Genomic Epidemiology of Meningococcal Vaccine Antigens in the United Kingdom



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Abstract

Vaccination against *Neisseria meningitidis* remains the optimal means to prevent invasive meningococcal disease (IMD), an important cause of septicaemia and meningitis disproportionately affecting infants and young children. Subcapsular protein-based vaccines were developed to offer broad protection against genetic lineages of meningococci responsible for endemic capsular group B IMD. As meningococci solely colonise the human oropharynx, the inherent variation in surface proteins potentially limits their efficacy as vaccine antigens. With increasing use of these vaccines worldwide, high-throughput genomic methods were required to analyse meningococcal protein diversity. The aims of this study were to determine the diversity and distribution of proteins in subcapsular protein-based vaccines Bexsero® and Trumenba® amongst meningococcal whole genome sequences (WGS) from disease and carriage isolates from the UK, 2010-2017. Using the principles underlying multilocus sequence typing, two novel genomic typing schemes were established on PubMLST.org *Neisseria* website. The Bexsero® Antigen Sequence Typing (BAST) and Outer Membrane Vesicle peptide Typing (OMVT) schemes were used to analyse ~3500 disease and ~650 carriage meningococcal WGS.

Using this comprehensive, national dataset, non-random distribution of BASTs and Bexsero® antigens was demonstrated, with a few variants occurring at high frequency. Strong, non-overlapping associations between OMVT clusters, BASTs, cc and genogroups were identified in disease and carriage meningococci, likely driven by host immune and metabolic selection. Understanding the population structure of subcapsular and outer membrane proteins has important implications when selecting antigens and variants for inclusion in existing or novel protein-based vaccines, for both meningococci

and other *Neisseria* species, including gonococci. The development of BAST typing coincided with Bexsero® vaccine introduction into the UK infant immunisation programme and detailed pre-vaccine genomic surveillance provided estimations of vaccine coverage (58.3%-60.3%) and identified low incidence of exact antigenic variants. Both typing methods are rapid, scalable and open-source, with the potential to optimise vaccine development, reformulation and implementation worldwide.

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Firstly, I wish to thank my supervisors Professor Martin Maiden and Professor Chris Tang, both of whom believed in my abilities long before I did. They have been of immense support and encouragement throughout this journey, helping me navigate the scientific landscape and to build my confidence as a researcher. I would also like to acknowledge the intellectual contributions of Professor Richard Moxon and Professor Ian Feavers, I have enjoyed and learned from our thought-provoking discussions. With that, I am grateful for the financial support for the DPhil through the Wellcome Biomedical and Clinical Research Fellowship.

I am indebted to all the members of the Maiden Lab, both past and present, for welcoming me into the group, helping me develop my scientific skills, and being all-round good company. Team *Neisseria*; Dr Holly Bratcher, Dr Odile Harrison, Dr Keith Jolley, Dr Jenny Maclennan, and Marianne Clemence have played a key role in increasing my knowledge of genomics, bioinformatics, microbiology, laboratory skills, as well as scientific rigor. Through the technical and practical support from this excellent multidisciplinary team, I was able to complete this body of work.

Words cannot describe the gratitude and love I have for my family. My inspirational Mum and Dad, who instilled the values of hard work and pursuit of excellence, have not both lived to see this particular endeavour to its completion, but I have no doubt that my Dad knew I would reach this point, enthused by his passion for science. I thank my Mum and my family for their boundless love and support through all life's endeavours. Finally, I thank my wonderfully patient husband, Peter. In these three years his love, eternal optimism and kindness has made everything possible.

Declarations

The work herein is my own except where explicitly stated in relevant chapters and in Contributions. No part of this work has been submitted for any other degree or professional qualification.

Where the work has been published during the course of DPhil study, this has been stated in the relevant chapters with the co-authors and contributions listed. Permission has been sought from co-authors of published papers, to use the work that I produced as part of my thesis, this includes both written text, figures, and tables (see Appendix IV – Co-author consent for re-use of published material in thesis).

Charlene Rodrigues

Contributions

This work made use of the PubMLST.org *Neisseria* website (http://pubmlst.org/neisseria/) developed by Keith Jolley and sited at the University of Oxford (Jolley and Maiden 2010). The development of this site has been funded by the Wellcome Trust and European Union. I am grateful to Dr Keith Jolley and Dr James Bray for their bioinformatics support assembling, uploading, and curating meningococcal genomes and genomic schemes and to the other curators, in particular Dr Odile Harrison and Dr Holly Bratcher.

A large, national collection of invasive meningococcal disease isolates from the UK was used in these studies. The Meningitis Research Foundation Meningococcus Genome Library (http://www.meningitis.org/research/genome) was developed by Public Health England, the Wellcome Trust Sanger Institute and the University of Oxford as a

collaboration. All culture-confirmed isolates from the UK underwent whole genome sequencing from 1 July 2010. I am grateful to the staff at all the reference laboratories, whose commitment to this endeavour allows this research to continue.

The development of the Bexsero® Antigen Sequence Typing scheme was conceived by Professor Martin Maiden and Professor Richard Moxon, and the typing scheme created by Dr Carina Brehony, prior to commencing the DPhil. I continued this work after Dr Brehony's departure from the research group (2015), and the final output is a joint first author paper containing the work from Chapter 2.

I would like to acknowledge the contribution from Dr Hannah Chan and Dr Caroline Vipond and guidance from Professor Ian Feavers (National Institute of Biological Standards and Control, UK) in developing the Outer Membrane Vesicle peptide Typing scheme described in Chapter 4. They provided the mass spectrometry data, upon which the proteins were selected, and Dr Chan proceeded to develop and curate the scheme alongside me.

The work in Chapter 5 is based on two retrospective carriage studies orchestrated in large part by Dr Jenny Maclennan. The meningococcal whole genome sequences from 2014/15 carriage study were available for use thanks to the UKMenCar4 study team. Archived samples from 1999 were used in this study, which were subsequently whole genome sequenced with the help of Dr Holly Bratcher.

I have received statistical support from Professor Noel McCarthy (Department of Zoology, University of Oxford) and consulted Dr Daniel Lunn (Department of Statistics, University of Oxford) on statistical modelling.

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Abbreviations

ACWY	Meningococcal vaccine against serogroups A, C, W, and Y
BAST	Bexsero® Antigen Sequence Type
BIGSdb	Bacterial Isolate Genome Sequence Database
bp	Base pair
cc	Clonal complex
cgMLST	Core-genome multilocus sequence typing
CI	Confidence interval
EC	Extracellular
Hib	*Haemophilus influenzae* type b
HGT	Horizontal genetic transfer
IM	Inner membrane
IMD	Invasive meningococcal disease
LOS	Lipooligosaccharide
MATS	Meningococcal Antigen Typing System
MRF-MGL	Meningitis Research Foundation Meningococcus Genome Library
MLST	Multilocus sequence typing
NEIS	*Neisseria* Sequence Typing Database prefix for nucleotide loci
NEISp	*Neisseria* Sequence Typing Database prefix for peptide loci
NG	Non-groupable on immunological serogrouping
NGS	Next generation sequencing
OMP	Outer membrane protein
OMV	Outer membrane vesicle
PBT	Positive bactericidal threshold (as part of the Meningococcal Antigen Typing System)
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PorA VR	Porin A variable region
PP	Periplasmic

rMLST	Ribosomal multilocus sequence typing
RP	Relative potency (as part of the Meningococcal Antigen Typing System)
SNP	Single nucleotide polymorphism
UK	United Kingdom
US	United States
ST	Sequence type
WGS	Whole genome sequencing

Protein names

FetA	Ferric enterochelin receptor
FbpA	Ferric binding protein A
fHbp	Factor-H binding protein
LbpA	Lactoferrin-binding protein A
MafA	Multiple adhesin family A
MtrE	Multiple transferable resistance protein E
Mip	Macrophage infectivity potentiator
NadA	*Neisseria* adhesin A
NHBA	Neisserial heparin-binding antigen
NspA	Neisserial surface protein A
OpcA	Outer membrane protein A
PilQ	Pilin Q
PorA	Porin A
PorB	Porin B
RmpM	Reduction-modifiable protein M
RplN	50S ribosomal protein L14
RpsG	30S ribosomal protein S7
RpsM	30S ribosomal protein S13
TbpA	Transferrin-binding protein
TBDR	TonB-dependent receptor

Chapter 1

Introduction and literature review

Part 1 Introduction to *Neisseria meningitidis* and invasive meningococcal disease

1.1. Carriage of *Neisseria meningitidis*

The Gram-negative diplococcus *Neisseria meningitidis* is an obligate human pathogen. Asymptomatic oropharyngeal carriage occurs in approximately 10% of the population, with carriage rates dependent on age and exposure to risk factors (Cartwright *et al.* 1987). A meta-analysis of carriage in countries with predominantly serogroup B and C invasive meningococcal disease (IMD), demonstrated increasing carriage with age from 4.5% in infants to 23.7% in 19 year olds, then declining in adulthood to 7.8% in 50 year olds (Figure 1-1) (Christensen *et al.* 2010). Risk factors that impact on carriage include; living in overcrowded settings, passive and active smoking, intimate contact (e.g. kissing), frequenting drinking establishments such as public houses or clubs, and intercurrent viral respiratory tract infection (Glover 1918, Stuart *et al.* 1989, MacLennan *et al.* 2006). Carriage rates are dynamic and have been documented as rising in students starting at university in the UK, from 6.9% on the first day of university term to 23.1% by day four (Neal *et al.* 2000). Carriage rates as high as 60-70% have been measured amongst military personnel, with outbreaks a frequent occurrence in both these settings (Caugant *et al.* 1992, Tyski *et al.* 2001).

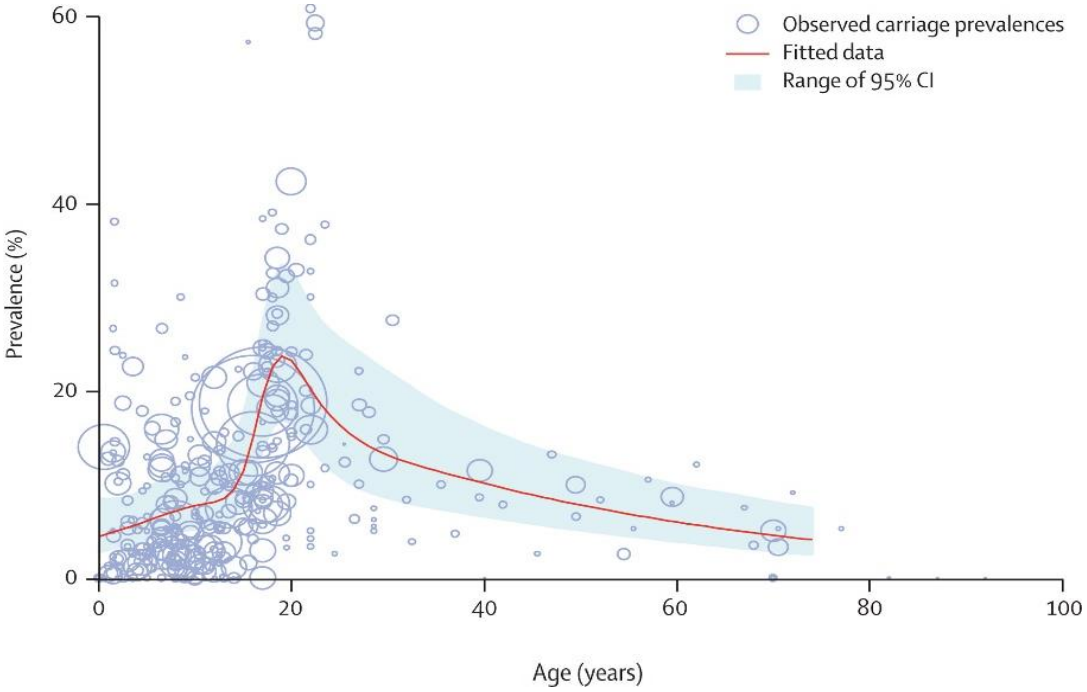


Figure 1-1 Meningococcal carriage estimated by age in European countries, or those with similar epidemiology.
Prevalence of meningococcal carriage from a meta-analysis of carriage studies, including those where nasopharyngeal swabs were directly plated after collection. Each circle represents a data point, where the size of the circle is proportional to study size. The three largest circles represent UK carriage studies (UKMenCar1-3) performed in 1999, 2000, and 2001. Reproduced unchanged with permission (Christensen et al. 2010).

Transmission of meningococci is by droplet spread through close contact with an asymptomatic carrier or diseased individual. Of those who carry meningococci, a very small number will develop IMD as the bacteria invade through the oropharyngeal epithelium into the systemic circulation, resulting in septicaemia and/or meningitis.

1.2. Susceptibility to invasive meningococcal disease

IMD can affect all age groups, but disproportionally affects infants and young children in industrialised settings. In the UK, the highest burden of disease is seen in infants and children aged 1-4 years, with a further peak of disease incidence in adolescents, those at highest risk of carriage (Figure 1-2) (Christensen *et al.* 2010, Public Health England 2015). With changing epidemiology of IMD since 2009, there has been increasing IMD in adults ≥65 years old (Ladhani *et al.* 2016).

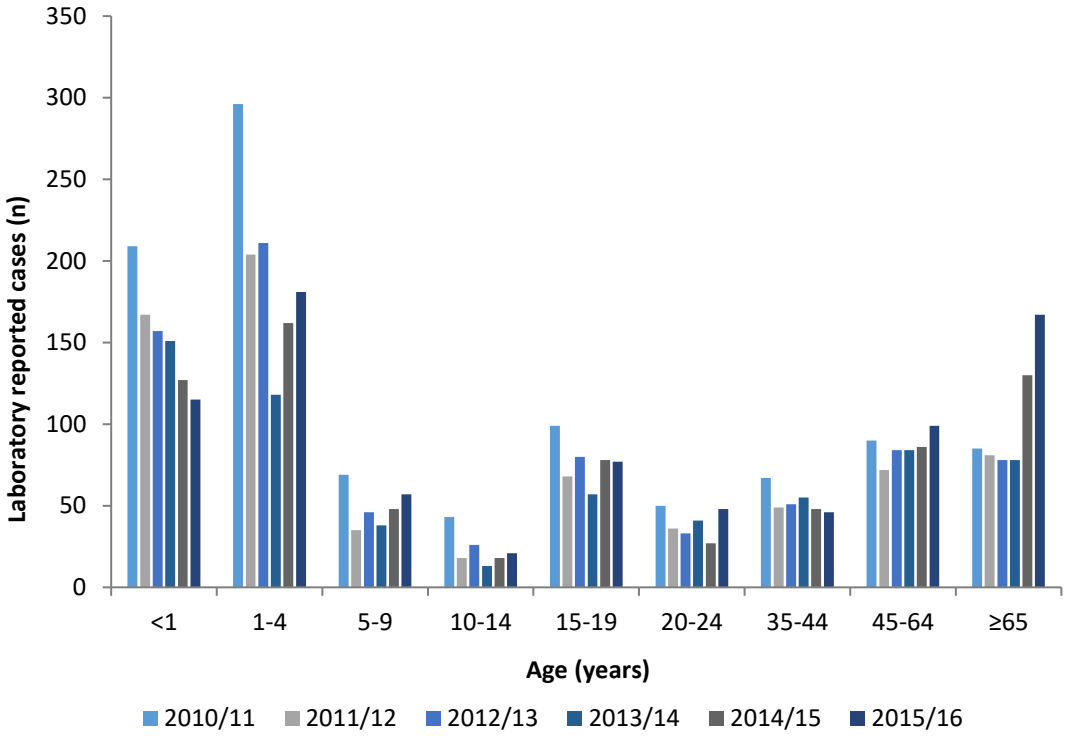


Figure 1-2 Age distribution of invasive meningococcal disease in England.
The number of laboratory confirmed cases of IMD in England for the last five epidemiological years, by age group. The highest burden of disease fell in infants, 1-4 year olds, 15-19 year olds and the ≥65 year olds, with increased IMD in ≥65 year olds since 2014/15. Data from (Public Health England 2017).

It is recognised that immunodeficient individuals are more susceptible to infection with *N. meningitidis*, due to inadequate innate immune responses in particular affecting the complement pathway. Rare, inherited defects in complement component C3, alternative pathway components factor D, properdin, factor H and factor I, and terminal complement pathway components C5-C9 predispose individuals to IMD, which can be severe or recurrent (Lewis and Ram 2014). This is due to their inability to generate a membrane attack complex, critical for the clearance of *N. meningitidis* from the blood through antibody-mediated complement dependent bactericidal killing. Acquired deficiencies in complement components also increase risk of IMD, and can occur through: impaired production (e.g. liver dysfunction), increased consumption (e.g. autoimmune disease), loss (e.g. nephrotic syndrome, protein-losing enteropathies), or

inhibition (e.g. Eculizumab; monoclonal antibody to C5). Similarly, individuals with splenic dysfunction (both anatomical and functional) are at increased risk of infection with encapsulated organisms *N. meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* due to impaired antibody production (Lewis and Ram 2014).

However, most IMD occurs in immunocompetent individuals and there are likely to be other factors within the host, the pathogen, or their interaction that increase susceptibility to invasive disease following oropharyngeal acquisition. Examples of host genetic polymorphisms identified to date, include the phagocytic Fcγ receptors IIa and IIIb affecting opsonophagocytosis of encapsulated organisms and host complement factor H and related proteins, which bind the meningococcal surface-protein factor H-binding protein (fHbp) to evade complement-mediated killing (Bredius *et al.* 1994, Davila *et al.* 2010).

1.3. Clinical progression to invasive meningococcal disease

In the England and Wales, approximately 2/100,000 population, will develop IMD as the bacteria invade through the oropharyngeal epithelium into the systemic circulation, causing septicaemia or through the blood-brain barrier, causing meningitis (Ladhani *et al.* 2012). Patients presenting early with IMD, within the first four to six hours, have non-specific symptoms including; fever, blanching or purpuric/petechial rash, vomiting, headache, malaise, high-pitched cry, lethargy, cold peripheries, limb pain, and pale or mottled skin (Centers for Disease Control and Prevention 2015). This makes timely recognition and treatment of IMD extremely challenging for medical practitioners (Thompson *et al.* 2006). Progression to meningococcal septicaemia can result in severe hypotension, renal impairment, peripheral limb ischaemia, adrenal haemorrhage (Waterhouse–Friderichsen syndrome), purpura fulminans, and multiorgan

failure. Further, patients can deteriorate very rapidly, with 5-6% mortality despite maximal medical interventions (Ladhani *et al.* 2012, Sridhar *et al.* 2015). Patients with suspected IMD should be treated with optimal fluid resuscitation and early empirical parenteral antibiotics, third generation cephalosporins are effective and used in the UK (cefotaxime or ceftriaxone). Patients of all ages with IMD may require intensive care management including: mechanical ventilation, inotropic and vasopressor support, and renal replacement therapy.

Amongst survivors of serogroup B IMD, one third will have significant long-term physical, cognitive or psychological sequelae (Salisbury *et al.* 2006, Viner *et al.* 2012). A case-control study of 245 IMD survivors in the UK, identified that they were more likely to suffer disabling limb amputations, bilateral sensorineural hearing loss of ≥40 decibels, and deficits in IQ, executive functioning, and memory when compared to age- and sex-matched controls (Viner *et al.* 2012).

Contacts of patients with IMD are at high risk of infection with the same invasive strain, given the transmission dynamics amongst household and close contacts. Therefore, in conjunction with local public health teams these individuals should be identified and provided with prophylactic antibiotics (ciprofloxacin or rifampicin), with the aim of eradicating carriage of the invasive strain. Prophylactic vaccination can also be offered to contacts of all ages in sporadic cases, clusters or outbreaks of serogroup A, C, W, or Y IMD; capsular group B vaccines are limited to serogroup B cluster contacts if the cluster strain is deemed vaccine-preventable (Public Health England 2013).

1.4. Microbiological diagnosis

The microbiological diagnosis of IMD follows the initial clinical suspicion and early empirical interventions. Blood cultures should be taken at presentation, with

cerebrospinal fluid culture if deemed safe to perform a lumbar puncture and clinical concern of meningitis. Positive cultures demonstrate morphologically Gram-negative diplococci, which are oxidase test positive and can ferment glucose and maltose. Serogrouping methods include a number of immunological assays using polyclonal/monoclonal antibodies, including slide agglutination, directed against capsular polysaccharides. Antibiotic susceptibilities can be determined from positive cultures, with minimal inhibitory concentrations reported routinely for penicillin, cefotaxime, rifampicin, and ciprofloxacin (Kaczmarski 2015). Genotypic methods include meningococcal polymerase chain reaction (PCR) of capsule biosynthesis genes, in particular *ctrA* and *siaD*, to identify meningococcal DNA and the capsular type using a blood specimen stored in EDTA (ethylenediaminetetraacetic acid) (Kaczmarski *et al.* 1998). This has the advantage of identifying the organism in patients who received pre-hospital antibiotic therapy (intramuscular benzylpenicillin in primary care), had parenteral antibiotics administered before cultures were drawn, or those with negative cultures.

1.5. Typing of meningococci

Since early work on germ theory, it was evident that there was variation in the degree of morbidity and mortality caused by organisms categorised as the same species. Additionally, diversity was not continuous within a species, for example meningococci have stable, distinctive genetic lineages (organisms that share a common ancestor) which persist through time (Maiden *et al.* 2013). Hence, developing typing methods became an important means to further distinguish microorganisms within species. Phenotypic methods are widely-used clinically, including the identification of capsular polysaccharides (*N. meningitidis*, *S. pneumoniae*, *H. influenzae*), biochemical tests

(catalase test, oxidase test), or functional characteristics (antibiotic resistance, toxin production). Twelve capsular groups have been identified for meningococci, which form the basis of successful polysaccharide capsular vaccines, hence the categorisation of meningococci by serogroup remains a common typing method (Harrison *et al.* 2013).

1.5.1. Molecular typing methods

The degree of resolution obtained with phenotypic methods, however, was insufficient for epidemiological surveillance of clinically important microorganisms. Molecular typing methods have superseded phenotypic methods in the public health microbiology setting, and are broadly categorised into non-sequence-based and sequence-based methods. Non-sequence based methods include; Pulsed-Field Gel Electrophoresis (PFGE); PCR-restriction fragment length polymorphisms (PCR-RFLP); Multiple Locus Variable number tandem repeat Analysis (MLVA); and Multilocus Enzyme Electrophoresis (MLEE). These methods all employ the principle of distinguishing between isolates by comparing selectively fragmented DNA or enzymatic patterns by electrophoretic mobility (Fournier *et al.* 2014). PFGE, PCR-RFLP, and MVLA target highly variable genetic regions, with resultant typing of rapidly accumulating variation, best suited to investigations of short term epidemiology, such as outbreaks or clusters of infections. MLEE provides better discrimination for global epidemiology or population biology as the selected metabolic housekeeping enzymes are under neutral selection, however it remains technically challenging and poorly comparable between laboratories (Enright and Spratt 1999, Maiden 2006).

1.5.2. Multilocus Sequence Typing (MLST)

Sequence-based methods can determine the variation in nucleotide sequences with high resolution which was first exploited using multilocus sequence typing (MLST). MLST

remains the most widely-used sequence-based method for typing bacteria, successful due to its highly discriminatory and unambiguous nature, when compared to previous techniques such as MLEE (Maiden *et al.* 1998). *N. meningitidis* was the first species for which MLST schemes were developed, providing a method that had temporal and geographical utility. To catalogue diversity, MLST of *N. meningitidis* uses seven housekeeping genes (*abcZ, adk, aroE, gdh, fumC, pdhC, pgm*), which exhibit stabilising selection for the conservation of metabolic function. By assigning unique alleles based on any genetic difference for each locus, it is possible to systematically index genetic diversity (Maiden *et al.* 2013). Every unique allelic profile, comprised of seven loci, is assigned a numerical sequence type (ST). STs can be grouped based on similarity to a central genotype to form clonal complexes (cc). A cc contains all STs with four or more loci similarity. The cc is of particular value in epidemiology due to its association with phenotypic characteristics. In particular for disease-causing meningococci, cc is associated with serogroup, hyperinvasive strains, type of disease, and vaccine antigens.

1.6. Epidemiology of invasive meningococcal disease

1.6.1. Global epidemiology

Six serogroups are responsible for the majority of invasive disease, based on capsular antigens A, B, C, W, X, and Y (Harrison *et al.* 2013). There is global variation in the predominant serogroup causing disease in different regions, but also the epidemiological pattern of endemic, hyperendemic, or epidemic disease. Serogroup B disease is endemic in many industrialised settings. Within Europe, the UK (incidence 1.44/100,000) and Ireland (2.08/100,000) have the highest rates of disease, for reasons that remain unclear (Sridhar *et al.* 2015). Other countries with endemic serogroup B disease include Canada (0.3/100,000); USA (0.16/100,000) which has regular

university outbreaks; Australia (0.99/100,000); and New Zealand (4.26/100,000) (Sridhar *et al.* 2015). Two particular serogroup B epidemics of note are those in Norway and New Zealand. Outer membrane vesicle (OMV) vaccines were successfully developed using epidemic strains; in Norway against cc32, P1.7,16 (vaccine deployed 1988-1991), and New Zealand against cc41/44, P1.7b,4 (vaccine deployed 2004-2008) to reduce disease burden (Bjune *et al.* 1991, Galloway *et al.* 2009).

In Latin America, IMD is inconsistently or sparsely reported. The 1987-1989 epidemic of serogroup B disease in Cuba (cc32, P1.19,15), led to the development of the first OMV vaccine (Sierra *et al.* 1991). Serogroup B and C continue to predominate in Brazil, Argentina, Uruguay, and Chile, the countries with the highest burden of IMD (Borrow *et al.* 2016). Since 2009 serogroup W cc11 (W:cc11) IMD has increased in Brazil, Argentina, and Chile, which spread globally, and was termed the South American/UK strain (Lucidarme *et al.* 2015).

