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Multicomponent meningococcal serogroup B vaccination elicits cross-reactive immunity in infants against genetically diverse serogroup C, W and Y invasive disease isolates

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ABSTRACT

Background: The multicomponent meningococcal serogroup B vaccine (4CMenB) is currently indicated for active immunization against invasive meningococcal disease caused by *Neisseria meningitidis* serogroup B (MenB). However, genes encoding the 4CMenB antigens are also variably present and expressed in strains belonging to other meningococcal serogroups. In this study, we evaluated the ability of antibodies raised by 4CMenB immunisation to induce complement-mediated bactericidal killing of non-MenB strains.

Methods: A total of 227 invasive non-MenB disease isolates were collected between 1 July 2007 and 30 June 2008 from England and Wales, France, and Germany; 41 isolates were collected during 2012 from Brazil. The isolates were subjected to genotypic analyses. A subset of 147 isolates (MenC, MenW and MenY) representative of the meningococcal genetic diversity of the total sample were tested in the human complement serum bactericidal antibody assay (hSBA) using sera from infants immunised with 4CMenB.

Results: Serogroup and clonal complex repertoires of non-MenB isolates were different for each country. For the European panel, MenC, MenW and MenY isolates belonged mainly to ST-11, ST-22 and ST-23 complexes, respectively. For the Brazilian panel, most MenC and MenW isolates belonged to the ST-103 and ST-11 complexes, respectively, and most MenY isolates were not assigned to clonal complexes. Of the 147 non-MenB isolates, 109 were killed in hSBA, resulting in an overall coverage of 74%.

Conclusion: This is the first study in which 147 non-MenB serogroup isolates have been analysed in hSBA to evaluate the potential of a MenB vaccine to cover strains belonging to other serogroups. These data demonstrate that antibodies raised by 4CMenB are able to induce bactericidal killing of 109 non-MenB isolates, representative of non-MenB genetic and geographic diversity. These findings support previous evidence that 4CMenB immunisation can provide cross-protection against non-MenB strains in infants, which represents an added benefit of 4CMenB vaccination.

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Abbreviations: list: 4CMenB, four-component meningococcal serogroup B vaccine; CFU, colony forming units; fHbp, factor H binding protein; hSBA, serum bactericidal antibody assay using human complement; IMD, invasive meningococcal disease; rLP2086, bivalent factor H binding protein vaccine; MATS, Meningococcal Antigen Typing System; Men, meningococcal serogroup; MLST, multilocus sequence typing; NadA, Neisseria adhesin A; NHBA, Neisserial heparin binding antigen; OMV, outer membrane vesicle; PorA, porin A; SBA, serum bactericidal antibody; ST, sequence type; US, United States.

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1. Introduction

Invasive meningococcal disease (IMD) remains a major public health concern worldwide. The majority of IMD is caused by six immunologically distinct *Neisseria meningitidis* serogroups: A, B, C, W, Y, and X [1,2], (hereafter referred to as MenA, MenB, MenC, MenW, MenY, and MenX). Quadrivalent MenACWY glycoconjugate vaccines have been approved for use for several years, and tailor-made vaccines composed of meningococcal outer membrane vesicles (OMV) have proven largely efficacious against MenB clonal outbreaks in Brazil, Norway, Cuba, New Zealand and France [3–7]. The introduction of an affordable and highly efficacious MenA glycoconjugate vaccine (*MenAfriVac*, Serum Institute of India) has drastically reduced the burden of MenA-IMD in the so-called sub-Saharan African meningitis belt [8,9].

Despite these impressive strides, meningococcal vaccination uptake in most developing countries has been fairly slow and a sampling of global IMD epidemiology suggests that all six serogroups still account for significant IMD-related morbidity and mortality. Despite the dramatic impact of MenA vaccination, sporadic outbreaks of both MenW and MenX IMD continue to plague sub-Saharan Africa and MenC outbreaks were recently reported in Niger [8,10]. MenB accounted for 36% of IMD burden in the United States (US) between 2006 and 2015, while MenY and MenC were together responsible for about half of IMD cases [11]. In Canada, while MenC, MenY and MenW invasive isolates are still present, MenB IMD dominates, contributing close to two thirds to the burden of IMD, with a particular threat in Ontario [12–14]. Countries of the Southern Cone region of South America (Argentina, Chile, Uruguay, and Paraguay) have an unusual distribution of meningococcal serogroups; MenW is recently making significant contributions to overall IMD in this region, even though MenB remains prominent [15]. During 2006–2010, the dominant meningococcal serogroups in Brazil and Central America were MenB and MenC, with only minimal contribution by MenY, whereas all three serogroups showed high prevalence in the Andean region [16]. MenY has recently made a significant impact in Europe and is the third most common serogroup responsible for IMD in this region, after MenB and MenC [17–19], while hypervirulent MenW strains have also emerged in several European countries [20,21]. Although little is known about the IMD epidemiology in Asia, MenA, MenC, MenW, and MenY are endemic in many different regions and sporadic outbreaks, particularly of MenA, have recently occurred [22–24].

