
Remoteness Area			
Major Cities of Australia	2928 (71.3)	2192 (81.3)	1142 (85.0)
Inner Regional Australia	680 (16.6)	339 (12.6)	148 (11.0)
Outer Regional Australia	320 (7.8)	76 (2.8)	26 (1.9)
Remote Australia	126 (3.1)	32 (1.2)	6 (0.4)
Very Remote Australia	47 (1.1)	3 (0.1)	1 (0.1)
Missing	6 (0.1)	53 (2.0)	20 (1.5)
Index of Socio-economic Disadvantage			
1 (most disadvantaged)	832 (20.3)	429 (15.9)	212 (15.8)
2	668 (16.3)	405 (15.0)	189 (14.1)
3	679 (16.5)	466 (17.3)	226 (16.8)
4	841 (20.5)	542 (20.1)	284 (21.1)
5 (least disadvantaged)	1081 (26.3)	799 (29.7)	412 (30.7)
Missing	6 (0.1)	54 (2.0)	20 (1.5)
Ethnicity			
Aboriginal	60 (1.5)	25 (0.9)	20 (1.5)
Torres Strait Islander	7 (0.2)	5 (0.2)	8 (0.6)
Caucasian	3063 (74.6)	1868 (69.3)	893 (66.5)
Asian	440 (10.7)	467 (17.3)	287 (21.4)
Middle Eastern	66 (1.6)	58 (2.2)	19 (1.4)
African	66 (1.6)	49 (1.8)	19 (1.4)
Pacific Islander	6 (0.2)	11 (0.4)	0 (0.0)
Other	231 (5.6)	131 (4.9)	62 (4.6)
Missing	168 (4.1)	81 (3.0)	35 (2.6)

* Ns (%) presented unless otherwise indicated.

Table 2. *N. meningitidis* carriage prevalence for 2018, 2019 and 2020 school leavers

***N. meningitidis* carriage**	**2018 (N = 4104) N (%)**	**2019 (N = 2690) N (%)**	**2020 (N = 1338) N (%)**	**Total (N = 8132) N (%)**
Disease-associated (A, B, C, W, X, Y)†	225 (5.48)	134 (4.98)	68 (5.08)	427 (5.25)
Any	393 (9.58)	227 (8.44)	106 (7.92)	726 (8.93)
Genogroup B	86 (2.10)	64 (2.38)	43 (3.21)	193 (2.37)
Genogroup A	0 (0)	0 (0)	0 (0)	0 (0)
Genogroup C	16 (0.39)	8 (0.30)	3 (0.22)	27 (0.33)
Genogroup W	21 (0.51)	4 (0.15)	3 (0.22)	28 (0.34)
Genogroup X	1 (0.02)	6 (0.22)	2 (0.15)	9 (0.11)
Genogroup Y	105 (2.56)	57 (2.12)	31 (2.32)	193 (2.37)
Genogroup E	23 (0.56)	7 (0.26)	4 (0.30)	34 (0.42)
Non-groupable*	168 (4.09)	93 (3.46)	38 (2.84)	299 (3.68)

* 'non-groupable' carriage is defined as failure to detect genogroup A, B, C, W, X, or Y, in those with porA detected. † Less than the individual serogroup total because more than one isolate has been detected in some participants.

Table 3 Unadjusted and adjusted analyses of *N. meningitidis* carriage in school leavers in 2020 and 2019 compared to 2018 (N=8132)

