Published in final edited form as:
Euro Surveill. 2015 ; 20(49): . doi:10.2807/1560-7917.ES.2015.20.49.30084.

Meningococcal vaccine antigen diversity in global databases

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Abstract

The lack of an anti-capsular vaccine against serogroup B meningococcal disease has necessitated the exploration of alternative vaccine candidates, mostly proteins exhibiting varying degrees of antigenic variation. Analysis of variants of antigen-encoding genes is facilitated by publicly accessible online sequence repositories, such as the *Neisseria* PubMLST database and the associated Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL). We investigated six proposed meningococcal vaccine formulations by deducing the prevalence of their components in the isolates represented in these repositories. Despite high diversity, a limited number of antigenic variants of each of the vaccine antigens were prevalent, with strong associations of particular variant combinations with given serogroups and genotypes. In the MRF-MGL and globally, the highest levels of identical sequences were observed with multicomponent/multivariant vaccines. Our analyses further demonstrated that certain combinations of antigen variants were prevalent over periods of decades in widely differing locations, indicating that vaccine formulations containing a judicious choice of antigen variants have potential for long-term protection across geographic regions. The data further indicated that formulations with multiple variants would be especially relevant at times of low disease incidence, as relative diversity was higher. Continued surveillance is required to monitor the changing prevalence of these vaccine antigens.

Introduction

Neisseria meningitidis, the meningococcus, a Gram-negative diplococcus, is a globally important causative agent of meningitis and septicaemia (severe sepsis), accounting for a significant amount of morbidity and mortality worldwide. However, it is frequently carried harmlessly in the human nasopharynx and can be considered part of the normal human

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Authors' contributions

CB and DH undertook the data analysis and interpretation and prepared the figures and tables. CB wrote the first draft of the manuscript and DH, JL, RB and MM contributed to discussions of results, interpretation of data and contributed to writing the manuscript.

Conflict of interest

DH was supported by the Meningitis Research Foundation. RB does contract research on behalf of his employer, Public Health England, for manufacturers of meningococcal vaccines including Baxter Biosciences, GlaxoSmithKline, Novartis Vaccines, Pfizer, and Sanofi Pasteur. MM has received grants and personal fees from Novartis outside the submitted work.
The other authors declare no competing interests.

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commensal micro-biota. Currently, no comprehensive vaccine exists against meningococcal disease due in large part to the structural similarity of serogroup B polysaccharide to polysaccharides associated with the human neural cell adhesion molecule (NCAM). This is thought to account for the poor human immune response against group B polysaccharide and also raises safety concerns [1]. Many subcapsular vaccine antigen candidates, especially proteins, have therefore been investigated, with the intention of producing serogroup B substitute formulations. Several such antigens have been incorporated into vaccine formulations that are in various stages of development.

First developed in the 1980s, outer membrane vesicle (OMV) vaccines were created to counter higher levels of disease incidence caused by particular serogroup B meningococci. These OMV vaccines contained the respective epidemic antigen variants of the outer membrane protein PorA of these meningococci as major immunogens and were successfully deployed in Norway (MenBvac), Cuba (VA-MENGOC-BC), and New Zealand (MeNZB) [2-4]. In the last decade however, many high-income regions such as Europe and North America have experienced a period of relatively low incidence of serogroup B meningococcal disease [5,6]. In such periods, when disease incidence is lower, but caused by more diverse meningococci, vaccines should ideally contain several components in order to attain the widest possible strain coverage. An example is the proposed NonaMen (RIVM, the Netherlands) vaccine formulation comprising nine PorA variants corresponding to the most prevalent disease-associated strains [7]. Alternatively, Bexsero, developed by Novartis, is a supplemented OMV vaccine which contains four components: PorA P1.7–2, 4, fHbp subvariant 1.1, NHBA variant 2, and NadA-3.8 subvariant [8]. This was licenced in Europe in 2013 and in the United States (US) in 2015 and has been included in the infant immunisation schedule in the United Kingdom (UK) since September 2015. The rLP2086 vaccine, Trumenba developed by Pfizer, which was licenced by the Food and Drug Administration in the US in 2014, is a bivalent recombinant vaccine based on two fHbp antigens from subfamily A and B (subvariants 3.45 and 1.55 respectively) [9].

