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**Title: Impact of meningococcal B (4CMenB) vaccine on pharyngeal *Neisseria meningitidis* carriage density and persistence in adolescents**

**Running title: Meningococcal carriage density**

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**Key words:** Carriage, Density, Vaccines, Risk factors, Public Health

**Summary:**

There was no reduction in *N. meningitidis* carriage density in 4CMenB vaccinated students compared to unvaccinated students 12 months post vaccination, despite increased carriage clearance. Higher *N. meningitidis* carriage density is associated with a longer duration of carriage.

## **Abstract**

### **Background**

Higher density of *Neisseria meningitidis* carriage may be associated with transmission of the meningococcus. Our aim was to establish the impact of 4CMenB vaccine on *N. meningitidis* carriage density.

### **Methods**

We compared 4CMenB vaccine to control among 913 South Australian students aged approximately 15-18 years in a cluster randomized trial who had *N. meningitidis* carriage at 12 months.

Oropharyngeal swabs were collected at baseline and 12 months later to detect *N. meningitidis* carriage. Colony forming units per millilitre (CFU/ml) were estimated by generating a standard curve that plotted qPCR cycle threshold values against log-normalized CFU.

### **Results**

Among the 913 students with *N. meningitidis* carriage at 12 months, there was no difference in mean carriage density between the vaccinated (n=434, 3.80 log CFU/ml [SD 1.29]) and control group (n=479, 3.73 log CFU/ml [SD 1.30]; p=0.51). Higher *N. meningitidis* carriage density at baseline was associated with an increase in the odds of persistent carriage at 12 months (n=504, odds ratio per 1.0 log CFU/ml increase in density = 1.36 [95% CI, 1.17, 1.58], p<0.001). Students with baseline carriage who were vaccinated had decreased persistent *N. meningitidis* carriage at 12 months compared to unvaccinated students (82/186 [31%] vs 105/186 [43%], odds ratio 0.60 [95% CI, 0.40, 0.90], p=0.01)

### **Conclusion**

4CMenB vaccine did not reduce carriage density of *N. meningitidis* 12 months post vaccination, despite increased carriage clearance. Higher carriage density is likely to enable transmission through prolonged periods of population exposure.

**Clinical Trials Registration**

Clinicaltrials.gov NCT03089086

## BACKGROUND

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis*, remains an important cause of morbidity and mortality worldwide.[1, 2] Serogroups A, B, C, W, X, or Y are most commonly associated with disease.[1] A high proportion of IMD cases are due to serogroup B, this is especially so in Australia, Europe, North America, and South America.[3]

Asymptomatic pharyngeal carriage of *N. meningitidis* is common in the general population.[4] A large meta-analysis reports that *N. meningitidis* carriage prevalence increases in children and peaks around 19 years of age at approximately 24%.[5] Carriage prevalence in university settings especially in the Americas and European region is consistently high.[6] Serogroup B has been responsible for 11 outbreaks of IMD among university students and close contacts between 2008 and 2017 in the United States (US).[7, 8] In each outbreak since 2013, recombinant meningococcal vaccines have been used, as well as chemoprophylaxis of close contacts.[8]

The relationship between carriage and disease is not entirely clear.[9] However, a study of 21,000 teenagers aged 15-19 years in the United Kingdom (UK) recently reported a halving of *N. meningitidis* carriage rates over a 15 year duration, consistent with the reduction in disease incidence.[10] Changes in carriage prevalence may occur though natural variation, alteration in risk factors for carriage such as smoking, and social contact, [5, 10, 11] or introduction of meningococcal vaccines.[12] Lower than anticipated carriage prevalence in Australian university students (6.2%) was thought to be associated with low rates of cigarette smoking.[13] Carriage prevalence of less than 10% has also been observed in other global adolescent populations over recent years.[14-18]

Two novel recombinant vaccines Bexsero 4CMenB (GSK) and Trumenba MenB-FHbp (Pfizer) are now licenced in USA, Canada, Europe, and Australia against serogroup B disease. Recent randomized controlled trials provide evidence that 4CMenB has limited impact on the carriage prevalence and acquisition of disease-causing *N. meningitidis* in adolescents, including group B.[15, 19] Clearance of existing carriage may be important in populations with very high carriage prevalence or for outbreak responses.

Reporting *N. meningitidis* carriage just as absence / presence may miss potentially important reductions in carriage density that could be relevant when investigating vaccine impact. This is because reducing the density of bacteria colonising the pharynx may reduce the risk of transmission. Density of *N. meningitidis* pharyngeal carriage varies, with the majority of those colonised having relatively small amounts of bacteria present.[20, 21] Pneumococcal conjugate vaccines have demonstrated a reduction in carriage density of vaccine-type strains of pneumococci in a small number of studies,[22-24] but not all.[25, 26] Mucosal immune responses to recombinant vaccines may be different to that of conjugate vaccines.[27] To date, the impact of meningococcal vaccines on carriage density has not been established.