Genogroup	Effect	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)*	p-value	Adjusted OR (95% CI)†	p-value
Disease-associated			0.63#		0.71#		0.25#
	2019 vs 2018	0.90 (0.73, 1.13)	0.37	0.91 (0.72, 1.14)	0.40	0.82 (0.64, 1.05)	0.12
	2020 vs 2018	0.92 (0.70, 1.22)	0.57	0.96 (0.71, 1.30)	0.79	0.82 (0.57, 1.17)	0.27
Any *N. meningitidis*			0.10#		0.19#		0.29#
	2019 vs 2018	0.87 (0.73, 1.03)	0.11	0.86 (0.72, 1.03)	0.10	0.86 (0.71, 1.04)	0.12
	2020 vs 2018	0.81 (0.65, 1.02)	0.07	0.85 (0.66, 1.09)	0.20	0.89 (0.66, 1.18)	0.42
Genogroup B			0.07#		0.14#		0.81#
	2019 vs 2018	1.14 (0.82, 1.58)	0.44	1.13 (0.80, 1.58)	0.49	0.98 (0.67, 1.42)	0.90
	2020 vs 2018	1.55 (1.07, 2.25)	0.02	1.51 (1.00, 2.29)	0.05	1.14 (0.69, 1.89)	0.60
Genogroup C‡			0.62#				
	2019 vs 2018	0.76 (0.33, 1.78)	0.53				
	2020 vs 2018	0.57 (0.17, 1.97)	0.38				
Genogroup W‡			0.04#				
	2019 vs 2018	0.29 (0.10, 0.84)	0.02				
	2020 vs 2018	0.44 (0.13, 1.47)	0.18				
Genogroup X‡			0.12#				
	2019 vs 2018	9.17 (1.10, 76.23)	0.04				
	2020 vs 2018	6.14 (0.56, 67.79)	0.14				
Genogroup Y			0.50#		0.67#		0.28#
	2019 vs 2018	0.82 (0.60, 1.14)	0.25	0.86 (0.61, 1.20)	0.37	0.77 (0.53, 1.10)	0.15
	2020 vs 2018	0.90 (0.60, 1.36)	0.62	0.94 (0.61, 1.47)	0.80	0.74 (0.44, 1.24)	0.25
Genogroup E			0.15#				
	2019 vs 2018	0.46 (0.20, 1.08)	0.08				
	2020 vs 2018	0.53 (0.18, 1.54)	0.24				
Non-groupable§			0.08#		0.15#		0.87#
	2019 vs 2018	0.84 (0.65, 1.09)	0.18	0.82 (0.62, 1.07)	0.15	0.93 (0.70, 1.25)	0.64
	2020 vs 2018	0.68 (0.48, 0.98)	0.04	0.72 (0.49, 1.07)	0.10	1.02 (0.65, 1.60)	0.93

* Adjusted for MenACWY vaccination status, cigarette smoking, smoked water pipe in last month, days out at pub/club in last week, people kissed in last week, ethnicity, drank alcohol in last month and presence of cold or sore throat (based on N=7575 observations). † Adjusted for the same variables as the original model plus age, timing of swab by quarter, remoteness area, socio-economic status (based on N=7513 observations). ‡ Adjusted analysis not performed due to small number of observations. § *PorA* detected and failure to detect genogroup A, B, C, W, X, or Y. # Global p-value from a multiple-parameter Wald test)

Table 4. Unadjusted and adjusted analyses of *N. meningitidis* carriage in 4CMenB vaccinated school leavers compared to 4CMenB unvaccinated school leavers

***N. meningitidis* genogroup**	**Vaccinated n (%)** (N = 4904)		**Unvaccinated n (%)** (N = 3228)		**Unadjusted OR (95% CI)**	**p-value**	**Adjusted OR* (95% CI)**	**p-value**	**Adjusted OR† (95% CI)**	**p-value**
Disease-associated	252	(5.14)	175	(5.42)	0.95 (0.78, 1.15)	0.58	0.85 (0.69, 1.05)	0.13	0.84 (0.68, 1.04)	0.11
Any group	421	(8.58)	305	(9.45)	0.90 (0.77, 1.05)	0.18	0.82 (0.70, 0.97)	0.02	0.83 (0.70, 0.98)	0.03
Group B	118	(2.41)	75	(2.32)	1.04 (0.77, 1.39)	0.81	0.91 (0.67, 1.24)	0.55	0.87 (0.63, 1.20)	0.39
Group C‡	13	(0.27)	14	(0.43)	0.61 (0.29, 1.30)	0.20				
Group W‡	11	(0.22)	17	(0.53)	0.42 (0.20, 0.91)	0.03				
Group X‡	8	(0.16)	1	(0.03)	5.27 (0.66, 42.18)	0.12				
Group Y	119	(2.43)	74	(2.29)	1.06 (0.79, 1.42)	0.70	0.96 (0.71, 1.30)	0.79	0.93 (0.68, 1.27)	0.65
Group E‡	17	(0.35)	17	(0.53)	0.66 (0.34, 1.29)	0.22				
Non-groupable§	169	(3.45)	130	(4.03)	0.85 (0.67, 1.07)	0.17	0.80 (0.63, 1.03)	0.08	0.84 (0.65, 1.08)	0.16

* Adjusted for MenACWY vaccination status, cigarette smoking, smoked water pipe in last month, days out at pub/club in last week, people kissed in last week, ethnicity, drank alcohol in last month and presence of cold or sore throat, (complete cases included in adjusted model n=7575). † Adjusted for the same as the previous model plus age, timing of swab by quarter, remoteness area, socio-economic status, (complete cases included in adjusted model n=7513). ‡ Adjusted analysis not performed due to small number of observations. § *PorA* detected and failure to detect genogroup A, B, C, W, X, or Y.