Over the past two decades, sequence-based molecular typing has become an intrinsic part of meningococcal disease surveillance and standardised typing methods and schemes have allowed for more comparability across reference and research laboratories in different countries [10-12]. For example, the European surveillance system (TESSy) of the European Centre for Disease Control (ECDC) (http://www.ecdc.europa.eu/en/activities/surveillance/Tessy) and the European Meningococcal Epidemiology in Real Time (EMERT) database (http://emgm.eu/emert/) include two typing antigens which are also vaccine candidates, PorA and FetA. Following the advent of whole genome sequencing (WGS) and its rapidly reducing costs, comprehensive investigations of the likely and actual impact of available or potential interventions may be made more easily. Publicly accessible online resources such as the *Neisseria* PubMLST database (http://pubmlst.org/neisseria/) and the Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL) (http://www.meningitis.org/research/genome), which contain molecular typing information from single genes up to many hundreds, and for many thousands of isolates, allow fine-scaled analyses, including investigation of the distribution of vaccine components. In this study, the PubMLST and MRF-MGL databases were used in an investigation of the distribution of

vaccine components in Bexsero, Trumenba, NonaMen, MenBvac, MeNZB and VA-MENGOC-BC.

Methods

This study made use of the public *Neisseria* PubMLST database http://pubmlst.org/neisseria/ and the MRF-MGL http://www.meningitis.org/research/genome which is hosted within it. The databases were accessed in August 2014. The MRF-MGL contained 1,344 *N. meningitidis* isolates which were all from England and Wales, covering all culture-confirmed cases of invasive meningococcal disease (IMD) from the epidemiological years 2010/11 to 2012/13. From the PubMLST database, we included 1,717 *N. meningitidis* isolates within the database which had assembled sequence data of at least 0.5 Mbp. This is the minimum amount of assembled sequence data that allows as complete an analysis of vaccine antigen distribution as possible. This multinational collection contained data on disease and carriage isolates from the years 1937 to 2014, from 53 countries, six continents and excluded MRF-MGL isolates (Table 1).

The PubMLST database is a publicly-accessible repository of isolate and typing information for several species including *N. meningitidis*. It contains a large set of global records of *Neisseria* genus isolates (33,019 in March 2014) spanning 100 years. It is a complete catalogue of known genotypic and phenotypic variation, date and location of isolation, and permits an estimation of the minimum lifespan of particular genotypes and deduced antigenic types. While the collection is not an epidemiologically coherent sample set as a whole, it does include several such datasets (including the MRF-MGL), and permits several types of investigation into the evolution and population biology of *N. meningitidis.*

The presence of components of each of the fHbp-containing vaccines, were analysed in the collections [8,9]. Before the development of a unified nomenclature scheme in which each unique allele is assigned a unique numerical identifier [13], separate schemes were developed which divided fHbp into either two subfamilies (subfamily A and B) or three variant families (variant families 1, 2 and 3) according to nomenclature system [14,15]. These schemes can be cross-referenced online (http://pubmlst.org/neisseria/fHbp/). Briefly, subfamily B is equivalent to variant family 1 and subfamily A incorporates both variant families 2 and 3. Peptides are then numbered with the variant family/subfamily name e.g. fHbp 1.1 is variant family 1 peptide 1 [13]. This is the fHbp nomenclature used throughout this paper. The recombinant vaccine Trumenba contains two fHbp antigen subvariants: peptide 45 (Pfizer nomenclature A05, subfamily A/variant family 3) and peptide 55 (Pfizer nomenclature B01, subfamily B/variant family 1). As well as Bexsero and Trumenba, other vaccine formulations analysed were: NonaMen, which contains nine PorA antigens variants (P1.7,16; P1.5–1,2–2; P1.19,15–1; P1.5–2,10; P1.12–1,13; P1.7–2,4, P1.22,14; P1.7–1,1; P1.18–1,3), MenBvac, (P1.7,16), MeNZB (P1.7–2,4) and VA-MENGOC-BC (P1.19,15) [4,7,16,17]. Analysis of the distribution of vaccine components among clonal complexes and patient age groups in the MRF-MGL was based on an exact match of deduced peptide sequences (‘sequence match’) to at least one component of each vaccine formulation investigated. The analysis was carried out on isolates of all genogroups (organisms with a *cps* region, encoding a capsule) and genogroup B isolates only (those containing a *cps*

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region encoding the group B polysaccharide capsule). For the purposes of this analysis is was assumed that such meningococci either had the capacity to express a capsule or had a very closely related ancestor which could [18]. It should be noted that OMV vaccines include other potentially immunogenic proteins not assessed here, although WGS allows for such analyses.

Simpson's index of diversity (*D*) was used to determine the diversity of each Bexsero vaccine antigen by age group in the MRF-MGL. The value of the index ranged from 0 to 1, with values nearer to 1 indicating greater diversity. Calculation of the index was performed as described previously [19]. The 95% confidence intervals (CIs) for the index were calculated as described previously [20].

