Characterization of meningococcal carriage isolates from Greece by whole genome sequencing: Implications for 4CMenB vaccine implementation

Konstantinos Kesanaopoulos, Holly Bratcher, Eva Hong, Athanasia Xirogianni, Anastasia Papandreou, Muhamed-Kheir Taha, Martin Maiden, Georgina Tzanakaki

To cite this version:

Konstantinos Kesanaopoulos, Holly Bratcher, Eva Hong, Athanasia Xirogianni, Anastasia Papandreou, et al.. Characterization of meningococcal carriage isolates from Greece by whole genome sequencing: Implications for 4CMenB vaccine implementation. PLoS ONE, Public Library of Science, 2018, 13 (12), pp.e0209919. 10.1371/journal.pone.0209919. pasteur-02489251

HAL Id: pasteur-02489251
https://hal-pasteur.archives-ouvertes.fr/pasteur-02489251
Submitted on 24 Feb 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Abstract

Herd protection, resulting from the interruption of transmission and asymptomatic carriage, is an important element of the effectiveness of vaccines against the meningococcus. Whilst this has been well established for conjugate polysaccharide vaccines directed against the meningococcal capsule, two uncertainties surround the potential herd protection provided by the novel protein-based vaccines that are used in place of serogroup B (MenB) polysaccharide vaccines (i) the strain coverage of such vaccines against carried meningococci, which are highly diverse; and (ii) the generation of a protective immune response in the mucosa. These considerations are essential for realistic estimates of cost-effectiveness of new MenB vaccines. Here the first of these questions is addressed by the whole genome sequence (WGS) analysis of meningococci isolated from healthy military recruits and university students in Greece. The study included a total of 71 MenB isolates obtained from 1420 oropharyngeal single swab samples collected from military recruits and university students on voluntary basis, aged 18–26 years. In addition to WGS analysis to identify genetic lineage and vaccine antigen genes, including the Bexsero Antigen Sequence Type (BAST), the isolates were examined with the serological Meningococcal antigen Typing System (MATS) assay. Comparison of these data demonstrated that the carried meningococcal population was highly diverse with 38% of the carriage isolates showed expression of antigens matching those included in the 4CMenB vaccine. Our data may suggest a limited potential herd immunity to be expected and be driven by an impact on a subset of carriage isolates.

Introduction

Neisseria meningitidis, the meningococcus, despite its propensity to cause invasive meningococcal disease (IMD) principally meningitis and septicaemia worldwide, is an obligate...
the commensal of the human nasopharynx, found asymptotically colonising approximately 10% of the human population [1,2]. Although the processes whereby asymptomatic carriage develops into invasive disease remains incompletely understood, genetic analysis of carriage and disease isolates shows that the diversity of carried meningococci is higher, with cases of invasive disease predominately caused by a relatively few genotypes, known as hyperinvasive lineages. These are recognised by multi-locus sequence typing (MLST) analyses as particular clonal complexes (cc) [1,3]. Colonisation is a prerequisite to disease and requires the bacteria to adhere to the mucosal surface, exploit locally available nutrients, and evade human immune responses. Multiple meningococcal factors facilitate colonization, including pilus-mediated attachment to the epithelial cell surfaces and the opacity-associated adhesins [4].

The immunochemical structure of the \textit{N. meningitidis} capsular polysaccharide defines 12 serogroups, but only six of these (serogroups A, B, C, W, Y and to lesser extent X) are responsible for most IMD [5]. For example, in the early 21st century in Europe, MenB accounted for 74% IMD cases, MenC for 16%, and MenY and MenW for 5% and 3% respectively [6]. Meningococcal capsule synthesis is encoded by the capsule operon region of the genome and particular capsules tend to be associated with given clonal complexes (ccs). Virtually all IMD isolates are capsulate, although very rare cases of IMD caused by non-capsulate meningococci occur worldwide and these organisms lack the \textit{cps} region, the so-called ‘capsule null’ (cnl) meningococci [7]. By contrast, many meningococcal carriage isolates are categorized as non-groupable (NG), by virtue of not expressing a capsule, and can be \textit{cnl}, have a down-regulated capsule synthesis, or be genetically damaged in the \textit{cps} region [8,9]. For instance, in a carriage study in Italy [10], the majority of NG isolates (72.7%) were \textit{cnl}, with other NG isolates containing down-regulated serogroup B capsule synthesis genes. Highly effective polysaccharide conjugate vaccines are available to control groups A, C, W, and Y IMD. Studies during meningococcal serogroup C conjugate (MCC) vaccine implementation demonstrated sustained protection for both vaccinated and non-vaccinated populations, showing both direct and herd protection induced by MCC vaccines [11,12].

The monovalent conjugate vaccine for MenC was introduced in Greece on January 2001 and included in the national immunization program in 2005 in older children and adolescents, with estimated vaccination coverage from 20.7% (2001) to 51.4% (2005) Since April 2011, the quadrivalent meningococcal conjugate vaccine (MCV4) was included in the national immunization programme as a booster dose in adolescents 11–16 years old [13].