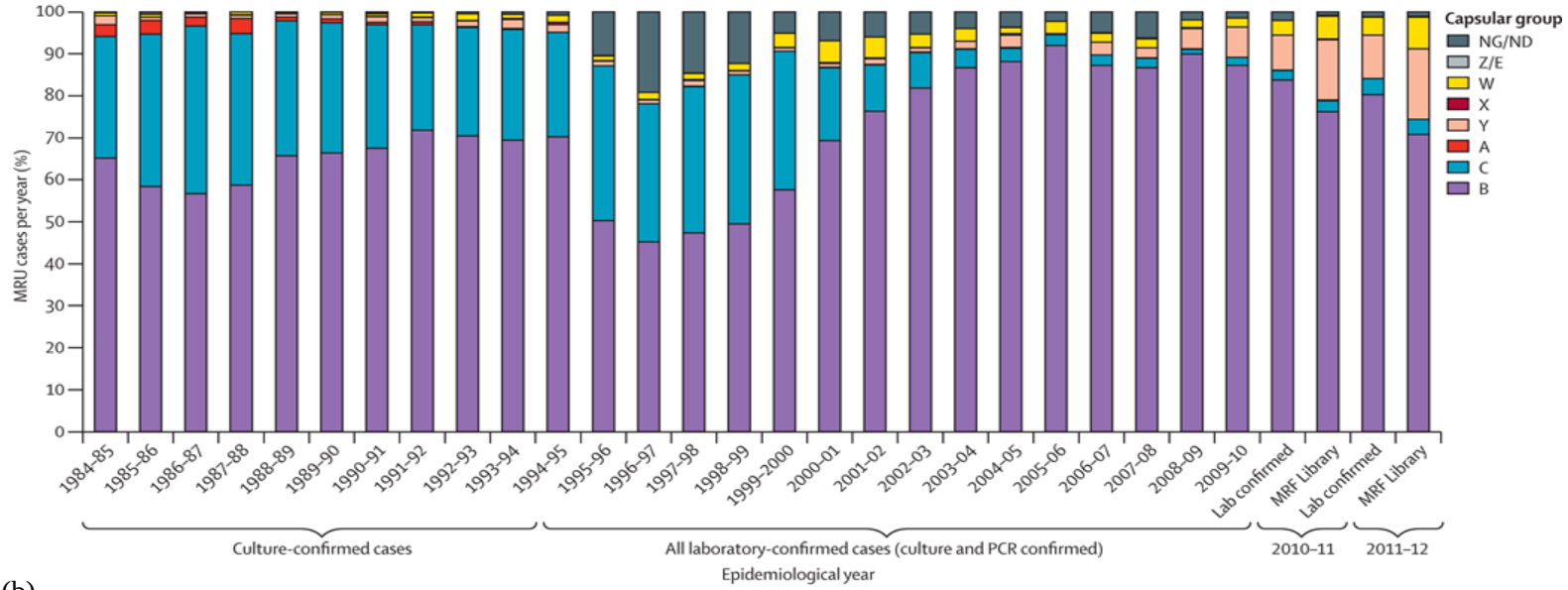
In the African meningitis belt, which includes the countries in sub-Saharan Africa between Senegal and Ethiopia, serogroup A was a major cause of cyclical, large epidemics, afflicting up to 1000/100,000 people per year. Following introduction of the affordable, tailor-made serogroup A conjugate polysaccharide vaccine (PsA-TT, MenAfriVac®) in 2010 and its progressive roll out across the belt, there has been a dramatic reduction in serogroup A IMD and carriage (Kristiansen *et al.* 2014, Trotter *et al.* 2017). Subsequently, there has been more serogroup C, W, and X IMD noted through established surveillance systems (Borrow *et al.* 2017). In South Africa, IMD incidence was 0.36/100,000 in 2014, and the predominant capsular group W, followed by B (Borrow *et al.* 2017).

In the Asia-Pacific region, the reporting of IMD is highly variable, with published incidences likely to underestimate the true burden of disease. For countries where surveillance systems are in place, China has reported a changing epidemiology, having had five large serogroup A epidemics prior to 1977. The introduction of plain polysaccharide vaccines against serogroup A (1980) and C (2008) mean that there has been a subsequent increase in serogroup B IMD, caused by cc4821 through capsule switching events (Chen *et al.* 2018, Li *et al.* 2018). Reporting of outbreaks, which are predominantly serogroup A and rarely B or C, remains the only data available for India. Japan has very low incidence IMD of 0.027/100,000, and rare outbreaks (Borrow *et al.* 2016).

1.6.2. **UK epidemiology**

In the UK, periods of hyperendemic disease occur sporadically on a background of low incidence endemic disease (Figure 1-3). There was very high incidence of IMD during the First and Second World Wars, which was likely caused by serogroup A and associated with living or aggregating in overcrowded communal spaces, poor recognition of the early symptoms, and inconsistent use of antibiotics (Millar *et al.* 2017). The next hyperendemic periods occurred between 1972-1975 with serogroup B serotype 2a strain, and in 1985 with serogroup B ST-32 (Public Health England 2013).

(a)



(b)

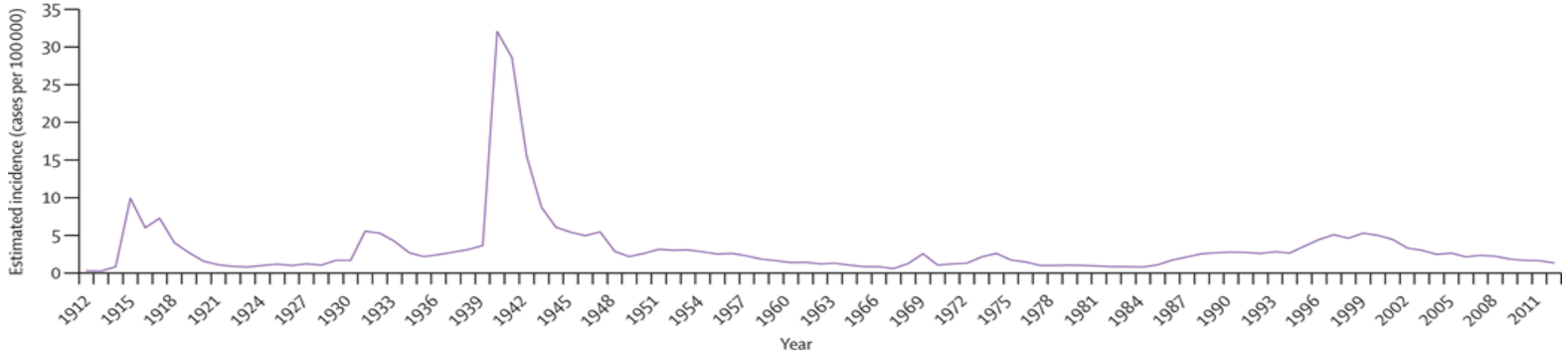


Figure 1-3 Invasive meningococcal disease epidemiology in England and Wales, 1912-2012.
(a) Representation of the proportion of capsular types from culture-confirmed and PCR-confirmed isolates from 1984/85-2011/12. Capsular types were identified using serology, PCR genotyping or WGS genogrouping.

(b) Estimated disease incidence in England and Wales based on Public Health England notification data from 1912-1997 and laboratory reports from 1998-2012. Figure reproduced unchanged under CC BY (Hill *et al.* 2015).

In the mid-1990s there was a marked increase in C:cc11 IMD with significant morbidity and mortality amongst adolescents and young adults. Since 2009, there has been an increase in the incidence of serogroup W:cc11 IMD, from 22 cases in 2009/10 to 176 in 2014/15 (Public Health England 2015). IMD caused by W:cc11 disproportionately affected the extremes of age; under five and over 65 year olds, with atypical presentations and high case fatality ratio (Ladhani *et al.* 2015, Campbell *et al.* 2016).

The burden of endemic IMD has been due to serogroup B for the last three decades (Figure 1-3) (Hill *et al.* 2015). Unlike the hyperendemic periods, which usually represent a clonal expansion of a novel strain, endemic disease is caused by multiple different genetic lineages of meningococci, clustered by MLST into ccs, which are influenced by secular changes (Maiden *et al.* 1998). There has been a progressive fall in overall serogroup B disease in England since 2000/01, when 1614/2343 cases were due to serogroup B compared with 418/724 in 2014/15, predominantly affecting infants and children under five (Table 1-1, Figure 1-2) (Hill *et al.* 2015, Public Health England 2015). The reasons for this decline in IMD were unclear, with no vaccine available against serogroup B IMD at that time. However, multi-centre carriage studies from the UK, demonstrated that the adolescent oropharyngeal carriage rate of meningococci has fallen since 1999 from 16.7% to 7.0% in 2014/15, which is likely due to changing social behaviours including a reduction in smoking cigarettes and socialising in public houses and clubs (Maclennan *et al.* 2016).

Table 1-1 Age distribution of laboratory confirmed invasive meningococcal disease from epidemiological year 2014/15 by serogroup and age (Public Health England 2015).

Age group	Serogroups					
	B n (%)	**C** n (%)	**W** n (%)	**Y** n (%)	**Other** n (%)	**Total**
<1 year	101 (24)	1 (4)	21 (12)	4 (4)	0	127
1-4 years	139 (33)	0	18 (10)	5 (5)	0	162
5-9 years	36 (9)	5 (18)	2 (1)	3 (3)	1 (11)	47
10-14 years	13 (3)	0	2 (1)	3 (3)	0	18
15-19 years	36 (9)	3 (11)	25 (14)	14 (15)	1 (11)	79
19-24 years	17 (4)	0	6 (3)	2 (2)	2 (22)	27
≥25 years	76 (18)	19 (68)	102 (58)	62 (67)	5 (56)	264
Total	418 (58)	28 (4)	176 (24)	93 (13)	9 (1)	724

1.7. Meningococcal vaccine development

1.7.1. Polysaccharide vaccines

There has been a strong commitment to vaccine development for all serogroups of IMD. In the 1960's, at the Walter Reed Army Institute of Research, Goldschneider *et al.* observed that the incidence of IMD was inversely proportional to the concentration of serum bactericidal antibodies to meningococci (Goldschneider *et al.* 1969). In particular, the recognition of low disease incidence in the neonatal period, when maternal antibody was present, and in adulthood after repeated immunising carriage states, was in contrast to high disease incidence occurring when physiological hypogammaglobulinaemia developed (Goldschneider *et al.* 1969). Further work by Goldschneider *et al.* identified the determinants of immunity and from this developed purified bacterial polysaccharide vaccines against serogroups C, A, W, and Y. (Goldschneider *et al.* 1969, Gotschlich *et al.* 1969). Polysaccharide C vaccines were introduced for all new US military recruits in 1971 after efficacy of 89.5% was demonstrated amongst adults in field trials (Gold and Artenstein 1971, Artenstein

1975). Importantly, the serum bactericidal antibody (SBA) assay, using a human complement source, remains the accepted correlate of protection against which meningococcal vaccines are tested, in view of the rarity of disease precluding efficacy trials.

Three decades later, these 'plain' polysaccharides were conjugated to carrier proteins, such as the diphtheria toxoid, tetanus toxoid, or cross-reactive material (CRM) 197 which stimulated T-cell dependent immune responses. The resultant effects of these conjugate polysaccharide vaccines demonstrated: improved immunogenicity in infants, those at highest risk of IMD; longer duration of protection through development of immunological memory; and the potential for preventing acquisition of meningococcal carriage (Pollard *et al.* 2009). At the time of writing, conjugate polysaccharide vaccines were available as: monovalent A, monovalent C, combined Hib and monovalent C, quadrivalent ACWY, with pentavalent ACWYX in development.

1.7.2. **Outer membrane vesicle vaccines**

Serogroup B capsular polysaccharides were poorly immunogenic and shared structural similarity to human neural tissues, raising concerns over autoimmune responses if used as a vaccine antigen (Finne *et al.* 1983). This prompted the investigation of alternative vaccine components. OMV vaccines were developed for use in Cuba, Norway, and New Zealand against serogroup B IMD epidemics, each primarily caused by a single strain (as defined by cc and Porin A (PorA) type) (Bjune *et al.* 1991, Sierra *et al.* 1991, O'Hallahan *et al.* 2009). OMV vaccines contain numerous proteins, in particular the highly diverse PorA, and lipooligosaccharide (LOS), but activity against strains heterologous to the epidemic isolate was minimal in adults and extremely limited in infants (Tappero *et al.* 1999, Holst *et al.* 2009). This, therefore, limited the use of OMV

vaccines outside the epidemic setting, for example in countries where endemic disease was caused my multiple ccs and PorA types. To address the breadth of PorA types in endemic disease, combination vaccines with multivalent PorA were developed, namely Hexamen™ and Nonamen™, which were under evaluation but have not progressed beyond clinical trials (Kaaijk *et al.* 2013).

1.7.3. Subcapsular protein-based vaccines

Due to the limitations imposed by the serogroup B polysaccharide capsule and OMV vaccines, the search for alternative vaccine candidates moved to subcapsular proteins. In an attempt to broaden coverage of multiple ccs, two vaccines were developed using subcapsular proteins. The 4CMenB vaccine Bexsero® (GlaxoSmithKline, Siena, Italy) combined three recombinant proteins discovered using 'reverse vaccinology', namely factor-H binding protein (fHbp), Neisserial heparin binding antigen (NHBA), and *Neisseria* adhesin A (NadA), with the OMV vaccine developed for the New Zealand outbreak, MeNZB™ (Serruto *et al.* 2012). The bivalent rLP2086 vaccine, Trumenba® (Pfizer, New York, US) contained two different variants of fHbp, independently discovered as a potential vaccine antigen (Jiang *et al.* 2010). These proteins are inherently diverse due to their surface-exposed regions and interaction with the host immune system.

It is appreciably more difficult to assess the efficacy of protein-based vaccines against *N. meningitidis* than for the polysaccharide or OMV vaccines. When considering the efficacy of protein-based vaccines, it is important to measure both host immunogenicity i.e. the extent to which the host's vaccine-induced antibodies can kill strains that both match and do not match the vaccine antigenic variants, and bacterial coverage i.e. the

proportion of circulating meningococci that would be prevented from causing disease by vaccine-induced antibodies.

1.7.3.1. Immunogenicity to protein-based vaccines

Immunogenicity to Bexsero® has been demonstrated in all age groups using strains that are matched to one of the vaccine variants including H44/76 (fHbp), 5/99 (NadA), and NZ98/254 (PorA), with a limited response to heterologous meningococci (Findlow *et al.* 2010, Kimura *et al.* 2011, Gossger *et al.* 2012, Santolaya *et al.* 2012). This information was necessary for demonstrating the efficacy of Bexsero®-induced antibodies, though there has been minimal serological testing of strains beyond the ones containing the exact vaccine antigenic variants. Hence, the protection afforded by this vaccine in regions where the vaccine antigenic variants are not frequently causing disease, remains unclear.

For Trumenba®, vaccine-induced protective antibody titres have been demonstrated in adolescents and adults, against a breadth of fHbp variants from both subfamilies A and B, not matching the vaccine peptides (Harris *et al.* 2017, Lujan *et al.* 2017, Ostergaard *et al.* 2017, Taha *et al.* 2017). Subfamily A and B fHbp variants are phylogenetically distinct, but the degree of cross-protective immune responses within subfamilies are the basis of this vaccine's utility.

1.7.3.2. Estimating bacterial coverage

Bacterial coverage provides information on the effect of the vaccine against diverse antigenic variants that differ from those contained in the vaccine. Ideally, performing the gold standard SBA with human complement on a panel of diverse meningococcal lineages representing regional epidemiology and serum from individual vaccinees would provide a suitable estimate. However, due to the practical limitations of this

approach including; time- and resource-intensity, poor comparability between laboratory protocols, and the large requirement of vaccinee sera and complement sources, two laboratory assays were developed to estimate the breadth of strain coverage. The Meningococcal Antigen Typing System (MATS) was devised for Bexsero® and Meningococcal Antigen Surface Expression (MEASURE) assay for Trumenba® (Donnelly *et al.* 2010, Donald *et al.* 2017). It is important to note that these assays remain surrogate measures for meningococcal coverage, as they assess meningococcal antigenic expression rather than antibody responses in individual vaccinees.

The MATS assay determines antigenic variants and reactivity of fHbp, NHBA, and NadA using a sandwich enzyme-linked immunosorbent assay (ELISA). The resultant relative potency (RP) is used to determine the likelihood of bactericidal killing, based on SBA assays performed with pooled toddler serum for a set of 57 reference strains, with a positive bactericidal threshold (PBT) defined for each antigen. If the RP is above the PBT, then the isolate is considered to be covered by Bexsero®. PorA is genotyped, and if variant P1.4 is present then the isolate is considered to be covered, with no further serological testing performed (Donnelly *et al.* 2010). The MATS assay estimated meningococcal coverage of 73% (95% CI 57–87) in England and Wales, using a collection of 535 isolates from 2007-08, which was important in achieving licensure of the vaccine in 2013 and inclusion into the UK national programme in 2015 (Vogel *et al.* 2013). A revised estimate of MATS coverage in England, Wales and Northern Ireland, was 66% (95% CI 52–80) for 251 meningococcal isolates from 2014/15 (Parikh *et al.* 2017). The MEASURE assay has been used to determine surface expression levels

of fHbp using monoclonal antibodies and flow cytometry, and to correlate this with meningococcal susceptibility in SBA (Donald *et al.* 2017).

1.7.4. UK vaccination schedule

The meningococcal vaccine schedule in the UK is continually adapted due to the changing epidemiology of IMD and the development of novel vaccines. The conjugate polysaccharide meningococcal C (MenC) vaccine was introduced in the UK in 1999 for infants with a catch up programme for all ≤18 year olds (Miller *et al.* 2001). This mass vaccination intervention was highly successful in inducing both direct and indirect protection, by reducing carriage of C:cc11 meningococci (Maiden *et al.* 2008). In 2006, a MenC booster dose was added to the schedule at 12 months of age, as there was evidence of waning immunity in the second year of life (Trotter *et al.* 2004). From 2006 to 2015, the infant schedule comprised a primary course at three and four months of age, followed by a booster dose at 12 months. In 2013, a further adolescent booster dose was added, to optimise direct and indirect protection and the four month dose of MenC was removed from the schedule, leaving a three month priming dose (Public Health England 2013).

Quadrivalent ACWY adolescent vaccination was implemented in the UK in August 2015 in response to the increasing W:cc11 disease; this replaced the adolescent MenC booster (Campbell *et al.* 2015). Quadrivalent ACWY was offered to all children aged 14-18 years and all <25 year olds attending university for the first time.

The 4CMenB vaccine Bexsero® was introduced into the UK national immunisation programme in September 2015, for all infants born after 1 July 2015, with a catch up to those born after 1 May 2015 (Ladhani *et al.* 2015). The schedule included a priming course at two and four months of age, followed by a booster at 12 months. In 2016, the

MenC dose at three months was removed from the immunisation schedule, on the rationale that serogroup C IMD incidence remained very low since 1999, the 12 month dose would protect toddlers, the herd immunity generated in adolescents should protect unvaccinated individuals, and Bexsero® may afford some protection against non-B serogroups (Public Health England 2013).

For those at heightened risk of IMD, such as individuals with functional or anatomical asplenia, complement deficiencies, or on complement inhibitor therapy (Eculizumab) vaccinations against A, C, W, Y, and B are advised (Public Health England 2013). Equally, individuals travelling to high risk areas should be advised accordingly, for example, recommendation of ACWY vaccination for travellers to the Hajj pilgrimage.

Part 2 Role of genomic epidemiology in informing vaccine strategy for prevention of bacterial meningitis, with a focus on *Neisseria meningitidis*

1.8. Public Dissemination of Work

The work from Part 2 was published in the open peer review platform F1000 Reviews in April 2018:

Rodrigues, C. M. C. and Maiden, M. C. J. (2018). A world without bacterial meningitis: how genomic epidemiology can inform vaccination strategy [version 1; referees:2 approved]. *F1000Research* 7(F1000 Faculty Rev): 401.

For the published paper, CMCR and MCJM conceptualised the idea, CMCR wrote the draft of the paper, and CMCR and MCJM revised and approved the final version.

1.9. Abstract

Bacterial meningitis remains an important cause of global morbidity and mortality. Though effective vaccinations exist and are being increasingly utilised worldwide, there are still many limitations to their efficacy due to bacterial diversity. Through genomic epidemiology, we can appreciate the bacterial population structure, which in turn affects transmission dynamics, virulence factors, and development of new vaccines. Here, we review what we have learned through genomic epidemiological studies following the rapid development of whole genome sequencing that can help to optimize preventative strategies for bacterial meningitis.

1.10. Introduction

Bacterial meningitis describes infection of the subarachnoid space with bacterial pathogens, resulting in inflammation of the brain linings (meninges), with significant morbidity and mortality worldwide. Bacteria reach the subarachnoid space through haematogenous or direct contiguous spread, where they replicate with resultant local meningeal inflammation and potential for involvement of the brain parenchyma (Figure 1-4). In addition, where haematogenous spread of bacteria causes meningitis, patients may also experience symptoms from the persistence of bacteria in the blood, septicaemia, which can rapidly develop into multiorgan failure and death.

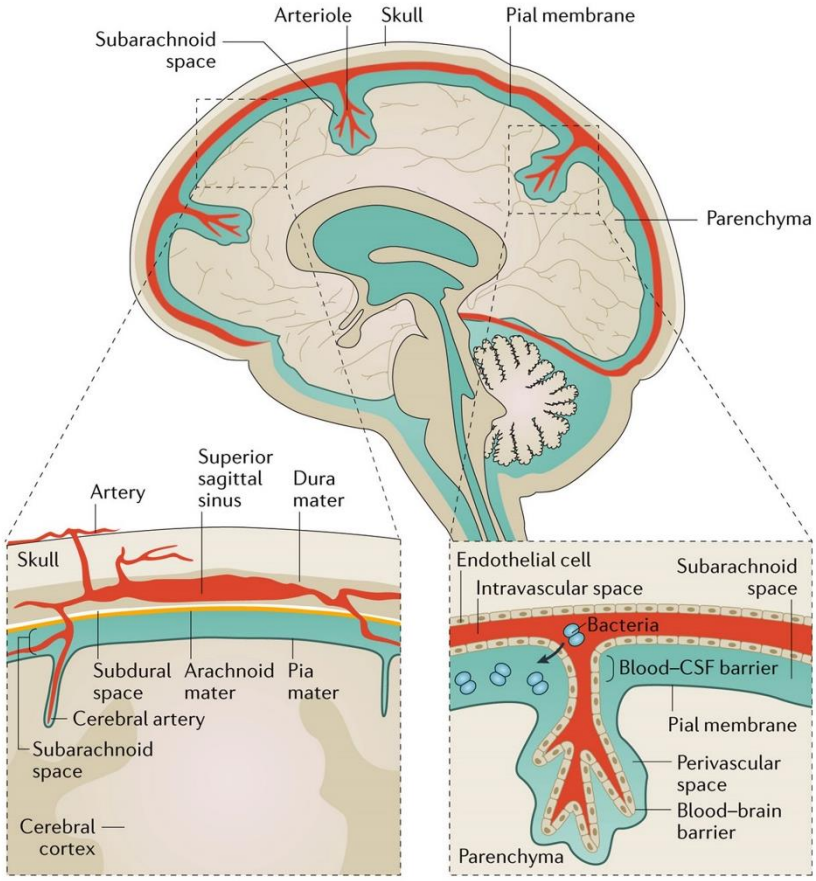


Figure 1-4 Anatomical representation of the human brain and meningeal structures affected by bacterial meningitis.
The bacteria access the subarachnoid space from the blood, crossing the blood-brain barrier. Here, they replicate and cause inflammation as the host attempts to control the developing infection. The ensuing inflammation and ongoing infection result in major morbidity and mortality. Figure reproduced unchanged with permission (van de Beek *et al.* 2016).

Depending on age, geography, immune system function, and vaccine implementation, the incidence rates and causative organisms of bacterial meningitis differ (Kasanmoentalib *et al.* 2013, Liu *et al.* 2015). In 2013, there were an estimated 303,500 deaths globally from meningitis, attributed to *S. pneumoniae* (n=79,100), *N. meningitidis* (n=65,700), *H. influenzae* type b (Hib) (n=64,400), and other causes (n=94,200) (Naghavi *et al.* 2015). Despite highly effective vaccination programmes against these pathogens, disease persists. Here, the role of genomic epidemiology in helping us to understand this problem will be discussed, as by studying transmission networks and bacterial biodiversity it is possible to improve existing vaccine use and the development of new ones.

1.10.1. **Global burden of bacterial meningitis**

Bacterial meningitis in neonates (infant <28 days of age) in the first seven days of life, is commonly caused by Group B Streptococcus and *Escherichia coli*, through vertical transmission from the birth canal and perineal region. Disease after the first week of life is spread by nosocomial or horizontal transmission, with *Listeria monocytogenes* and *S. pneumoniae* also contributing to disease (Gaschignard *et al.* 2011, Okike *et al.* 2014). Vaccination programmes against Hib, *N. meningitidis* and *S. pneumoniae* have greatly altered the epidemiology of bacterial meningitis in older children and adults over the last three decades (Table 1-2). *H. influenzae* meningitis is extremely rare in countries with high Hib vaccine uptake, but cases can occur in unvaccinated individuals, or with non-b serotypes. *S. pneumoniae* and *N. meningitidis* cause the majority of disease; with meningococcus predominating in older children and adolescents, and pneumococcus predominating in adults. Other causes include; *L. monocytogenes* (elderly or

immunocompromised), *Staphylococcus aureus* (co-existing endocarditis), and *H. influenzae* (co-existing otitis or sinusitis) (Thigpen *et al.* 2011, van de Beek *et al.* 2016).