Results

Current vaccine antigens in the MRF-MGL collection: invasive meningococcal disease isolates from England and Wales, 2010/11 to 2012/13

Within the MRF-MGL collection, NadA was significantly the least diverse peptide antigen examined (*D* = 0.39; 95% CI: 0.36–0.42) compared with: PorA variable region 1 (VR1) (*D* = 0.87; 95% CI: 0.86–0.88), PorA VR2 (*D* = 0.92; 95% CI: 0.92–0.93), fHbp (*D* = 0.91; 95% CI: 0.91–0.92) and NHBA (*D* = 0.91; 95% CI: 0.91–0.92). However, a total of 1,025 (76.3%) isolates were inferred to be missing the *nadA* gene as it was not found in their genomes (Table 2). A total of 1,102 of 1,344 (82.0%) isolates contained exact peptide sequence matches to components of any of the vaccines investigated (Figure 1). Isolates belonging to the two most prevalent clonal complexes (cc) in the MRF-MGL, ST-41/44cc and ST-269cc, had most sequence matches (302 and 181 instances, respectively). Exact matches to NonaMen components were found in 20 different clonal complexes. In total there were 1,077 of 1,344 isolates (80.1%) with genomes that encoded the PorA VR1 or VR2 antigen variants included in the NonaMen formulation. ST-41/44cc isolates comprised 294 of these 1,077 (27.3%), a consequence of their association with PorA subtype P1.7–2,4.

Unlike the other vaccine formulations, exact matches to Trumenba vaccine components were found in a single clonal complex (ST-213cc). Exact amino acid sequence matches to at least one of the four Bexsero vaccine antigen variants were present in 378 of 1,344 (28.1%) isolates, which belonged to 10 clonal complexes. Again, ST-41/44cc contained the majority of matches (276 isolates) due to its association with NHBA peptide 2 and PorA VR2 P1.4, but only two isolates of ST-269cc contained an exact match to any Bexsero antigen peptide variant. None of the major hyperinvasive clonal complexes, except for a single genogroup A ST-5cc isolate, contained an exact match to NadA subvariant 8, which was found in 13 ST-174cc isolates. However, 65.7% of ST-32cc isolates contained Bexsero fHbp variant family 1 peptide 1, consistent with derivation of this component from MC58, an isolate of the same complex [21].

In total, 37.0% of genogroup B isolates contained at least one exact match to any Bexsero vaccine component. For the other vaccines, the proportion of isolates with at least one exact match to vaccine antigen variants were NonaMen: 82.7%, MeNZB: 21.8%, MenBvac: 7.5% and VA-MENGOC-BC: 7.3%. When cross-protection among NadA peptide alleles

belonging to variants NadA-1 and NadA-2/3 and among fHbp peptide alleles belonging to family 1 was assumed, the overall number of isolates containing an exact match to Bexsero antigen variants more than doubled to 847 isolates, or 63.0% of the MRF-MGL.

Vaccine antigen distribution by age group

Vaccine peptide diversity and distribution in the MRF-MGL were assessed by age group (Table 2, Figure 2). For all antigens, peptide diversity was lowest among meningococci isolated from patients younger than one year or 65 years and older. Significant differences in peptide diversity, those where the 95% CIs did not overlap, were seen. For example, fHbp diversity (D = 0.93; 0.91–0.95) was significantly greater in isolates from patients aged 25 to 29 years than in isolates from patients 65 years and older (D = 0.85; 0.81–0.89). Apart from isolates from patients 65 years and older, where PorA P1.7–2 predominated, and those from patients 40 to 64 years of age, where NHBA and PorA P1.7–2 were equally prevalent (15 occurrences), NHBA-2 was the most frequently identified Bexsero antigen peptide in all age categories (data not shown). PorA P1.7–2 was the second most prevalent vaccine subvariant in age groups 25 to 39 years and younger. The fHbp 1.1 subvariant was found at the lowest proportions in patients younger than one year and in those 65 years and older. NadA-3.8 was absent from isolates from patients aged one to four years and 15 to 24 years, and was identified in less than 2% of isolates from all other age groups except the group 65 years and older, where it was present in 3.9% of isolates. Thus, isolates possessing at least one of the four Bexsero antigen peptide variants were found at the highest proportions in patients aged one to four and five to 14 years (114/315 (36.2%) and 34/99 (34.3%) isolates, respectively), and at the lowest proportions in patients aged 65 years and older (37/207 (17.9%) isolates). The greatest proportion of genogroup B isolates with at least one exact amino-acid sequence match was identified in patients aged 15 to 24 years (48/107 (44.9%) genogroup B isolates), and the least was in patients younger than one year (88/277 (31.8%) genogroup B isolates). The association of ST-41/44cc and less common lineages with patients younger than one year meant that 30.3% of all isolates contained at least one exact peptide match. Isolates possessing at least NadA-1 or NadA-2/3, fHbp variant family 1, PorA P1.4, or NHBA-2 allele were found at the highest proportion in patients aged five to 14 years (72/99 (72.7%) isolates). Of the vaccine formulations assessed, NonaMen antigen peptide variants were the most frequently identified in isolates of all age groups (Figure 2), ranging from 76.3% of patients aged 40 to 64 years to 82.1% of patients 65 years and older. A total of 81.7% of all isolates and 82.3% of genogroup B isolates from patients younger than one year possessed at least one NonaMen PorA variant. The Trumenba vaccine antigen peptide subvariants fHbp 3.45 and fHbp 1.55 were not found in isolates from patients aged five to 14 years (n = 99).