The impact of 4CMenB vaccine on carriage are important considerations for cost-effectiveness, implementation of vaccine programs, and future vaccine design. The aims of this study are to: 1) establish the impact of 4CMenB vaccine on *N. meningitidis* carriage density, 2) identify if carriage density is associated with persistence of carriage, 3) establish the impact of 4CMenB vaccine on carriage persistence, and 4) identify associations between participant characteristics and carriage density.

## **METHODS**

### **Study Design**

This analysis was derived from an investigator led, cluster randomized controlled trial designed to examine the impact of 4CMenB vaccine on carriage of disease-causing meningococci in adolescent school students.[15, 28] Participating schools were randomized to 4CMenB vaccination at baseline (intervention) or 12 months (control). The trial was sponsored by The University of Adelaide, designed and overseen by an Independent Scientific Advisory Committee and conducted in South Australia between 2017 and 2018 with support by a research grant from GlaxoSmithKline. Ethical approval was granted by the Women's and Children's Health Network Human Research Ethics Committee (HREC/16/WCHN/140).

### **Procedures/Measurements**

All 260 secondary schools in metropolitan, rural, and remote South Australia were invited to participate in the trial. Year 10 and 11 students were followed up in schools at 12 months, whereas the year 12 students only provided baseline data.

Oro-pharyngeal swabs were collected from the posterior pharynx using a standardised technique by trained nursing and medical staff using sterile flocked swabs at baseline (2017), and repeated at 12 months for year 10 and 11 students (2018).[15] The swabs were placed into vials containing 2 mL of skim milk, tryptone, glucose, and glycerol (STGG) transport medium (Thermo Scientific). A questionnaire designed to assess risks factors for carriage was completed prior to each swab collection.[28]

Each specimen was subjected to real-time PCR (rt-PCR) screening for the presence of specific meningococcal DNA using *porA* gene detection as described previously.[15] Samples



with cycle threshold (Ct) values  $\leq 50$  were considered detected. Further rt-PCR analysis was used on *porA* positive specimens to determine the genogroup of *N. meningitidis* detected (A, B, C, W, X, Y). They were classified as non-groupable if the capsule biosynthesis genes for these genogroups were not detected. Specimens with a positive *porA* PCR were cultured and resulting strains of *N. meningitidis* had whole genome sequencing analysis using methods described elsewhere.[13] Clonal complex is assigned based on the sequence type of the *Neisseria meningitidis* by their similarity to a central allelic profile according to the PubMLST database.[29]

To convert Ct values to an estimate of colony forming units (CFU) per ml, *N. meningitidis* *siaD* B positive stock control was cultured out and a suspension equal to a 0.5 McFarland turbidity standard was made. Serial dilutions were performed and plated out (4 plates per dilution), these were then diluted 1:1 with lysis buffer. Samples were then extracted and subjected to *porA* PCR (8x per dilution). A standard curve was plotted using qPCR Ct values against log-normalized value of colony forming units of *N. meningitidis*.

### **Statistical Analysis**

As this analysis is exploratory and involves the secondary use of data already collected in the high school RCT, no pre-specified sample size calculation was undertaken.

To investigate the effects of 4CMenB vaccination on carriage density, the mean difference in log CFU/ml between groups was estimated in students with carriage detected at 12 months. CFU/ml estimations were highly positively skewed so log transformations were used to better approximate the normal. Comparisons were made for any *N. meningitidis*, disease-causing *N. meningitidis*, and non-groupable *N. meningitidis* carriage density. The analyses were conducted using linear regression, with generalized estimating equations (GEE) used

to account for clustering at the school level. The association between overall carriage density at baseline and persistence of carriage 12 months later (yes/no) was evaluated using logistic GEEs, with adjustment for treatment group. All analyses were performed using Stata v14.[30]

## RESULTS

### Characteristics of participants

Baseline characteristics of the students that participated in the randomised control trial are described in Supplementary Table 1. 237 schools agreed to participate and 24,269 year 10 and 11 students (approximately 15-16 years) and 10,220 year 12 students (approximately 17-18 years of age) were enrolled following informed consent. Swabs were collected at visit one between 1<sup>st</sup> April - 30<sup>th</sup> June 2017, and visit two 1<sup>st</sup> February - 13<sup>th</sup> of July 2018. Both periods encompass autumn and the first month of winter. The mean length of time between swab 1 and swab 2 was 366 days (SD 24 days). Carriage data were available for 21,126 year 10 and 11 students at 12 months (87%) following the withdrawal of 43 students and loss to follow-up of 3,100 students. Overall *N. meningitidis* carriage at 12 months was 4.32%. Students with carriage at 12 months (n=913) were included in this density analysis (Figure 1). Of the 913 in this cohort, 423 (46%) were in year 10 and 490 (54%) were in year 11. For the analysis of baseline density and persistence of carriage, 504 participants (of a total of 658 who had carriage at baseline) who returned at 12 months were included, with 186/504 (37%) showing persistent carriage (Figure 1). Carriage density was similar for any *N. meningitidis* between 2017 (n=658, 3.70 log CFU/ml [SD 1.35]) and 2018 (n=913, 3.76 log CFU/ml [SD 1.30]).