Table 5. Characteristics associated with carriage prevalence of disease-associated genogroups of *N. meningitidis* (A, B, C, W, X, Y) in South Australian school leavers

Characteristic	Level	Frequency	(%)	Adjusted OR‡ (95% CI)	p-value
Timing of swab (quarter)	Jan-March	218 / 4790	(4.55)	1	0.12†
	April-June	124 / 2105	(5.89)	1.17 (0.90, 1.51)	0.24
	July-Sept	74 / 1064	(6.95)	1.46 (1.06, 2.00)	0.02
	Oct-Dec	11 / 173	(6.36)	1.33 (0.66, 2.65)	0.42
Remoteness area	Major cities of Australia	324 / 6254	(5.18)	1	0.11†
	Inner Regional Australia	51 / 1165	(4.38)	0.82 (0.59, 1.13)	0.22
	Outer Regional Australia	33 / 422	(7.82)	1.67 (1.07, 2.61)	0.02
	Remote Australia	9 / 164	(5.49)	0.97 (0.45, 2.09)	0.93
	Very Remote Australia	3 / 49	(6.12)	1.00 (0.22, 4.50)	1.00
Index of Relative Socioeconomic	1 (most disadvantaged)	54 / 1471	(3.67)	1	0.37†
Disadvantage (IRSD) quintile	2	58 / 1260	(4.60)	1.09 (0.72, 1.66)	0.68
	3	62 / 1370	(4.53)	1.07 (0.72, 1.61)	0.74
	4	98 / 1665	(5.89)	1.32 (0.91, 1.93)	0.15
	5 (least disadvantaged)	148 / 2287	(6.47)	1.34 (0.93, 1.94)	0.12
Gender	Male	178 / 3223	(5.52)	1	0.64†
	Female	247 / 4864	(5.08)	0.91 (0.73, 1.13)	0.38
	Other*	0 / 9	(0.00)		
	Rather not say	1 / 17	(5.88)	1.42 (0.18, 11.45)	0.74
Age in years	-			1.19 (0.99, 1.42)	0.06
Current cold or sore throat	No	377 / 7296	(5.17)	1	
	Yes	43 / 737	(5.83)	0.93 (0.65, 1.32)	0.68
Antibiotics	None	377 / 7189	(5.24)	1	0.46†
	Stopped in last month	25 / 430	(5.81)	0.91 (0.58, 1.43)	0.68
	Stopped in last week	12 / 167	(7.19)	1.32 (0.69, 2.53)	0.39
	Currently taking	9 / 248	(3.63)	0.62 (0.30, 1.29)	0.20
MenACWY vaccination status	Unvaccinated	411 / 7765	(5.29)	1	
	Vaccinated	16 / 367	(4.36)	0.74 (0.43, 1.28)	0.28
Cigarette smoking	No	407 / 7891	(5.16)	1	
	Yes	18 / 208	(8.65)	0.94 (0.53, 1.69)	0.85
Smoked e-cigarette last week	No	415 / 7836	(5.30)	1	
	Yes	9 / 179	(5.03)	0.81 (0.40, 1.65)	0.56
Smoked water pipe last month	No	394 / 7713	(5.11)	1	
	Yes	31 / 349	(8.88)	1.49 (0.96, 2.30)	0.07
Days out at pub/club last week	0	148 / 4538	(3.26)	1	
	1 or more	274 / 3463	(7.91)	1.56 (1.23, 1.97)	<0.001
People kissed in last week	0	175 / 4838	(3.62)	1	
	1 or more	245 / 3044	(8.05)	2.22 (1.67, 2.94)	<0.001
Currently in a relationship	No	250 / 5422	(4.61)	1	
	Yes	172 / 2594	(6.63)	0.73 (0.55, 0.96)	0.02
Ethnicity	Caucasian	358 / 5816	(6.16)	1	0.01†
	Asian	26 / 1193	(2.18)	0.49 (0.31, 0.76)	0.002
	Aboriginal or Torres Strait Islander	7 / 122	(5.74)	1.29 (0.58, 2.90)	0.53
	Other	28 / 718	(3.90)	0.73 (0.47, 1.13)	0.16
Drank alcohol last month	No	39 / 2367	(1.65)	1	
	Yes	383 / 5641	(6.79)	2.33 (1.59, 3.40)	<0.001
Work status	Full-time work	35 / 471	(7.43)	1	0.06†
	Part-time work	42 / 583	(7.20)	0.87 (0.52, 1.46)	0.60
	Part-time work + study	87 / 1359	(6.40)	1.00 (0.63, 1.59)	0.99
	Full-time study	236 / 4791	(4.93)	0.90 (0.59, 1.38)	0.64
	Not working or studying	22 / 632	(3.48)	0.56 (0.31, 1.02)	0.06
	Other	2 / 245	(0.82)	0.17 (0.04, 0.71)	0.02
Number of persons/room	0-1	246 / 4018	(6.12)	1	0.04†
	>1 to ≤ 2	171 / 3850	(4.44)	0.76 (0.61, 0.94)	0.01
	>2	4 / 103	(3.88)	0.77 (0.23, 2.59)	0.67
Used mouthwash in last month	No	344 / 6602	(5.21)	1	
	Yes	80 / 1473	(5.43)	0.99 (0.75, 1.29)	0.92
Other smokers at home	No	327 / 6368	(5.14)	1	
	Yes	92 / 1559	(5.90)	1.12 (0.86, 1.45)	0.39

* Excluded from analysis due to insufficient variation in outcome. † Global p-value from a multiple-parameter Wald test (applicable for categorical characteristics with three or more levels). Analysis adjusted for all characteristics in the table, based on N=7260 participants with complete data for all included risk factors.