The 4CMenB vaccine, a recombinant multicomponent vaccine (Bexsero, GSK), has Marketing Authorization Approval in many countries including EU/EEA, Australia, Canada, and the USA. The vaccine was not available until 2014, at which time IMD due to MenB accounted for 74% of IMD disease in most European countries. This is, in part, due to vaccination programmes that targeted serogroups A, C, W, and Y. The 4CMenB vaccine is available in Greece since 2015. According to the National Immunisation Programme is recommended only for high risk groups; however, paediatricians are offering vaccination privetly the past three years. In this low incidence setting, the cost-effectiveness of 4CMenB vaccine use in young adults depended on it being able to generate direct and indirect protection, as seen with the MCC vaccines [6]. In a study where conjugate serogroup A, C, W, and Y (MACWY) or 4CMenB vaccine were used in UK university students, immunisation reduced overall carriage of \textit{N. meningitidis}, with MenACWY affecting only the targeted capsular groups, whereas 4CMenB had a broader but modest effect regardless of capsule group [14]. More information on the impact of 4CMenB vaccine on carriage is required to support widespread use of 4CMenB and other protein based vaccines, which can be described as ‘serogroup B-substitute vaccines’, as they are used in place of vaccines that contain serogroup B polysaccharide.
In the early 21st century, the proportion of MenB meningococci recovered in carriage studies ranged from 2.2% to 43.9% in Europe [3], with a study in Greece showing that MenB was predominant (34.9%) among groupable carriage isolates [14]. The aim of the present study was the characterisation of the 71 asymptotically carried capsule group B (MenB) isolates obtained from healthy young adults by serogrouping/genogrouping, whole genome sequencing (WGS), and Meningococcal antigen Typing System (MATS) assay which is based on the serum antibody bactericidal activity rather than mucosal immunity, in order to identify the meningococcal genotypes circulating among military recruits and University students. Data on serogroup, sequence type (ST), antigen variability, Bexsero Antigen Sequence Type (BAST) and the MATS results established baseline data and indicated the possible impact of 4CMenB immunization on asymptomatic transmission in this setting.

Material and methods

Isolation and identification of N. meningitidis

The isolates examined were the serogroup B subset of isolates recovered from healthy young adults (military recruits or university students) enrolled in a previous carriage study [14]. All participants - in addition to the approval by the Ethics Committee from the National School of Public Health—responded ad hoc to a structured self-administrated questionnaire and a written informed consent form was signed as previously described [14]. The pharyngeal swabs were immediately plated on New York City medium (OXOID LTD, Basingstoke, Hampshire, England) and incubated at 37°C in the presence of 5% CO₂. Cultured plates were examined at 24 and 48 hours for suspected Neisseria colonies. Identification procedures included Gram stain, oxidase test, and a rapid carbohydrate utilization test. All N. meningitidis identified colonies were stored at -70°C in Heart Infusion Broth with 20% glycerol. In addition, the supernatant from a heat killed cell suspension was prepared and stored at -20°C using 1 μl of overnight culture suspended in 200 μl of PCR grade water, vortexed, heated at 100°C for 10 min, and centrifuged at 20,000 g for 12 min for further confirmation for the presence of the porA gene, by PCR amplification as described previously [15].

Capsular identification

Serogroup and genogroup was determined for all isolates. Serogrouping was carried out by slide agglutination test (Remel Europe Ltd. UK) according to manufacturer’s instructions and genogrouping determined by the implementation of a multiplex PCR targeting specific capsule group genes, as described previously [16].

Molecular characterization (MLST, PorA, FetA)

All 71 MenB isolates along with 56 of 127 meningococcal isolates belonging to a genogroup, were characterised by ‘finetyping’ (MLST and PorA and FetA typing), as described previously [17] using the PubMLST.org/neisseria database (http://pubmlst.org/neisseria/) [14]. Sequence types (ST) were defined and grouped into Clonal Complexes (ccs). PorA genotyping for variable regions 1 and 2 (VR1 and VR2) was performed as described previously [15] and compared with the variable sequences in the Neisseria PorA database (http://pubmlst.org/neisseria/PorA/). Similarly, the FetA variable region was also obtained for all the typable isolates, as previously described [18], and compared with variable sequences in the Neisseria FetA database (http://pubmlst.org/neisseria/FetA/).

Genomic DNA extraction was performed using GenElute Bacterial Genomic DNA Kit (SIGMA, Germany) following the manufacturer’s instructions. Briefly, one microliter of
18-hour culture was suspended in 180 μl of Lysis Solution T and the extraction included the optional RNase A treatment step. The eluate was stored at -20°C until sequencing. Whole genome libraries were created using Nextera XT DNA Library Preparation Kit (Illumina FC-131-1096), using Double Indexing Strategy (Nextera XT Index Kit v2 FC-131-200x) according to the manufacturer’s instructions. Each genome assembly was annotated using the pubMLST. or/neisseria sequence definition database and included the loci defining the MLST, BAST, antigen finetyping, and cgMLST v1.0 schemes.