Vaccine candidate antigen variants in PubMLST/neisseria database

There were 38 isolates in the PubMLST *Neisseria* collection with the fHbp 3.45 subvariant and none with the 1.55 subvariant (Table 3). A total of 36 of the 38 isolates that possessed the 3.45 subvariant were ST-213cc and were all from the UK. Those which had a date of isolation recorded in the database were from between 1999 and 2012. The Bexsero subvariant fHbp 1.1 was found in 73 isolates in the dataset. Of these, 66 were ST-32cc and 47 of 73 were serogroup B. They were found over a time span of 40 years from 1969 to 2009 and on all continents. NadA-3.8 was found in 41 isolates. Of those, 17 were ST-174cc

and 16 were ST-5cc; 16 were serogroup Y, 16 serogroup A and five were serogroup B. They were found across 49 years from 1963 to 2012 and on all continents except Oceania. NHBA variant 2 was found in 121 isolates and 98 of these were ST-41/44cc. Of the 121 isolates for which serogroup data were recorded, 66 were serogroup B, 12 were serogroup C and 11 were non-groupable. NHBA variant 2 was found on all continents except Asia and Africa and across 37 years from 1976 to 2013.

Cross-protection among fHbp variant family 1 and NadA-1, NadA-2 and NadA-3 variant family members has been described [22,23], prompting an analysis of their distribution. There were 873 fHbp variant family 1 peptide variants and these were found across the whole time span of the collection (77 years) from 1937 to 2014 and on all continents. Of these, 349 (40.0%) were ST-11cc, 98 (11.2%) were ST-41/44cc, and unassigned sequence types (STs) accounted for 92 (7.4%) of the isolates. Of the 873, 241 were serogroup W, 159 were serogroup B, 113 were serogroup C and 76 were serogroup A. There were 709 isolates that were NadA-1, 2 and 3 variant family members. These spanned 51 years of the collection from 1963 to 2014 and were found on all continents. The majority were ST-11cc (n = 568; 80.1%), 368 were serogroup W and 135 were serogroup C.

Discussion

Since its introduction in the 1990s, sequence-based molecular typing has established a role in the clinical microbiology laboratory, replacing or complementing existing phenotypic typing methods. WGS is the latest sequencing technology and, as costs continue to decrease, will become more commonplace in clinical and reference laboratories [24-26]. WGS provides definitive sequence-level resolution with widespread applications including molecular epidemiology, surveillance, vaccine design and vaccine implementation monitoring [27]. To be useable by physicians and public health specialists, databases will need to use uniform nomenclature and be interoperable and compatible with other databases such as those that contain phenotypic information [28].

As WGS databases such as PubMLST are generic and scalable, they enable detailed deduction of potential coverage and preliminary assessment of the impact of given vaccine formulations on the meningococcal population, thus informing further work such as phenotypic assays [29]. This requires the assembly of representative collections of meningococcal isolate genomes. The MRF-MGL is an exemplar of a representative, contemporary, curated, publicly accessible database containing many hundreds of genomes and was expressly established as a resource for the meningococcal research and public health communities. It is embedded within the PubMLST database, which has been running for many years as a community resource and repository for isolate and characterisation data. The MRF-MGL is the most comprehensive epidemiological sample of meningococcal WGS currently available, allowing an assessment of vaccine antigen distribution among disease cases.

Molecular epidemiology using phenotypic or genotypic data has been used to inform vaccine design: tailor-made OMV vaccines were designed to contain the respective outbreak strain PorA variant [2-4]; and the broad-spectrum multivalent Hexamen/Nonamen

formulation was based on the most prevalent PorA serosubtypes documented in the Netherlands at the time of its development [30]. One of the earliest uses of genome data in vaccine design was in the discovery of novel meningococcal vaccine candidates by ‘reverse vaccinology’ based on a single isolate genome [31]. Several of these genome-derived antigens are components of vaccines at various stages of clinical development and deployment at the time of writing [8,9].