1.10.2. **Bacterial diversity – carriage and immune selection**

With the exception of *L. monocytogenes*, the bacteria principally responsible for causing meningitis are carried asymptomatically as members of a healthy microbiota. Group B Streptococcus is found in the vaginal tract of up to 20% of women; universally *E. coli* is found in the gut; *S. aureus* on the skin; and *S. pneumoniae, N. meningitidis*, and *H. influenzae* in the oropharynx. There are likely to be interactions between the host immune system and the microbiota, albeit not clearly understood, that result in structured diversity of bacterial populations. This diversity is observed amongst microorganisms of the same species, as bacteria have stable, distinctive lineages (organisms that share a degree of genetic similarity) which persist through time. Even within these lineages, bacteria vary genotypically (genetic constitution) and phenotypically (observable characteristics), with the potential to alter protein expression states through phase variation. Thus, to appreciate the degree to which populations are structured, by clonal descent or host immune selection, requires the ability to study genome-wide variation in representative bacterial samples that appropriately reflect the biodiversity (Gupta *et al.* 1996). Only by understanding the host bacterial population can we start to identify the strains (bacteria that share a similar genotype) that cause disease, strains that may afford some host protection by contributing towards host metabolic and immunological function, and the most suitable target population for preventative measures such as vaccines.

Table 1-2 Characteristics of the three main pathogens that cause bacterial meningitis worldwide.

	Haemophilus influenzae	*Streptococcus pneumoniae*	*Neisseria meningitidis*
Bacterial features	Gram-negative coccobacillus. Six capsular types recognised (a-f). Type b (Hib) predominated in invasive disease. Since vaccination began in 1990, non-b types have been causing disease.	Gram-positive cocci in chains. Over 90 serotypes based on capsular polysaccharides identified. Prevalence varies by region, with 10 serotypes responsible for 62% of disease.	Gram-negative diplococcus. 12 recognised polysaccharide capsular groups. Disease caused predominantly by six serogroups (A, B, C, W, X, Y).
Carriage	Carried in the oropharynx as part of the normal commensal. Prior to vaccination, Hib was carried predominantly by young children.	Carried in the oropharynx as part of the normal commensal. Varies by age and geographical distribution, but ranges 5-60%.	Carried in the oropharynx as part of the normal commensal. Varies by age and geographical distribution, but ranges from 1-40%.
Disease	Invasive disease with Hib manifests with meningitis, epiglottitis, pneumonia, septic arthritis, cellulitis, osteomyelitis and septicaemia. Non-b type disease can cause similar infections, increasingly noted in Europe.	Invasive disease in upper and lower respiratory tract (mastoiditis, pneumonia), central nervous system (meningitis, cerebral abscess) and septicaemia and localised upper respiratory tract (otitis, sinusitis).	Invasive meningococcal disease comprising septicaemia and meningitis can affect all age groups. Early disease is non-specific and deterioration of clinical condition can occur very rapidly.
Diagnostic methods	Isolation from a sterile site by culture (chocolate agar). Serotyping with either slide-agglutination or serotype-specific PCR.	Isolation of *S. pneumoniae* from a normally sterile site – by culture or PCR. Serological testing using antibody reactivity to capsular polysaccharides for determination of serotype.	Isolation of *N. meningitidis* from a normally sterile site – by culture or meningococcal PCR (*ctrA*). Serological testing using antibody reactivity to capsular polysaccharides for determination of serogroup.

	Haemophilus influenzae	*Streptococcus pneumoniae*	*Neisseria meningitidis*
Age group affected	Prior to vaccination, Hib was the leading cause of bacterial meningitis in children under five years.	Young children and the elderly at highest risk of invasive disease.	Infants and under five year olds at highest risk, adolescents have increased risk compared to other adults. Epidemics can affect all age groups in susceptible populations.
Global epidemiology	Disease occurs sporadically worldwide, but the use of Hib has greatly reduced the incidence. Hib vaccine has been introduced in 190 countries since 1990, with global infant coverage of 70% in 2017.	Disease occurs sporadically worldwide, with invasive pneumococcal disease amongst the main causes of global mortality through pneumonia, meningitis and sepsis deaths. Introduction of pneumococcal vaccines has had a major impact on the disease caused by these serotypes, but non-vaccine serotypes still cause disease.	Occurs as both epidemic and endemic disease, variable on geographic region. The "Meningitis Belt" in sub-Saharan Africa had cyclical epidemics prior to introduction of conjugate polysaccharide A vaccine. Industrialised countries (Europe, Australasia, North America) have low incidence endemic disease. Outbreaks have been associated with mass gatherings e.g. university students, military recruits, Hajj pilgrims; resulting in targeted vaccinations of high risk groups.
Morbidity (meningitis)	Hearing impairment, neurological sequelae in 15-30%.	Hearing impairment, neurological sequelae.	Psychological, cognitive or physical sequelae in 30%.

	Haemophilus influenzae	*Streptococcus pneumoniae*	*Neisseria meningitidis*
Mortality (meningitis)	3-6%	8% in children, 22% in adults.	5-17%
Vaccines available	Conjugate polysaccharide capsular type b vaccine. No vaccines are available against non-b strains.	Pneumococcal conjugate vaccine - 7 valent (PCV7) 4, 6B, 9V, 14, 18C, 19F and 23F. PCV10 - 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. PCV13 - 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. Pneumococcal polysaccharide vaccine - 23 valent (PPV23) 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.	Conjugate polysaccharide vaccines against A, C, W, Y (monovalent or quadrivalent). Pentavalent conjugate vaccines including serogroup X are in development. Multi-peptide vaccines (4CMenB and recombinant lipoprotein rLP2086) against serogroup B. Plain polysaccharide vaccines against A, C, W, Y. Outer membrane vesicle vaccines against serogroup B epidemic disease.

1.10.3. **Persistence of bacterial meningitis despite vaccination**

The diversity of *S. pneumoniae* and *N. meningitidis* challenges the continued success of vaccines and the elimination of bacterial meningitis caused by these organisms. Both bacteria exhibit high rates of horizontal genetic transfer (HGT) resulting in distinct, non-overlapping genetic lineages, with varying degrees of pathogenicity. Each genetic lineage or ST (based on MLST) can exhibit a variety of polysaccharide capsular types and undergo capsule switching, with hyperinvasive lineages most likely to cause disease regardless of capsular type (Yazdankhah *et al.* 2004). Until 2013, all licensed vaccines for prevention of bacterial meningitis pathogens were based on polysaccharide capsular antigens, key virulence factors as both acapsulate streptococci and meningococci very rarely cause invasive disease. Prevention of disease by capsular group was beneficial but allowed bacteria from hyperinvasive lineages who switched capsule to persist through carriage and ongoing transmission between hosts (Figure 1-5, Figure 1-6).

The first pneumococcal polysaccharide conjugate vaccine (PCV7) included seven capsular serotypes subsequently increasing to PCV10 and PCV13, with further iterations in development (Table 1-2). This was based on the serotypes most frequently causing disease, but some capsular types were antigenically related resulting in a degree of cross-protection. With ~90 serotypes identified worldwide, the development of a universal vaccine appears challenging.

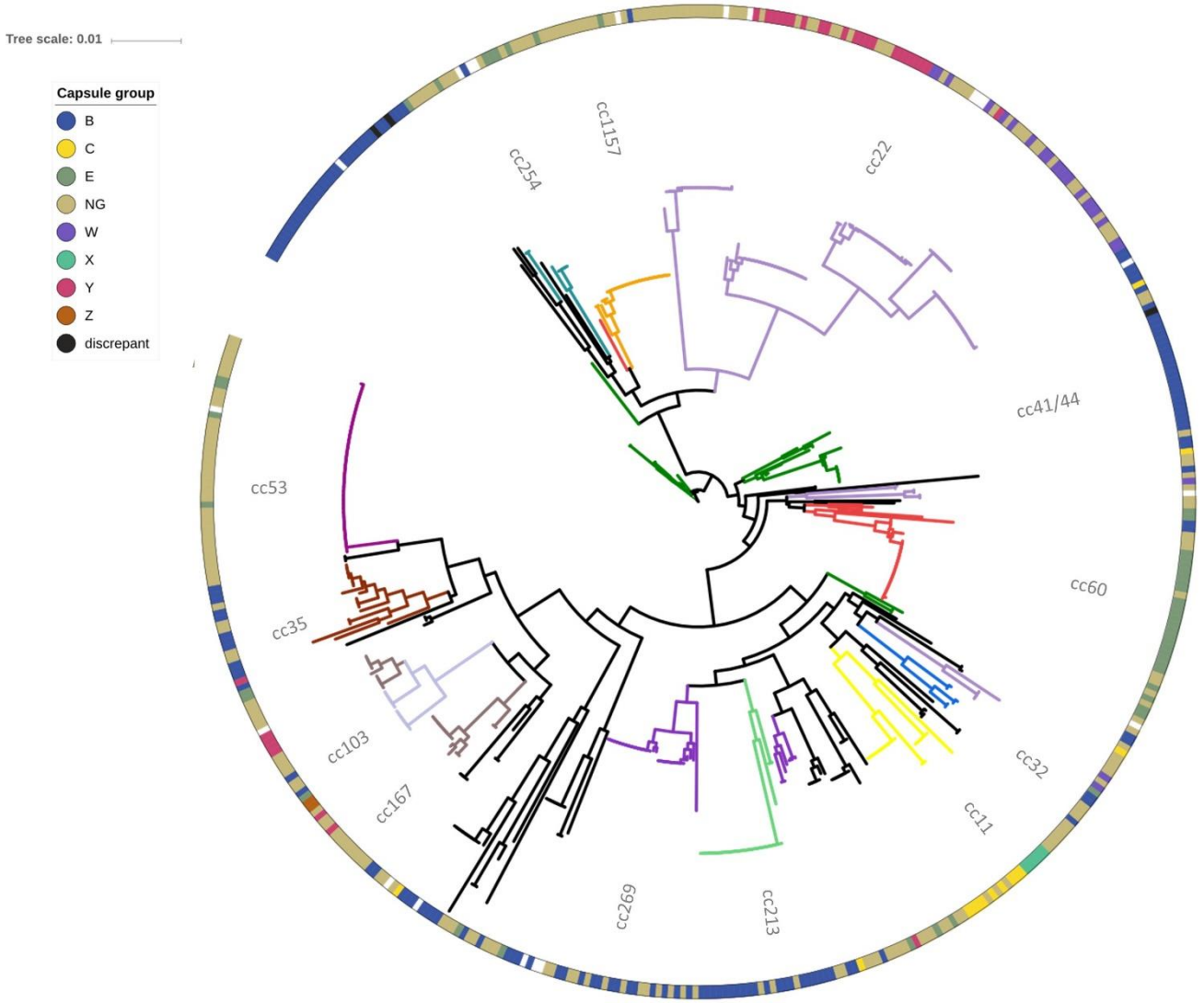


Figure 1-5 Population structure of *Neisseria meningitidis* carriage isolates, demonstrating the diversity of genotype and capsular types.
Allele-based phylogeny of 498 *N. meningitidis* carriage isolates from the UK obtained in 1999, generated using seven MLST loci. Genotypes, described as ccs, are shown by coloured clades on the tree branches. Capsular group is displayed on the peripheral band, with data derived from serological typing and genotyping, "discrepant" isolates had non-concordant results. Phylogeny is visualised using Interactive Tree of Life software (Letunic and Bork 2016). Figure reproduced unchanged under CC BY (Rodrigues and Maiden 2018).

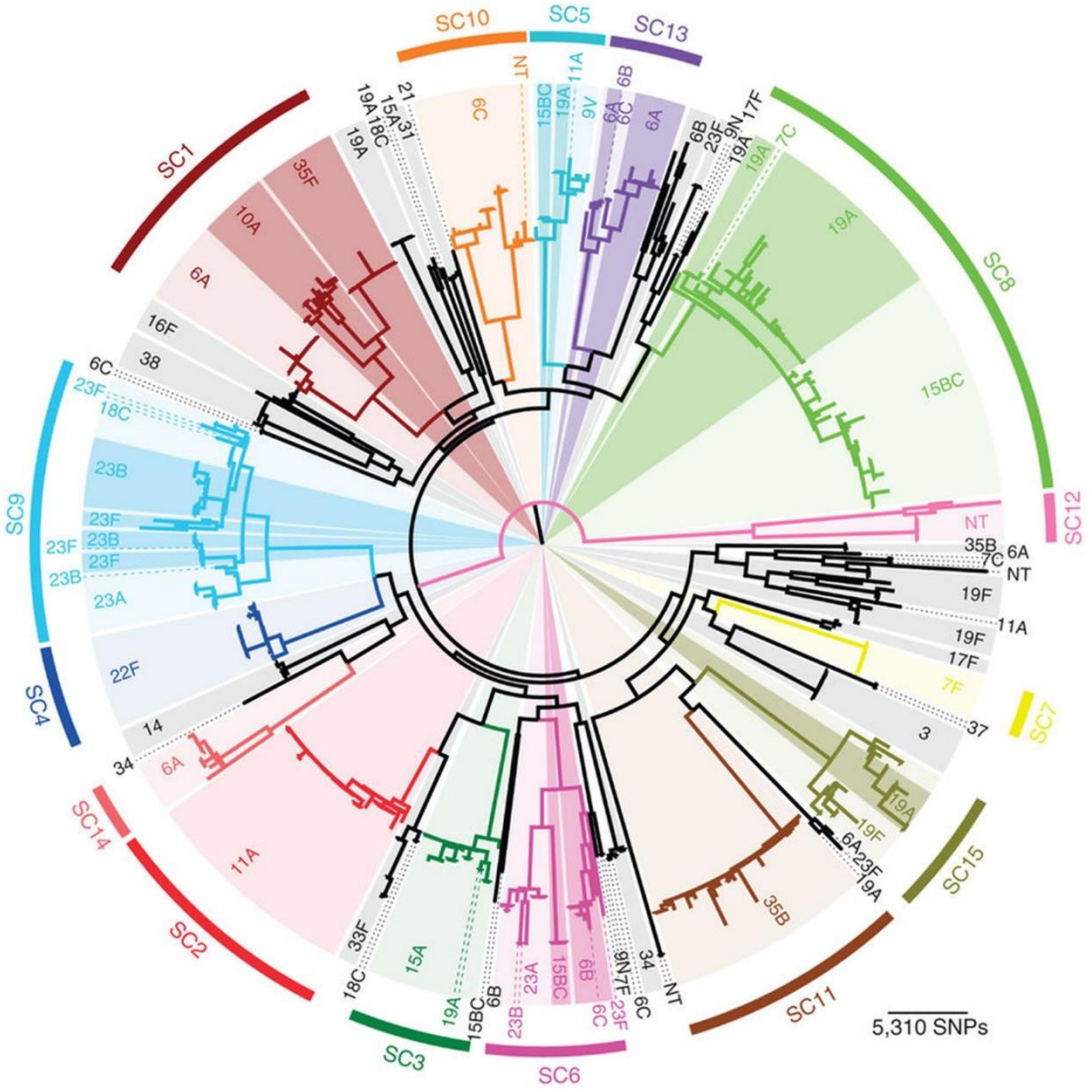


Figure 1-6 Population structure of *Streptococcus pneumoniae* carriage isolates, demonstrating the diversity of genotype and capsular types.
Maximum likelihood phylogeny of 616 *S. pneumoniae* carriage isolates from the US from 2001 and 2007, generated using 106,196 polymorphic sites within 1194 core genes. Monophyletic sequence clusters are shown and labelled peripherally. Within each sequence cluster, differential shading represents the different serotypes. Figure reproduced unchanged with permission (Croucher *et al.* 2013).

There are only 12 recognised meningococcal capsular types, with six serogroups causing the majority of disease (Table 1-2). Conjugate vaccines against serogroups A, C, W, and Y are used extensively worldwide (Salisbury *et al.* 2006). Until 2013, there was no licensed vaccine against serogroup B, a major cause of meningitis in industrialised countries. Hence, genetic lineages that were non-vaccine types continue to be carried in the host oropharynx and transmitted to cause disease in susceptible populations. Furthermore, through the extensive recombination of these pathogens, newly emerging genotypes occur with the propensity to cause invasive disease. The introduction of bacteria with a novel genotype into an immunologically susceptible population can result in epidemic disease.

1.11. Genomic epidemiology

Genomic epidemiology aims to achieve "systematic investigation of how natural genomic variation affects the clinical outcome of disease" (Chokshi *et al.* 2006). The utility of this methodology, in the prevention of bacterial meningitis lies in understanding transmission networks, population structure of bacterial pathogens and epidemiology; using this to inform vaccine development, implementation, and post-vaccine surveillance (Figure 1-7) (Gardy and Loman 2017).

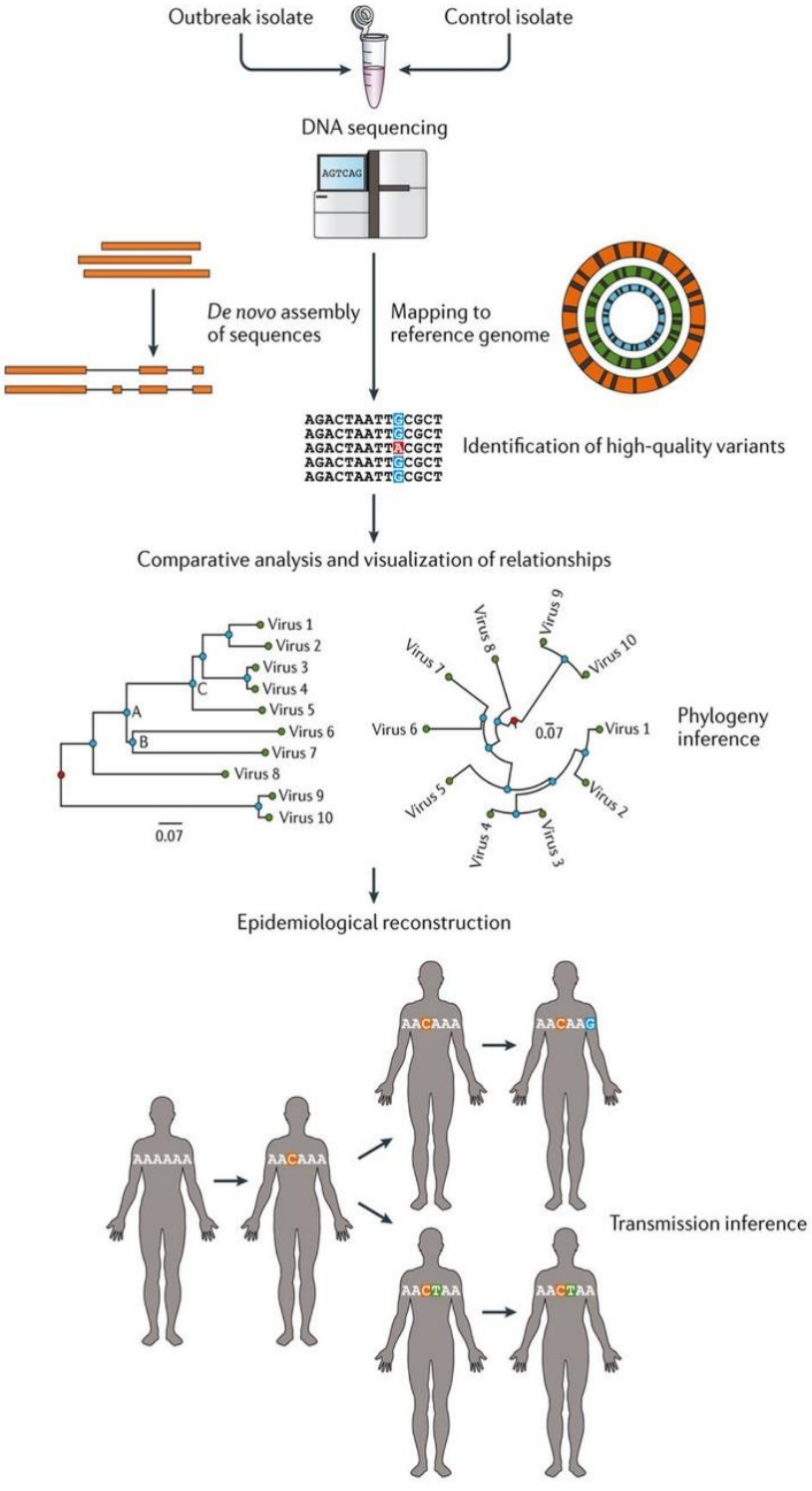


Figure 1-7 Schematic diagram demonstrating the process and utility of genomic epidemiology. Initially microbial isolates undergo WGS. The WGS can be assembled *de novo* or by mapping to a reference. Bioinformatics platforms enable the uploaded WGS to be annotated and allow users (microbiologists, bioinformaticians, public health officials, and clinicians) to analyse the genes of interest by visualising phylogenetic relationships and associating these with appropriate and relevant metadata. The example of outbreak tracing is used here but this can be extrapolated to many areas of health and disease. Figure reproduced unchanged with permission (Gardy and Loman 2017).

1.11.1. **Next Generation Sequencing technology**

Genomic epidemiological studies are increasingly feasible due to the generation of high-quality microbial genomes through Next Generation Sequencing (NGS) with benchtop sequencers including; Illumina, Ion Torrent, and Pacific Biosciences. The use of portable sequencing devices, such as the MinION, have been used in the field for Ebola and Zika virus epidemics, but at the time of writing still had higher error rates than other NGS platforms, although the flexibility exists to use the technology most suited to the research or clinical question, or location (Faria *et al.* 2016, Quick *et al.* 2016). Genomic epidemiology is scalable, for consideration of bacterial diversity, representative isolate collections are required, often large-scale studies requiring a global, collaborative approach, whereas outbreak tracing requires smaller collections of disease isolates or food sources. The cost of sequencing genomes has been rapidly falling since its commercial inception, but the challenge now lies in developing bioinformatic techniques for systematically analysing these genomes which are inexpensive, standardised, highly reproducible worldwide, and easily accessible to microbiologists, epidemiologists, and clinicians (Sabat *et al.* 2013).

1.11.2. **Bioinformatic approaches**

The most widely used method for typing bacteria is MLST, successful due to its highly discriminatory, portable and unambiguous nature, when compared to previous techniques such as MLEE (Maiden *et al.* 1998). Genetic lineages are most frequently defined by MLST which uses housekeeping genes, to catalogue diversity (Maiden *et al.* 1998). By assigning unique alleles based on any single genetic difference at each locus, from individual nucleotide differences to recombination events, it is possible to systematically index genetic diversity, not influenced by the rate of vertical or

horizontal transmission (Maiden *et al.* 1998). However, the limitations of seven locus MLST are apparent when studying relatively monomorphic bacteria or diversity among sublineages within species (Achtman 2004, Bratcher *et al.* 2012). This has been overcome by using the hierarchical and scalable gene-by-gene approach with assembled whole genome sequences (WGS) (Maiden *et al.* 2013). Following the founding principles of MLST but employing more loci, ribosomal MLST (rMLST), core-genome MLST (cgMLST), or whole-genome MLST (wgMLST), can increase resolution to identify genetic diversity (Figure 1-8).

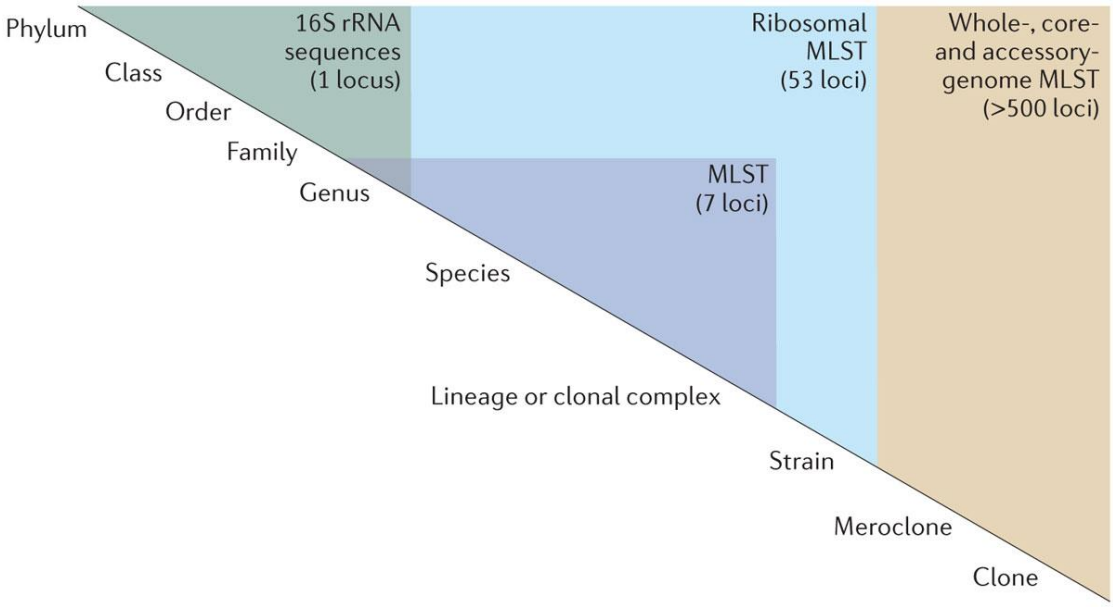


Figure 1-8 Representation of the hierarchical nomenclature schemes employing multilocus sequence typing principles and whole genome sequencing data.
Following the founding principles of MLST but employing more loci, rMLST, cgMLST, or wgMLST, can increase resolution within species to identify genetic relatedness. Whereas, using seven loci can determine resolution suitable for national or international surveillance, WGS can be of particular value in outbreak investigations. Reproduced unchanged with permission (Maiden *et al.* 2013).

Furthermore, antigenic typing allows characterisation of virulence factors including; capsule loci, pili, and antibiotic resistance genes. Alternative approaches include single nucleotide polymorphism (SNP) typing. Raw WGS data is used to map short reads onto a reference sequence, following which the SNPs can be identified (SNP calling). The SNPs are collated to form a SNP address, identifying closely-related isolate clusters within a given collection, which can be visualised using phylogenetic trees and interpreted with additional epidemiological data. This method can be performed rapidly, easily, and sensitively but is dependent on specialist software, reference genomes and sequencing platforms, which limits portability among sites (Fournier *et al.* 2014).