### **Carriage density (vaccinated vs. unvaccinated)**

Carriage density of any *N. meningitidis* at 12 months was similar (Figure 2) between the vaccinated (n=434, 3.80 log CFU/ml [SD 1.29]) and control group students (n=479, 3.73 log CFU/ml [SD 1.30], difference = 0.07 [95% CI -0.13 to 0.26]; p=0.51). The observed densities of disease-causing *N. meningitidis* by individual genogroups (B, C, W, and Y) and for non-groupable *N. meningitidis* were also similar between the vaccinated and control students (Table 1). Of the 10,220 year 12 students participating in the high school RCT, 3,719 (36.4%) provided a 12-month swab as part of a separate cross-sectional school leaver study. A sensitivity analysis that included an extra 351 year 12 students with carriage detected at 12 months was consistent with the analysis of year 10s and 11s (Supplementary Table 2).

There was no significant difference in the carriage density of the two most common Clonal Complex groups causing invasive disease in South Australia, group B Clonal Complex 41/44 (vaccinated n=29, 4.23 log CFU/ml [SD 1.32] versus unvaccinated n=26, 4.07 log CFU/ml [SD 1.36], difference 0.15 [95% CI, -0.51 to 0.82]; p=0.65) and group B Clonal Complex 32 (vaccinated n=28, 4.65 log CFU/ml [SD 0.83] versus unvaccinated n=24, 4.69 log CFU/ml [SD 1.13], difference -0.04 [95% CI, -0.55 to 0.47]; p=0.88).

### **Density at baseline and carriage persistence (carriage persistence vs. loss)**

Approximately 37% (186/504) of participants with *N. meningitidis* carriage at baseline had persistent carriage at 12 months. For each one unit increase in the baseline log density measure, independently of treatment group the odds of having persistent *N. meningitidis* carriage increased by 36% (n=504, OR 1.36 [95% CI, 1.17 to 1.58], <0.001). A similar association was also present for disease-causing *N. meningitidis* carriage density (Table 2). The majority of the students who had persistent carriage also retained the same genogroup

at the 12 month visit as the baseline visit (group B 43/58 [74%], group C 2/2 [100%], group W 1/4 [25%], group Y 24/35 [69%], and non-groupables 77/88 [88%]).

Independent of baseline carriage density, students with baseline carriage who were vaccinated had decreased *N. meningitidis* carriage persistence at 12 months compared to unvaccinated students (Table 3). The decreased persistence was mostly in non-groupable *N. meningitidis*. Students with carriage detected at baseline who did not return for a 12 month swab (n=154) were more likely to be in the vaccinated group, smoke cigarettes and/or water-pipes, be older or have kissed someone in the last week compared to those who returned at 12 months. However, adjusting for these (predominantly fully observed) predictors of loss to follow-up in a sensitivity analysis had little impact on results.

#### **Risk factors for carriage density**

In a multivariable model, higher year level and kissing one or more people in the last week were associated with higher carriage density of any *N. meningitidis* at baseline. Identifying as 'other' ethnicity was associated with a reduced carriage density (Table 4).

#### **DISCUSSION**

This secondary analysis of students with carriage from a large cluster-randomized controlled trial did not find evidence of an impact of 4CMenB vaccine on *N. meningitidis* carriage density. Students with higher carriage density at baseline were more likely to have persistent carriage of *N. meningitidis* at 12 months. Although not an unexpected finding, it adds weight to the importance of reducing carriage density. Not only will reducing carriage density potentially decrease the risk of transmission, it is likely to also shorten the duration of carriage.

Salivary antibodies are thought to be key to reducing acquisition and colonization of *N. meningitidis*. Salivary IgA and IgG antibodies have been shown to peak at approximately 1 month and wane considerably 6 to 12 months following meningococcal conjugate and polysaccharide vaccination.[27, 31, 32] In a longitudinal study in the United Kingdom, pharyngeal swabs and saliva samples were taken from 416 students (295 completed three visits). Students received 4CMenB at visit 1 and 2, mean carriage density fell from 70 gene copies/ml in Sept-Dec to 34 gene copies/ml in Jan-March.[33] This small uncontrolled pilot study had a 3 month interval between vaccination and swabbing. In our cohort, it is possible that we may have missed a shorter impact on carriage density due to the longer 12 month interval between swabs.