The temporal and geographic spread of antigen variants and combination of variants, with some existing for long time periods and across several continents, demonstrated the stability of antigen clonal complex relationships and therefore the potential longevity of appropriate vaccine formulations [32,33]. Previous work using the PubMLST database collection [32] demonstrated the longevity of PorA VR types and their association with clonal complex which we here extend to other antigens available from WGS. For example, the most frequent strain type (PorA:FetA:cc) P1.5,2:F3–6:cc11 had a minimum lifespan of 49 years and was found on three continents (Europe, North America and South America) [32]. In the present analysis, multicomponent vaccines exhibited more potential to protect against isolates represented in the MRF-MGL than vaccine formulations containing one or a few components, although this did not take into account any potential cross-protection that may be offered by any particular vaccine antigen. Europe and North America are experiencing low rates of meningococcal disease at present [5,6], and in the absence of a dominant epidemic clone accounting for disease, a multicomponent vaccine formulation would be required to cover most disease [34,35]. Since a differential distribution of clonal complex and antigens has been demonstrated among different age groups and those at highest risk (the under one year-olds) are less at risk from lineages that affect the next peak of disease incidence, late adolescents, multicomponent vaccines are likely to be most appropriate, especially in a period of low incidence [36]. Therefore, comprehensive molecular epidemiology and surveillance is required in order to maximise the coverage of a given vaccine formulation. Continuous surveillance will be required to track changes in epidemiology that may need vaccine reformulation.

While genotypic data can provide valuable information on the potential utility of vaccines, the evaluation of antigen expression and potential cross-reactivity is fundamental to gauging the actual success of a given formulation. Assays have been developed and expression studies carried out that attempt to predict the coverage of various meningococcal vaccine antigens in the population [14,37]. One assay, the ELISA-based meningococcal antigen typing system (MATS) was developed to predict the strain coverage of the Bexsero vaccine [37]. Based on a panel of invasive serogroup B-associated meningococcal isolates from several European countries it was estimated that it could protect against 78% of serogroup B cases and against a panel of serogroup B invasive isolates from Greece up to 90% [38,39]. One of the features of the PubMLST database is that phenotypic information such as MATS data can be added to isolate or allele data so that phenotypic and genotypic information may be associated allowing further analyses.

Conclusion

Highly variable pathogens require detailed characterisation to appropriately tailor clinical and public health responses such as treatment, immunisation, outbreak control, and novel vaccine design. This is especially true for organisms such as meningococci, in particular those that express the serogroup B polysaccharide, given that a universal capsular vaccine is unavailable. Well-characterised isolate collections can easily be investigated for any number of vaccine formulations and vaccine candidates when they are housed within databases embedded with analysis tools which can handle phenotype and genotype data including WGS. This high level of characterisation and molecular epidemiology provides a foundation for further phenotypic analyses so that a fuller picture of potential vaccine effectiveness can be seen. Detailed characterisation and monitoring is particularly relevant in periods of low incidence, such as experienced in high-income regions at present, as multivalent vaccines may be most appropriate and also most adaptable should changes in the meningococcal population occur. This rationale for vaccine formulation using molecular epidemiology may be applied to any pathogen and will become more readily applicable as well characterised datasets like the MRF-MGL and PubMLST become increasingly available. A combination of detailed genotypic characterisation and phenotypic investigation offer the best hope of producing vaccines with the widest possible coverage.

Acknowledgements

This publication made use of the Meningitis Research Foundation Meningococcus Genome Library (http://www.meningitis.org/research/genome) developed by Public Health England, the Wellcome Trust Sanger Institute and the University of Oxford as a collaboration. The project is funded by Meningitis Research Foundation. It also made use of the Neisseria PubMLST website (http://pubmlst.org/neisseria/) developed by Keith Jolley and sited at the University of Oxford (BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 2010;11(1):595). The development of this site has been funded by the Wellcome Trust and European Union.