The recent licensure of two MenB vaccines, rLP2086 (MenB-fHbp; *Trumenba*, Pfizer) and 4CMenB (*Bexsero*, GSK) in several countries worldwide adds much needed armament to the meningococcal vaccine tool chest and brings us a step closer to universal serogroup coverage. These vaccines were licensed based on the efficacy inferred from their ability to elicit complement dependent, antibody-mediated bacterial killing activity as measured by the serum bactericidal antibody (SBA) assay using human complement (hSBA). rLP2086 is a bivalent factor H binding protein (fHbp) vaccine indicated for adolescents 10–25 years of age in the US and individuals as of 10 years of age in Europe [25–27]. 4CMenB is a four-component MenB vaccine formulation containing the *Neisseria* adhesin A (NadA), fHbp and the Neisserial heparin binding antigen (NHBA). The latter two antigens are included in the vaccine as fusions to GNA2091 and GNA1030 respectively. All antigens, identified by reverse vaccinology [28,29], are present in the vaccine in combination with the OMV obtained from the New Zealand outbreak strain. The immunodominant antigen of the OMV is the porin A protein (PorA) P1.4 [30]. 4CMenB is currently indicated for active immunization against MenB-caused IMD for individuals 10–25 years of age in the US and from two months of age and older in Europe and several countries worldwide [31–34]. As the

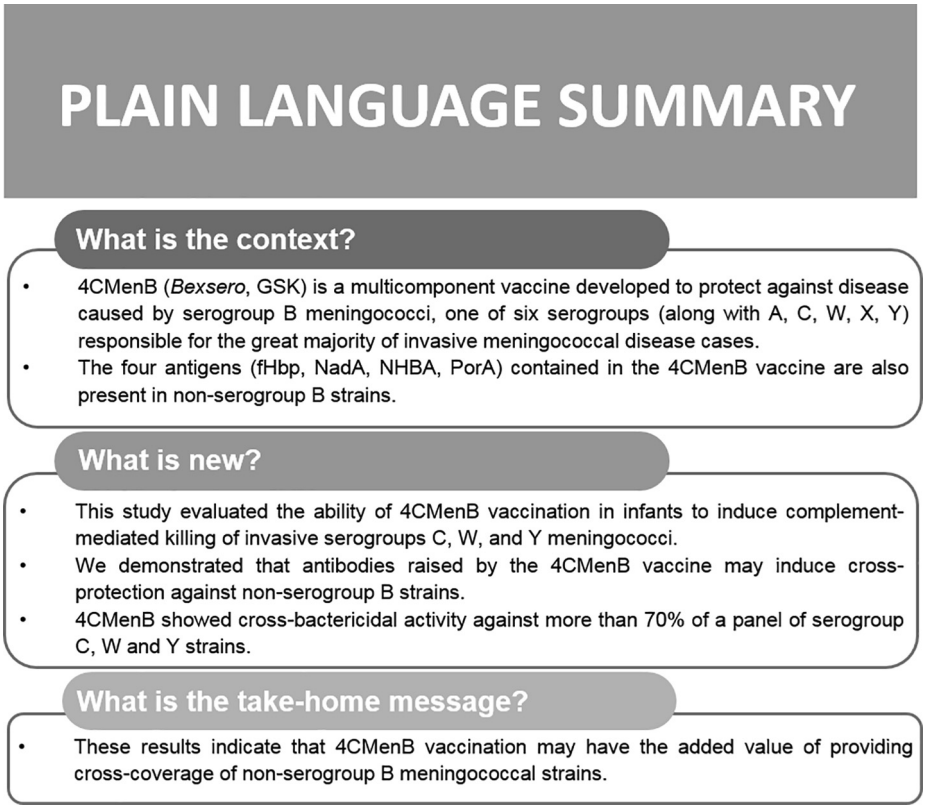


Fig. 1. Plain language summary.

4CMenB vaccine antigens are also variably present and expressed in non-MenB strains [35–39], a certain level of cross-serogroup protection might be expected in individuals vaccinated with 4CMenB. However, the sole presence of the genes is not an adequate indicator of possible vaccine strain coverage. Moreover, no published data are available on the expression of 4CMenB antigens in all the other serogroups. Consequently, cross-serogroup protection afforded by 4CMenB vaccination has not been estimated to date.

MenB strain coverage by 4CMenB is evaluated using the Meningococcal Antigen Typing System (MATS), a predictive tool that measures both antigenic similarity and antigen expression levels of the vaccine antigens in MenB isolates [38,40]. However, since the link between bactericidal killing measured by hSBA and MATS was established using a panel of MenB isolates, MATS as a predictive tool cannot *a priori* be applied to coverage assessments of isolates belonging to serogroups other than MenB.

In the present study, we used hSBA to evaluate the susceptibility of MenC, MenW, and MenY isolates collected in several European countries and Brazil to bactericidal killing using pooled immune sera from infants immunized with 4CMenB. This analysis provides new evidence for the potential benefit of 4CMenB in pan-serogroup meningococcal immunisation campaigns, in the context of the unpredictable and rapidly-evolving epidemiology of IMD.