Risk factors for *N. meningitidis* carriage in adolescents are reasonably well established but rarely examined in relation to carriage density. The association of kissing at least one person in the last week and higher carriage density is similar to carriage studies that have assessed the impact of social factors on carriage prevalence.[10, 11, 15] The numbers of participants smoking cigarettes, e-cigarettes, and water pipes were too small to draw any conclusions for these activities. Unlike previous studies that have investigated social risk factors for carriage,[10, 11, 18] age/year of schooling remained associated with carriage density after adjusting for social factors. This is similar to the findings of the RCT, where school year remained associated with carriage after adjusting for social risk factors.[15] In a carriage study in The Gambia, pharyngeal swabs were collected from 999 pupils aged 10 to 18 years with no association identified between age and *N. meningitidis* density.[34]

With only one 12 month time point in this study it is not possible to assess the average duration of carriage for participants. Approximately 37% of participants with carriage at

baseline had carriage detected at 12 months. The mean duration of carriage calculated in a longitudinal study of Belgian children was approximately 11.7 months. Serogroup B and C had the longest mean duration of carriage, 16.2 months and 18 months respectively.[35]

There was minimal change of genogroup in those with persistent carriage in our cohort.

It is of interest that there is a modest increase in carriage clearance in vaccinated students compared to unvaccinated students who had carriage at baseline. However, the clearance is greatest for non-groupable *N. meningitidis*. An exploratory analysis reported the risk of non-groupable *N. meningitidis* carriage was 29% lower in the vaccinated group than control group at 12 months.[15] Our finding indicates that the reduction at 12 months is due to a combination of clearance of carriage, as well as reduced acquisition. It remains unclear what role non-groupable *N. meningitidis* have in preventing acquisition of disease-causing *N. meningitidis*. Whilst it's possible that non-groupable carriage has some role in producing protective SBA titers, it is generally accepted that the most important correlate of protection against meningococcal infection is the presence of serum bactericidal activity against the invasive strain.[36, 37] In populations with low carriage prevalence similar to South Australia, it is likely that a much larger impact on clearance would be required to result in significantly reduced carriage prevalence. This is especially the case with carriage acquisition being similar between the vaccinated and unvaccinated students at 12 months following vaccination in our cohort.[15]

With saliva IgA and IgG circulation likely to peak approximately one month after vaccination, it is possible that this resulted in increased clearance of *N. meningitidis* from oral mucosa, without a lasting impact on acquisition after the initial antibody peak. Loss to follow up of approximately 23% of the participants who had carriage detected at baseline means that

caution needs to be taken when assessing the outcomes in relation to persistent carriage. The loss of 23% is close to double that of the year 10 and 11 cohort as a whole (12%).[15] Similar to this study, in a pilot longitudinal study we have previously reported that students at higher risk of carriage are more likely to be lost to follow up.[13] It is possible that carriage clearance is over estimated with those more likely to have carriage at 12 months excluded from this analysis. However, results were unchanged when predominantly fully observed baseline predictors of missing data were adjusted for.

The greatest limitation when examining carriage density is the variability in sampling and testing. The sensitivity of swabbing is estimated to be between 60–83%. [38] We used standard operating procedures and trained medical and nursing staff in swab technique to maximize consistency. Whilst some variation is to be expected, it is not likely to favor either the vaccinated or control groups.

In this study, the majority of participants had carriage density >50 CFU/ml, which is higher than previously reported.[20, 21] This is most likely due to inter-laboratory variation, including the use of *porA* primers designed specifically for this study, to maximise detection of *N. Meningitidis* carriage. Previous density studies have often used *ctrA* qPCR or *sodC* qPCR, which potentially have less sensitivity when used as the sole target primer. [39, 40] Within this study sample, the same qPCR testing methods were used and therefore differences between the vaccinated and unvaccinated groups are not affected.

Overall, the results of this study show that higher density is associated with persistent carriage. Important risk factors for higher carriage density are increasing year of schooling, and kissing one or more people in the last week. There appears to be no indication that

vaccinating students will reduce carriage density of disease-causing *N. meningitidis* 12 months following vaccination.

## NOTES

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**Conflict of Interest.** HM is supported by a NHMRC CDF APP1155066. HM is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer, Sanofi, Seqirus. and Merck). HM's and MM's institution receives funding for investigator led studies from Industry. HM and MM receive no personal payments from Industry. AF's institution is in receipt of research funding from GlaxoSmithKline, Pfizer and consultancy fees from Alios BioPharma/Johnson & Johnson, BioNet-Asia, Takeda, and VBI Vaccines. 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. LL, AK, AL, MT, TS, and RA report no conflict of interest.

**Author contributions.** HM, MM, AL, and AF designed the study. LW, MT, and AL performed rt-PCR. LL conducted WGS. MM, LW, LL, and MT acquired and entered data. MM and TS



conducted the analysis. All named authors were involved in the interpretation of data, critically reviewing the content, and have approved the final version for publication.