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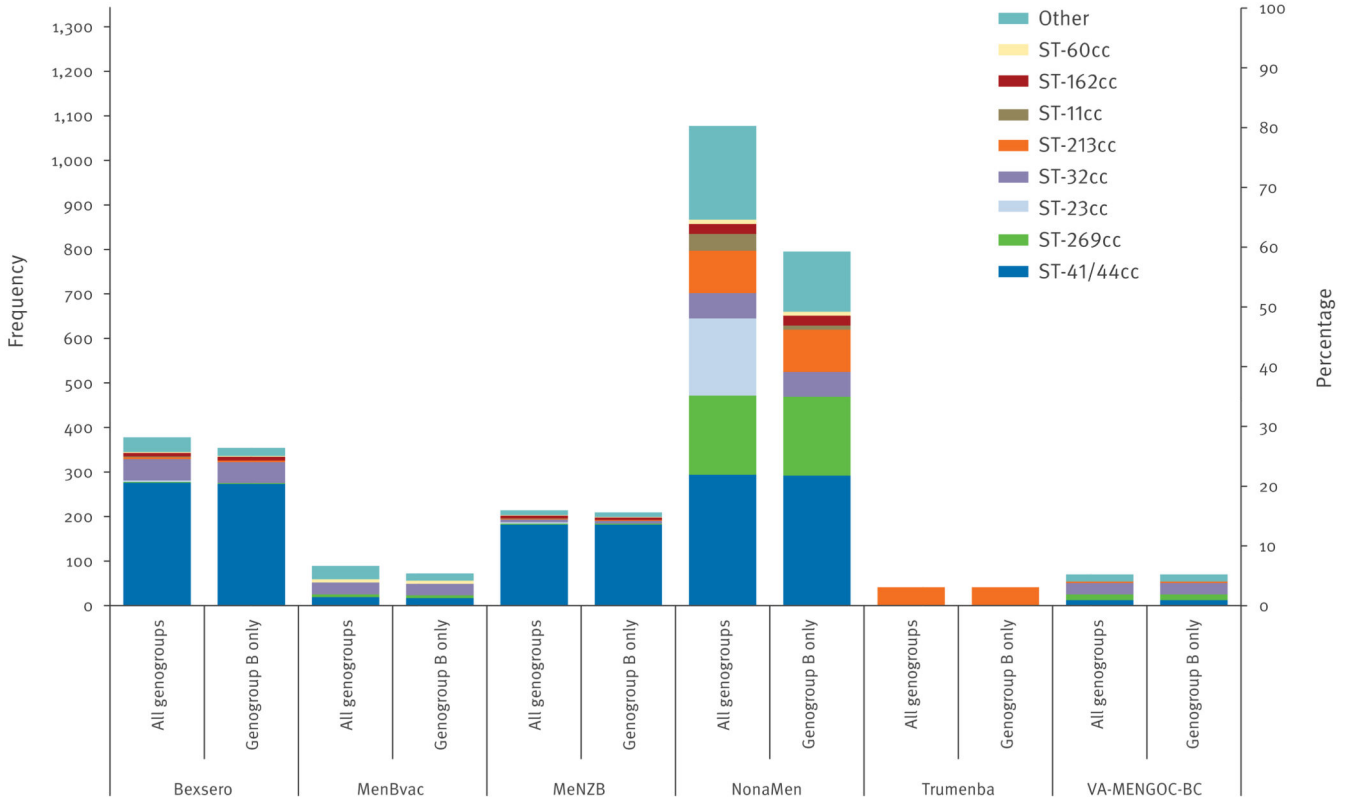

Figure 1.

Distribution of clonal complexes in meningococcal disease isolates in the MRF-MGL collection with exact peptide matches to at least one antigen of various vaccines, England and Wales, 2010/11–2012/13 (n = 1,344)

MRF-MGL: Meningitis Research Foundation Meningococcus Genome Library.

Invasive meningococcal disease isolates from England and Wales from epidemiological years 2010/11 to 2012/13 inclusive of all genogroups or genogroup B only. ‘Other’ includes uncommon ccs and unassigned STs. Secondary y-axis indicates percentage of total number of isolates.

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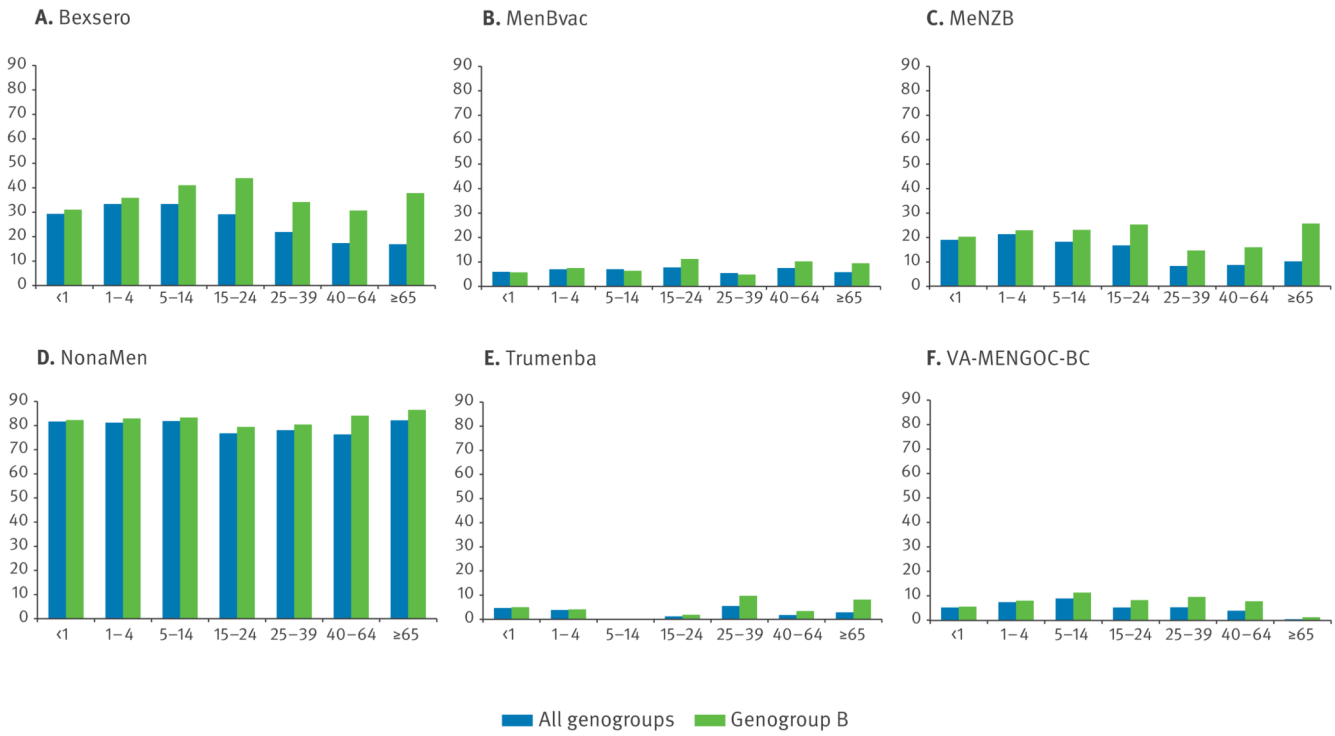