**4CMenB (Bexsero, GSK) vaccine antigen sequence typing (BAST)**

Nucleotide sequences of *fhbp*, *nhba*, *nadA*, and *porA* variable regions 1 and 2 were obtained by WGS analysis as previously described [19]. Alleles and the corresponding protein variants were assigned using the *Neisseria* sequence definition database (http://pubmlst.org/neisseria/).

**Meningococcal antigen typing system (MATS)**

To determine the proportion of strains expected to be covered by 4CMenB all isolates were analyzed by MATS ELISA. MATS ELISA was carried out at the Meningococcal Reference Laboratory, Institut Pasteur (Paris, France), one of the accredited reference laboratories that participated in the MATS standardization process [20, 21]. MATS ELISA values were calculated as antigen-specific relative potencies compared with MenB reference strains expressing each vaccine antigen [20, 22].

Predicted coverage using MATS-PBT (Positive Bactericidal Threshold) was calculated as described previously using the threshold that was established for invasive isolates [20, 21, 22]. The presence of at least one antigen with a relative potency greater than its MATS-PBT relative potency value (0.012 for *fhbp*, 0.294 for NHBA and 0.009 for NadA) or the presence of PorA VR2 P1.4 (the VR2 present in the OMV-NZ component of 4CMenB) was considered sufficient for an isolate to be covered by 4CMenB. Strains that did not meet these criteria were considered not covered. Estimates of the 95% confidence intervals (95% CI) for the MATS-PBTs were derived on the basis of overall assay repeatability and reproducibility (0.014–0.031 for *fhbp*, 0.169–0.511 for NHBA, 0.004–0.019 for NadA) [22]. These intervals defined the 95% CI of strain coverage by 4CMenB.

**Genetic diversity and association analysis**

The diversity of each vaccine component was assessed using the Simpson’s index of diversity (D) [19, 23] and was calculated for each antigen using the Comparing Partitions Online Tool (http://www.comparingpartitions.info/index.php?link=Tool). A D index value near one indicated high diversity and values <1 indicated lower diversity. Cramer’s V coefficient was used to assess the association of the BAST antigenic variants with clonal complex, using SPSS version 20, with values from 0, indicating no association, to 1, indicating complete match.

**Results**

**Capsular typing**

Among the 180 *N. meningitidis* isolates recovered from 1420 pharyngeal swabs, 71 were identified as MenB either by slide agglutination test (45/71, 63.4%) or PCR (26/71; 36.6%) and included in the present study for further analysis. All 71 isolates possessed genes encoding the group B capsule (genogroup B). A complete *csb* gene (NEIS2161) sequence was assembled in 64 of the 71 (90%) isolates. In the remaining seven isolates an identifiable *csb* gene was present, although the *cps* region was incompletely assembled. Among the 26 capsule genogroup B PCR
positive isolates not expressing serogroup B phenotypically, 18 (69%) presented sequence insertions or deletions switching off the gene expression (genetically phase variable ‘off’), as were four isolates identified as serogroup B by the slide agglutination test.

**Sequence typing: MLST, porA, fetA**

The 71 MenB isolates were distributed into 8 distinct clonal complexes (cc). The 41/44cc was the most frequent (21/71; 30%) followed by 35cc (17/71; 23.9%) and 213cc (10/71; 14.1%). A total of 11 meningococci (15.5%) exhibited STs that were not assigned to any known cc (“Fig 1”). PorA variable region genotyping revealed variability for the two variable regions VR1 and VR2, with 36 combinations. The most frequent combinations were: P1.22–1,14 (13/71; 18.3%); P1.22,14 (10/71; 15.4%); P1.22,14–6 (7/71; 9.8%); and P1.22,9 (5/71; 7%). The two most frequent PorA VR1 types were 22 (27/71; 38%) and 22–1 (16/71; 22.5%) while the most frequent PorA VR2 type was 14 (35.2%). The PorA variant P1.7–2,4, present in the 4CMenB vaccine, was present in seven genomes (7/71; 9.9%) and 1 (1/71; 1.4%) for VR1 and VR2 respectively (S1 Table). The fetA gene was present in all 71 genomes, encoding 16 different FetA variable regions. Variants F1-5 (15/71; 21.1%), F5-5 (13/71; 18.3%), and F4-1 (10/71; 14.1%) were the most frequently observed. The fetA gene was incompletely assembled in five isolates and in an additional isolate the gene contained a mutation introducing a stop codon and, consequently, no peptide variant was assigned in this case (“S1 Table”).