Fig. 1 summarizes our findings and their clinical relevance and impact on the vaccinated population.

2. Materials and methods

2.1. Non-MenB meningococcal isolates and isolate characterization

A total of 227 European non-MenB isolates (hereafter referred to as the Euro-3 panel) were collected in the period 1 July 2007–30 June 2008 by reference laboratories in England and Wales (Public Health England, Manchester), Germany (University of Würzburg) and France (Institut Pasteur, Paris) and analysed in this study. The isolates from England and Wales and Germany represented all non-MenB isolates collected during this period in these countries. The isolates collected in France represented one third of the total invasive MenC, MenW and MenY collected during the same period, with every third isolate of each serogroup being selected for inclusion in the Euro-3 panel, according to the date of reception at the reference laboratory.

The panel of 41 Brazilian isolates (Brazilian panel) was selected as a representative sampling of the phenotypes observed in the overall endemic non-MenB IMD burden in Brazil, out of the 428 isolates (377 MenC, 29 MenW and 22 MenY isolates) collected during the year 2012 by the Brazilian Meningitis, Pneumonia and Pneumococcal Infections Bacteriology Center at the Adolfo Lutz Institute in São Paulo. One hundred MenB isolates were also collected in this period.

All tested strains were invasive disease isolates originating from meningitis and/or septicaemia and were isolated from cerebrospinal fluid or blood samples.

All isolates were characterised by multilocus sequence typing (MLST) by each reference laboratory as described previously [41]. PorA serosubtype (or genosubtype if serosubtype was not available), fHbp and NHBA genotypes, and the presence of the *nadA* gene were also assessed.

2.2. Serum bactericidal antibody assay using human complement

The hSBA assays were performed as previously described [42], with minor modifications. Bacteria were sub-cultured overnight on chocolate agar plates, resuspended in liquid Multipurpose Handling Medium (Irvine Scientific) and grown until an optical density of 0.25 at 600 nm. SBA titres were determined as the last dilution that resulted in at least a 50% reduction in colony forming units (CFU) relative to the number of CFU present at the beginning of the bactericidal reaction (i.e. 50% of T0). Human plasma obtained from volunteer donors under informed consent was selected for use as complement source for an isolate only if it did not reduce the number of CFU of that isolate relative to T0 when added to the assay at a final concentration of 50%. The final assay mixture contained 25% complement-preserved human plasma.

The following control and test serum pools were prepared using samples from participants in various clinical trials:

- As a positive control pool, sera from 10 randomly-selected adolescents who received a single dose of a MenACWY conjugate vaccine (*Menveo*, GSK) were pooled (NCT00518180, [43]). Sera from pre-vaccination and one month post-vaccination were pooled separately.
- Sera from 20 to 40 randomly selected infants who received three doses of 4CMenB at 2, 4, and 6 months of age and a booster dose at 12 months of age were pooled (NCT00944034, NCT00847145, [44]).
- Pre-immunisation sera from a separate group of 180 infants enrolled in study NCT00657709 [44] were also pooled and used as a negative control.

2.3. Ethics

An informed consent form which included permission for potential re-use of the biological samples was obtained for participants in all the clinical trials.

3. Results

3.1. MLST analysis of the non-MenB meningococcal isolates

All isolates were classified on the basis of serogroup, clonal complex, 4CMenB antigenic sequence (fHbp, NHBA and PorA), and on the presence/sequence of the NadA coding gene.

3.1.1. Euro-3 panel

The 78 isolates from England and Wales included 16 MenC, 28 MenY, and 24 MenW isolates (Fig. 2); the 93 isolates from Germany included 75 MenC, 14 MenY, and three MenW isolates; and the 56 isolates from France comprised 39 MenC, eight MenY and nine MenW isolates. The serogroups and clonal complex repertoires within the Euro-3 panel are presented in Fig. 2A and B. Overall, MenC represented more than half of the isolates (57%, 130 isolates), followed by MenY (22%, 50 isolates) and MenW (16%, 36 isolates). Most MenC isolates belonged to the ST-11 complex, most MenW isolates to the ST-22 complex, while MenY isolates were mainly ST-23, ST-167 or ST-174 complexes; these five clonal complexes collectively represented 78% of the strain panel.