Effective bioinformatics platforms are required to enable the storage and analysis of WGS data. Databases that allow sharing of WGS include the publicly available Bacterial Isolate Genome Sequence Database (BIGSdb) platform (PubMLST.org) (Figure 1-9), the International Nucleotide Sequence Database Collaboration comprising of the National Center for Biotechnology Information (NCBI), the European Nucleotide Archive (ENA) and the DNA Databank of Japan (Jolley and Maiden 2010, Maiden *et al.* 2013). Global surveillance systems using standardised typing methods have incorporated sequence-based technology, e.g. European Centre for Disease Prevention and Control European surveillance system (TESSy) and US Centers for Disease Control and Prevention PulseNet. In the UK, the Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL) is a repository of WGS of all culture-confirmed meningococcal isolates from 2010 onwards, hosted on PubMLST.org *Neisseria* website (Hill *et al.* 2015). Here, examples of how genomic epidemiology has informed preventative strategies for infections caused by *N. meningitidis* will be discussed.

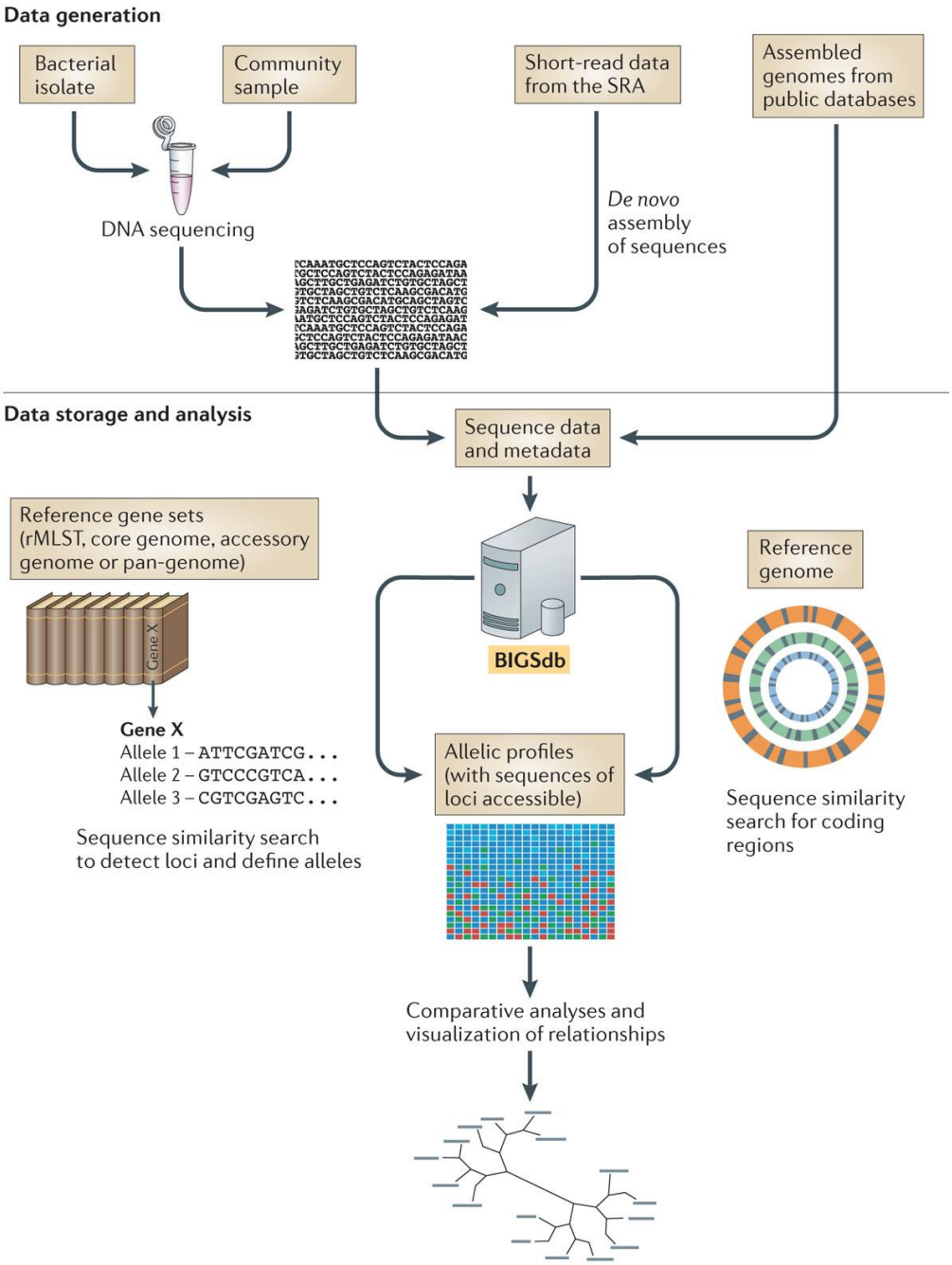


Figure 1-9 Illustrative representation of the gene-by-gene approach to whole genome sequence analysis using the Bacterial Isolate Genome Sequence Database (BIGSdb) platform.
Following DNA sequencing, the short reads are assembled *de novo* using bioinformatics pipelines. These genomes, or those from public databases, can be stored with their associated metadata and subsequently analysed using BIGSdb. Reproduced unchanged with permission (Maiden *et al.* 2013).

1.12. Genomic epidemiology of *N. meningitidis*

1.12.1. **Transmission dynamics**

1.12.1.1. Learning from carriage studies

As acquisition is the prerequisite of invasive disease, it is crucial to understand the transmission cycle and impact of preventative interventions to interrupt carriage and thereby induce herd immunity. The UK MenC vaccine programme, introduced in 1999, was successful by inducing both direct and indirect protection, reducing oropharyngeal carriage of serogroup C and genogroup C isolates. This was the first demonstration of how MLST could be rapidly employed in carriage studies for pre- and post-vaccine surveillance (Maiden *et al.* 2008). Subsequently, serogroup B disease has persisted, caused by multiple ccs, though rates of disease have been declining naturally since 2000. A large follow up carriage study of UK adolescents was done in 2014/15, which identified a national reduction in meningococcal carriage rates by 58.1% (Maclennan *et al.* 2016).

1.12.1.2. Neisseria species interactions in the oropharynx

The factors determining progression from oropharyngeal carriage to invasive disease remain incompletely understood. It has been postulated that interactions amongst other closely-related species, may be implicated. WGS has been exploited to develop an accurate and affordable method of speciation of the *Neisseria* genus, for use in resource-poor settings. With rMLST, sequencing of 53 loci to determine species produces reproducible, unambiguous results, however this may be technically challenging in regions lacking the necessary resources. Instead, amplifying and sequencing a single 413 base pair (bp) fragment of the *rplF* gene produces results that are consistent with the phylogeny constructed with rMLST (Bennett *et al.* 2014). This rapid and cost-

effective *rplF* assay has been used in large MenAfriCar studies in the African meningitis belt, identifying 10.2% carriage of *Neisseria* species, with point prevalence of *Neisseria lactamica* 5.6%, *N. meningitidis* 3.6%, *Neisseria polysaccharea* 0.6%, *Neisseria bergeri* 0.2% and *Neisseria subflava* 0.05% (MenAfriCar Consortium 2015, Diallo *et al.* 2016). However, this markedly varied by country across the belt (Figure 1-10). By age, *N. lactamica* was carried at the highest rate of 14.1% by 1-4 year olds and *N. meningitidis* was carried at the highest rate of 5.2% by 5-14 years olds (Diallo *et al.* 2016). Further, there was a mean 4.7 year delay in acquisition of *N. meningitidis* following carriage of *N. lactamica* (Coen *et al.* 2000). Although the underlying mechanisms are yet to be elucidated, this observation has implications for intervention strategies. Since 2010, the conjugate polysaccharide A vaccine has been progressively implemented across the African meningitis belt, with a dramatic reduction in hyperepidemic meningococcal disease and carriage in vaccinated and unvaccinated individuals (Diomande *et al.* 2015).

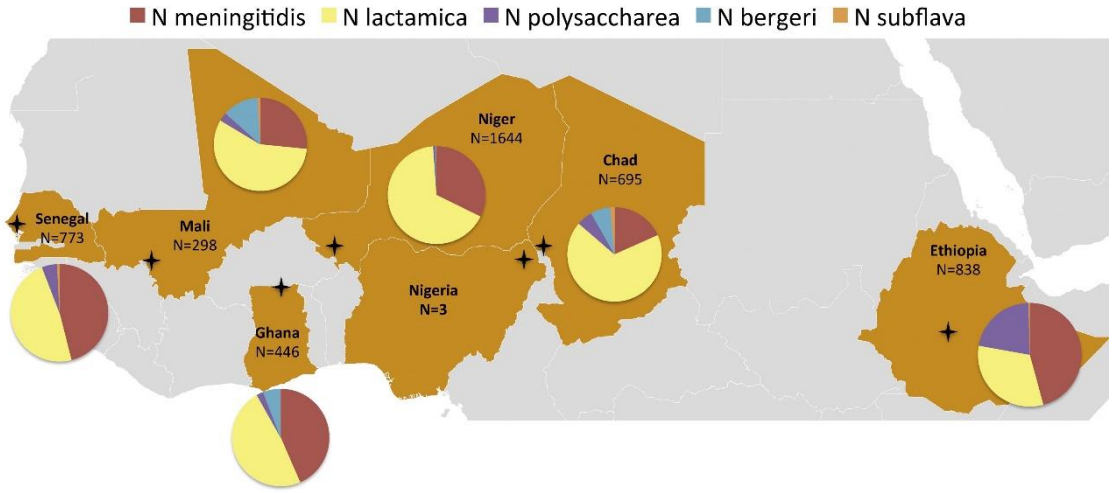


Figure 1-10 Distribution of *Neisseria* species from pharyngeal carriage across the meningitis belt in sub-Saharan Africa.
These studies analysed carriage of individuals aged 0-4 years, 5-14 years, 15-29 years and >30 years. The proportions of the different species vary markedly in cross-sectional carriage surveys, which investigated seven countries across the belt. Crosses indicate the sampling area for the respective countries. Figure reproduced unchanged under CC BY (Diallo et al. 2016).

1.12.1.3. Manipulating the nasopharyngeal niche

It is recognised that the age-specific rate of meningococcal carriage and invasive disease is inversely proportional to the rate of colonisation with *N. lactamica* (Gold *et al.* 1978, Cartwright *et al.* 1987, Olsen *et al.* 1991). Alternative vaccination strategies have been investigated which exploit this observation. Nasal inoculation with live *N. lactamica* was performed in UK university students, whose baseline *N. meningitidis* carriage rate was 22.4% (control group) and 24.2% (inoculated group) (p=0.778). New colonisation with *N. lactamica* occurred from two weeks after inoculation, and in those carrying *N. lactamica*, carriage of meningococci fell from baseline of 24.2% (36/149) to 14.7% (n=21/143) (p=0.006). This effect could be explained through the displacement of resident *N. meningitidis* soon after colonisation with *N. lactamica*, or in those not colonised with either *Neisseria* species to start with, the colonisation with *N. lactamica* could inhibit acquisition of *N. meningitidis*. There remained a group of patients persistently colonised with *N. meningitidis* despite *N. lactamica*, challenge, suggesting that displacement can be inhibited. The serogroup distribution was not characterised, so it cannot be determined whether this effect was related to all serogroups; however, the effect was seen across clonal complexes as determined through WGS (Deasy *et al.* 2015).

1.12.2. **Extent of capsular group B vaccine coverage**

1.12.2.1. Estimating 4CMenB coverage

The serogroup B polysaccharide capsule was poorly immunogenic and showed structural similarity to human tissue, raising concerns about autoimmunity. Protein-based vaccines were developed to address this issue, namely 4CMenB (Bexsero®) and bivalent recombinant lipoprotein rLP2086 (Trumenba®) (Jiang *et al.* 2010, Serruto *et*

al. 2012). In September 2015, Bexsero® was introduced for infants in the UK immunisation schedule at two, four, and 12 months of age. This vaccine contains multiple subcapsular proteins, including: fHbp, NHBA, NadA, and OMV containing PorA, and efficacy should be considered in terms of host immunogenicity and bacterial coverage. Bacterial coverage estimates using MATS assay, which assesses the phenotypic and functional properties of circulating meningococci, estimated coverage of 73% (95% CI 57-87%) in England and Wales (Vogel *et al.* 2013). The MATS assay can only be performed by specialist laboratories, is expensive, time and labour intensive and relies on pooled infant serum. Genomic analysis of vaccine antigens was done using a novel method, Bexsero® Antigen Sequence Typing (BAST), implemented on the PubMLST.org *Neisseria* database (see section 2.4.2). A collection of 2016 UK isolates of all serogroups from 2010/11 and 2013/14, from MRF-MGL, were analysed and exact matches to the vaccine estimated coverage between 22.8–30.8%, increasing to 58.3%-60.3% when considering potentially cross-reactive antigens (see 2.5.4) (Vogel *et al.* 2013, Brehony *et al.* 2016). A genotype-phenotype correlation model using the seven MLST loci to predict MATS coverage of serogroup B isolates, predicted coverage of 66%, the same as a subsequent revision of MATS coverage for contemporaneous isolates (2014/15) (see 2.5.5) (Brehony *et al.* 2016, Parikh *et al.* 2017).

1.12.2.2. Pre- and post-vaccine surveillance

A pre-vaccine genomic surveillance study, including 3073 UK disease isolates, identified the incidence of Bexsero® antigenic variants to be very low; fHbp:1, 13.4%; NHBA:2,13.8%; NadA:8, 0.8%; and PorA VR2:P1.4,10.9% (see section 3.5.6) (Rodrigues *et al.* 2018). This suggested that if the vaccine is to be effective, it would need to be through cross-protective immune responses or through alternative

mechanisms involving other OMV proteins, not well understood to date. It is imperative that ongoing surveillance is in place to monitor the secular changes in cc distribution that underlie many changes in antigenic variants, informing reformulation where necessary.

Ten months after vaccine implementation, vaccine efficacy of Bexsero® was estimated by Public Health England at 82.9% (95% CI 24·1–95·2) (Parikh *et al.* 2016). Two years after implementation, disease in the cohort of infants who had been vaccinated with high uptake (92.6% with two doses) in 2016/17 represented 12% of the serogroup B burden compared to 19% in 2015/16, when they would not have been fully protected, and 24% in 2014/15 pre-vaccine implementation (Public Health England 2016, Public Health England 2017). It was, at that time, difficult to disentangle vaccine benefit from natural variation in disease rate, as serogroup B, C, and Y disease all decreased in 2016/17 compared to 2015/16, on an overall downward trend for over a decade (Public Health England 2016, Public Health England 2017).

1.12.2.3. 4CMenB (Bexsero®) vaccines and uncertain impact on carriage

The use of Bexsero® has been restricted to infants in the UK and Ireland in national immunisation programmes, due to the uncertain impact on carriage. Herd immunity would undoubtedly prevent disease in other age groups who bear the overall burden of disease, thereby also improving vaccine cost-effectiveness and likelihood of implementation in endemic regions. Observation from a US university outbreak, where a close contact of young people who had been vaccinated with two doses of Bexsero®, acquired the same outbreak strain and died, suggested that carriage was not eliminated by vaccination (McNamara *et al.* 2015). The largest study to date of 2968 UK university students, comparing Bexsero® to ACWY vaccine and unvaccinated controls,

demonstrated a modest effect on carriage of all *N. meningitidis* with 18.2% (95% CI 3.4-30.8) reduction and on serogroup B disease-associated STs with 12.6% (95% CI -15.9-34.1) at least three months after vaccination (Read *et al.* 2014). At the time of writing, multi-centre carriage studies were underway in South Australia and the UK to more comprehensively address the impact of protein vaccines on carriage of meningococci. Genomic techniques were being used to capture population structure of carried meningococci in adolescents before and after targeted vaccination with Bexsero® and Trumenba®.

1.12.3. **Emergence of new strains**

1.12.3.1. Expansion of serogroup W South American/UK strain

The global increase in serogroup W disease has been of clinical concern since 2012. Genomic epidemiological studies of this serogroup W strain identified it as belonging to lineage 11.1, distinct from the Hajj strain and most closely-related to isolates causing IMD in Brazil, Chile, and Argentina, that subsequently spread worldwide (Figure 1-11) (Lucidarme *et al.* 2015). Detailed characterisation using WGS of MRF-MGL isolates in the UK, have identified that most disease after 2013 was due to isolates varying by only 30 loci, likely recombination events, demonstrating microevolution of the aggressive genotype (Lucidarme *et al.* 2016). The disease caused by this W strain has been particularly severe, affecting all age groups with atypical manifestations; pneumonia (12%), septic arthritis (7%) and epiglottitis/supraglottitis (4%) as well as septicaemia (49%), meningitis (12%) and both (16%) (Ladhani *et al.* 2015, Campbell *et al.* 2016). There were several adolescents presenting with gastrointestinal symptoms, with a high case fatality ratio (Campbell *et al.* 2016).

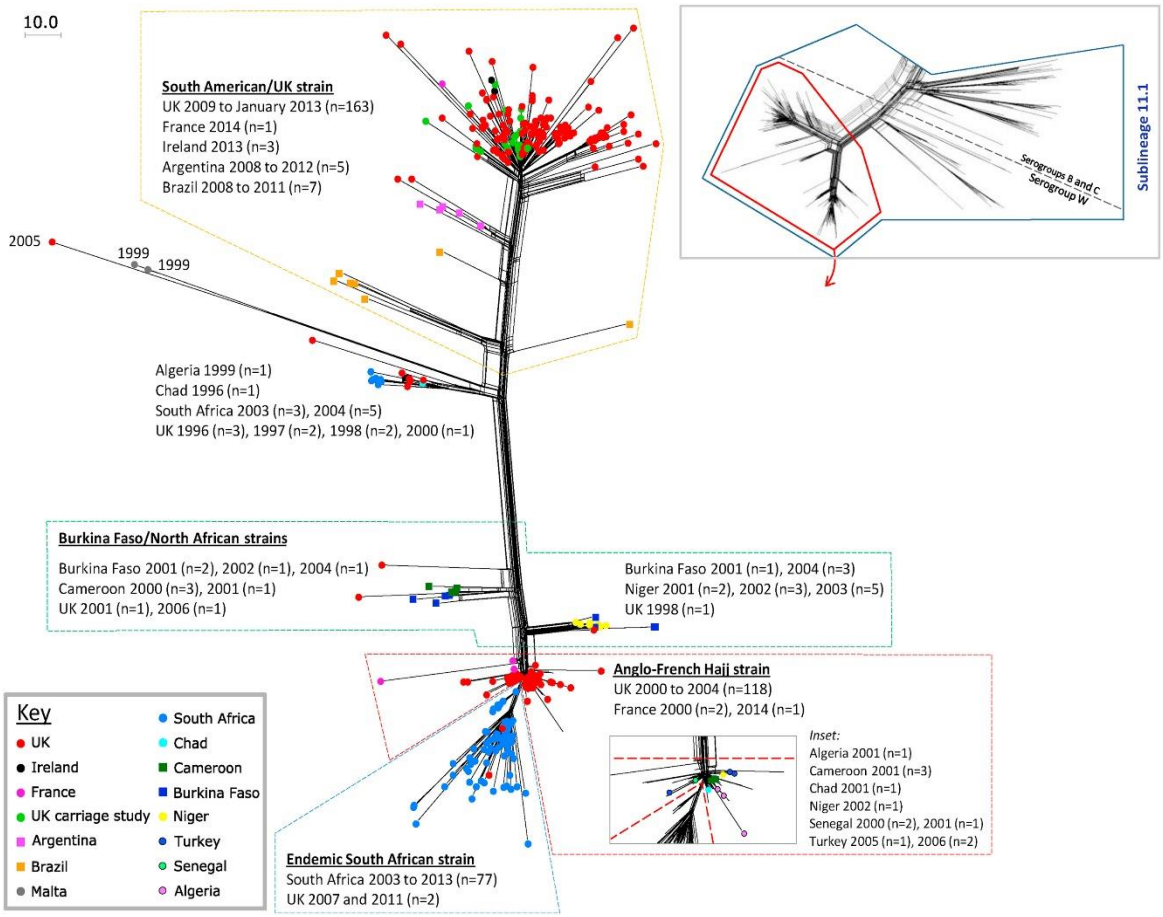


Figure 1-11 Geotemporal distribution of lineage 11.1 serogroup W isolates in global collections. The inset panel shows a Neighbour-net phylogenetic network of sublineage 11.1 and the distribution of capsular groups within it. Global disease isolates with serogroup W capsular antigens have been analysed using the meningococcal core genome (cgMLST, consisting of 1546 loci) and visualised with a Neighbour-net network in the main figure, allowing high-resolution discrimination between isolates. The South American/UK strain has been expanding since 2012 and is seen to be distinct from the Hajj strain. Figure reproduced unchanged under CC BY (Lucidarme *et al.* 2015).

In response to the emergence of this hyperinvasive genotype, conjugate meningococcal ACWY vaccine was introduced into the UK national immunisation schedule in August 2015 for adolescents, historically those with highest carriage rates (Christensen *et al.* 2010, Campbell *et al.* 2015). A recent study amongst UK university students with baseline 33% carriage rate of *N. meningitidis*, showed a modest reduction of 36.2% (CI 15.6-51.7) in carriage of serogroups C, W, and Y at least two months after conjugate ACWY vaccination (Read *et al.* 2014). Although, this effect on carriage is relatively

limited, it may be due to reducing acquisition rates and therefore impact on invasive disease incidence.

1.13. Conclusion

Genomic epidemiology of disease-causing bacteria has far-reaching implications for promoting human health and preventing disease. The ability to perform such studies has been accelerated with the ease, rapidity, and affordability of WGS, which provides the computational challenges to develop analysis tools for non-bioinformaticians. Considering bacterial meningitis, both *S. pneumoniae* and *N. meningitidis* have been extensively studied in this regard. Genomic epidemiology of *N. meningitidis* has increased our understanding of carriage and transmission, interactions with other commensals, distribution of vaccine antigens in national surveillance and emergent strains. This information in turn has helped to shape vaccination strategies worldwide to reduce the burden of this potentially devastating disease.

1.14. Thesis Outline and Aims

The development of vaccines against *N. meningitidis* remains the optimal method of prevention of IMD, in view of the non-specific clinical signs early in the disease, the rapid progression to fulminant sepsis or meningitis, and the high morbidity and mortality despite optimal healthcare interventions. The rationale to use alternative vaccine candidates to polysaccharide capsule or OMV proteins is clear, due to the concern regarding autoimmunity and narrow breadth of coverage respectively. The use of subcapsular protein-based vaccines to target serogroup B disease has its own limitations, and the inherent variability of meningococcal surface-exposed proteins has been the subject of this body of work.

The study of meningococcal WGS has been enabled by progressive initiatives such as the MRF-MGL, which after eight epidemiological years, held almost 4000 meningococcal genomes. This unique collection facilitated the application of genomic methodologies developed through this DPhil study on a national scale. This was particularly pertinent to the UK, as the 4CMenB vaccine, Bexsero®, had been implemented into the infant immunisation schedule in September 2015. The evidence base at the point of introduction demonstrated that infants had good immunogenicity to meningococci that possessed the vaccine variants and 73% of meningococcal strains in the UK were estimated to be covered by this vaccine (Findlow *et al.* 2010, Vogel *et al.* 2013). This DPhil study began in October 2015, in parallel with the vaccine introduction in the UK, the population-based experiment of vaccine efficacy. Due to the limitations in the assessment of protein-based vaccine immunogenicity and coverage estimates, and the antigenic variation of meningococcal surface-proteins, this work addressed methods

of cataloguing this diversity and demonstrated their utility in informing vaccine strategy.

In Chapter 2, a novel genomic typing method was developed to analyse the recombinant antigens present in the 4CMenB vaccine Bexsero®. These proteins (fHbp, NHBA, NadA and PorA) were selected to broaden the coverage of meningococci and were considered key vaccine antigens. Hosted on PubMLST.org *Neisseria* website, the BAST scheme provides a stable nomenclature to describe combinations of vaccine antigens. The scheme is publicly-available for use worldwide, allowing rapid, scalable and informative region-specific genomic estimates of coverage.

In Chapter 3, the BAST scheme was used to demonstrate the systematic examination of a national collection of disease-causing meningococci from the UK. This work provides important information about contemporaneous circulating strains from 2010/11 to 2015/16. This pre-vaccine surveillance study provided a baseline frequency distribution of each antigen and BAST for the five years preceding national vaccine introduction, allowing comparisons to be made in subsequent years. Understanding the circulating antigenic variants in the UK was important in interpreting the UK vaccine efficacy and mechanisms through which Bexsero® provides protection.

Vaccine efficacy from the UK was reported at 82.9% (95% CI 24·1–95·2), 10 months after introduction of Bexsero® into the immunisation schedule. This was surprising given the degree of meningococcal coverage expected from genomic estimates and genotype-phenotype modelling (see Chapter 2), and MATS estimates from 2014/15. In light of this, methods to catalogue the OMV components of Bexsero®/MeNZB™ were developed in Chapter 4, creating a typing scheme of the most abundant OMPs to assess their diversity and distribution, given their potential role as minor or synergistic

antigens. This work provides an insight into the population structure of the BAST and OMV proteins, with implications for vaccine design.

As carriage is the prerequisite to invasive disease, in Chapter 5 the typing schemes developed in Chapter 2 and 4 were used to analyse the diversity and distribution of these proteins in a longitudinal collection of carried meningococci from Oxfordshire. The antigenic variants present in carried meningococci, both those that were capable of causing disease (largely encapsulated with B, C, W, X, Y, or Z) and those that very rarely cause disease and comprise part of a healthy, functional microbiota (capsule E, L or capsule null) were determined. The impact of protein-based vaccine-induced antibodies on carried meningococci still remains uncertain, but as we await large carriage studies to report in South Australia and the UK, this genomic study provides a baseline frequency distribution of vaccine antigens.