**4CMenB molecular typing**

The fHbp gene (NEIS0349), encoding the fHbp antigen, was present and was in-frame in all 71 genomes, with 17 distinct peptides encoded. The most common fHbp peptides were 19 (n = 21/71; 29.5%) and 16 (n = 17/71; 24%) (“Fig 2”). Peptide 1, which is included 4CMenB fHbp vaccine, was not found in any of the isolates examined. All three fHbp peptide variant families were present [24, 25]. Variant family 2 was observed more frequently (74.6%; 53/71), followed by variant family 1 (12.7%; 9/71) and variant family 3 (8/71; 11.2%). Among the variant family 2 fHbp peptides 19 and 16 were most frequently identified, while, peptide 45 of the family variant 3 (included in the Trumenba vaccine, Pfizer), was identified in 9.9% (7/71) of isolates. (“Fig 3”). Twenty-six different NHBA peptides were identified, of which 16 (62%) were present in a single isolate (“S1 Table”). The most frequent was peptide 21 (15/71; 21.1%), followed by peptide 18 (8/71; 11.2%), while, NHBA peptide 2, included in the 4CMenB vaccine, was observed in 4 isolates (5.6%) (“Fig 4”). The NadA gene (NEIS1969) was present in 12 isolates (6.9%), nine of which contained a frameshift mutation resulting in a premature stop codon. Of the three genomes with assigned peptides (1, 21, and 100), none was identified to the 4CMenB vaccine target, peptide 8 (“S1 Table”).

**Bexsero antigen sequence type (BAST)**

The pubMLST.org/neisseria database hosts the BAST (Bexsero Antigen Sequence Type) scheme for 4CMenB antigens and BAST types were assigned for 65 of the 71 isolates (91.5%). Forty-six (46) different BASTs were identified, 40/65 (61.5%) of which were present in only one isolate. BAST-257 was observed most frequently (9/65, 13.8%) followed by BAST-224, BAST-583, and BAST-933 (4/65, 6.1% each), representing 32.3% of the isolates (“Fig 5”). Nine BASTs were present in isolates with an ST not associated with a defined clonal complex (cc) and a 10th BAST that was similarly in isolates with an ST was not assigned to a cc. The remaining BASTs, including five with incomplete profiles, were associated with eight ccs: BAST-257 (n = 9) with 35cc; BAST-933 (n = 4), BAST-586 (n = 2), and BAST 1200 (n = 2) with 41/44cc; BAST-224 (n = 4) with 213cc; and BAST-583 (n = 3) with 269cc (“Fig 6”).
Meningococcal antigen typing system (MATS) phenotype

The MATS analysis for 70 of the 71 MenB isolates predicted coverage by at least one antigen (fHbp, NHBA, NadA, or PorA) for 38% (27/70) of the isolates, with the NHBA antigen providing the highest contribution (16/70; 22.9%). Cross protective fHbp variants were present in 5 isolates (5/70; 7.1%), with 4 in combination with NHBA (4/70; 5.7%). NadA coverage was
detected in 1 isolate (1/70; 1.4%), while PorA (1/70, 1.4%) was found in combination with NHBA (“Table 1”).

fHbp-1 sub-variants were found in all 9 isolates showing detectable expression levels of the fHbp antigen (alone or with the NHBA combination) while 1 of the 3 isolates expressing the NadA peptide 1 had an RP value above the detection threshold for this specific antigen. Simpson’s index of diversity (D) indicated that NHBA was the most diverse antigen (0.926 [0.892–0.959 CI 95%]), followed by PorA VR2 (0.847 [0.773–0.922 CI 95%]), fHbp (0.845 [0.79–0.9 CI 95%]), PorA VR1 (0.796 [0.727–0.866 CI 95%]), and NadA (0.083 [1.000–0.173]). Using the
Cramer’s V coefficient calculation an association was observed between BAST-257 and clonal complex ST-35.

**Discussion**

At the time of writing IMD, particularly that caused by serogroup B meningococci, was incompletely controlled by immunisation, largely because of the antigenic and genetic diversity of *Neisseria meningitidis*. In particular, there were uncertainties as to the ability of the protein-based vaccines to generate herd protection (immunity), especially given the diversity of meningococci meningococci isolated from asymptomatic carriers [1].