3.1.2. Brazilian panel

The panel included 20 MenY (49%), 16 MenW (39%), and five MenC (12%) isolates. From a molecular epidemiology perspective, a relevant peculiarity was the fact that all Brazilian MenC isolates belonged to the ST-103 complex and all MenW isolates belonged to the ST-11 complex (Fig. 2C, D), whereas in the Euro-3 panel, the majority of MenC and MenW isolates belonged to the ST-11 and ST-22 complexes, respectively. The clonal complex repertoires of MenY isolates were also different between the Euro-3 and the Brazilian panel, since Brazilian MenY isolates belonged to various clonal complexes (ST-32, ST-22, ST-23 and ST-174), while most

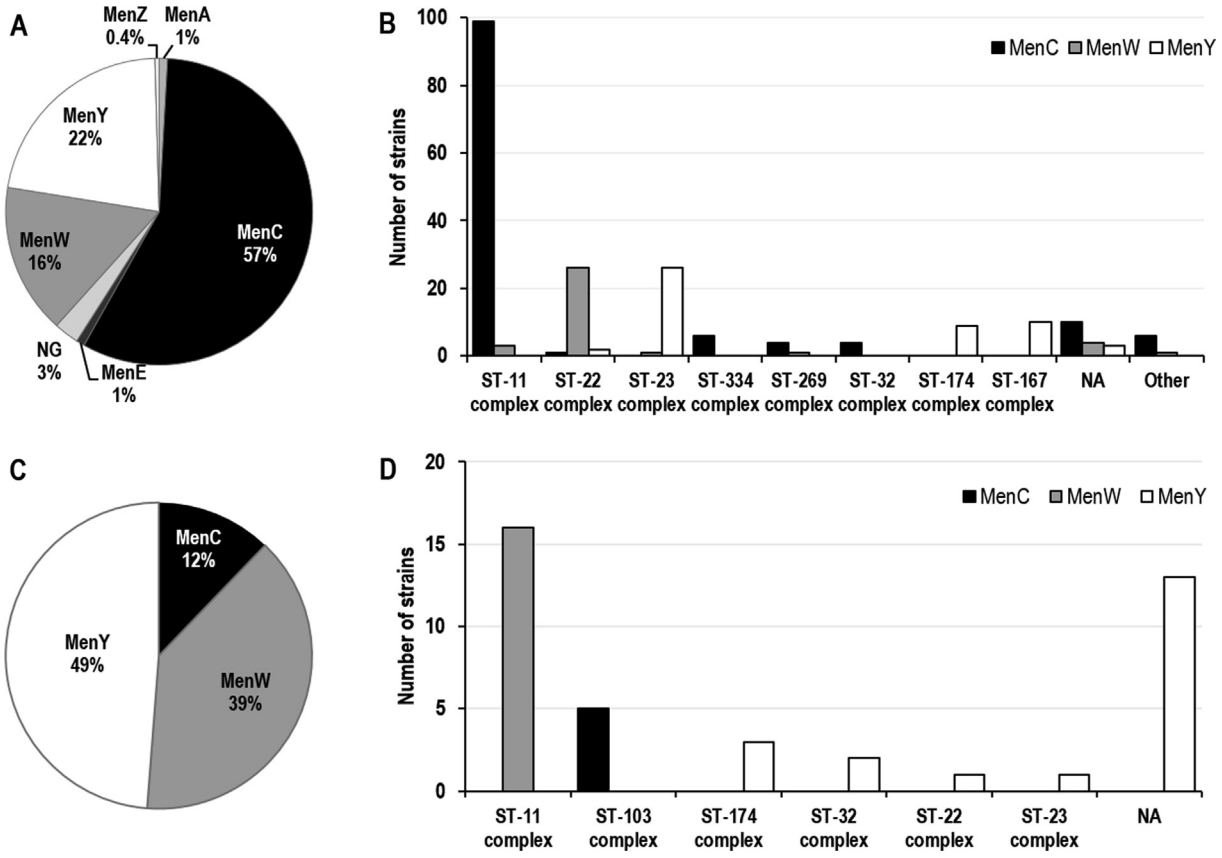


Fig. 2. Serogroup distribution and clonal complexes of non-MenB isolates in the Euro-3 (A and B) and Brazilian panels (C and D), respectively. Footnotes: Men, meningococcal serogroup; NG, non-groupable; ST, sequence type. Note: "Other" indicates a ST for which less than 1% of isolates were identified: • considering all serogroup: ST-103; ST-1; ST-5; ST-41/44: ST-60; ST-1157; ST-18; ST-865; ST-87. • considering C, W and Y serogroup: ST-103; ST-41/44; ST-18; ST-865; ST-8; ST-5. "NA" indicates a ST which was not assigned to any clonal complex or not defined: ST-3015, ST-10568, ST-10569, ST-6525, ST-10322, ST-877, ST-7952, ST-8373, ST-8353, ST-8308, ST-8454, ST-6675, ST-9294.

European MenY isolates belonged to the ST-23 complex (Fig. 2C, D).

3.1.3. 4CMenB antigen diversity in the Euro-3 and Brazilian panels

4CMenB antigen genotypes for each isolate in the Euro-3 and Brazilian panels were determined. The fHbp variant and subvariant repertoires, the NHBA peptide diversity and the *nadA* presence/absence genotype distributions are presented in Fig. 3A (Euro-3 panel) and 3B (Brazilian panel). In both panels, the fHbp variant 2 was found prevalent, whereas variant 3 was the least represented. A relevant high diversity of fHbp subvariants was identified in both panels. More than 11 NHBA peptides were identified, with NHBA-20 and NHBA-29 being the most common. The *nadA* gene was present in 38% and 49% of the Euro-3 and Brazilian isolates, respectively (Fig. 3). Only two isolates harboured the vaccine homologous PorA variable region (VR)2 subtype 4: a non-groupable isolate from England and Wales and a Brazilian MenY isolate.