Chapter 2

Development of Bexsero® Antigen Sequence Types (BASTs) for rapid, high-throughput analysis of meningococcal whole genome sequences

2.1. Public Dissemination of Work

These findings were published in the peer-reviewed journal Vaccine in April 2016:

Brehony, C., Rodrigues, C. M., Borrow, R., Smith, A., Cunney, R., Moxon, E. R. and Maiden, M. C. (2016). Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation. *Vaccine* 34(39): 4690-4697.

For the published paper, MCJM and ERM conceptualised the idea. CB developed the methods for sections 2.4.1, 2.4.2, 2.4.3, and 2.4.4, wrote the methods sections 2.4.3 and 2.4.4, performed data analysis and wrote sections 2.5.2 and 2.5.3. CMCR developed the methods for sections 2.4.1, 2.4.2, and 2.4.5, performed data analysis for sections 2.5.1, 2.5.4, and 2.5.5, wrote the abstract, introduction, methods sections 2.4.1, 2.4.2, and 2.4.5, results sections 2.5.1, 2.5.4, and 2.5.5, all discussions, and conclusion. RB, AS, and RC provided genome sequences from England and Wales, Scotland and Republic of Ireland reference units respectively. All authors reviewed and approved the final version.

Work from this chapter was accepted for oral presentation at:

British Paediatric Allergy, Immunity & Infection Group Winter Research Meeting, London, (December 2016), runner up prize presentation.

Work from this chapter was accepted for poster presentation at:

Federation of Infection Societies Annual Conference and Healthcare Infection Society 10th International Conference, Edinburgh, UK (November 2016).

2.2. Abstract

Serogroup B is the only major disease-associated capsular group of *N. meningitidis* for which no protein-polysaccharide conjugate vaccine is available. This has led to the development of multi-component protein-based vaccines that target serogroup B invasive meningococcal disease (IMD) including Bexsero®, which was implemented for UK infants in 2015, and Trumenba®. Given the diversity of meningococcal protein antigens, post-implementation surveillance of IMD isolates, including characterisation of vaccine antigens, is essential for assessing the effectiveness of such vaccines. WGS, as realised in the MRF-MGL, provides a rapid, comprehensive, and cost-effective approach to this. To facilitate the surveillance of the antigen targets included in Bexsero® (fHbp, PorA, NHBA and NadA) for protective immunity, a Bexsero® Antigen Sequence Type (BAST) scheme, based on deduced peptide sequence variants, was implemented in the PubMLST.org *Neisseria* database, which includes the MRF-MGL and other isolate collections. This scheme enabled the characterisation of vaccine antigen variants and here the invasive meningococci isolated in Great Britain and Ireland in the epidemiological years 2010/11 to 2013/14 are analysed. Many unique BASTs (647) were present, but nine of these accounted for 39% (775/1966) of isolates, with some temporal and geographic differences in BAST distribution. BASTs were strongly associated with other characteristics, such as serogroup and cc, and a significant increase in BAST-2 was associated with increased prevalence of serogroup W clonal complex 11 meningococci. Potential coverage was assessed by the examination of the antigen peptide sequences

present in the vaccine and epidemiological dataset. There were 22.8-30.8% exact peptide matches to Bexsero® components and predicted coverage of 66.1%, based on genotype-phenotype modelling for 63.7% of serogroup B isolates from 2010/14 in UK and Ireland. While there are many caveats to this estimate, it lies within the range of other published estimates.

2.3. Introduction

Invasive meningococcal disease (IMD) caused by Gram-negative organism *Neisseria meningitidis*, remains an important cause of morbidity and mortality worldwide (Greenwood 2007). In high income regions IMD is normally endemic at low incidence and is associated with serogroups B, C, W and Y, although higher incidence outbreaks occur periodically (Caugant and Maiden 2009). Protein-polysaccharide conjugate vaccines have been successfully implemented against the major disease-associated serogroups in high and low income settings, with the exception of serogroup B (Sow *et al.* 2011, Ali *et al.* 2014). The immuno-chemical similarity of the serogroup B capsule and human cell surface polysaccharides, results in poor immune responses and safety concerns (Finne *et al.* 1983). Consequently, development of 'group B' vaccines has concentrated on sub-capsular antigens, using either OMVs, purified proteins, or both (Tan *et al.* 2010). Bexsero® was licensed in Europe in 2013 and by the end of 2015, two vaccines, Bexsero® and Trumenba®, were licensed in the USA (Jiang *et al.* 2010, Serruto *et al.* 2012). Bexsero®, included in the UK infant immunisation schedule in September 2015, combines the protein antigens factor-H binding protein (fHbp), *Neisseria* adhesin A (NadA), neisserial heparin-binding antigen (NHBA) and PorA with an outer membrane vesicle (OMV) from the MeNZB™ vaccine (Holst *et al.* 2003, Serruto *et al.* 2012, Ladhani *et al.* 2015).

Molecular typing approaches are important for IMD diagnosis in the absence of a confirmed culture (Centers for Disease Control and Prevention 2012, Drew *et al.* 2012); as approximately half of IMD cases are diagnosed solely by PCR in the UK (Heinsbroek *et al.* 2013). Nucleotide sequence-based typing of meningococci has many applications (Maiden and Harrison 2016), including post-vaccination surveillance, where multilocus

sequence typing (MLST) established that the meningococcal C conjugate vaccine significantly reduced carriage of the outbreak strain in the UK population (Maiden *et al.* 2008). The application of rapid, cost-effective bacterial whole genome sequencing (WGS), achieved with the Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL), enables high resolution characterisation of clinical isolates at >95% of loci simultaneously (Hill *et al.* 2015). Analysis of MRF-MGL data from 2010/11 onwards, publicly accessible on the PubMLST.org *Neisseria* database, made a major contribution to UK vaccination policy, by determining that increases in serogroup W IMD in the UK were due to clonal expansion of an aggressive strain first reported in South America (Jolley and Maiden 2010, Campbell *et al.* 2015, Lucidarme *et al.* 2015).

WGS for disease-causing meningococci in Great Britain and Ireland provides detailed information on vaccine antigen sequence variants and enables inference of vaccine coverage. However, WGS does not identify the extent of immunological cross-reactivity of antigens, especially those with similar but distinct peptide sequences. Therefore, enhanced post-implementation surveillance of Bexsero® currently employs the Meningococcal Antigen Typing System (MATS) assay, developed to predict strain coverage of Bexsero® (Donnelly *et al.* 2010). Based on serogroup B IMD isolates, strain coverage of 73% in England and Wales was predicted (Vogel *et al.* 2013). Here, the diversity and structure among disease-causing *N. meningitidis* isolates from the UK and Ireland over four years (2010/14) was characterised, defining a reproducible method for typing Bexsero® vaccine antigens. As a demonstration of the utility of WGS, a model for predicting the likely MATS result from genotype was developed.

2.4. Materials and Methods

2.4.1. Genome collections

A total of 2016 genomes were analysed, representing all culture-confirmed cases of IMD for epidemiological years 2010/11 to 2013/14 from Great Britain and Ireland: England (n=1602); Wales (n=120); Scotland (n=114); Northern Ireland (n=47); and Republic of Ireland (n=133) the latter two grouped as 'Ireland' for analysis. There were 19 non-groupable isolates, the remainder were serogroups A (n=1), B (n=1393), C (n=88), E (n=5), W/Y (n=4), W (n=202), X (n=2), Y (n=301), and Z (n=1). The genomes were hosted on PubMLST.org *Neisseria* public database. Embedded tools within PubMLST were used to analyse the presence of Bexsero® vaccine antigens (fHbp, NadA, NHBA and PorA), diversity, association with clonal complex (cc), and geographical and temporal spread.

2.4.2. Implementation and curation of Bexsero® Antigen Sequence Type (BAST) scheme

A curated sequence type scheme for Bexsero® antigens was established within PubMLST.org *Neisseria* database to provide a robust, objective method of comparing the vaccine antigens. This enables easy comparison among datasets collected in different temporal or geographical regions (Mowlaboccus *et al.* 2016). Previously established nomenclatures for each component were used, with every unique peptide sequence for each Bexsero® antigen (fHbp, NHBA, NadA, PorA VR1 and PorA VR2) assigned a unique identification number (Russell *et al.* 2004, Brehony *et al.* 2009, Bambini *et al.* 2014). Although not included in the MATS assay, PorA VR1 was included in the BAST scheme, for additional discrimination among vaccine antigens. Each unique combination of these peptide sequences identifiers in an isolate was assigned an arbitrary number

(Bexsero® Antigen Sequence Type or BAST) in order of discovery, as sequence types (STs) are assigned in MLST (Maiden 2006). Assigning peptide sequence variant numbers was complicated where the gene sequence encoding an antigen was absent or did not encode an expressed protein. In the absence of protein expression or functional studies for these loci in all isolates, the most biologically plausible interpretation of sequence data guided the nomenclature. The allele designation 0 (null) was used where absence of the locus encoding a protein in an isolate was confirmed. In those cases where an indel mutation in the *nadA* gene resulted in the gene being phase variable 'off', the potential NadA peptide identifier for phase variable 'on' was used. For genomes with frameshift mutations in fHbp, NadA, or NHBA that rendered the resultant protein truncated, peptide designation 0 (null) was assigned.

2.4.3. **Analysis of antigen diversity and recombination**

Simpson's index of diversity (*D*) assessed the diversity of each protein, ranging from zero to one, with values nearer one indicating greater diversity (Hunter and Gaston 1988, Grundmann *et al.* 2001). Cramer's V coefficient measured the association of vaccine antigens and BASTs with cc and was calculated using the 'cramersV' function in the 'lsr' package in R 3.1.1. (R Core Team 2015). Rarefaction curves of BAST and peptide types were produced using the 'rarefaction' function in the 'vegan' package in R. A rarefaction curve is created by repeatedly re-sampling at random from a collection of N individuals and subsequently plotting the average number of species/types represented by N individuals. Analysis of recombination in the Bexsero® vaccine antigen genes was carried out using the ClonalFrameML program (Didelot and Wilson 2015). ClonalFrameML uses a maximum likelihood approach for phylogenetic reconstruction while taking into account recombination.

2.4.4. **Analysis of population structure**

The index of association (I_A) determined the level of linkage equilibrium, the random association of alleles at various loci, in sequence data (Maynard Smith *et al.* 1993). The 'standardised index of association', I^S_A, detected linkage disequilibrium among the BAST loci using the START2 program 0.9.0 beta (Hudson 1994, Jolley *et al.* 2001). The *f** metric measured the amount of non-overlapping structure in the combinations of antigens (Buckee *et al.* 2010). A script written and executed in R calculated the *f** metric.

Wrights fixation index, F_{ST}, investigated geographic and temporal structuring. The F_{ST} value ranged from zero (no differentiation among groups, indicative of gene flow), to one (complete differentiation and presence of structure in the population). Significant genetic differentiation among groups of isolates (years/regions) and the contribution of geotemporal factors were assessed by AMOVA (Excoffier *et al.* 1992). F_{ST} and AMOVA were calculated with Arlequin version 3.1 (Excoffier and Lischer 2010). The chi squared test and chi squared test for trend detected changes in BAST over time and calculated with 'prop.test' and 'prop.trend.test' functions respectively in the 'stats' package in R version 3.2.0.

2.4.5. **Genotype-phenotype associations**

The BAST scheme is a stable nomenclature that acts as a reference which can be correlated with functional assays, for example MATS, which is based on comparison of isolates to reference strains, not individual serum bactericidal assays (Donnelly *et al.* 2010). A genotype-phenotype association was modelled using 508 invasive serogroup B disease isolates from England and Wales from 2007/08 with previously published genomic and MATS data (Vogel *et al.* 2013). The pilot studies using BAST genotype

alone were not statistically valid in view of the small sample size (n=508) and high dimensionality of the BAST variable alone (217 levels). Instead, the seven MLST loci (due to associations between cc and BAST) were used with MATS coverage outcome, based on the presence of at least one antigen whose relative potency (RP) was greater than the positive bactericidal threshold (PBT) (NadA 0.009, fHbp 0.021, NHBA 0.294) or presence of PorA VR2:4 or both (Vogel *et al.* 2013). A Projection Pursuit Regression (PPR) model was employed to address the high dimensionality in explanatory variables. This was fitted to the data based on overall coverage outcomes of 0 (not covered) or 1 (covered), using RStudio Version 0.99.893. To internally validate the PPR model, 10% of data points were randomly removed and their coverage score predicted, with 80% accuracy for correct predictions. The PPR model was used to predict the coverage of STs in the 2007/08 dataset and these were applied to serogroup B 2010/14 isolates (n=1393).

2.5. Results and Discussion

2.5.1. BASTs identified in IMD isolates from Great Britain and Ireland

Complete BASTs were assigned to 1966 of the 2016 isolates (97.5%) with only 50 (2.5%) isolates with incomplete BASTs. Within the 1966 isolates, 647 BASTs were identified, the nine most frequent accounted for 775 (39.4%) isolates (Figure 2-1). A total of 475 BASTs were each represented by single isolates. The most frequent BAST was BAST-2 (fHbp 22; NHBA 29; NadA 6; PorA VR 1:5; VR 2:2), which accounted for 137 (7.0%) isolates. Each of the vaccine components exhibited peptide sequence variation, with fHbp and NHBA being the two most diverse antigens (Table 2-1). These data demonstrate that, although there is high diversity in these antigens and therefore many BASTs, a limited number of BASTs occur at high frequency (Figure S1, Appendix I – Supplementary figures). Taking into account secular changes, this indicates that a relatively small number of Bexsero® vaccine antigens could achieve high coverage of IMD isolates. There

were no occurrences of BAST-1, an exact match to the vaccine antigens (Serruto *et al.* 2012).

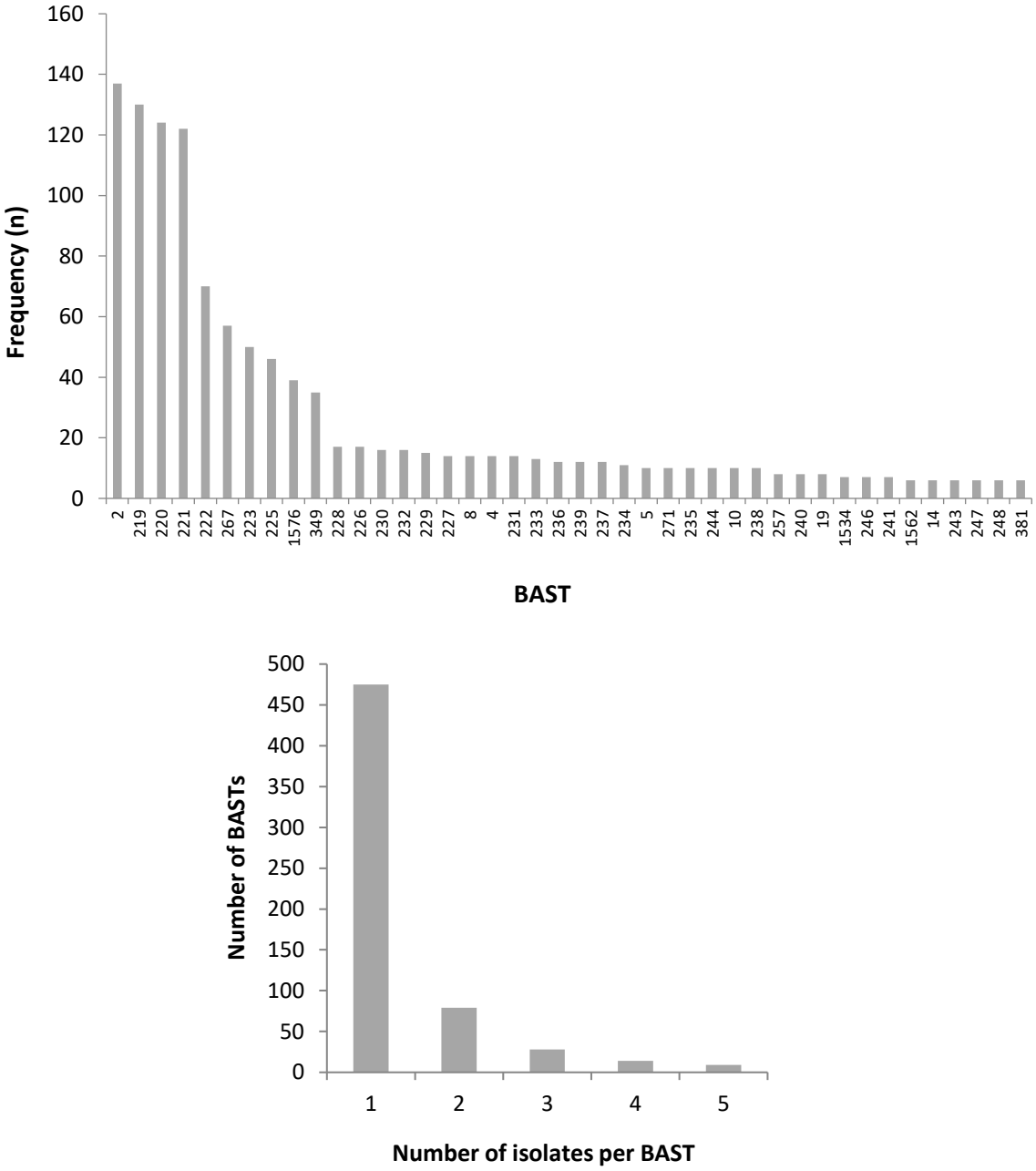


Figure 2-1 Frequency distribution of Bexsero® Antigen Sequence Types (BAST) in an invasive meningococcal disease collection from the UK and the Republic of Ireland, 2010/11 to 2013/14.
(a) The x-axis indicates the BAST, each numbered BAST-2, BAST-219, etc. Each BAST is a unique combination of peptide sequences of the four Bexsero® vaccine antigens: fHbp; NHBA; NadA and PorA. The y-axis is the frequency of each BAST. The BASTs shown are those with >5 isolates (b) Isolates for which the BAST frequency was five or fewer. The x-axis is the number of isolates and the y-axis the frequency of BASTs. Thus, for example, 475 distinct BASTs were represented by single isolates. Figure reproduced unchanged under CC BY (Brehony *et al.* 2016).

Table 2-1 Bexsero® antigen diversity and associations in meningococcal genome collection from Great Britain and Ireland between epidemiological years 2010/11 to 2013/14.

Simpson's index of diversity (*D*) assessed the diversity of each protein, ranging from zero to one, with values nearer one indicating greater diversity (Hunter and Gaston 1988, Grundmann *et al.* 2001). Cramer's V coefficient measured the association of vaccine antigens and BASTs with clonal complex (cc), with values nearer one indicating stronger association. The *f** metric measured the amount of non-overlapping structure in the combinations of antigens (Buckee *et al.* 2010). The 'standardised index of association', I^S_A, detected linkage disequilibrium among the BAST loci.

Peptide	No. of isolates	No. of variants	Index of diversity (*D*) (95% CIs)	Cramer's V (association with cc)	*f** metric				Standardised Index of association (I^S_A)			
					fHbp	NadA	NHBA	PorA VR1	fHbp	NadA	NHBA	PorA VR1
fHbp	2003	165	0.920 (0.915-0.925)	0.651								
NadA	436	17	0.688 (0.643-0.732)	0.489	0.614				0.735			
NHBA	1984	140	0.914 (0.909-0.919)	0.548	0.321	0.521			0.506	0.647		
PorA VR1	2010	39	0.871 (0.864-0.878)	0.716	0.229	0.633	0.255		0.330	0.648	0.380	
PorA VR2	2009	87	0.919 (0.915-0.923)	0.497	0.211	0.492	0.273	0.471	0.345	0.635	0.442	0.597
BAST	1966	647	0.974 (0.971-0.977)	0.946								

2.5.2. **Associations of BAST components with clonal complex**

There were strong non-overlapping associations between BAST and cc as determined by MLST (Figure 2-2, Figure S2) (Maiden 2006). For example, all isolates with BAST-2 (22; 29; 6; 5; 2) (n=137) and BAST-235 (13; 20; 0; 5-1; 10-8) (n=10) belonged to cc11. The nine most frequently occurring BASTs were associated with five ccs, with each cc exhibiting a major BAST (Figure 2-2). The association of individual vaccine antigens and ccs was supported statistically by Cramer's V coefficient (Table 2-1), where values approaching one support an association with cc.

Such non-random, non-overlapping associations of cc and antigens can be due to clonal descent or immune selection, as predicted by strain structure models (Gupta *et al.* 1996, Gupta and Anderson 1999). Meningococcal populations have been shown to have a fundamentally non-clonal population structure, as a consequence of extensive HGT (Gupta and Maiden 2001). The effect of recombination events on a population was measured with I^S_A, (Maynard Smith *et al.* 1993), with values ranging from zero (non-clonal) to unity (clonal). For the whole isolate collection, I^S_A was 0.301 consistent with a non-clonal population dominated by a limited number of dominant, hyperinvasive lineages. The overall I^S_A value in the dataset remained consistent over the first three years (average=0.297) but increased to 0.403 in 2013/14, most likely reflecting the increase in W:cc11 associated BAST-2. When this BAST was removed from the collection the I^S_A value decreased to 0.283. This non-random association also held for all pairwise comparisons of antigens with values ranging from 0.330 for the association of fHbp and PorA VR2 to 0.735 between fHbp and NadA. The effects of HGT resulting in intergenic recombination was detected in each of the antigen genes by ClonalFrame (Figure S3 (a-d)), not discussed further here (Didelot and Wilson 2015).

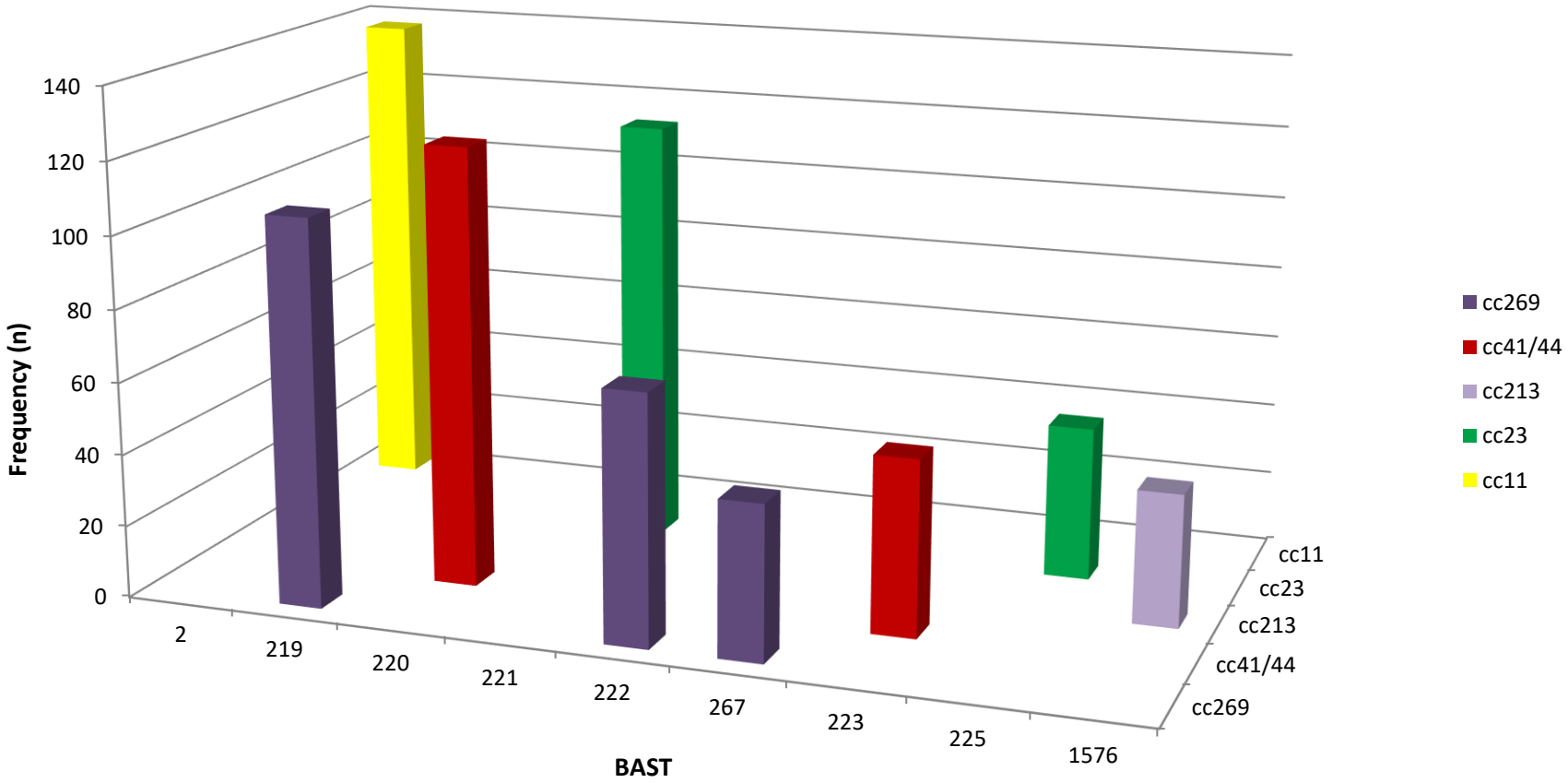


Figure 2-2 Frequency distribution of Bexsero® Antigen Sequence Typing (BAST) by clonal complex in invasive meningococcal disease isolates in the epidemiological years 2010/11 to 2013/14.
The nine most frequently occurring BASTs (n=775/2016, 39.4%) circulating in Great Britain and Ireland from 2010/14 are shown on the x axis which are structured by clonal complex (cc) (z axis), for example BAST-2 is only found in isolates of cc11. Cc269 has three predominant BASTs, 219, 222 and 267 not found in other ccs. Figure reproduced unchanged under CC BY (Brehony *et al.* 2016).