Previous studies have shown that the introduction of monovalent meningococcal group C polysaccharide conjugate vaccine (MCC) in national immunization programs reduced MenC IMD through herd protection, by reducing the transmission of the epidemic strain among asymptomatic carriers [11,12]. Similarly, the national introduction of a strain-specific outer membrane vesicle vaccine (MeNZB) in New Zealand reduced IMD cases from the epidemic clone, with limited evidence for cross-protection [26] with a potential impact on the acquisition of carriage of the epidemic strain [27]. Similar impact on the acquisition of carriage of an epidemic isolate was also suggested for another OMV vaccine (MenBVac) [28]. The impact of meningococcal vaccines on asymptomatic transmission is an important aspect in the evaluation of the impact of new meningococcal vaccines on disease, particularly their cost-effectiveness [29]. Recently, a multicomponent protein-based vaccine (4CMenB, Bexsero), designed to target MenB disease in the absence of a vaccine against the group B capsule, was licensed in many European countries including Greece; however, a carriage study in UK has shown that the potential broad additional effect on commensal *Neisseria* and non-disease associated *N.*
meningitis cannot be predicted [14]. While no effect on carriage was observed 1 month after completion of the vaccine course, this effect was from 3 months after dose two of 4CMenB. This effect was observed for all meningococci but not specifically of serogroup B isolates. This global effect could be expected as this vaccine does not target the capsule but surface exposed proteins that can be shared by the isolates regardless the serogroup. However, an effect on serogroup B should be analysed on the basis of the expression data (e.g. MATS as performed in our study) but that still require a “correlate of protection” for carriage isolates.

No correlate of protection against acquisition of carriage at the mucosal surfaces was available to this study, and this prevented this issue being addressed directly, by assaying the mucosal immunity against meningococcal isolates; however, data from animal models have demonstrated the detection of immune response in the mucosal secretions for protein-based vaccines, which correlated with the prevention against intranasal colonization by isolates showing multiple matching with vaccine antigen [28,30]. Our data clearly show that the expression of antigens of the 4CMenB vaccine in carriage isolates as suggested by the MATS data.

Among the isolates that possessed a \( \text{cps} \) region encoding the group B capsule (genogroup B, MenB), over a third (\( n = 26, 36.6\% \)) were defined as phenotypically non-groupable (NG) by agglutination, suggesting down-regulation of capsule expression during carriage. This is consistent with many previous studies including a recent investigation in Italy [10], where 40% of

| Antigen Combination | Number of isolates (%) | coverage of each antigen combination (%) | % coverage of combined antigen group |
|---------------------|------------------------|-----------------------------------------|-----------------------------------|
| No antigen          | 43 (61.4)              | 0                                       | 0                                 |
| fHbp\(^{b}\)         | 5 (7.1)                | 7.1%                                    | 31.4%                             |
| NHBA\(^{c}\)         | 16 (22.9)              | 22.9%                                   |                                   |
| NadA\(^{d}\)         | 1 (1.4)                | 1.4%                                    |                                   |
| PorA\(^{e}\)+NHBA    | 1 (1.4)                | 1.4%                                    | 7.1%                              |
| fHbp +NHBA           | 4 (5.7)                | 5.7%                                    |                                   |

\(^{a}\)MATS: Meningococcal Antigen Typing System, 
\(^{b}\)fHbp: factor H binding protein, 
\(^{c}\)NHBA: Neisseria Heparin Binding Protein, 
\(^{d}\)NadA: Neisseria Adhesion A, 
\(^{e}\)PorA: outer membrane Protein A

https://doi.org/10.1371/journal.pone.0209919.t001
group B carrier isolates were NG. A total of 21 (81%) of the NG isolates exhibited intact capsule operons, with 18 (69%) containing a csb gene predicted to be phase variable off. This demonstrates the importance of using WGS data to investigate such isolates, as this is the most practical and cost-effective means of determining the presence and expression status of the capsule operon. The majority of genogroup B isolates analysed in this study were represented by clonal complexes 41/44cc, 35cc, 32cc, 213cc, 269cc, and 162cc. This was also consistent with previous observations from a variety of high-income countries [10, 32]. As the relationship between asymptomatic carriage and the development of invasive disease remains incompletely understood, it is important to collect isolates belonging to hyperinvasive genotypes worldwide.

The peptide sequences of the principal 4CMenB vaccine antigens, summarized by the BAST type, were extracted from the assembled WGS data using the tools integrated into the https://pubmlst.org/neisseria/ [19]. As has been reported previously for disease isolates [19, 33], each of the vaccine antigens exhibited extensive sequence variation among the 71 genogroup B isolates, with NHBA being the most diverse antigen ("Table 1"). The strong correlation of cc with both BAST and individual antigen variant, such that in the absence of antigen data cc could be used as a surrogate for likely cross protection, was consistent with several studies of invasive disease isolates [19, 33, 34]. Similarly consistent with studies of IMD isolates, exact matches to the vaccine antigen variants were rare: PorA VR2 (P1.4) was found in only one isolate belonging to 41/44 cc; NHBA peptide 2 was found in five isolates (7.6%); the fHbp target peptide 1 and NadA target peptide 8 were not present in this collection of isolates. The prevalence of PorA VR2 P1.4 (1, 1.4%) was considerably lower to that was found among invasive isolates (7%) in Greece [35]. Consequently, any impact of the 4CMenB vaccine on carriage isolates will depend on immunological cross protection of the type assessed in the MATS assay.