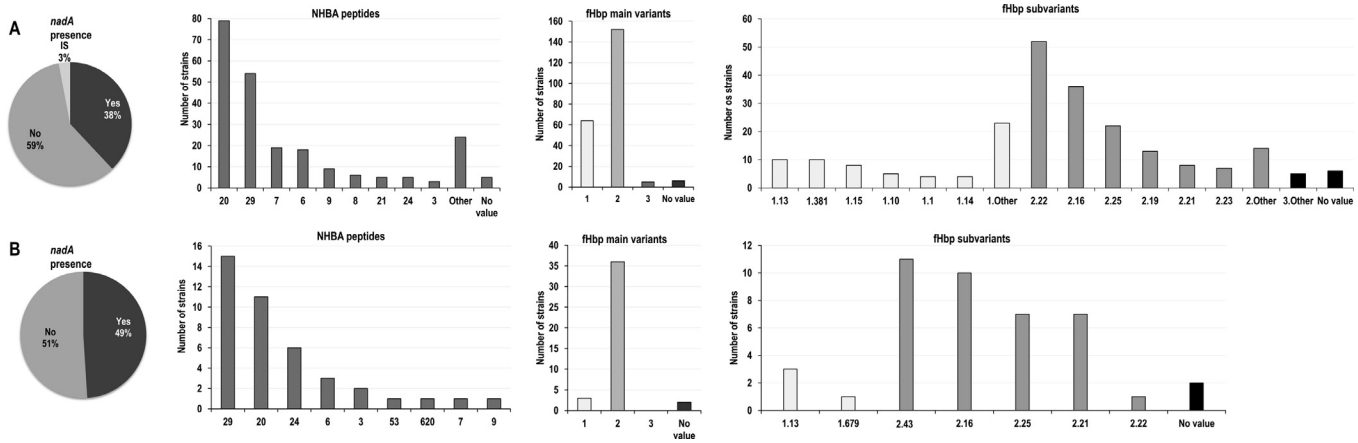


Fig. 3. 4CMenB antigenic diversity in the Euro-3 (A) and Brazilian (B) strain panels. Footnotes: *nadA*, *Neisseria* adhesin A gene; *IS*, insertion sequence-disrupted gene; NHBA, neisserial heparin binding antigen; fHbp, factor H binding protein. Note: "No value" refers to new fHbp variants and subvariants or NHBA peptides. fHbp subvariants listed as "Other" preceded by 1, 2 or 3 refers to subvariants other than those listed and present in less than 1% of isolates.

Three MenC isolates isolated in England and Wales as well as one German MenC isolate harboured a 1 bp deletion within the *fHbp* gene, causing a frameshift mutation and the introduction of a premature stop codon.

3.2. Unbiased selection of isolates for analysis in hSBA

In order to select a fully randomised strain panel to be tested in hSBA, which might represent respective balance of all genetic diversity and antigenic profiles of MenC, MenY and MenW isolates, we applied a random, unbiased selection to isolates from each serogroup of the Euro-3 panel, followed by a statistical evaluation to confirm adequate representation. The selection of the 20 Brazilian isolates to be evaluated in hSBA was made at the reference laboratory Adolfo Lutz Institute in São Paulo on the basis of the epidemiological relevance of each genotype, with no further support of statistical analysis.

A total of 147 isolates were analysed in the hSBA assay. The distribution of clonal complexes, fHbp variants, NHBA peptides, and *nadA* presence/absence genotype distributions among the subpanels of selected isolates corresponding to each non-MenB isolate, in comparison with the total strain collection is shown in Supplementary Fig. 1A (Euro-3 panel) and 1B (Brazilian panel). The Fisher's exact test for count data was applied to investigate a possible bias in the selection of the isolates for hSBA. There were no significant differences observed between the Euro-3 MenC and MenW isolates selected for hSBA testing and those not selected ($p > 0.05$), confirming that the selected isolates adequately represented the genotype diversity of the whole strain panel. The slight difference between MenY isolates was attributed to the high complement sensitivity of the isolates which resulted in some genotypes not being fully represented in the final subset tested in hSBA (p-values ranging from 0.03 to 0.05 for clonal complex, NHBA and *nadA* distributions).