The non-overlap in antigen repertoires predicted by strain structure models was assessed with the *f** metric (Buckee *et al.* 2010). Values towards one indicate a non-overlapping structure representing high cross-immunity amongst antigen variants and immune selection maintaining antigenic combinations (Gupta *et al.* 1996), while values towards zero indicate non-overlapping antigen repertoires and therefore no cross-immunity amongst antigen variants. Similar to the I^S_A values, the pairwise *f** metric calculations produced values ranging from 0.211 for the fHbp and PorA VR2 pair to 0.614 for fHbp and NadA. The absence of NadA in 1537/2016 (76.2%) isolates and restriction to a small number of lineages is likely to account for the higher I^S_A and *f** metric values compared to the other antigens comparisons. In addition to being consistent with these antigens contributing to the development of natural immunity to the meningococcus, this structure provides an additional means of assessing potential vaccine coverage. Taken together, the patterns of variants within the BASTs in this collection, the distribution of individual antigen variants, and their relationship to cc were consistent with the behaviour of immunodominant antigens in a recombining population as envisaged in strain structure models, which supports the use of combinations of these antigens in vaccine formulations (Gupta and Maiden 2001, Buckee *et al.* 2010).

2.5.3. **Distribution of major BASTs by year and geographical region**

There was a similar distribution of the major BASTs for each epidemiological year (Figure 2-3), with the exception of a significant increase related to BAST-2 (cc11) (χ^2 test for trend; $p<0.005$) which increased in proportion from 2.3% (n=13/569)) in 2010/11 to 15.6% (n=66/422) in 2013/14. In the UK, these meningococci caused concern due to atypical and severe clinical presentation and genomic analyses demonstrated their close relationship to South American W:cc11 isolates (Ladhani *et al.* 2015, Lucidarme *et al.*

2015), leading to the implementation of vaccines including conjugate serogroup W polysaccharides. Other notable changes in the nine predominant BASTs were decreases in the two ccs to which most serogroup B strains belong, from 2010/11 to 2013/14. These were cc269 and associated BAST-219 which declined from 7.9% (n=45/569) to 2.6% (n=11/422) (χ^2 test; $p<0.005$) and cc41/44 and associated BAST-220 which declined from 8.3% (n=47/569) to 4.5% (n=19/422) (χ^2 test; $p<0.05$).

There was variation in the geographical distribution of BASTs (Figure 2-3). For example, at the time of sampling BAST-2 was more prevalent in England (122/1566, 7.8%) and Wales (9/114, 7.9%) than in either Scotland (2/114, 1.8%) or Ireland (4/174, 2.3%). BAST-219 ranged in prevalence from 3.4% of isolates in Ireland to 14.0% in Wales. The eighth most common type in the whole dataset, BAST-225, associated with cc23, was not found in Irish disease isolates. Pairwise comparisons of the four regions using F_{ST} values obtained from BASTs were different from the null hypothesis, indicating a significant ($p<0.05$) subdivision among all regions. It is well-known that the major hyperinvasive ccs are present worldwide and are stable over time but that their prevalence varies among countries (Achtman 1995, Muros-Le Rouzic *et al.* 2004, Brehony *et al.* 2007, Trotter *et al.* 2007). These data are consistent with a generally stable population of invasive meningococci, that varies over time, with occasional large changes in disease incidence caused by the introduction of a novel clone.

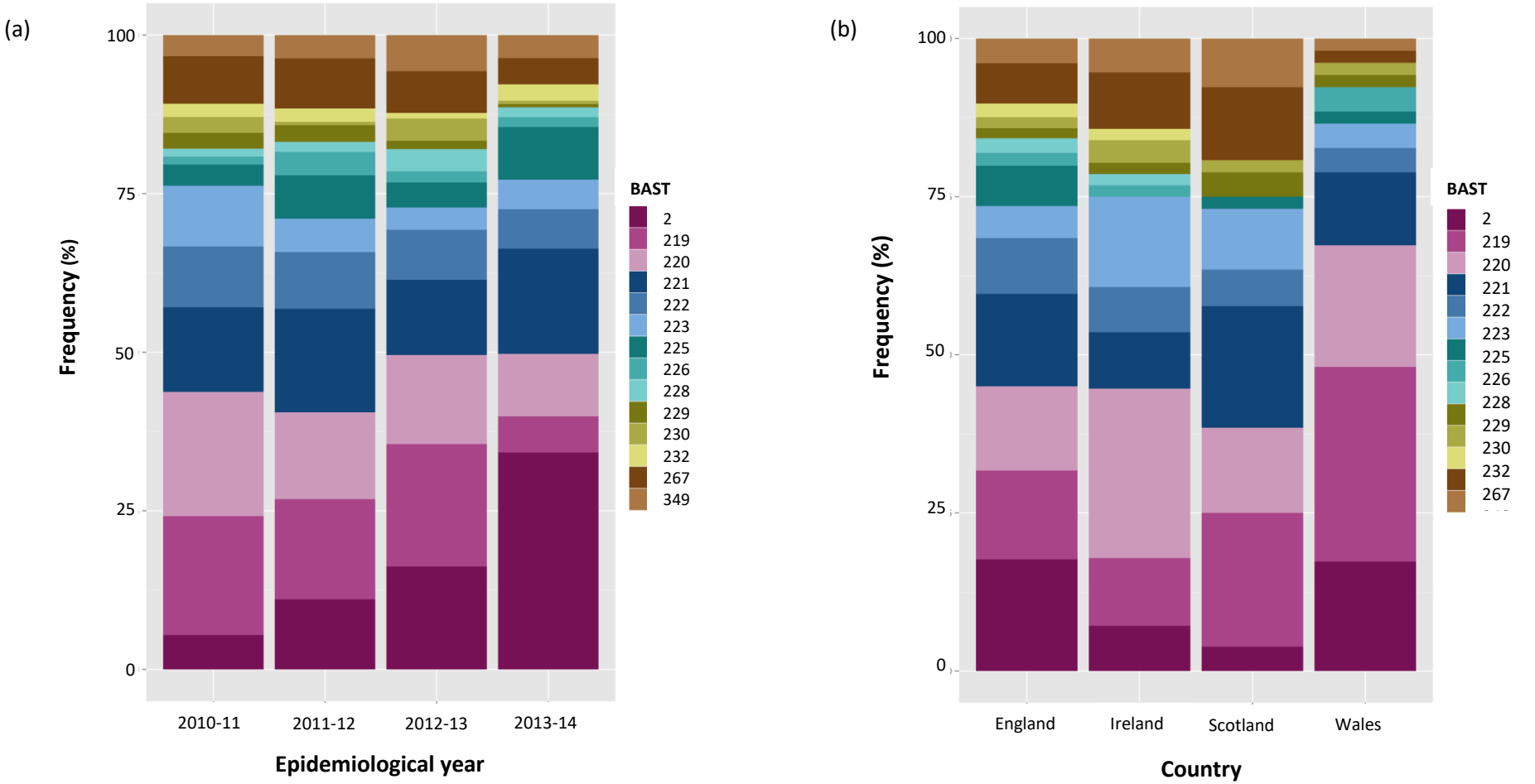


Figure 2-3 Bexsero® Antigen Sequence Type (BAST) frequency by epidemiological year and by region.
Comparison of the relative numbers of BASTs represented by more than 15 isolates by epidemiological year and region from IMD-causing isolates n=2016 (Great Britain and Ireland) during each of the four epidemiological years 2010/11 (n=582), 2011/12 (n=463), 2012/13 (n=532) and 2013/14 (n=439). **(b)** Percentage of BASTs represented by more than 15 isolates from 2016 IMD-causing isolates from England (n=1602); Ireland (Northern Ireland and Republic of Ireland; n=180); Scotland (n=114) and Wales (n=120). Figure reproduced unchanged under CC BY (Brehony *et al.* 2016).

2.5.4. **Genotypic matches to the Bexsero® vaccine antigens**

Isolates were compared at four loci within the vaccine BAST-1, fhbp:1, NHBA:2, NadA:8 and PorA VR2:4, as PorA VR2 is included in the MATS assay where PorA VR1 is not (Donnelly *et al.* 2010). No isolates in the dataset had an exact match to the vaccine BAST-1. There were two peptide matches in 74/582 (12.7%) isolates from 2010/11 which reduced to 31/439 (7.1%) in 2013/14 (Figure 2-4); however, it has been proposed that immunogenicity to only one of the four vaccine targets is sufficient to protect against disease (Medini *et al.* 2015). Over the four years, the proportion of isolates with at least one vaccine antigen matching to BAST-1 decreased from 179/582 (30.8%) to 100/439 (22.8%). Predictions based on antigen peptide sequence alone therefore indicated that a large proportion of disease-causing isolates would not be covered by the vaccine; however, this does not take into account the potential contribution to protection afforded by antigen cross-reactivity.

To account for this, MATS vaccine antigen coverage, which acts as a proxy based on recombinant antigens, was used to identify cross-reactive variants. The fHbp variants considered cross-reactive were: 1.1; 1.4; 1.13; 1.14; 1.15; 1.37; 1.232 (Vogel *et al.* 2013). As there was no correlation between nucleotide sequence variants and relative potency of NadA, presence of any NadA variant was considered cross-reactive for the purposes of this estimate. The presence of PorA VR1:7-2 was included due to the potential immunogenicity of this antigen. Due to lack of phenotypic data regarding the immunogenicity of NHBA variants, this antigen was excluded from this analysis. The proportion of isolates possessing at least one cross-reactive vaccine antigen ranged from 58.3%-60.3% (Figure 2-4). We conclude that the remaining 39.7-41.7% would likely be

dependent on protection through antibodies to NHBA, for which there is a lack of published information.

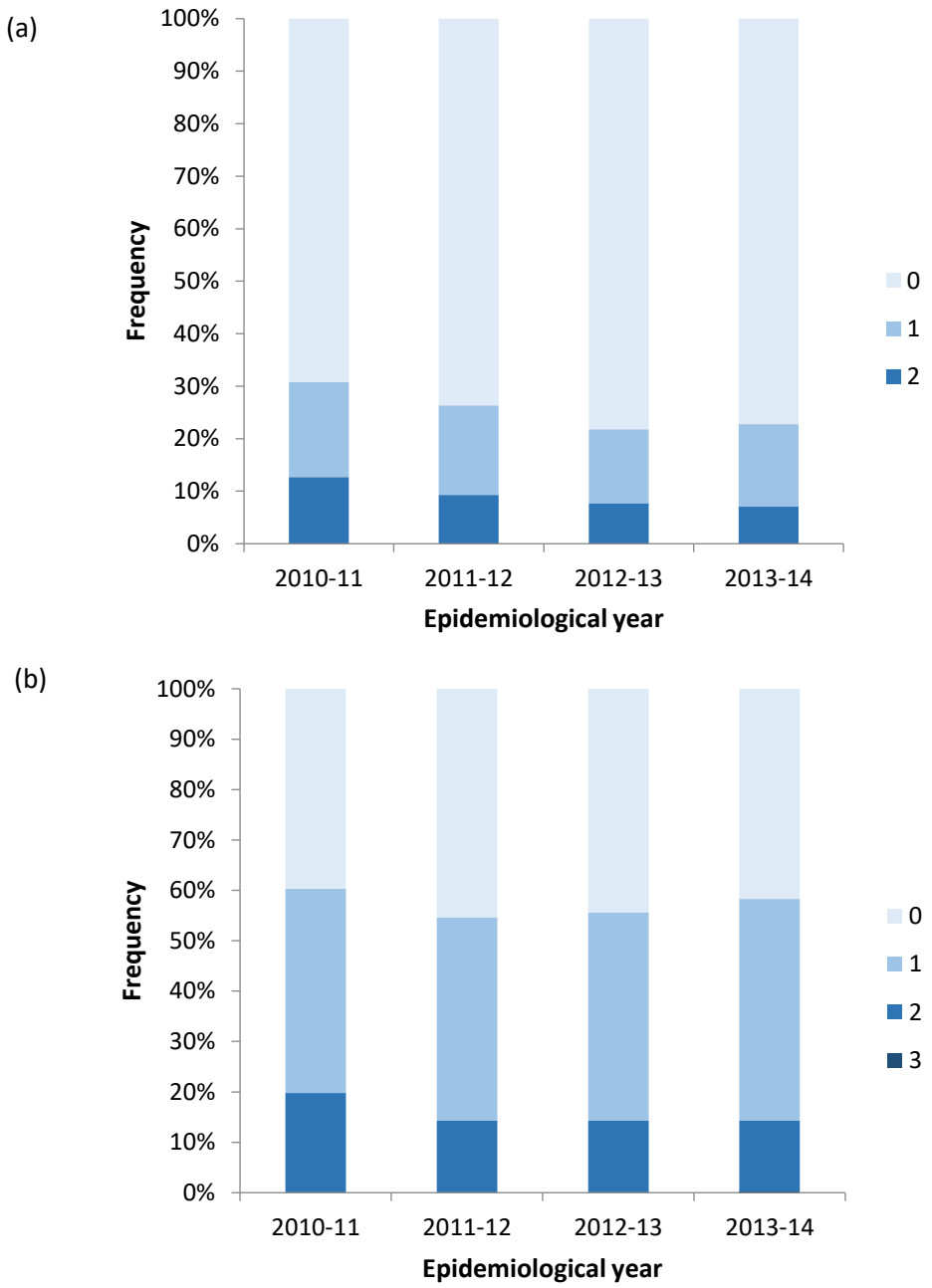


Figure 2-4 Bexsero® Antigen Sequence Type (BAST) matches of 2016 invasive meningococcal disease isolates in the epidemiological years 2010/11 to 2013/14 to the Bexsero® vaccine variants.
(a) Percentage of isolates whose BASTs match BAST-1 (the antigen profile in the Bexsero® vaccine) at up to four loci (fHbp 1, NHBA 2, NadA 8, PorA VR2 4) by epidemiological years 2010/11 to 2013/14. The number of matches is shown by the different shades of 0, 1 or 2 matches. (b) Percentage of isolates whose Bexsero® Antigen Sequence Types (BASTs) any antigenic variants considered to be cross-reactive (fHbp variant 1 peptides, any NadA peptide, PorA VR1 7.2) by epidemiological years 2010/11 to 2013/14. The number of matches is shown by the different shades of 0, 1, 2 or 3 matches. Figure reproduced under CC BY, colours adapted from the original (Brehony *et al.* 2016).

Vaccine coverage in England and Wales in 2007/2008 was estimated using the MATS assay (Vogel *et al.* 2013). This identified 73% (95% CI 57-87%) of isolates that possessed at least one vaccine antigen exceeding the relative potency threshold or presence of PorA VR P1.4 or both. Vogel *et al.*, deem this a conservative estimate, owing to limitations imposed by using pooled toddler sera as opposed to adult or adolescent sera, and lack of consideration of the co-operation and interactions of minor antigens or those below the positive bactericidal threshold (Vogel *et al.* 2013). As the isolates used in this analysis of BASTs were collected between three and seven years after the time period for which coverage of 73% was estimated, secular changes in the bacterial population may contribute to these differences, but it may also be attributable to differences in the approaches used.

2.5.5. **Genotype-phenotype associations**

Using increasingly available WGS, BAST provides a first step in identifying potential vaccine coverage. As this metric does not take into account cross-protection and phenotypic expression, it is important to correlate BAST analysis with functional assays, such as MATS, and ultimately with disease incidence in immunised and unimmunised individuals. The latter are more difficult to quantify robustly, therefore assumptions were made, but these associations were based on the stable BAST nomenclature. To demonstrate the potential utility of linking genotype and phenotype, published data from 2007/08 (Vogel *et al.* 2013) was used to fit a PPR model using the seven MLST loci (given the strong associations between cc and BAST) and vaccine coverage outcomes (0=not covered, 1=covered). The predicted coverage for each ST in 2007/08 was applied to serogroup B 2010/14 isolates for which an ST was available (n=1393). There were 887/1393 (63.7%) isolates for which coverage could be predicted, the remaining 506 had STs that were not present in 2007/08. For 2010/14, the predicted coverage for individual

epidemiological years ranged from 63.0-73.0%. The mean coverage estimate for 2010/14 was 66.1% (95% CI 61.5-70.7%). The proportion of isolates for which prediction was possible diminished over the four years; 68.3% in 2010/11 to 51.6% in 2013/14. In this study, the limitation of using BAST as opposed to ST, was the variation in number of unique BASTs, 217 in 2007/08 (0.43 BASTs/isolate) which increased to 647 in 2010/14 (0.33 BASTs/isolate). As more MATS data becomes available for WGS isolates in larger datasets, this statistical modelling may become possible. The difference in coverage for a given ST, as indicated by the MATS assay, where the host response is assumed to be constant, presumably reflects inter-strain variation in antigen expression. As Bexsero® surveillance continues, this model therefore provides predictions controlling for the host immune responses, a potentially added complexity encountered when analysing the impact of isolates causing disease in vaccinated individuals. It also allows estimations of vaccine coverage for isolates where STs are already available in the absence of WGS. For example, the EUMenNet collection (2413 isolates across 17 countries from 2000/2002) had 1240 isolates for which coverage could be predicted, and was estimated at 88.8% (95% CI 85.8-91.8%) (Brehony *et al.* 2007).

2.6. Conclusions

In this large and representative collection of IMD isolates from Great Britain and Ireland between 2010/14, the diversity of BASTs, their correlation with genetic structure, and variability over time and by geographic region has been shown. This analysis demonstrates a means of achieving rapid and scalable surveillance of isolates from IMD for Bexsero® vaccine antigens. The number of BASTs not previously present in these regions, demonstrated how the diversity of IMD isolates alters over time (Vogel *et al.* 2013); however, a number of BASTs were relatively stable, W:cc11 associated BAST-2

was an exception. Based on these analyses, mean estimates of vaccine coverage were 66.1% (95% CI 61.5-70.7%), but further disease surveillance occurring in newly vaccinated infants will inform about cross-reactivity and coverage. Finally, BAST provides a tool for studying the distribution of vaccine antigens present in the stable and persistent ccs in any population, presenting the opportunity for reformulation of the current Bexsero® vaccine if and when required.

Chapter 3

Genomic surveillance of 4CMenB (Bexsero®) vaccine antigenic variants among invasive meningococcal disease isolates in the United Kingdom, 2010/11-2015/16

3.1. Public Dissemination of Work

These findings were published in the peer reviewed journal Emerging Infectious Diseases in April 2018:

Rodrigues, C. M. C., Lucidarme, J., Borrow, R., Smith, A., Cameron, J. C., Moxon, E. R. and Maiden, M. C. J. (2018). Genomic Surveillance of 4CMenB Vaccine Antigenic Variants among Disease-Causing *Neisseria meningitidis* Isolates, United Kingdom, 2010-2016. *Emerging Infectious Diseases* 24(4): 673-682.

For the published paper, MCJM, CMCR, and ERM conceptualised the idea, CMCR analysed the data and wrote the manuscript. JL, RB, AS, and JCC provided genome sequences from reference units in England, Wales, Scotland, and Northern Ireland. All authors reviewed and approved the final version.

Work from this chapter was accepted for oral presentation:

European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria (April 2017).

European Society for Paediatric Infectious Diseases Annual meeting, Madrid, Spain (May 2017).

The European Meningococcal Disease Society Conference (EMGM), Prague, Czech Republic (September 2017).

Work from this chapter was accepted for poster presentation at:

Applied Bioinformatics and Public Health Microbiology Conference, Cambridge, UK (May 2017).

Academy of Medical Sciences Spring Meeting for Clinician Scientists in Training, London, UK (February 2017).

3.2. Abstract

In September 2015, 4CMenB meningococcal vaccine (Bexsero®) was introduced into the UK infant immunisation programme, in the absence of phase 3 trial information. Understanding the impact of the programme requires enhanced surveillance of invasive meningococcal disease (IMD) isolates and comparison with pre-vaccination isolates. Bexsero® Antigen Sequence Types (BASTs) were used to analyse whole genome sequences (WGS) of 3073 pre-vaccine UK disease-causing isolates obtained between 2010/11-2015/16. The isolates exhibited 803 BASTs among 31 ccs and frequencies of Bexsero® antigen peptide variants were: fHbp:1, 13.4%; NHBA:2,13.8%; NadA:8, 0.8%; and PorA VR2:P1.4,10.9%. In 2015/16, serogroup B isolates showed the highest proportion (35.7%) of exact matches to at least one Bexsero® component. Serogroup W isolates showed the highest proportion (93.9%) of putatively cross-reactive variants of Bexsero® antigens. The results highlighted the likely importance of cross-reactive antigens. BAST surveillance of meningococcal WGS data is rapid, scalable, and portable, enabling international comparisons of isolates.

3.3. Introduction

Neisseria meningitidis is an accidental human pathogen, carried asymptomatically in the oropharynx of 1-40% of the population, depending on age and social behaviour (MacLennan *et al.* 2006, Christensen *et al.* 2010). In England and Wales, ~2/100,000 people per annum develop invasive meningococcal disease (IMD) comprising septicaemia, meningitis, or both (Ladhani *et al.* 2012, Sridhar *et al.* 2015). Patients presenting early have non-specific symptoms, but can deteriorate rapidly, with case fatality ratios of 5-17% and physical and psychological sequelae in one third of survivors (Salisbury *et al.* 2006, Ladhani *et al.* 2012, Viner *et al.* 2012). Consequently, vaccination represents the optimal strategy for IMD prevention.

Meningococci are commonly characterised according to their expressed capsular polysaccharides, defining serogroups, with six (A, B, C, W, X, and Y) causing the majority of IMD. The capsular antigens are major virulence factors and efficacious A, C, W, and Y polysaccharide-based vaccines are used worldwide; however, serogroup B capsular polysaccharides are poorly immunogenic and share structural similarity to carbohydrates found in human tissues (Finne *et al.* 1983). The first serogroup B vaccines were derived from outer membrane vesicles (OMV) for use in epidemics caused by single strains defined by genotype and PorA type (Holst *et al.* 2009). The genotype, or clonal complex (cc) determined by MLST, groups related organisms and is important for categorising IMD phenotype, antimicrobial resistance, and vaccine antigens (Maiden *et al.* 1998, Jolley and Maiden 2014).

The UK and Republic of Ireland are among the high-income countries with highest IMD incidence (Hill *et al.* 2015). The UK has low-incidence endemic disease and periods of hyperendemicity, which changes with frequency of hyperinvasive genotypes (Hill *et al.* 2015). Historically, endemic serogroup B IMD predominated caused by multiple ccs,

especially hyperinvasive lineages cc41/44, cc269, cc213, and cc32 (Hill *et al.* 2015). In the 1990s, hyperendemic serogroup C IMD caused by cc11 (C:cc11) prompted introduction of infant MenC conjugate vaccination and a catch-up campaign, reducing disease and carriage of C:cc11 (Maiden *et al.* 2008). The UK experienced another period of hyperendemicity from 2012 with increasing W:cc11, lineage 11.1 meningococci, first identified in South America (Lucidarme *et al.* 2015).

Owing to the diversity of ccs responsible for IMD, OMVs derived from single ccs, do not offer sufficiently broad protection. Alternative vaccine candidates have been developed comprising sub-capsular proteins prevalent among many *N. meningitidis* strains; however, their protective potential has been complicated by meningococcal diversity (Gupta *et al.* 1996, Gupta and Anderson 1999, Tan *et al.* 2010). Two vaccines were licensed in 2013: 4CMenB (Bexsero®) for infants in Europe; and bivalent recombinant lipoprotein rLP2086 (Trumenba®) for 10-25 year olds in the US (Jiang *et al.* 2010, Serruto *et al.* 2012, Ladhani *et al.* 2016). The 4CMenB vaccine contains multiple proteins including: fHbp, NHBA, NadA, and PorA P1.7-2,4 from the New Zealand OMV vaccine (MeNZB™) (Serruto *et al.* 2012). The rLP2086 vaccine contains two fHbp variants, one from each of subfamily A (A05) and subfamily B (B01) (Jiang *et al.* 2010).

Bexsero® was implemented into the UK immunisation schedule from 1 September 2015, as a two-dose priming course for infants at two and four months and a 12 month booster, intended as an efficacious strategy for those at highest risk within the constraints of cost-effectiveness (Ladhani *et al.* 2016). As with previously licensed meningococcal vaccines, efficacy studies were precluded due to the rarity of IMD, so the MATS assay was developed, which estimated 73% (95% CI 57-87) vaccine coverage of UK isolates (Donnelly *et al.* 2010, Vogel *et al.* 2013). An appreciation of meningococcal antigenic diversity and persistence over time is required to inform the degree and longevity of

coverage provided by such protein vaccines. Real-time, continuous, and high-throughput methods are needed to determine the characteristics of circulating meningococci on a national scale, especially the frequency distribution of vaccine antigens. This can be done rapidly and reproducibly utilising WGS of IMD isolates, publicly-available through PubMLST.org *Neisseria* database (Jolley and Maiden 2010). A novel nomenclature, BAST, was devised to describe Bexsero® antigenic variants (Brehony *et al.* 2016). There were strong, non-overlapping associations between BAST and cc, with estimated 58.3-60.3% Bexsero® coverage including the antigenic variants in Bexsero® or cross-reactive variants (Brehony *et al.* 2016). Here, the genomic diversity of Bexsero® vaccine antigens has been catalogued using web-accessible platforms incorporating BAST. The study provides a reference point for changes in population structure of IMD-causing meningococci in the UK prior to Bexsero® introduction.

3.4. Materials and Methods

There were 3073 meningococci isolated from culture-confirmed UK IMD cases between epidemiological (1 July-30 June) years 2010/11-2015/16. For the purposes of this study, the pre-vaccine period includes 2015/16 as Bexsero® implementation started on 1 September 2015 and many infants were not fully vaccinated during the peak IMD season (December 2015-February 2016). The 3073 isolates represented ~55% of laboratory-confirmed cases of IMD (Table 3-1), as recovery of isolates reflects: differential survival in artificial media; susceptibility to antibiotics given prior to venepuncture; or small volume paediatric blood cultures.