Figure 2.

Percentage distribution of age groups in meningococcal disease isolates in the MRF-MGL collection with exact peptide matches to at least one antigen of various vaccines, England and Wales, 2010/11–2012/13 (n = 1,344)

MRF-MGL: Meningitis Research Foundation Meningococcus Genome Library.

Invasive meningococcal disease isolates from England and Wales from epidemiological years 2010/11 to 2012/13 inclusive of all genogroups and genogroup B only.

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Table 1

Characteristics of the PubMLST global *Neisseria meningitidis* collection, 1937–2014 (n = 1,717)

Sample source	Number of isolates	Country	Number of isolates	Year of isolation	Number of isolates	Clonal complex	Number of isolates
Disease	926	United Kingdom	1,143	Unknown	319	ST-11cc	677
Carriage	112	South Africa	178	1937	1	ST-41/44cc	154
Unknown	679	Czech Republic	53	1940	1	ST-269cc	102
		Greece	47	1941	1	ST-32cc	88
Serogroup	Number of isolates	Norway	36	1947	1	ST-23cc	79
Unknown	596	United States	25	1962	1	ST-213cc	52
W	413	Canada	22	1963	5	ST-1cc	47
B	278	Ireland	20	1964	3	ST-22cc	46
C	256	Unknown	15	1965	1	ST-60cc	44
A	82	Spain	15	1966	5	ST-53cc	35
Y	46	Niger	13	1967	3	ST-1157cc	28
NG	40	Burkina Faso	12	1968	2	ST-35cc	22
E	2	Germany	12	1969	3	ST-5cc	21
X	2	The Netherlands	12	1970	8	ST-174cc	19
Z	2	France	11	1971	1	ST-8cc	19
		China	11	1973	1	ST-167cc	18
Continent	Number of isolates	Cameroon	8	1974	2	ST-162cc	15
Europe	1,373	Malta	7	1975	9	ST-4cc	14
Africa	234	Turkey	6	1976	6	ST-198cc	10
North America	49	Brazil	5	1977	4	ST-865cc	10
Asia	30	Chile	4	1978	4	ST-18cc	9
Unknown	15	Denmark	4	1979	5	ST-254cc	8
South America	10	New Zealand	4	1980	4	ST-103cc	6
Oceania	6	Algeria	3	1981	1	ST-4821cc	5
		Chad	3	1982	1	ST-1136cc	4
		Gambia	3	1983	3	ST-1117cc	3
		Italy	3	1984	6	ST-178cc	3
		Mali	3	1985	20	ST-334cc	3
		Morocco	3	1986	10	ST-364cc	3
		Portugal	3	1987	9	ST-461cc	3
		Russia	3	1988	19	ST-181cc	2
		Senegal	3	1989	6	ST-282cc	2
		Australia	2	1990	5	ST-37cc	2
		Cuba	2	1991	4	ST-613cc (lactamica)	2
		Finland	2	1992	5	ST-175cc	1
		Ghana	2	1993	58	ST-212cc	1