3.3. Bactericidal activity of 4CMenB infant sera against non-MenB isolates

To provide insight into the potential coverage of non-MenB meningococci by 4CMenB-elicited antibodies, we tested the MenC, MenW and MenY isolates in hSBA using pooled immune sera from infant vaccinees. Pooled pre-immunization sera were used as neg-

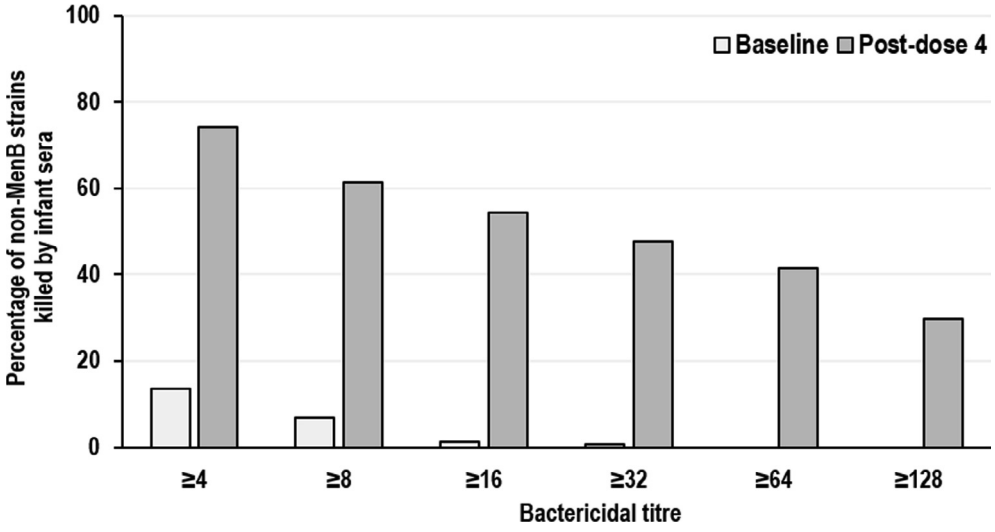


Fig. 4. Percentage of non-MenB isolates killed in human complement serum bactericidal activity assay by infant 4CMenB-elicited immune sera at different bactericidal titres. Footnotes: MenB, meningococcal serogroup B.

Table 1
Number of non-MenB isolates killed in hSBA by infant 4CMenB-elicited immune sera at different bactericidal titres in the Euro-3 and Brazilian panels.

SBA titres	MenC		MenW		MenY		Total	
	Baseline	Post-vac	Baseline	Post-vac	Baseline	Post-vac	Baseline	Post-vac
Euro-3 panel	N = 76		N = 28		N = 23		N = 127	
≥4	4 (5%)	50 (66%)	2 (3%)	21 (75%)	11 (48%)	21 (91%)	17 (13%)	92 (72%)
≥8	2 (3%)	35 (46%)	0	19 (68%)	8 (35%)	20 (87%)	10 (8%)	74 (58%)
≥16	0	29 (38%)	0	18 (64%)	2 (9%)	17 (74%)	2 (2%)	64 (50%)
≥32	0	26 (34%)	0	14 (50%)	1 (4%)	15 (65%)	1 (1%)	55 (43%)
≥64	0	24 (32%)	0	14 (50%)	0	12 (52%)	0	50 (39%)
≥128	0	21 (28%)	0	12 (43%)	0	10 (43%)	0	43 (34%)
Brazilian panel	N = 4		N = 7		N = 9		N = 20	
≥4	0	1 (25%)	0	7 (100%)	3 (33%)	9 (100%)	3 (15%)	17 (85%)
≥8	0	0	0	7 (100%)	0	9 (100%)	0	16 (80%)
≥16	0	0	0	7 (100%)	0	9 (100%)	0	16 (80%)
≥32	0	0	0	6 (86%)	0	9 (100%)	0	15 (75%)
≥64	0	0	0	4 (57%)	0	7 (78%)	0	11 (55%)
≥128	0	0	0	0	0	1 (11%)	0	1 (5%)

Men, meningococcal serogroup; hSBA, human complement serum bactericidal activity assay; N, number of isolates tested in hSBA with the 4CMenB-elicited immune sera; post-vac, post-vaccination (post-dose 4).

ative control, while a serum pool from adolescents vaccinated with a quadrivalent MenACWY glycoconjugate vaccine was used as positive control.

Bactericidal activity of the infant sera against MenC, MenW, and MenY isolates showed that 74.1% of the non-MenB isolates tested were killed by infant sera, with hSBA titres ranging from ≥4 to ≥128 (Fig. 4; Table S1). The percentage of isolates killed (hSBA titres ≥4) by infant pre-immune sera was 13.6%. A more detailed analysis on the number of isolates killed in the hSBA, classified by serogroup is reported in Table 1.

4. Discussion

A full evaluation of meningococcal vaccine performance must account for the proportion of likely-to-be-encountered endemic meningococcal strains that are covered by a vaccine-elicited immune response. As the antigens included in the 4CMenB vaccine are also carried by non-MenB strains, some level of cross-serogroup protective immunity might be expected. Here we show the ability of sera from infants vaccinated with 4CMenB to induce bactericidal killing of 109 of the 147 tested isolates representative of genetic and geographic diversity of MenC, MenY and MenW isolates. The selection of the isolates tested in hSBA was unbiased, and highly representative of the genotype diversity of the whole panel of 268 strains. The relative distribution of non-MenB serogroup strains in the two panels was different, with MenC (57% of strains) being predominant in the European panel and MenY (49% of strains) in the Brazilian one.