Table 3-1 Geographic and serogroup distribution of the culture-confirmed UK isolates that have undergone whole genome sequencing, by epidemiological year.
The middle columns indicate the isolates that underwent whole genome sequencing (WGS) compared to all laboratory-confirmed cases of invasive meningococcal disease (IMD) (this includes culture-confirmed and PCR confirmed cases) from England, Wales and Northern Ireland (Public Health England) and Scotland (Public Health Scotland) by year, overall the data included in this study represented 55.4% of all laboratory-confirmed cases in England and 55.9% in Scotland. NG=non-groupable.

Year	WGS invasive meningococcal disease isolates					Public Health England laboratory-confirmed cases		Public Health Scotland laboratory-confirmed cases		Serogroup distribution of WGS isolates								
	Regions of the United Kingdom					Total cases (n)	WGS isolates (%)	Total cases (n)	WGS isolates (%)									
	England	**Wales**	**Scotland**	**Northern Ireland**	**Total**					**A**	**B**	**C**	**E**	**NG**	**W**	**W/Y**	**X**	**Y**	**Z**
2010/11	464	37	36	13	550	1009	46.0	76	47.4	1	423	13	1	5	27	2	1	76	1
2011/12	370	28	30	11	439	730	50.7	56	53.6		311	18	1	4	32	1		72	
2012/13	415	29	34	13	491	769	54.0	66	51.5		325	29		7	51	1		78	
2013/14	367	27	30	12	436	636	57.7	60	50.0		240	24		1	92	2		77	
2014/15	483	19	55	15	572	724	66.7	65	84.6		283	23		5	172	2		87	
2015/16	488	23	62	12	585	805	60.6	99	62.6		238	34	2	8	197	2		104	
Total	**2587**	**163**	**247**	**76**	**3073**	**4673**	**55.4**	**442**	**55.9**	**1**	**1820**	**141**	**4**	**30**	**571**	**10**	**1**	**494**	**1**

3.4.1. **Genomic analysis**

WGS determination was part of the Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL) initiative (Hill *et al.* 2015). Genomes were assembled using Velvet and VelvetOptimiser, uploaded and available through the PubMLST.org *Neisseria* database and annotated using *Neisseria* Sequence Typing Database numbers (NEIS) for all loci. Analysis was undertaken using the gene-by-gene approach with BIGSdb to determine ST, cc, and strain designation (Jolley and Maiden 2010, Maiden *et al.* 2013). Each isolate had associated provenance and phenotype data including year, serogroup, and region.

The BASTs were assigned as previously described (Brehony *et al.* 2016). Nucleotide sequences of *fhbp*, *nhba*, *nadA* and *porA* variable regions 1 and 2 (VR1 and VR2) were translated to deduce peptide sequences and variant numbers assigned using established nomenclatures (Russell *et al.* 2004, Brehony *et al.* 2009, Bambini *et al.* 2014). Unique combinations of the five components were assigned a BAST number in order of discovery; BAST-1 corresponds to the vaccine constituents: fHbp:1, NHBA:2, NadA:8, PorA:7-2,4 (Brehony *et al.* 2016). Data were manually curated to confirm the absence of *fhbp*, *nhba*, *nadA*, and *porA*, and isolates were assigned peptide designation 0 (null). Where nucleotide sequences contained a frameshift mutation, peptide designation 0 (null) was assigned. Peptide variants were not assigned if the complete gene was not available due to sequencing or assembly issues (Bratcher *et al.* 2014, Brehony *et al.* 2016). For assessment of Bexsero® antigenic variants expected to be prevented by vaccination (coverage), the isolates genotypic profile (BASTs) was compared to vaccine antigenic variants. The term "exact match" describes isolates possessing at least one of four Bexsero® antigenic variants (fHbp:1, NHBA:2, NadA:8, PorA VR2:4). The term "cross-reactive match" describes isolates possessing at least one variant that can potentially be

recognised by Bexsero®-induced antibodies, demonstrating a possible cross-protective immune response in humans. These variants were previously identified through MATS analysis, at present the most extensively used method for assessing qualitative and quantitative differences in antigens (Vogel *et al.* 2013, Abad *et al.* 2016, Parikh *et al.* 2017). Variants considered putatively cross-reactive, fHbp peptides 4, 13, 14, 15, 37, 232 and NadA variants 1 or 2/3. NadA peptides were included due to potential discrepancies between *in vitro* and *in vivo* NadA expression (Fagnocchi *et al.* 2013, Vogel *et al.* 2013). Cross-reactive NHBA peptides were not included, due to lack of data on the breadth of peptides covered by Bexsero®. Genomic analysis has not been used to infer protein expression or immunological cross-reactivity per se. All statistical analyses were performed using R version 3.2.4. Simpson's index of diversity (*D*) was calculated using the 'Vegan' package in R to assess diversity of BASTs, with values nearer one indicating greater diversity.

3.4.2. **Nomenclature of antigenic variants**

There are three systems for classifying fHbp variants. The first described three variants (1, 2, and 3), based on sequence similarity and cross-reactivity in serum bactericidal antibody (SBA) assays (Masignani *et al.* 2003). The second described two subfamilies: A and B (Fletcher *et al.* 2004). The third peptide nomenclature, used here as part of the BAST scheme, assigned arbitrary numerical integers to unique deduced peptide sequences, independent of variant/subfamily (Brehony *et al.* 2009). The NHBA peptide variants were assigned arbitrarily to unique peptide sequences. The updated nomenclature for NadA described four variants based on peptide sequence homology: NadA-1; NadA-2/3; NadA-4/5; and NadA-6 (Bambini *et al.* 2014). PorA nomenclature was based on nucleotide and peptide sequence homology and recognised the previous serological

classification; P1. followed by VR1 family-variant, VR2 family-variant, e.g. P1.7-2,4 (Russell *et al.* 2004). All are available on PubMLST.org *Neisseria* website.

3.5. Results

3.5.1. Distribution of Bexsero® vaccine antigens in the UK

For 2922 isolates (95.1%), MLST, deduced from WGS data, identifed 645 STs with 2866 isolates (93.3%) assigned to one of 31 ccs. There was variation in cc distribution over the six years, with a tenfold increase in cc11 and decreases in cc41/44 (172 to 56, 50.0%) and cc269 (108 to 52, 51.8%), which accounted for most serogroup B isolates (Figure 3-1).

3.5.2. fHbp peptide

An *fhbp* gene was present in 3065 (99.7%) isolates, absent in five (1.6%) and unassigned in three (0.1%) due to incomplete sequence assembly. Across all serogroups, variant 2 fHbp peptides increased across the six years, but most markedly in 2014/15 (313/572, 54.7%) and 2015/16 (347/585, 59.3%). From 2010/11 to 2012/13 variant 1 peptides were predominant, from 2013/14 onwards variant 2 peptides were more frequent than variant 1 (Figure 3-2). Overall, there were 207 unique fHbp peptides in the collection, which belonged to: variant 1 (n=109); variant 2 (n=43); variant 3 (n=54) and one between variants 2 and 3.

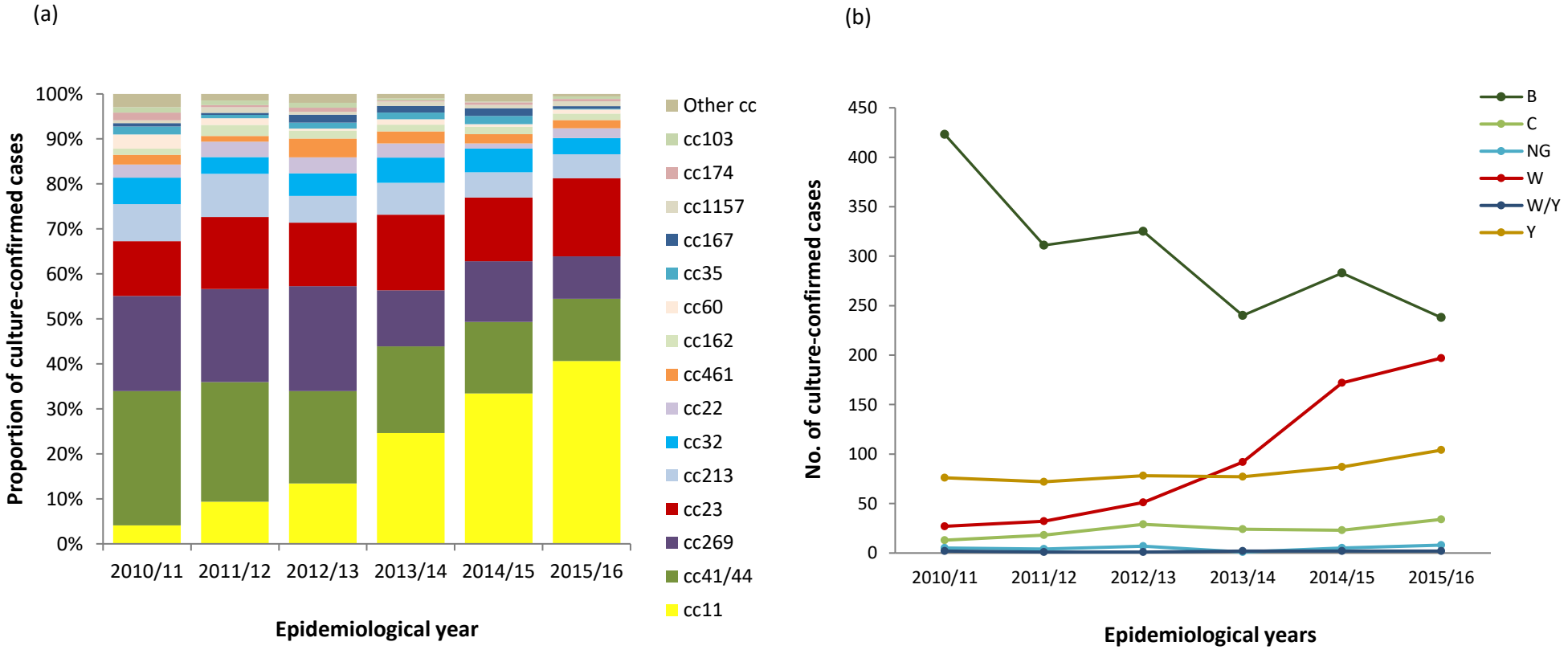


Figure 3-1 Clonal complex (cc) and serogroup distribution of invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
(a) Proportional contribution of each cc to disease-causing culture-confirmed meningococcal isolates by epidemiological year. "Other cc" indicates ccs that were found in <20 isolates during the 6-year study period. (b) Distribution of isolate serogroups by epidemiological year. Serogroups shown had >10 isolates during the 6-year study period. Serogroups with <10 isolates (A, E, X, and Z) are shown in Table 3-1. W/Y, serogroups combined because of inconclusive serogrouping results. Figures reproduced under CC BY, (a) unchanged, (b) colours adapted from original version (Rodrigues *et al.* 2018).

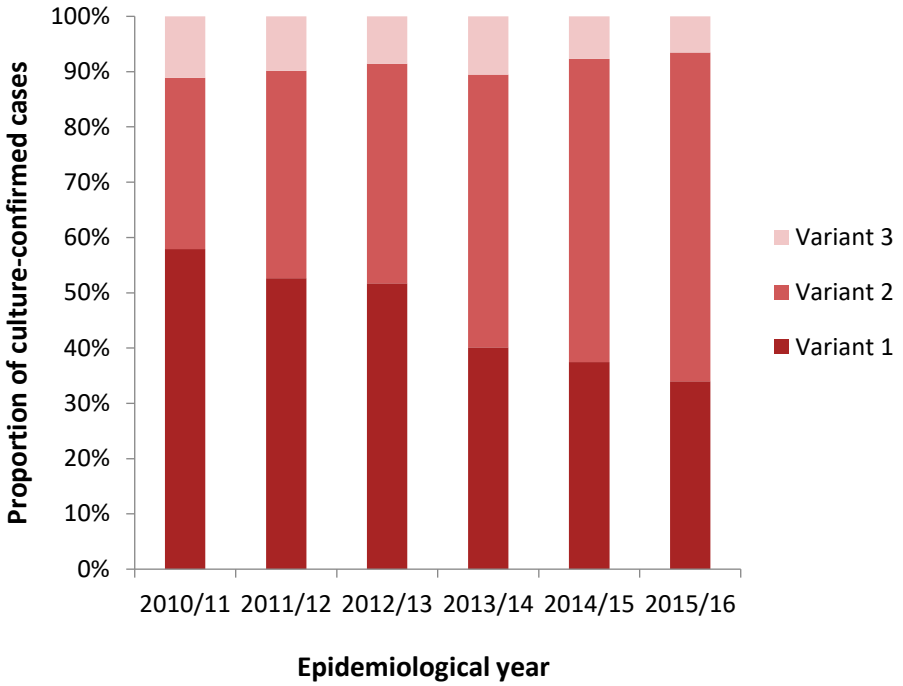


Figure 3-2 Distribution of fHbp antigenic variants among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
Proportion of isolates with fHbp variants 1, 2, and 3 by epidemiological year. Peptide 1 (a variant 1 member) is found in the Bexsero® vaccine, and cross-reactive variants included in this analysis are all variant 1 peptides. Number of isolates with fHbp peptides assigned by year were: 2010/11=549, 2011/12=437, 2012/13=491, 2013/14=436, 2014/15=571, 2015/16=583. Figure reproduced with colours adapted from original version under CC BY (Rodrigues *et al.* 2018).

Variant 1 peptides were present in 1389/3065 (45.3%) isolates and five peptides accounted for 1144/1389 (82.4%) isolates: 4 (399, 28.7%), 13 (329, 23.7%), 15 (184, 13.2%), 14 (127, 9.1%), and 1 (105, 7.6%). The contribution of isolates possessing these five variants among IMD isolates fell from 278/550 (50.5%) in 2010/11 to 154/585 (26.3%) in 2015/16. Peptide 4 was predominantly associated with B:cc41/44, whereas peptides 13 and 15 were mainly associated with B:cc269. Peptide 1, present in Bexsero®, was present in 105 isolates, predominantly B:cc32, decreasing in incidence from 25/550 (4.5%) isolates in 2010/11 to 13/585 (2.2%) in 2015/16. Variant 2 peptides were present in 1404/3065 (45.8%) isolates; six peptides (22, 25, 19, 16, 21, 24) accounted for 1323/1404 (94.2%). Peptide 22 (predominantly W:cc11) increased 13-fold from 15 isolates in 2010/11 to 198 isolates in 2015/16. Peptide 25, associated with Y:cc23, was consistently present through the period. Variant 3 peptides were present in 274/3065

(8.9%) isolates. Peptides 45, 47, and 31 were the most frequently occurring (predominantly B:cc213 and B:cc461).

3.5.3. **NHBA peptide**

An *nhba* gene was present in 2986 (97.2%) isolates, 87 isolates were unassigned an alleleic variant due to incomplete sequence assembly. There were 163 unique NHBA peptides and four isolates were assigned null (0), considered unlikely to express functional proteins owing to frameshift mutations in *nhba* coding region. The most frequently occurring peptide was 29 (568/2986, 19.0%), which increased seven-fold throughout the study period, associated with W:cc11. The second most common was peptide 2, present in Bexsero®, which decreased in incidence from 111/550 (20.2%) isolates in 2010/11 to 50/585 (8.5%) in 2015/16, most belonging to B:cc41/44 (411/424). Other important NHBA variants were 7, 17, 21, 20 and 18 which, with 29 and 2, accounted for 2258/2986 (75.6%) isolates (Figure 3-3).

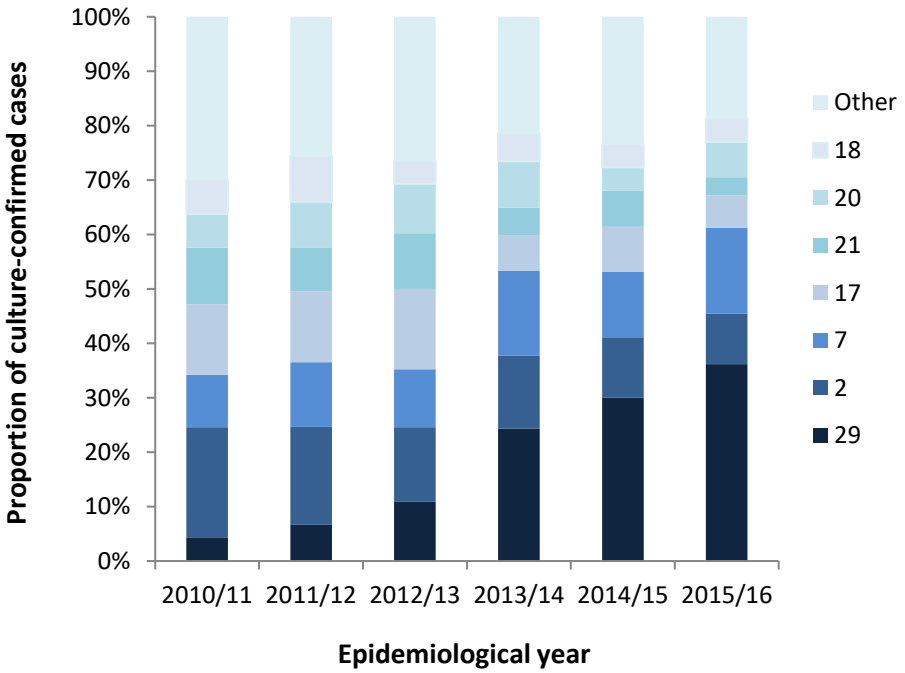


Figure 3-3 Distribution of NHBA antigenic variants among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
Proportion of isolates with the seven most prevalent NHBA peptides by epidemiological year; all other peptide variants are in "other." Peptide 2 is contained in Bexsero®. Number of isolates with NHBA peptides assigned by year were: 2010/11=549, 2011/12=438, 2012/13=488, 2013/14=411, 2014/15=557, 2015/16=539. Figure reproduced unchanged under CC BY (Rodrigues *et al.* 2018).

3.5.4. NadA peptide

There were 2036 isolates (66.3%) in which *nadA* was absent, 34 (1.1%) with an insertion element and 38 (1.2%) unassigned an alleleic variant due to incomplete sequence assembly. A total of 2128 (69.2%) isolates were assigned NadA peptide null (0) due to absence of *nadA* genes or presence of frameshift mutations or insertion elements disrupting the coding sequence. Of the prevalent ccs, NadA peptide was absent from all isolates belonging to: cc41/44; cc23; cc22; cc162; cc35; cc167; cc103; and cc282. Of the 907 (29.5%) isolates with NadA peptides present, there were 135 (4.4%) NadA-1 variants, 592 (19.3%) NadA-2/3 variants and 180 (5.9%) NadA-4/5 variants (Figure 3-4); there were no NadA-6 variants.

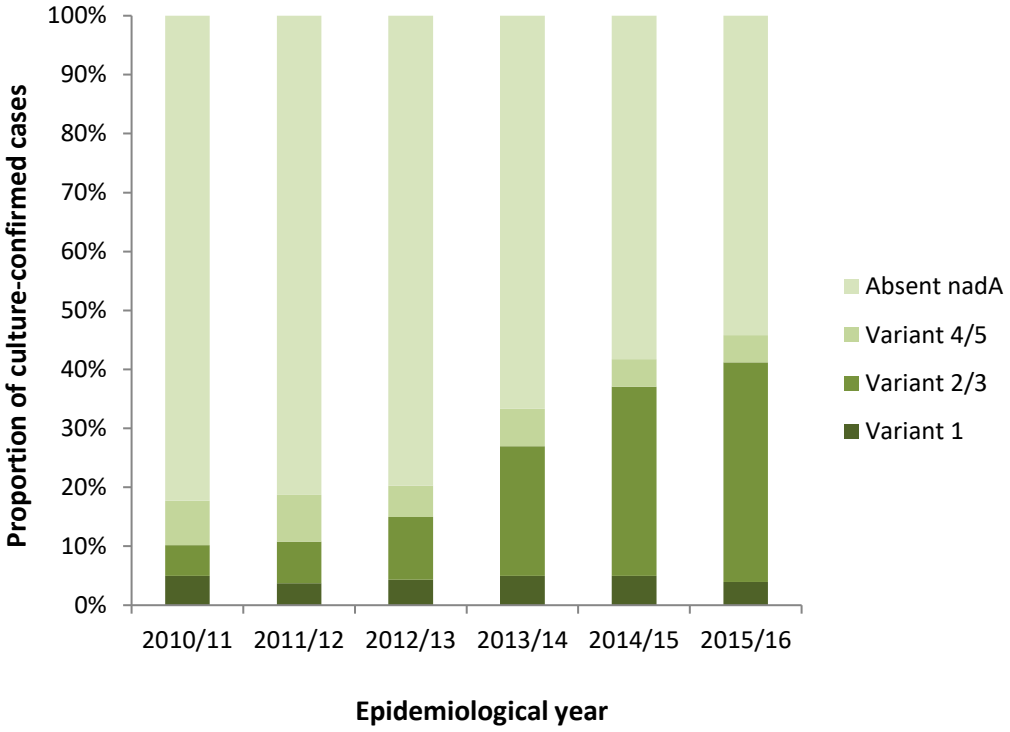


Figure 3-4 Distribution of NadA antigenic variants among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
Proportion of isolates with NadA variants 1, 2/3, and 4/5 by epidemiological year; there were no isolates with NadA variant 6. Peptide 8 (variant 2/3) is contained in Bexsero®. Number of isolates with NadA peptides assigned or *nadA* was absent by year were: 2010/11=5491, 2011/12=427, 2012/13=488, 2013/14=423, 2014/15=561, 2015/16=561. Figure reproduced with colours adapted from original version under CC BY (Rodrigues *et al.* 2018).

The proportion of isolates with NadA peptides increased from 96/550 (17.5%) in 2010/11 to 257/585 (43.9%) in 2015/16. Amongst these, there were 23 unique peptides. The most common, peptide 6 (NadA-2/3), increased from 14/550 (2.5%) isolates in 2010/11 to 187/585 (40.0%) isolates in 2015/16. Peptide 79 (NadA-4/5, containing a homopolymeric tract resulting in phase variation) was the second most common, designated as "phase variable on", occurring in 164 isolates predominantly B:cc213. Peptide 8 (NadA-2/3), found in Bexsero®, was present in 0.8% (26/3073) of isolates (Y:cc174, B:269, B:cc60, B:cc18, A:cc5).

3.5.5. **PorA**

The *porA* gene was present in 3011/3073 (98.0%) isolates. For 54 isolates, no nucleotide sequence allele was assigned due to incomplete sequence assembly. For 3064 (99.7%) isolates, predicted VR1 and VR2 peptides were obtained. PorA VR2 variant 4 was found in 336 (10.9%) isolates, with decreasing incidence from 90/547(16.5%) isolates in 2010/11 to 38/585 (6.5%) isolates in 2015/16. PorA VR2 variant 4 containing isolates were predominantly B:cc41/44 (302/336; 89.9%) but also B:cc162, B:cc269, B:cc213, B:cc32 and B:cc60 and were associated with PorA VR1 variants; 7-2 (n=330), 17 (n=2), 12-3 (n=2), 22 (n=1) and 22-1 (n=1) (Figure 3-5).



Figure 3-5 Distribution of PorA antigenic variants among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
Frequency distribution of PorA VR1 (top horizontal axis) and PorA VR2 (left vertical axis) variants. Variants shown were those that had >20 isolates in the collection from the UK during 2010/11–2015/16. Bexsero® contains the MeNZB™ OMV vaccine components, including variants PorA P1.7–2,4. Coloured boxes show the frequency of isolates from highest (green) to lowest (red). The total number of isolates with the given variants are shown in grey on the bottom and rights axes. Figure reproduced unchanged under CC BY (Rodrigues *et al.* 2018).

3.5.6. **Bexsero® Antigen Sequence Type (BAST)**

A BAST was determined for 2917 isolates (94.9%), with 803 unique BASTs identified. The ratio of BASTs per isolate was calculated by dividing the number of unique BASTs by the number of isolates. This ratio decreased from 0.42 in 2010/11 to 0.26 in 2015/16. Simpson's index of diversity (*D*) ranged from 0.976 in 2010/11 to 0.902 in 2015/16 (Table 3-2). There was strong, non-overlapping association of BAST and cc (Figure 3-6).

Table 3-2 Number of unique Bexsero® Antigen Sequence Types (BASTs) and measures of diversity including ratio of BASTs/isolate and Simpson's index of diversity (*D*), for invasive meningococcal disease isolates by epidemiological year.