All three fHbp variant families were present among the 71 carriage isolates. Consistent with the findings of other carriage studies, variant family 2 was more abundant than variant family 1 [10, 32]. No cross reactivity of the 4CMenB vaccine antibodies with isolates containing variant family 2 or 3 peptides, was observed ("S1 Table"); however, 90% of the isolates containing fHbp variant family 1 peptides were MATS RP positive ("S1 Table"). Similar data indicating the expression of fHbp family 1 among 95% of carriage isolates harbouring this variant were reported from France using ELISA [36]. There were 26 different peptides for NHBA, and only 5 isolates (7.7%) contained the 4CMenB vaccine variant, peptide 2. The presence of the NHBA peptide 2 among group B meningococci from carriers is 30%, lower than the presence of this peptide among IMD group B isolates in Greece (10.1%) [35]. This is in contrast with a recent carriage study from Spain, which found comparable levels of NHBA peptide 2 among carriers and IMD associated group B isolates of 3% and 4% for carrier and invasive group B isolates respectively [32].

While BAST data demonstrated a high diversity of the fHbp, NHBA, NadA, and PorA antigens, this was mainly due to fHbp and NHBA, and a limited number of BASTs occurred at high frequency [37]. BAST-1 (fHbp-1, NHBA-2, NadA-8, PorA P1.7–2,4), corresponding to the 4CMenB vaccine components, was absent from this dataset which was consistent with the fact that the vaccine formulation was assembled from meningococci from multiple ccs. MATS estimated the overall potential 4CMenB vaccine coverage at 38.6%, which was substantially lower than the estimated coverage for MenB IMD isolates (89.2%) from Greece for 1999–2010 [35]. A total of eight isolates (11.4%) predicted to be covered by MATS contained capsule group B-encoding cps regions but were non-groupable (NG) by serological methods. One limitation of this study was that no correlation had been established between the level of antibodies needed for protection at the mucosal level and the levels of expression of the antigens targeted by the 4CMenB.
In conclusion, this study demonstrates the utility of WGS data in the characterisation of meningococcal carriage isolates to assess the prevalence of vaccine antigens, including the presence and expression of polysaccharide and protein antigens. The BAST antigens found among carried isolates were highly diverse, which was reflected by a low level of predicted cross-protection of 4CMenB against carried MenB isolates. As the majority of carriage MenB isolates do not belong to hyperinvasive linages, this observation does not necessarily preclude the use of 4CMenB to generate herd immunity against IMD. To affect disease rates, it will be important to disrupt the transmission of hyperinvasive meningococci; indeed, it may well be preferable that the transmission of less invasive variants of any meningococcal group is not impacted by immunisation. At the time of writing, a number of large-scale vaccination and carriage investigations were underway and the data generated from these, using approaches similar to those reported here, will provide a definitive answer as to the impact of this vaccine on meningococcal transmission.

Supporting information
S1 Table. Molecular characterization, capsular typing, 4CMenB vaccine molecular typing and MATS of the meningococcal group B carriage isolates included in the study.

Author Contributions
Data curation: Konstantinos Kesanopoulos, Holly B. Bratcher, Martin C. J. Maiden.

Formal analysis: Georgina Tzanakaki.

Funding acquisition: Georgina Tzanakaki.

Investigation: Konstantinos Kesanopoulos, Holly B. Bratcher, Eva Hong, Athanasia Xirogianni, Anastasia Papandreou, Muhamed-Kheir Taha, Georgina Tzanakaki.

Methodology: Eva Hong, Athanasia Xirogianni, Anastasia Papandreou, Muhamed-Kheir Taha, Martin C. J. Maiden, Georgina Tzanakaki.

Project administration: Georgina Tzanakaki.

Supervision: Martin C. J. Maiden, Georgina Tzanakaki.

Writing – original draft: Konstantinos Kesanopoulos, Holly B. Bratcher, Martin C. J. Maiden, Georgina Tzanakaki.

Writing – review & editing: Muhamed-Kheir Taha, Martin C. J. Maiden, Georgina Tzanakaki.

References
1. Caugant DA, Maiden MCJ. Meningococcal carriage and disease—population biology and evolution. Vaccine 2009; 27 Suppl 2:B64–70. https://doi.org/10.1016/j.vaccine.2009.04.061 PMID: 19464092

2. Trotter Caroline L MMC. Handbook of Meningococcal Disease Management. Springer. ADIS; 2016. https://doi.org/10.1007/978-3-319-28119-3

3. Soriano-Gabarró M, Wolter J, HogeA C, Vyse A. Carriage of Neisseria meningitidis in Europe: a review of studies undertaken in the region. Expert Rev Anti Infect Ther 2011. https://doi.org/10.1586/eri.11.89 PMID: 21905785