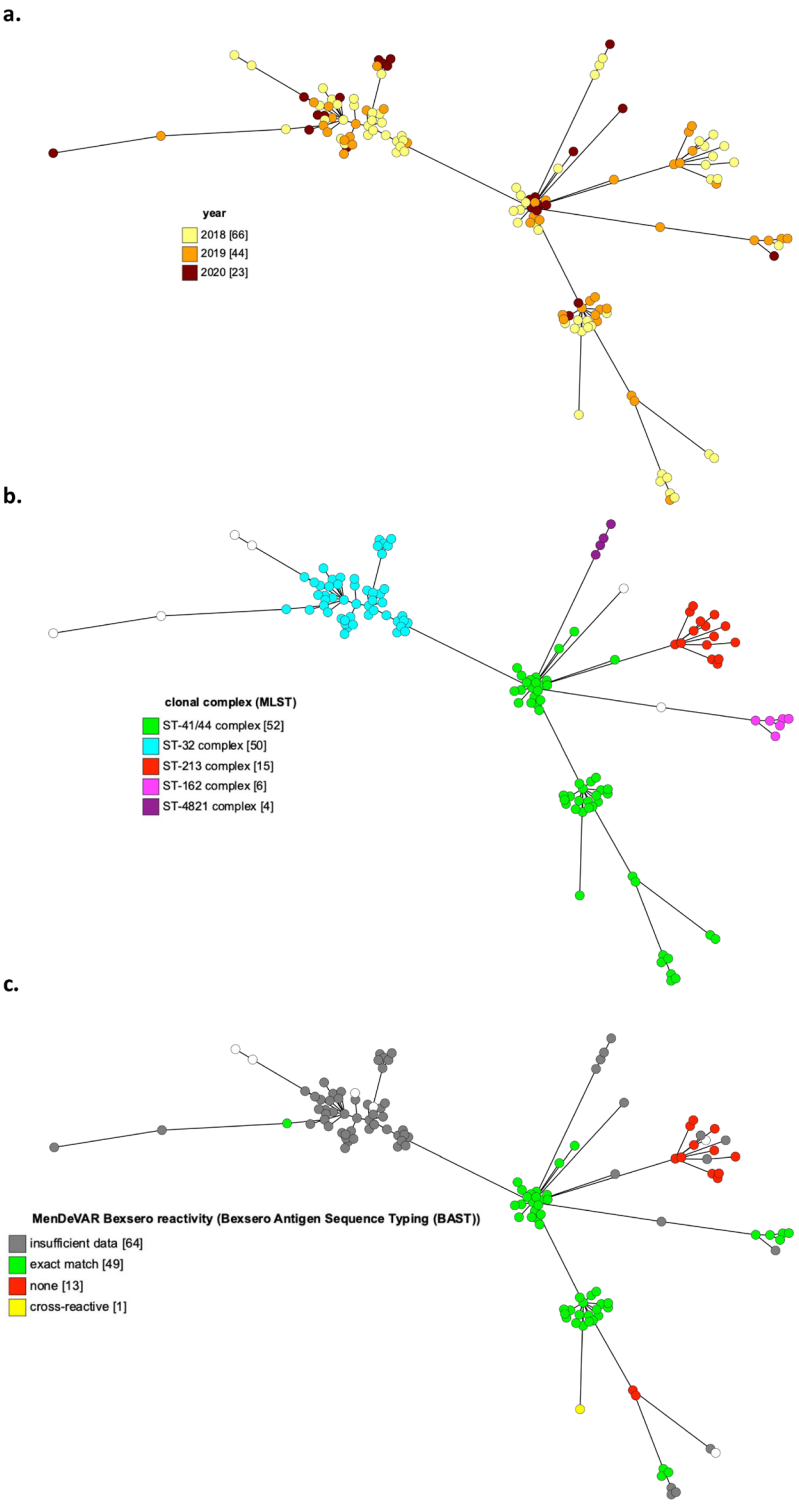

Figure 1. GrapeTree analysis using cgMLST v1.0 on the PubMLST.org/neisseria website of 133 genogroup B meningococci isolated from the 726 meningococcal positive participants. Annotated by: a. year of isolation; b. clonal complex; c. MenDeVar index.[43] Open circles indicate isolates with no value (i.e. unassigned to a cc or MenDeVAR).