**Trademarks.** Bexsero is a trademark of the GSK group of companies, Trumenba is a trademark of Pfizer.

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**Table 1: Carriage Density (CFU/ml) of vaccinated vs unvaccinated year 10 and 11 students at 12 months.**

<b>Outcome</b>	<b>Vaccinated n</b>	<b>Vaccinated mean log CFU/ml (std)</b>	<b>Unvaccinated n</b>	<b>Unvaccinated mean log CFU/ml (std)</b>	<b>Mean Difference (95% CI)*</b>	<b>P-Value</b>
<b>Any carriage</b>	434	3.80 (1.29)	479	3.73 (1.30)	0.07 (-0.13 to 0.26)	0.51
<b>Disease-causing†</b>	255	3.95 (1.24)	250	3.91 (1.19)	0.04 (-0.19 to 0.27)	0.72
- Genotype B	124	3.98 (1.20)	114	4.01 (1.23)	-0.03 (-0.33 to 0.27)	0.85
- Genotype C	12	3.65 (0.89)	7	4.22 (0.90)	-0.58 (-1.39 to 0.23)	0.16
- Genotype W	17	3.35 (1.46)	18	4.15 (1.14)	-0.80 (-1.60 to 0.01)	0.05
- Genotype Y	95	4.05 (1.28)	113	3.76 (1.16)	0.29 (-0.15 to 0.73)	0.20
<b>Non-groupable</b>	179	3.57 (1.33)	229	3.53 (1.38)	0.04 (-0.28 to 0.36)	0.80

\* Linear regression, with generalized estimating equations used to account for clustering at the school level, † Disease-causing refers to all capsular genotypes identified (B, C, W, X, Y).

**Table 2: Odds of persistent carriage at 12 months per 1.0 log CFU/ml increase in baseline density in year 10 and 11s.**

<b>Outcome†</b>	<b>Baseline carriage</b>	<b>Persistent carriage n (%)</b>	<b>Odds Ratio (95% CI)</b>	<b>P-Value</b>
<b>Any Carriage</b>	504	186 (37%)	1.36 (1.17 to 1.58)	<0.001
<b>Disease-causing</b>	255	98 (38%)	1.29 (1.03 to 1.62)	0.03
- Genotype B	138	58 (42%)	1.18 (0.87 to 1.60)	0.28
- Genotype Y	99	35 (35%)	1.35 (0.96 to 1.90)	0.08
<b>Non-groupable</b>	249	88 (35%)	1.40 (1.16 to 1.69)	<0.001

\* Logistic regression, with generalized estimating equations used to account for clustering at the school level, within individuals, and adjusted for treatment group. † Groups C and W were not included due to their small numbers.



**Table 3: Proportion of year 10 and 11 students with persistent *N. meningitidis* carriage (yes/no) at 12 months, by vaccinated and unvaccinated groups.**

<b>Outcome</b>	<b>Vaccinated n (%)</b>	<b>Unvaccinated n (%)</b>	<b>Odds Ratio (95% CI)</b>	<b>P-value</b>
<b>Any carriage</b>	81 (31)	105 (43)	0.60 (0.40 to 0.90)	0.01
<b>Disease-causing</b>	44 (36)	54 (41)	0.83 (0.50 to 1.37)	0.46
- Genogroup B	28 (38)	30 (46)	0.73 (0.37 to 1.44)	0.36
- Genogroup C	1 (100)	1 (17)	-	-
- Genogroup W	1 (17)	3 (38)	0.33 (0.26 to 4.25)	0.40
- Genogroup Y	15 (35)	20 (36)	0.96 (0.45 to 2.05)	0.93
<b>Non-groupable</b>	37 (27)	51 (46)	0.43 (0.23 to 0.80)	0.01

Logistic regression, with generalized estimating equations used to account for clustering at the school level and within individuals.