4. Hill DJ, Griffiths NJ, Borodina E, Virji M. Cellular and molecular biology of Neisseria meningitidis colonization and invasive disease. Clin Sci 2010; 118:547–64. https://doi.org/10.1042/CS20090513 PMID: 20132098
5. Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, et al. Description and Nomenclature of Neisseria meningitidis Capsule Locus. Emerg Infect Dis J 2013; 19:566. https://doi.org/10.3201/eid1904.111799 PMID: 23628376

6. Whittaker R, Dias JG, Ramliden M, Kódmón C, Economopoulou A, Beer N, et al. The epidemiology of invasive meningococcal disease in EU/EEA countries, 2004–2014. Vaccine 2017; 35:2034–41. https://doi.org/10.1016/j.vaccine.2017.03.007 PMID: 28314560

7. Ganesh K, Allam M, Wolter N, Bratcher HB, Harrison OB, Lucidarme J, et al. Molecular characterization of invasive capsule null Neisseria meningitidis in South Africa. BMC Microbiol 2017; 17:40. https://doi.org/10.1186/s12866-017-0942-5 PMID: 28222677

8. Hammerschmidt S, Muller A, Stilmann H, Muhlenhoff M, Borrow R, Fox A, et al. Capsule phase variation in Neisseria meningitidis serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD): Correlation with bacterial invasion and the outbreak of meningococcal disease. Mol Microbiol 1996; 20:1211–20. https://doi.org/10.1111/j.1365-2958.1996.tb02641.x PMID: 8809773

9. Dolan-Livengood JM, Miller YK, Martin LE, Urwin R. Genetic Basis for Nongroupable. Public Health 2003;30322.

10. Gasparini R, Comanducci M, Amicizia D, Ansaldi F, Orsi A, et al. Molecular and serological diversity of Neisseria meningitidis carrier strains isolated from Italian students aged 14 to 22 years. J Clin Microbiol 2014; 52:1901–10. https://doi.org/10.1128/JCM.03584-13 PMID: 24684565

11. Maiden MCJ, Ibzar-Pavon AB, Urwin R, Gray SJ, Andrews NJ, Clarke SC, et al. Impact of Meningococcal Serogroup C Conjugate Vaccines on Carriage and Herd Immunity. J Infect Dis 2008; 197:737–43. https://doi.org/10.1086/527401 PMID: 18271745

12. Ramsay ME, Andrews NJ, Trotter CL, Kaczmarski E. Herd immunity from meningococcal serogroup C conjugate vaccine in England: database analysis. BMJ Br Med J 2003; 326:365–6. https://doi.org/10.1136/bmj.326.7385.365

13. Tryfinopoulou K, Kesanopoulos K, Xirogianni A, Marmaras N, Papandreu A, Papaevangelou V et al. Meningococcal Carriage in Military Recruits and University Students during the Pre MenB Vaccination Era in Greece (2014–2015). PLoS One 2016; 11:e0167404. https://doi.org/10.1371/journal.pone.0167404 PMID: 27907129

14. Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: An observer-blind, phase 3 randomised clinical trial. Lancet 2014; 384:2123–31. https://doi.org/10.1016/S0140-6736(14)60842-4 PMID: 25145775

15. Russell JE, Jolley KA, Feavers IM, Maiden MCJ, Suer J. PorA variable regions of Neisseria meningitidis. Emerg Infect Dis 2004; 10:674–8. https://doi.org/10.3201/ed0403.030247 PMID: 15200858

16. Drakopoulou Z, Kesanopoulos K, Sioumala M, Tambaki A, Kremastinou J, Tzanakaki G. Simultaneous single-tube PCR-based assay for the direct identification of the five most common meningococcal serogroups from clinical samples. FEMS Immunol Med Microbiol 2008; 53:178–82. https://doi.org/10.1111/j.1574-695X.2008.00406.x PMID: 18623625

17. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA 1998; 95(6):3140–5 PMID: 9501229

18. Thomson EA, Feavers IM, Maiden MC. Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. Microbiology. 2003; 149 (7) 1849–58.

19. Brehony C, Rodrigues CMC, Borrow R, Smith A, Cunney R, Moxon ER, et al. Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation. Vaccine 2016; 34:4690–7. https://doi.org/10.1016/j.vaccine.2016.08.015 PMID: 27521232

20. Pikaytis B, Stella M, Bocodiloufu G., et al. Inter-laboratory standardization of the sandwich ELISA designed for MATS, a rapid, reproducible method for estimating the strain coverage of investigational vaccines. Clin Vaccine Immunol. 2012; 19(10) 1609–17. https://doi.org/10.1128/CVI.00202-12 PMID: 22875603

21. Vogel U, Taha MK, Vazquez J, Findlow J, Claus H, Stefanelli P et al. Predicted strain coverage of meningococcal multicomponent vaccine in Europe: a qualitative and quantitative assessment Lancet Infect Dis Feb 2013 (https://doi.org/10.1016/S1473-3099(13)70006-9)