This represents the first study in which such a high number of non-MenB isolates has been tested in hSBA to assess bactericidal killing induced by a MenB vaccine. Overall, 74.1% of MenC, MenW, and MenY isolates collected in three European countries and Brazil were killed by pooled sera from infants immunized with 4CMenB. The overall percentages of strains killed in hSBA at different dilutions tended to be higher in the Brazilian panel than in the Euro-3 panel. However, this comparison is hindered by the differences in serogroup and antigen repertoire. In addition, its significance is limited by the relatively small number of isolates in the Brazilian panel. Nevertheless, afforded strain coverage was up to 64% for MenC, up to 80% for MenW, and up to 94% for MenY. In both panels, the percentage of MenY strains killed by pre-vaccination sera was higher than for MenC or MenW. While the reasons for this phenomenon are not clear, the MenW and MenY capsular polysaccharides were previously reported to enhance activation of the alternate pathway of complement and consequently, susceptibility to killing [45]. Moreover, the higher SBA observed pre-vaccination had no impact on strain coverage, as ≥4-fold increases in hSBA titers were observed for the majority of MenY strains in both panels, thus demonstrating a clear effect of 4CMenB immunization. As in the case of MenB strains, the correlation between SBA titers at one month post-vaccination and long-term protection is not known. Nevertheless, ≥76% of vaccinated infants still maintain hSBA titers ≥4 against at least one 4CMenB vaccine antigen for up to 24–36 months post-vaccination [46]. Further studies will be needed to evaluate the long-term protection afforded by vaccination with 4CMenB against non-MenB strains. The killing in hSBA of >74% of non-MenB strains, implies that the 4CMenB vaccine antigens are accessible to antibody binding in most of the non-MenB strains, independently of the chemical structure of the capsular polysaccharide. Of note, for all isolates, SBA was only tested after a full vaccination series (four doses), but we cannot exclude that protection could happen even after a lower number of immunizations.

The use of MenB-derived MATS data to predict potential strain coverage for non-MenB is yet to be explored, thus precluding the use of MATS to estimate the coverage of non-MenB isolates. Therefore, we assessed the susceptibility of non-MenB strains to hSBA killing induced by pooled vaccine immune sera as a more direct means of measuring non-MenB strain coverage. The use of serum pools (versus individual serum analyses) as a surrogate of overall population responses was already described [47]. In that study, the investigators demonstrated a strong correlation between pooled hSBA titres and the mean of the individual titres for sera composing the pool, and that the hSBA performed on pooled sera predicts individual seroprotection.

A recent study also evaluated hSBA killing induced by rLP2086 immune sera against 236 non-MenB isolates collected from the US, England and Wales, the Netherlands and Africa. Only six isolates, one for MenA, MenC, MenY and MenX and two MenW isolates, belonging to the ST-11 and ST-22 complexes, were tested. The panel of selected isolates may not be fully representative of the fHbp variant expression level, seeing that only strains with moderate fHbp expression levels were considered for testing and that fHbp expression can vary considerably across meningococcal strains (≤15-fold for MenB) [48]. Moreover, bactericidal activity was evaluated using 30 adolescent sera and expressed as the percentage of subjects with positive bactericidal titer, with the seroresponse rate ranging from 28% to 100% depending on the isolate used [49]. In our study, we tested 147 isolates, belonging to MenC, MenY or MenW serogroups, which were representative of the genetic and antigenic diversity of circulating invasive disease isolates, thus allowing a more precise estimation of 4CMenB's potential coverage of non-MenB strains. We demonstrated that the immune sera of infants vaccinated with 4CMenB were able to kill 109 non-MenB isolates, resulting in a coverage of 74%.

Coverage of the non-MenB strains in this panel as well as in the field is likely mediated by antibodies targeting multiple antigens. For MenC, the predominance of NHBA peptides 20 and 29 among the isolates deposited at the PubMLST *Neisseria* website (http://www.pubmlst.org/neisseria, data extracted on September 2018) (present in 66% of the deposited MenC isolates) was also observed in the MenC collection used for this study. In both the PubMLST database and the panel of this study, 38% of MenC isolates were represented by fHbp variant 1 peptides. While 47% of PubMLST MenC isolates harboured the *nadA* gene, in the panel studied here, 55% harboured an intact locus. Collectively, these data suggest that a similarly robust coverage of endemic MenC by 4CMenB-elicited immunity may be expected in the field.

For MenY, NHBA was the sole vaccine antigen with potential cross-reactivity in the majority of isolates tested in the hSBA, since all isolates carried fHbp variant 2 or 3 peptides and were almost all negative for *nadA* and mismatched for PorA P1.4. These results are consistent with the majority of MenY isolates deposited in the PubMLST database. The relatively high coverage of up to 94% (30/32) of MenY isolates might indicate NHBA as the most effective vaccine component, and suggest that anti-NHBA induced antibodies might be bactericidal against MenY strains, acting alone or in synergy with antibodies directed against non-PorA OMV antigens and/or with anti-fHbp antibodies. The study estimating the coverage of non-MenB strains by rLP2086 also described a lower median fHbp expression level for MenY strains, when compared with MenA, MenC, MenW and MenX isolates [49]. However, the reliability of this comparison might be limited by the differences between the strain panels used in the two studies.