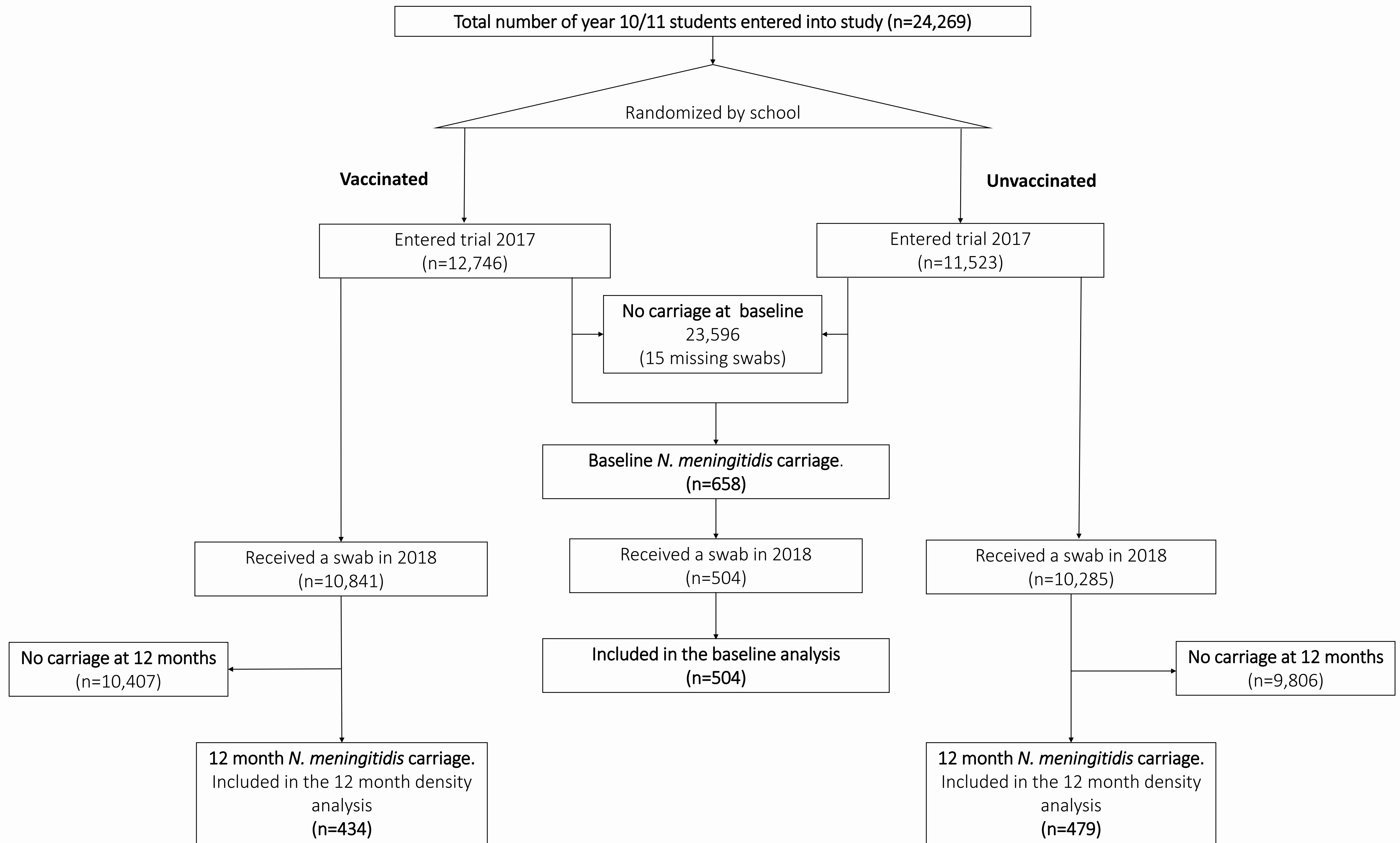
**Table 4: Multivariable analysis of risk factors for carriage density of any *N. meningitidis* in 10s 11s and 12s at baseline (n=1,222)**

Characteristic	Level	Number (%)	Log CFU/ml mean (SD)	Adjusted log CFU/ml Mean Difference (95% CI)	Adjusted P-value
Year of schooling	10	281 (23)	3.49 (1.32)	1	0.02*
	11	378 (31)	3.85 (1.35)	0.32 (0.10 to 0.55)	0.004
	12	563 (46)	3.85 (1.29)	0.23 (0.01 to 0.45)	0.04
Sex	Female	633 (52)	3.75 (1.34)	1	
	Male	589 (48)	3.78 (1.30)	0.04 (-0.12 to 0.20)	0.61
School ICSEA category <sup>†</sup>	<970 (low)	262 (21)	3.55 (1.27)	1	0.11*
	970 to 1020 (medium)	357 (29)	3.79 (1.32)	0.21 (0.00 to 0.43)	0.05
	>1020 (high)	603 (49)	3.84 (1.34)	0.20 (-0.03 to 0.42)	0.09
School size	<60 students/year	200 (16)	3.86 (1.36)	1	0.34*
	60 to 119 students/year	403 (33)	3.80 (1.31)	-0.12 (-0.44 to 0.19)	0.44
	>119 students/year	619 (51)	3.71 (1.32)	-0.21 (-0.51 to 0.09)	0.17
School location	Metropolitan	861 (70)	3.78 (1.31)	1	
	Rural	361 (30)	3.72 (1.35)	-0.07 (-0.29 to 0.15)	0.53
Antibiotics	Not taken in the past month	1,031 (85)	3.78 (1.31)	1	0.17*
	Taken in the last month	80 (7)	3.90 (1.53)	0.13 (-0.23 to 0.49)	0.49
	Stopped in the last week	43 (4)	3.72 (1.27)	-0.10 (-0.53 to 0.34)	0.66
	YES, currently taking	58 (5)	3.38 (1.36)	-0.38 (-0.72 to -0.03)	0.03
Current cold or sore throat		327 (27)	3.73 (1.40)	0.00 (-0.17 to 0.18)	0.96
Cigarette in the last week		96 (8)	3.67 (1.19)	-0.06 (-0.41 to 0.29)	0.75
E-cigarette in the last week		39 (3)	3.63 (1.27)	-0.05 (-0.41 to 0.32)	0.80
Water-pipe in the last week		116 (10)	3.78 (1.27)	0.11 (-0.20 to 0.42)	0.49
Out in last week		452 (37)	3.92 (1.33)	0.12 (-0.06 to 0.31)	0.20
Kissing in last week		487 (41)	3.93 (1.31)	0.22 (0.05 to 0.40)	0.01
Boarding student		54 (4)	3.68 (1.44)	-0.15 (-0.55 to 0.25)	0.47
Ethnicity	Caucasian	903 (76)	3.84 (1.31)	1	0.04*
	Aboriginal/TSI <sup>‡</sup>	61 (5)	3.53 (1.32)	-0.09 (-0.49 to 0.31)	0.66
	Asian	64 (5)	3.44 (1.42)	-0.36 (-0.74 to 0.03)	0.07
	Other <sup>§</sup>	165 (14)	3.57 (1.29)	-0.23 (-0.45 to -0.02)	0.03