22. Donnelly J, Medini D, Bocodiloufu G, Biolchi A, Ward J, Frasch C, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci 2010; 107:19490–5. https://doi.org/10.1073/pnas.1013758107 PMID: 20962280

23. Hunter PR, Gaston M. Numerical index of the discriminatory ability of typing systems: an application of Simpson β*™’s index of Numerical Index of the Discriminatory Ability of Typing Systems: an
24. Masignani V, Comanducci M, Giuliani MM, Bambini S, Adu-Bobie J, Arico B, et al. Vaccination against Neisseria meningitidis using three variants of the lipoprotein GNA1870. J Exp Med. 2003 Mar 17; 197 (6):789–99. https://doi.org/10.1084/jem.20021911 PMID: 12642606

25. Brehony C, Wilson DJ, Maiden MC. Variation of the factor H-binding protein of Neisseria meningitidis. Microbiology. 2009 Dec; 155(Pt 12):4155–69. https://doi.org/10.1099/mic.0.027995-0 Epub 2009 Sep 3. PMID: 19729409

26. Arnold R, Galloway Y, McNicholas A, O’Hallahan J. Effectiveness of a vaccination programme for an epidemic of meningococcal B in New Zealand. Vaccine 2011; 29:7100–6. https://doi.org/10.1016/j.vaccine.2011.06.120 PMID: 21803101

27. Holmes JD, Martin D, Ramsay C, Ypma E, Oster P. Combined administration of serogroup B meningococcal vaccine and conjugated serogroup C meningococcal vaccine is safe and immunogenic in college students. Epidemiol Infect 2008; 136(6):790–9. https://doi.org/10.1017/S0950268807009211 PMID: 17678558

28. Delbos V, Lemee L, Benichou J, Berthelot G, Deghmane AE, et al. Impact of MenBvac, an outer membrane vesicle (OMV) vaccine, on the meningococcal carriage. Vaccine 2013: 31: 4416–4420 https://doi.org/10.1016/j.vaccine.2013.06.080 PMID: 23856330

29. Marshall H, Wang B, Wesselingh S, Snape M, Pollard AJ. Control of invasive meningococcal disease: is it achievable? Int J Evid Based Health. 2016 Mar; 14(1):3–14.

30. Buckwalter CM, Currie EG, Tsang RSW, Gray-Owen SD (2017) Discordant Effects of Licensed Meningococcal Serogroup B Vaccination on Invasive Disease and Nasal Colonization in a Humanized Mouse Model. J Infect Dis 215: 1590–1598 https://doi.org/10.1093/infdis/jix162 PMID: 28368526

31. Blackwell CC, Tzanakaki G, Kremastinou J., Weir D.M., Vakalis N., Elton R.A. et al. Factors affecting carriage of Neisseria meningitidis among Greek military recruits. Epidemiology and Infection 1992; 108: 441–448. PMID: 1601077

32. Abad R, Medina V, Fariñas M del C, Martínez-Martínez L, Bambini S, Dari A, et al. Potential impact of the 4CMenB vaccine on oropharyngeal carriage of Neisseria meningitidis. J Infect 2017; 75:511–20. https://doi.org/10.1016/j.jinf.2017.09.021 PMID: 28987549

33. Rodrigues CMC, Lucidarme J, Borrow R, Smith A, Cameron JC, Moxon ER, Maiden MCJ. Genomic Surveillance of 4CMenB Vaccine Antigenic Variants among Disease-Causing Neisseria meningitidis Isolates, United Kingdom, 2010–2016. Emerg Infect Dis. 2018; Apr; 24(4):673–682. https://doi.org/10.3201/eid2404.171480 PMID: 29553330

34. Mowlaboccus S, Mullally CA, Richmond PC, Howden BP, Stevens K, Speers DJ et al. Differences in the population structure of Neisseria meningitidis in two Australian states: Victoria and Western Australia. PLoS One. 2017 Oct 24; 12(10):e0186839. https://doi.org/10.1371/journal.pone.0186839 PMID: 29065137

35. Tzanakaki G, Hong E, Kesanopoulos K, Xirogianni A, Bambini S, Orlandi L, et al. Diversity of Greek meningococcal serogroup B isolates and estimated coverage of the 4CMenB meningococcal vaccine. BMC Microbiol 2014; 14. https://doi.org/10.1186/1471-2180-14-111 PMID: 24779381

36. Lemee L, Hong E, Etiennie M, Deghmane AE, Delbos V, Terrade A, et al. Genetic diversity and levels of expression of factor H binding protein among carriage isolates of Neisseria meningitidis. PloS One 2014; 9(9):e107240 https://doi.org/10.1371/journal.pone.0107240 PMID: 25247300

37. Serruto D, Bottomley MJ, Ram S, Giuliani MM. The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: Immunological, functional and structural characterization of the antigens. Vaccine 2012; 30: B87–97 https://doi.org/10.1016/j.vaccine.2012.01.033 PMID: 22607904