For MenW, variant 2 was by far the predominant fHbp variant in the strains included in the present study, which is in line with the fHbp genotype of most of the MenW isolates deposited in PubMLST. Although the great majority of MenW isolates of the Euro-3 panel belong to the ST-22 complex and do not harbour *nadA*, the MenW isolates of the Brazilian panel belonged instead to ST-11 and carried the *nadA* gene. Most MenW isolates deposited

in the PubMLST database belong to the ST-11 complex and carry the *nadA* gene. The Brazilian MenW isolates included in the present study are genetically similar to six MenW isolates (all W:P1.5,2:ST-11) isolated in the United Kingdom in 2011–2012 and shown to be killed by sera of infants vaccinated with 4CMenB [37]. On the basis of the antigenic repertoire we can assume that in the case of the Euro-3 MenW ST-22 complex, NHBA is likely to be the bactericidal antigen, whereas for the Brazilian ones, a significant contribution of anti-NadA antibodies to killing is also expected. These data are of interest in view of the incidence of MenW worldwide and of the recent emergence and expansion in Europe, of MenW/ST-11 isolates that may have emerged from the South American MenW ST-11 isolates [50].Although two of the remaining globally prevalent serogroups were not studied here, there is some evidence suggesting that a reasonable level of cross-protection against MenA and MenX may be expected. All MenA isolates available in-house analysed thus far (>20) express the cross-reactive fHbp variant 1 and the NadA vaccine antigen and are killed in bactericidal assays by 4CMenB human sera (unpublished results). Indeed, data on MenA isolates deposited in PubMLST reveal that >97% harbour fHbp variant 1 and about 70% carry the *nadA* gene. MenX is quickly emerging as a significant public health threat in West Africa [51,52]. Data available from the PubMLST database show that while only 21% harbour the *nadA* gene and few express PorA P1.4 (2.1%), most contain the fHbp variant 1 (76%) and NHBA peptides 358, 359 or 114 (56%). The evidence that epidemic MenX isolates emerging and spreading in the African meningitis belt are likely to be covered by 4CMenB comes from a study assessing nine MenX isolates isolated in Africa and tested against 4CMenB-elicited immune sera from adult, adolescent, and infant vaccinees [35]. All nine isolates were killed with high hSBA titres regardless of age group. Notably, two endemic MenX local isolates collected in France which are genetically different from the African isolates, were not killed.

While our results are illustrative of the potential mechanism underlying cross-reactive 4CMenB-elicited immunity against non-MenB isolates, it is not possible to directly extrapolate a global perspective on non-MenB strain coverage in other regions not included in this analysis. However, given the present observations and the available genetic landscape of well-characterised clinical isolates from all serogroups (e.g. molecular epidemiology data available at the PubMLST database), it would be reasonable to expect similar cross-reactivity (and consequently cross-protection) against a large proportion of non-MenB isolates circulating worldwide.

5. Conclusion

This represents the first study in which a large collection of non-MenB isolates has been analysed in a hSBA assay, using sera from individuals immunized with 4CMenB. 4CMenB vaccination was able to induce bactericidal killing of 109 (74%) non-MenB isolates, indicating that vaccination campaigns using 4CMenB would also impact non-MenB IMD burden, which should be considered in health economic planning. The data described herein suggest that the inclusion of 4CMenB in a targeted pan-serogroup vaccination regimen, in combination with available vaccines against MenA, MenC, MenW and MenY, would not only provide coverage of endemic MenB strains, but may also enhance efficacy and coverage across non-MenB isolates. Implementation of 4CMenB vaccination will provide real-world evidence on its beneficial effect on non-MenB strains.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: MM and MC were, AB, GDA, ST, BB, MG, SB, SoB, MMG, RR and MP are employees of the GSK groups of companies. SoB has a patent pending (WO 2015014922 A2). PB was an employee of PRA Health Sciences c/o GSK at the time of study. MKT received grants from Pfizer and the GSK group of companies and has a patent issued (630133). JL and RB have performed contract research on behalf of Public Health England for the GSK group of companies, Pfizer, and Sanofi Pasteur. APSdL reports personal fees from Pfizer and Sanofi-Pasteur. HC and UV's institution received research grants from Novartis. MCOG and EH have nothing to disclose.

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Authors contributions

AB, BB, ST performed the bactericidal assays and contributed to data analysis. SB, GDA, MM, MC, MG and MMG contributed to the analysis of the genetic data. MCOG, APSL, HC, UV, MKT, EH, RB and JL provided the isolates, genotyping and phenotyping data and contributed to data discussion. SoB performed the statistical analysis, MMG, MC and RR contributed to the data interpretation and discussion, MP and PB designed the study and wrote the manuscript. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors reviewed and commented critically on the drafts of the manuscript for important intellectual content and gave final approval to submit for publication.

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Data sharing statement

To request access to patient-level data and documents for this study, please submit an enquiry via www.clinicalstudydatarequest.com.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.09.050.

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