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Sample source	Number of isolates	Country	Number of isolates	Year of isolation	Number of isolates	Clonal complex	Number of isolates
		Iceland	2	1994	7	ST-231cc	1
		India	2	1995	1	ST-4240/6688cc	1
		Japan	2	1996	39	ST-750cc	1
		Philippines	2	1997	35	Unassigned STs	157
		Argentina	1	1998	62		
		Austria	1	1999	51		
		Djibouti	1	2000	100		
		Israel	1	2001	101		
		Ivory Coast	1	2002	34		
		Pakistan	1	2003	52		
		Poland	1	2004	56		
		Saudi Arabia	1	2005	38		
		Sudan	1	2006	36		
		Switzerland	1	2007	41		
		Thailand	1	2008	29		
				2009	42		
				2010	33		
				2011	168		
				2012	167		
				2013	69		
				2014	19		

cc: clonal complex.

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Table 2

Allelic diversity of Bexsero vaccine antigens per patient age group, MRF-MGL invasive meningococcal disease isolate genomes, England and Wales, 2010/11–2012/13 (n = 1,344)

Age (years)	NadA D (95% CI)	NHBA D (95% CI)	FHBP D (95% CI)	PorA VR1 D (95% CI)	PorA VR2 D (95% CI)
< 1 (n = 300)	0.31 (0.24–0.38)	0.88 (0.86–0.91)	0.89 (0.87–0.91)	0.82 (0.78–0.85)	0.88 (0.86–0.90)
1–4 (n = 315)	0.39 (0.33–0.46)	0.88 (0.86–0.90)	0.90 (0.88–0.92)	0.84 (0.81–0.87)	0.92 (0.91–0.94)
5–14 (n = 99)	0.33 (0.21–0.44)	0.91 (0.88–0.94)	0.91 (0.88–0.94)	0.88 (0.85–0.92)	0.91 (0.89–0.94)
15–24 (n = 168)	0.42 (0.33–0.52)	0.90 (0.88–0.92)	0.90 (0.88–0.92)	0.88 (0.85–0.90)	0.93 (0.92–0.95)
25–39 (n = 73)	0.50 (0.36–0.64)	0.92 (0.90–0.95)	0.93 (0.91–0.95)	0.84 (0.79–0.90)	0.93 (0.91–0.96)
40–64 (n = 173)	0.42 (0.33–0.51)	0.91 (0.89–0.93)	0.90 (0.88–0.92)	0.87 (0.84–0.90)	0.92 (0.91–0.94)
≥ 65 (n = 207)	0.41 (0.32–0.49)	0.87 (0.84–0.90)	0.85 (0.81–0.89)	0.82 (0.78–0.86)	0.91 (0.89–0.92)
Total (n = 1,344)	**0.39 (0.36–0.42)**	**0.91 (0.91–0.92)**	**0.91 (0.91–0.92)**	**0.87 (0.86–0.88)**	**0.92 (0.92–0.93)**

CI: confidence interval; MRF-MGL: Meningitis Research Foundation Meningococcus Genome Library.

Diversity determined by Simpson's Diversity Index

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Table 3

Characteristics of Bexsero and Trumenba antigens in PubMLST *Neisseria* database

Antigen	Number of isolates	Dominant clonal complex(es) n (%)	Dominant serogroup(s) n (%)	Minimum lifespan (years)	Observed time period	Continents found
fHbp subvariant 3.45 (A05)[a]	38	ST-213: 36 (94.7)	Unassigned: 36 (94.7)	13	1999–2012	Europe
fHbp subvariant 1.55 (B01)[a]	0	NA	NA	NA	NA	NA
fHbp variant family 1/ subfamily B	873	ST-11: 349 (40.0), ST-41/44: 98 (11.2), Unassigned: 92 (7.4)	W: 241 (27.6), B: 159 (18.2), C: 113 (12.9), A: 76 (8.7)	77	1937–2014	Africa, Asia, Europe, North America, Oceania, South America
fHbp subvariant 1.1	73	ST-32: 66 (90.4)	B: 47 (64.4)	40	1969–2009	Africa, Asia, Europe, North America, Oceania, South America
NadA-3.8 subvariant	41	ST-174: 17 (41.5), ST-5: 16 (39.0)	Y: 16 (39.0), A: 16 (39.0), B: 5 (12.2)	49	1963–2012	Africa, Asia, Europe, North America, South America
NadA-1,2,3 variants	709	ST-11: 568 (80.1)	W: 368 (51.9), C: 135 (19.0)	51	1963–2014	Africa, Asia, Europe, North America, Oceania, South America
NHBA variant 2	121	ST-41/44: 98 (80.9)	B: 66 (54.6), C: 12 (9.9), NG: 11 (9.1)	37	1976–2013	Europe, North America, Oceania, South America

NA: not available; NG: non-groupable.

[a]A05, B01: Pfizer nomenclature.