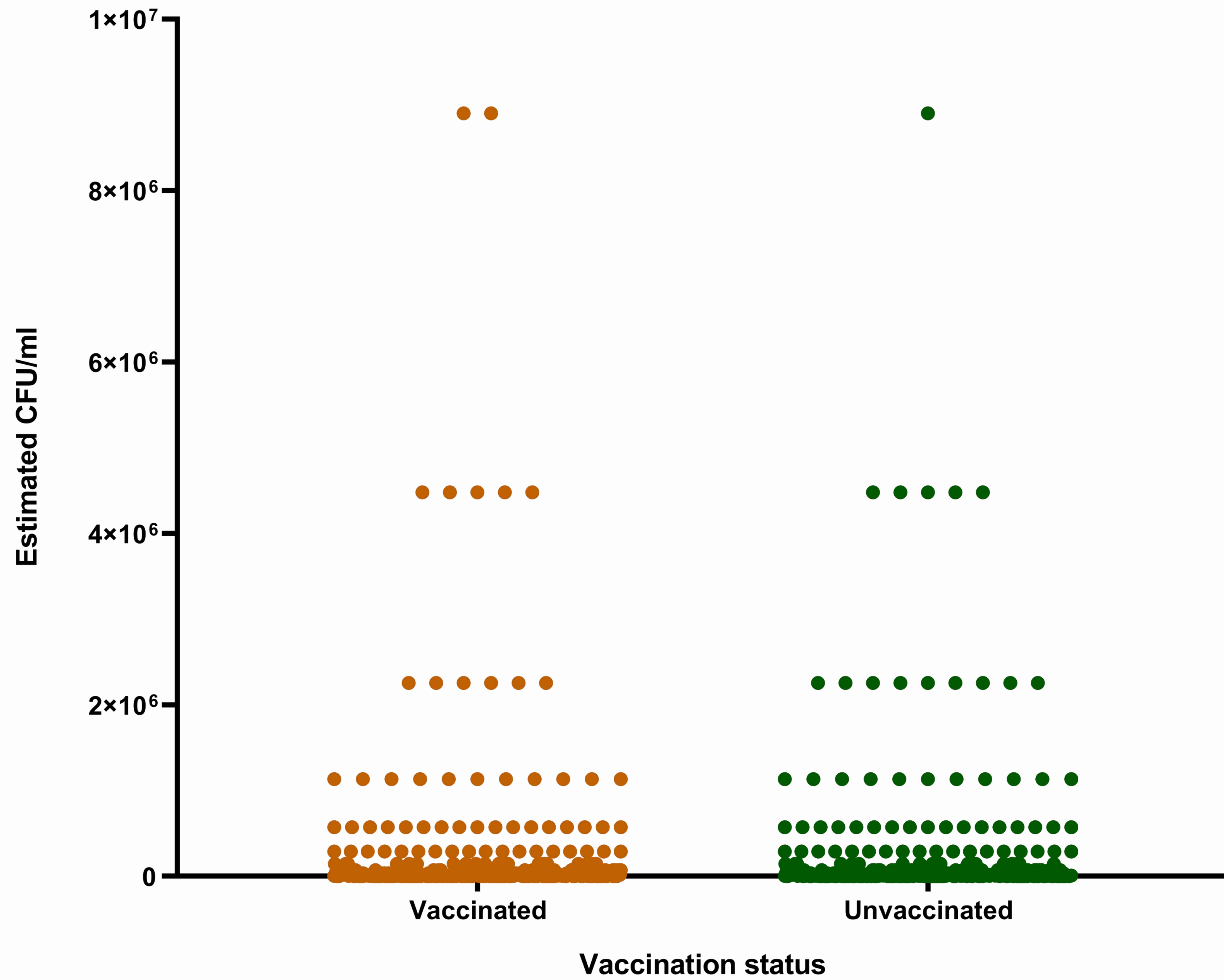
\* Global p-value, † Index of Community Socio-Educational Advantage, ‡ Aboriginal and/or Torres Strait Islander, § Combined with Middle Eastern, Pacific Islander, and African because of the small numbers in each category.

**Figure 1: Flow of subjects recruited in the study and included in the density analysis.**

**Figure 2: Estimated CFU/ml density by vaccination status at 12 months**



Density by vaccination status



## Supplementary Appendix

**Supplementary Table 1. Baseline characteristics for year 10 and 11 students.**

<b>Characteristic</b>	<b>Vaccinated group N=12746</b>	<b>Unvaccinated group N=11523</b>
<b>School ICSEA category: n (%)*</b>		
<970 (low)	2175 (17.06)	2471 (21.44)
970 to 1020 (medium)	3763 (29.52)	3601 (31.25)
>1020 (high)	6808 (53.41)	5451 (47.31)
<b>School size: n (%)</b>		
<60 students/year (small)	2112 (16.57)	1536 (13.33)
60 to 119 students/year (medium)	4181 (32.80)	3903 (33.87)
>119 students/year (large)	6453 (50.63)	6084 (52.80)
<b>School location: n (%)</b>		
Metropolitan	9829 (77.11)	8147 (70.70)
Rural	2917 (22.89)	3376 (29.30)
<b>Age - years: mean (sd)</b>		
	15.6 ( 0.7)	15.6 ( 1.2)
<b>Sex: n (%)</b>		
Female	6670 (52.33)	5795 (50.29)
Male	6076 (47.67)	5728 (49.71)
<b>Year of schooling: n (%)</b>		
10	6576 (51.59)	6188 (53.70)
11	6170 (48.41)	5335 (46.30)
<b>Smoking (cigarettes/day): n/ total n (%)</b>		
None	12457/12666 (98.35)	11273/11454 (98.42)
1-5	153/12666 ( 1.21)	140/11454 ( 1.22)
6-10	37/12666 ( 0.29)	32/11454 ( 0.28)
>10	19/12666 ( 0.15)	9/11454 ( 0.08)
<b>Currently taking antibiotics: n/ total n (%)</b>		
	620/12589 ( 4.92)	574/11374 ( 5.05)
<b>Boarding student: n/ total n (%)</b>		
	340/12686 ( 2.68)	190/11469 ( 1.66)
<b>Smoked e-cigarette in last week: n/ total n (%)</b>		
	127/12626 ( 1.01)	127/11,408 ( 1.11)
<b>Smoked water-pipe in last week: n/ total n (%)</b>		
	369/12626 ( 2.92)	281/11406 ( 2.46)
<b>Ethnicity: n/ total n (%)</b>		
Caucasian	9089/12509 (72.66)	7962/11314 (70.37)
Aboriginal and/or Torres Strait Islander	366/12509 (2.93)	313/11314 (2.77)
Asian	1216/12509 (9.72)	1173/11314 (10.37)
Other	1838/12509 (14.69)	1866/11314 (16.49)

\* Index of Community Socio-Educational Advantage

## Supplementary Appendix

Supplementary Table 2: Carriage Density (CFU/ml) of Vaccinated vs Unvaccinated year 10, 11, 12 students at 12 months.

<i>Outcome</i>	<i>Vaccinated n</i>	<i>Vaccinated mean log CFU/ml (std)</i>	<i>Unvaccinated n</i>	<i>Unvaccinated mean log CFU/ml (std)</i>	<i>Mean Difference * (95% CI)</i>	<i>P- Value</i>
<b>Any carriage</b>	(607)	3.96 [1.33]	(657)	3.92 [1.36]	0.04 (-0.14 to 0.21)	0.67
<b>Disease-causing†</b>	(360)	4.12 [1.24]	(345)	4.11 [1.27]	0.01 (-0.19 to 0.21)	0.93
- Genotype B	(164)	4.12 [1.24]	(152)	4.16 [1.25]	-0.03 (-0.30 to 0.23)	0.80
- Genotype C	(19)	3.98 [0.94]	(16)	4.25 [1.40]	-0.26 (-1.02 to 0.50)	0.50
- Genotype W	(25)	3.65 [1.50]	(29)	4.27 [1.17]	-0.62 (-1.27 to 0.03)	0.06
- Genotype Y	(147)	4.22 [1.23]	(151)	4.04 [1.29]	0.18 (-0.18 to 0.54)	0.32
<b>Non-groupable</b>	(247)	3.73 [1.41]	(312)	3.72 [1.43]	0.01 (-0.25 to 0.30)	0.91

\* Linear regression, with generalized estimating equations used to account for clustering at the school level, † Disease-causing refers to all capsular genotypes identified (B, C, W, X, Y).