Year	Unique BASTs (n)	Total isolates (n)	BASTs/isolate	Simpson's index of diversity (*D*)
2010/11	229	550	0.42	0.976
2011/12	187	439	0.43	0.977
2012/13	226	491	0.46	0.976
2013/14	181	436	0.42	0.960
2014/15	199	572	0.35	0.924
2015/16	155	585	0.26	0.902
Total	**803**	**3073**	**0.26**	**0.963**

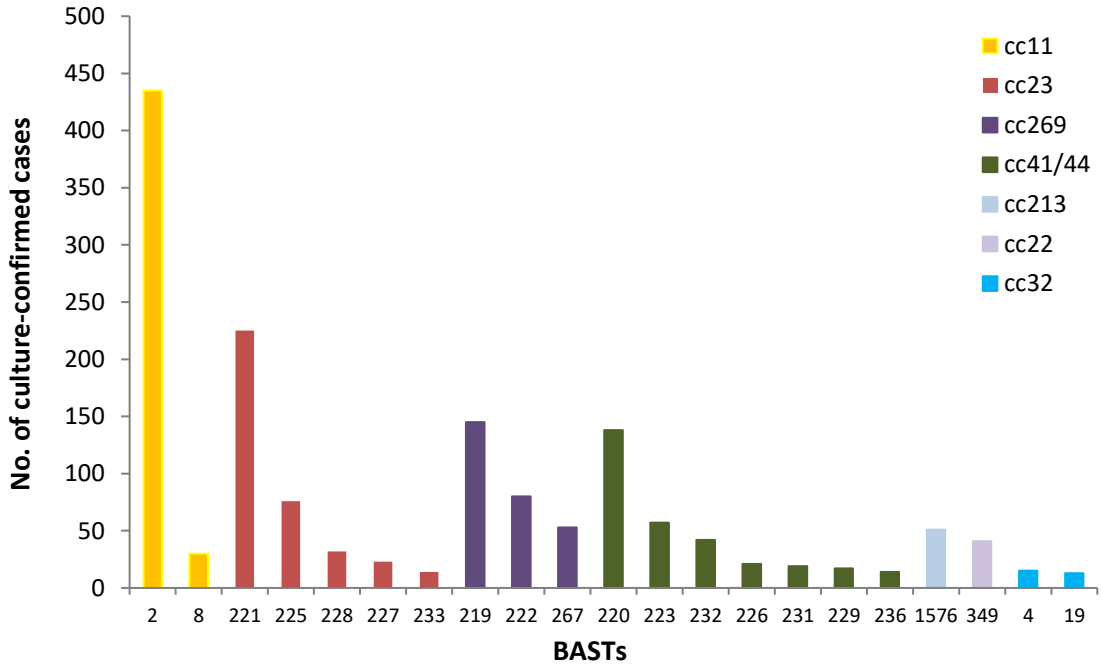


Figure 3-6 Non-overlapping association of Bexsero® Antigen Sequence Type (BAST) and clonal complex among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.
Frequency distribution of BAST by clonal complex (cc) for the seven most frequently found ccs that represent 82.4% (2,533/3,073) of culture-confirmed invasive meningococcal disease isolates. BAST-220, -223, -4, and -19 contain an exact match with BAST-1. BAST-2, -8, -219, -222, -232, -226, -231, -229, and -236 contain a potentially cross-reactive match with BAST-1. Figure reproduced unchanged under CC BY (Rodrigues *et al.* 2018).

When comparing BAST prevalence pre-implementation (cases from 1 July 2010 to 1 September 2015) and post-implementation (cases from 1 September 2015 to 30 June 2016), there were statistically significant increases in BAST-2 (cc11), BAST-221 (cc23), BAST-232 (cc41/44), and BAST-8 (cc11), and decrease in BAST-220 (cc41/44) (Figure 3-7).

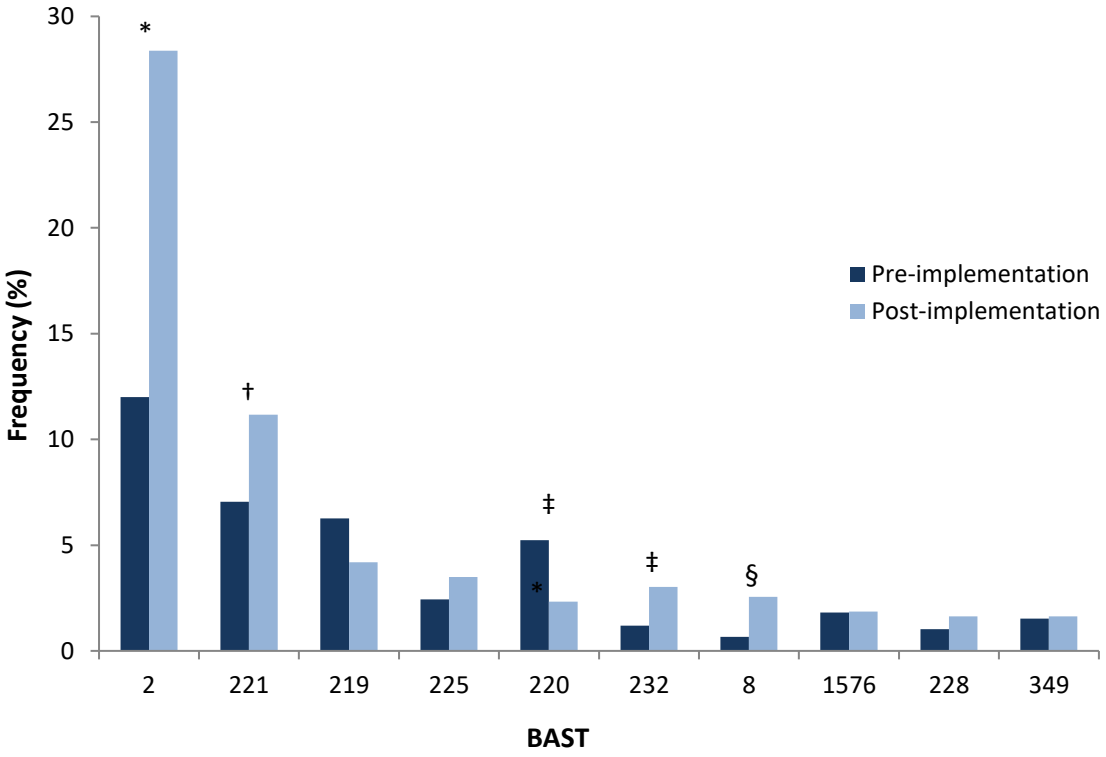


Figure 3-7 Changes in Bexsero® Antigen Sequence Type (BAST) prevalence before and after Bexsero® implementation among invasive meningococcal disease isolates, United Kingdom, 2010/11–2015/16.

Frequency of BASTs is shown for the period before implementation of Bexsero® vaccine, July 2010–August 2015 (dark blue), and after implementation, September 2015–June 2016 (light blue). The most frequently occurring BASTs pre-implementation were 2¶, 221, 219¶, 220#, 222¶, 267, 225, 223#, 1576, and 349. The most frequent BASTs post-implementation were 2¶, 221, 219¶, 225, 220#, 232¶, 8¶, 1576, 228, and 349. *p<0.00001; †p<0.01; ‡p<0.05; §p<0001; BAST¶ contains a potentially cross-reactive match to BAST-1; BAST# contains an exact match to BAST-1. BASTs with significant changes pre-implementation and post-implementation were BAST-2 (fHbp 22, NHBA 29, NadA 6, PorA VR1:5, and PorA VR2:2), p<0.00001; BAST-221 (25; 7; 0; 5–1; 10–1), p = 0.006; BAST-220 (4; 2; 0; 7–2; 4), p = 0.02; BAST-232 (4; 2; 0; 12–1; 16), p = 0.01; and BAST-8 (22; 29; 121; 5; 2), p = 0.0005. Figure reproduced unchanged under CC BY (Rodrigues *et al.* 2018).

3.5.7. **BAST distribution by serogroup**

Although Bexsero® is licensed for serogroup B IMD, its components may be present on the surface of meningococci, independent of capsular type, therefore distribution of Bexsero® antigens was analysed by serogroup. Serogroup B isolates had the highest proportion of exact matches to at least one Bexsero® antigen, with 155/423 (36.6%) isolates in 2010/11 and 85/238 (35.7%) isolates in 2015/16, predominantly cc41/44. The proportion of isolates with exact or potentially cross-reactive antigens was 293/423 (69.3%) isolates in 2010/11 and 149/238 (62.6%) isolates in 2015/16, representing: cc269; cc41/44; cc32; cc213; cc60; cc1157; cc18; cc162; cc11; cc461; cc35; cc167; and cc254. The potentially cross-reactive variants included: fHbp peptides 4, 13, 14, and 15 (956/1144) and NadA peptide 1 (118/1144).

For serogroup C, 8/141 isolates had at least one exact match to Bexsero® components, but varied each year depending on predominant cc. In 2010/11 and 2014/15 there were 2/13 (15.4%) and 3/23 (13.0%) matches respectively to Bexsero® components, with isolates belonging to cc32. There were no isolates with exact matches in 2011/12 and 2015/16 (predominantly cc11). The proportion of isolates also possessing potentially cross-reactive antigens increased from 4/13 (30.8%) in 2010/11 to 25/34 (91.3%) in 2014/15. The antigenic variants were fHbp (50 isolates, predominantly peptide 13), and NadA (50 isolates, predominantly peptides 121, 127, and 1), largely reflecting secular changes in cc distribution with increases in cc11, cc269, cc32, and cc174.

Of 571 serogroup W isolates, there was one cc11 isolate (0.2%) with an exact match to Bexsero® components (fHbp;1). When including potentially cross-reactive antigens, matches were 14/27 (51.9%) in 2010/11 rising to 185/197 (93.9%) in 2015/16. This was due to the presence of NadA peptide 6 in 500/571 (87.6%) isolates and the presence of fHbp 13 in 7/571 (1.2%) isolates, all of which belonged to lineage 11.1, W:cc11.

Serogroup Y disease isolates showed exact matches to Bexsero® components in 7/76 (9.2%) isolates in 2010/11 falling to 3/104 (2.9%) in 2015/16. When potential cross-reactive antigens were included, matches ranged from 9/76 (11.8%) in 2010/11 to 3/104 (2.9%) in 2015/16, representing: cc174; cc23; cc22; and cc11.

3.6. Discussion

The requirement for vaccines to protect against serogroup B meningococci caused by multiple ccs, led to the development of multi-peptide vaccines such as 4CMenB (Bexsero®) and bivalent rLP2086 (Trumenba®) (Jiang *et al.* 2010, Serruto *et al.* 2012). Bexsero® was introduced into the UK infant immunisation schedule in September 2015, supported by data from the MATS assay that estimated 73% IMD isolate coverage in England and Wales in 2007-08 (Vogel *et al.* 2013). Here, a comprehensive evaluation of the frequency distribution of Bexsero® antigen peptide variants in IMD isolates has been reported, using a national collection of WGS of culture-confirmed cases from 2010/11-2015/16. The frequency distribution of individual vaccine antigens was correlated with the distribution of meningococcal ccs in the UK over time. Between 2010/11 and 2015/16, the high diversity of 803 BASTs and 31 ccs emphasised the broad coverage required of peptide-based vaccines if they are to protect against endemic disease caused by multiple ccs. Indeed, the most frequently occurring BASTs (20 representing 44.1% of serogroup B isolates) could be used to inform future vaccine formulation.

Bexsero® components correspond to BAST-1; fHbp:1, NHBA:2, NadA:8, PorA VR1:7-2, PorA VR2:4. In this study, the incidence of individual BAST-1 antigens in 2010/11-2015/16 UK serogroup B IMD cases was: fHbp 5.4% (99/1820); NHBA 23.0% (419/1820); NadA 0.3% (5/1820), PorA VR2 18.4% (335/1820). The low levels of exact antigenic variants found in Bexsero® implies that host immunogenicity to cross-reactive antigens would be necessary to provide the level of protection required by a national

vaccination programme, although this is also dependent on adequate protein expression, which cannot be determined solely from genomic analysis. *In vitro* studies comparing bactericidal killing of various fHbp variant 1 peptide-expressing meningococci (peptides 1, 2, 3, 4, 5, 10, 12, 13, 14, 15) with post-vaccination sera, found that cross-reactivity was present in the sera of vaccinated adults, but functional activity in infants was limited to peptides 1 and 2 following immunisation at two, four, and six months (Brunelli *et al.* 2011). Surface protein expression is also an important determinant of bactericidal killing. For fHbp, when heterologous bactericidal activity was tested, mouse antisera to peptide 1 produced positive titres against closely-related peptide 4 regardless of expression level. For more distantly-related peptides, such as peptide 15, higher protein expression was required (Biagini *et al.* 2016).

At the time of writing, the most extensive estimation of cross-reactivity data for Bexsero® had been collected using the MATS assay. This assay quantifies the expression and antigenic similarity of fHbp, NHBA, and NadA by sandwich ELISA and identifies PorA serosubtype by sequencing for each isolate (Vogel *et al.* 2013, Medini *et al.* 2015). During development, the antigen measurements for fHbp, NHBA, and NadA were correlated to bactericidal killing with relative potency (RP) against 57 reference isolates tested by SBA assay, determining likelihood of bacterial killing. Isolates possessing PorA VR P1.4 peptide were considered to be covered, without further serological testing (Medini *et al.* 2015). Contemporaneous MATS estimates for UK coverage of 2014/15 IMD isolates was 66% (95% CI 52-80) (Parikh *et al.* 2017). The presence, cross-reactivity and expression levels of antigenic variants in the meningococcus alone does not, however, directly measure its susceptibility to bactericidal killing, which is also dependent on host innate and adaptive immune responses, a function not measured by the

MATS assay. Therefore, this assay remains a surrogate for estimating functional activity against cross-reactive antigenic variants.

Among the UK isolates examined here, the most frequent peptide variant 1 fHbp peptides were 4, 13, 15, 14 and 1. For most fHbp peptide 1 isolates tested by MATS, their RP lies above the PBT and these are predicted to be killed by the pooled sera from Bexsero®-vaccinated toddlers used in the assay (Vogel *et al.* 2013). However, for other fHbp variant 1 peptides, there is marked variation in the coverage estimates by MATS for isolates with the same peptide variant. Two European MATS studies identified the RP for peptide 4 isolates to be most consistently above PBT, but RP for peptide 13, 14, and 15 isolates span the PBT (Vogel *et al.* 2013, Abad *et al.* 2016). The degree of protection afforded by Bexsero® immunisation will be observed through post-implementation enhanced surveillance. With two-dose vaccine uptake at 88.6%, early reports of vaccine efficacy were estimated at 82·9% (95% CI 24·1–95·2) (Parikh *et al.* 2016). If these high efficacy estimates, albeit with wide confidence intervals, continue to show protection beyond that predicted by genotypic, phenotypic or functional estimates, then it may be that synergistic activity or minor antigens need to be considered, neither of which are quantified by MATS or BAST.

Coverage of non-serogroup B isolates by Bexsero®-induced immunity, was of especial interest in the UK, due to increasing IMD cases caused by W:cc11 from 2012, with high morbidity and mortality (Ladhani *et al.* 2015). The principle change in this analysis was the increase of BAST-2 (22; 29; 6; 5; 2), a direct consequence of W:cc11 clonal expansion. Although conjugate ACWY vaccine was introduced in August 2015, this was targeted to teenagers, the age group with increased disease and highest risk of carriage (Campbell *et al.* 2015). There is a paucity of supporting immunological evidence for the role of Bexsero® in protection against-non-serogroup B isolates, but in a small case series

of six W:cc11 isolates, all BAST-2, human SBA assay responses of ≥1:32 were observed using pooled serum from infants vaccinated with three doses of Bexsero® (Ladhani *et al.* 2016). Therefore, some protection may be provided to Bexsero®-vaccinated infants and toddlers.

3.7. Conclusion

Following implementation of Bexsero® into the UK immunisation schedule, long-term vaccine effectiveness will be established by enhanced IMD surveillance accompanied by characterisation of meningococcal isolates (Parikh *et al.* 2016). The methods employed here are rapid, standardised, open-source, and readily applied to different settings (Brehony *et al.* 2016, Mowlaboccus *et al.* 2016). The use of WGS, in the MRF-MGL in the UK, to extract vaccine antigenic variant data enables rapid isolate characterisation, permitting surveillance of circulating meningococci, monitoring of secular changes and the impact of all meningococcal vaccines (Hill *et al.* 2015, Brehony *et al.* 2016). These data serve as a reference point against which the effects of the national Bexsero® programme can be compared and it highlights the reliance on cross-reactive variants to maintain effective protection. Finally, such data will be invaluable in development of novel vaccine formulations that ensure continued coverage

Chapter 4

Typing complex meningococcal vaccines to understand diversity and population structure of key vaccine antigens

4.1. Public Dissemination of Work

Work from this chapter was accepted for oral presentation at:

The European Meningococcal Disease Society Conference (EMGM), Prague, Czech Republic (September 2017).

British Paediatric Allergy, Immunity & Infection Group Winter Research Meeting, London, (December 2017).

Work from this chapter was accepted for poster presentation at:

American Society for Microbiology Microbe Conference, Atlanta, USA (June 2018).

International Pathogenic *Neisseria* Conference, Monterey, USA (September 2018).

4.2. Abstract

Vaccines to prevent capsular group B meningococcal disease have been based on proteins, as the polysaccharide capsule was deemed unsuitable due to its similarity to human tissues. Outer membrane vesicle (OMV) vaccines have been targeted at specific capsular group B epidemics in Cuba, Norway, and New Zealand, with the major immunogen determined as porin A. Subcapsular protein vaccines aim to increase breadth of coverage of meningococcal strains, by the inclusion of protein variants from many different clonal complexes (ccs). Using rapid and scalable genomic techniques, it is possible to study the diversity of OMV and subcapsular proteins in relevant

meningococcal populations. The 24 most abundant proteins were identified from mass spectrometry of OMV vaccine MeNZB™. Amino acid sequences for these proteins were catalogued within the PubMLST *Neisseria* database as part of the Outer Membrane Vesicle peptide Typing (OMVT) scheme. Of the 24 proteins included, there was variation in the extent of diversity and association with ccs from the most conserved peptides FbpA (NEISp0578) and putative periplasmic proteins (NEISp1063) to the most diverse TbpA (NEISp1690). There were 1752 OMVTs identified amongst 2492/3506 isolates. OMVTs were further grouped into clusters (identical at ≥18 peptide sequences), 45.3% of isolates were assigned to one of 27 OMVT clusters. Both OMVTs and clusters were strongly associated with cc, genogroup and recombinant Bexsero® antigens. The OMVT scheme represents an open-access, web-based tool for the systematic analysis of the multiple components of OMV vaccines, demonstrating that combinations of OMV proteins exist in discrete, non-overlapping combinations associated with both genogroup and Bexsero® Antigen Sequence type (BAST). This highly structured population of disease-causing meningococci is consistent with proposed effects of host immune selection and competition between allelic variants on meningococcal population structure. This has implications for future vaccine assessment, and development, especially the choice of antigen combinations. The methodology is portable and will facilitate region-specific whole genome sequence interrogation, allowing informed choices about vaccine development or implementation.

4.3. Introduction

Ideal vaccines comprise a single antigenically-conserved component of the target pathogen, preferably against a virulence determinant. The highly successful tetanus and diphtheria toxoid vaccines epitomise this approach, with a major impact on disease globally as part of the World Health Organisation Expanded Programme on Immunisation (WHO EPI) (Okwo-Bele and Cherian 2011). Similarly, the conjugate polysaccharide vaccines against *Haemophilus influenzae* type b (Hib) and certain capsular variants of *Neisseria meningitidis*, the meningococcus, and *Streptococcus pneumoniae*, have been highly successful in eliminating the diseases they cause, largely through herd immunity effects (Maiden 2013). A number of more complex vaccines have also been successful but a several of these, including BCG the widely used tuberculosis vaccine, or those comprising killed whole cells such as whole cell pertussis and certain typhoid and cholera vaccines, are more difficult to define and control, often achieving imperfect disease control (Davenne and McShane 2016). Antigenically diverse pathogens continue to challenge vaccine design, and it is necessary either: (i) to identify protective antigens that are invariant, as has been attempted with the RTS,S malaria vaccine, or (ii) to include multiple components, perhaps varying composition over time, as has been done with influenza vaccines over many years (Gordon *et al.* 1995).

There is a limited repertoire of meningococcal polysaccharide capsules associated with invasive meningococcal disease (IMD), identified as serogroups A, B, C, X, W, and Y, potentially simplifying vaccine formulation (Stephens *et al.* 2007). While vaccines based on one or a few capsular polysaccharides have been deployed successfully since the 1960s, with notable success achieved by protein-polysaccharide conjugate vaccines, a comprehensive anti-capsular vaccine has been precluded by concerns as to the safety and efficacy of formulations that include the serogroup B polysaccharide (Finne *et al.* 1983).

Attempts to generate 'serogroup B substitute' vaccines have consequently focussed on subcapsular antigens, especially proteins, but these are highly variable. Meningococcal diversity extends beyond antigen genes, with a very wide range of distinct genotypes defined by variation in 'housekeeping genes' that encode essential cytoplasmic metabolic functions. This diversity is captured by multilocus sequence typing (MLST) approaches, including conventional MLST, ribosomal MLST (rMLST), and core genome MLST (cgMLST) (Maiden *et al.* 1998, Maiden *et al.* 2013). These approaches have shown that both genetic and antigenic diversity of this organism is structured into clusters of related organisms, which share a common ancestor (lineages). These clusters are recognised by conventional seven-locus MLST as clonal complexes (ccs) (Bratcher *et al.* 2014). The ccs are correlated with clinical phenotypes, including the propensity to cause disease and its severity, and antigenic characteristics including capsular and subcapsular antigens (Urwin *et al.* 2004, Yazdankhah *et al.* 2004). Comprehensive genomic typing schemes have been developed that catalogue serogroups, ccs and principle vaccine antigens (Jolley *et al.* 2007).

The multiple variants of the subcapsular antigens included in serogroup B substitute vaccines do not necessarily generate cross-reactive protective immune responses against a wide range of distinct meningococci (Feavers and Maiden 2017). Two main approaches have been adopted to overcome this problem: (i) the generation of strain-specific vaccines that target epidemics caused by particular meningococcal strains; (ii) the identification of conserved antigens that offer broad protection. The most widely used strain-specific vaccines have been based on meningococcal outer membrane vesicles (OMVs) which contain outer membrane proteins (OMPs) as principle antigens, especially the PorA porin (Stephens *et al.* 2007). An investigational Nonamen™ vaccine included nine different PorA proteins in a single OMV background to increase population coverage (Kaaijk *et*

al. 2013). 'Second generation' meningococcal B substitute vaccine development, using both conventional and 'reverse vaccinology' approaches, identified fHbp as a leading contender for a single, conserved vaccine antigen, leading to licensure of two vaccines: Bexsero® (4CMenB); and Trumenba® (rLP2086) (Giuliani *et al.* 2006, Vernikos and Medini 2014, Zlotnick *et al.* 2015). The fHbp protein is, however, highly variable in meningococcal populations, so the effectiveness of the protection offered, not fully established at the time of writing, is dependent on the generation of cross-protective immune responses (Brehony *et al.* 2009, Feavers and Maiden 2017).

The bivalent Trumenba® vaccine, containing two lipidated fHbp variants was licensed for use in the US in 10-25 year olds in 2014 and for those aged ≥10 years in Europe in 2017; however, as of summer 2018, Bexsero® vaccine was the only serogroup B substitute vaccine licensed, in 2013, for use in infants. This vaccine was introduced into the UK infant immunisation programme in 2015, undergoing post-implementation assessment and comprising three recombinant meningococcal proteins: fHbp, NHBA; and NadA; together with the MeNZB™ OMV that contained PorA as a principal antigen (Serruto *et al.* 2012, Ladhani *et al.* 2016, Parikh *et al.* 2016). The Meningococcal Antigen Typing System (MATS) immunological assay had been used to assess potential cross-reactivity of toddler antibodies to the principal Bexsero® components and the Bexsero® Antigen Typing Scheme (BAST) developed to assess the sequence variability of the vaccine components from meningococcal whole genome sequence (WGS) data (Donnelly *et al.* 2010, Vogel *et al.* 2013, Brehony *et al.* 2016, Rodrigues *et al.* 2018). At the time of writing, however, no means were available to systematically asses the other protein components of the OMV, which could potentially contribute to cross-immunity generated by this vaccine. Here we describe the development of such a scheme, the Outer Membrane Vesicle peptide Typing (OMVT) scheme, and investigate its value in

supporting the assessment of vaccine impact and the improvement of vaccine formulation.

4.4. Materials and Methods

4.4.1. Outer Membrane Vesicle peptide Typing (OMVT) scheme development and nomenclature

Twenty-five proteins were identified as representing the most highly-abundant proteins present in the OMV from Bexsero® and MeNZB vaccines (meningococcal isolate NZ98/254), using mass spectrometry (Figure 4-1, Table 4-1). This included work carried out at the National Institute of Biological Standards and Control (Appendix II – Mass spectrometry data for OMVT development), alongside previously published work (Tani *et al.* 2014). Although the Pilin E (PilE) protein was amongst this group, it was excluded from the scheme due to high frequency (>60%) of sequencing or assembly difficulties due to the highly repetitive DNA regions and high diversity of this antigen, with multiple copies in the meningococcal genome. Twenty-four proteins were taken forward and defined using a novel peptide-sequence based nomenclature. Each of the 24 peptides was been given the prefix NEISp, referring to the peptide sequence deduced from the corresponding nucleotide locus (designated with a NEIS prefix). Each peptide was numbered according to its equivalent NEIS number. For example, *porA* has a nucleotide locus NEIS1364, and peptide locus NEISp1364. This collection of 24 loci form the Outer Membrane Vesicle peptide Typing (OMVT) scheme which is hosted on the PubMLST *Neisseria* database (https://pubMLST.org/neisseria/) (Hill *et al.* 2015). The closed reference genome NZ05/33, an epidemic strain from the same New Zealand outbreak as the MeNZB™ vaccine strain (NZ98/254), sharing 99.4% sequence identity (1595/1605 core genome loci), was used to identify variant 1 amino acid sequence for each peptide

included in the scheme. Every unique amino acid sequence was assigned a different variant number, in order of discovery.

4.4.2. **Peptide variant designation and caveats**

For each component of the OMVT scheme, peptide variants were assigned from deduced peptide sequences of the respective NEIS locus. Where the NEIS locus was a complete coding sequence (i.e. containing a start and terminal stop codons with the number of base pairs (bp) being a multiple of three and no internal stop codons), each unique peptide sequence was assigned an arbitrary variant number in order of identification. Where the gene contained a mutation generating a frameshift, indel, or an internal stop codon more than 50bp from the consensus stop codon, then variant 0 (null) was assigned, following the deduction that no functional protein would be produced from such loci. Where internal stop codons occurred less than 50bp from the consensus stop codon, the peptide variants were assigned. Where there could be no NEIS or NEISp variant deduced, due to incomplete sequencing or genome assembly, no variant was assigned.

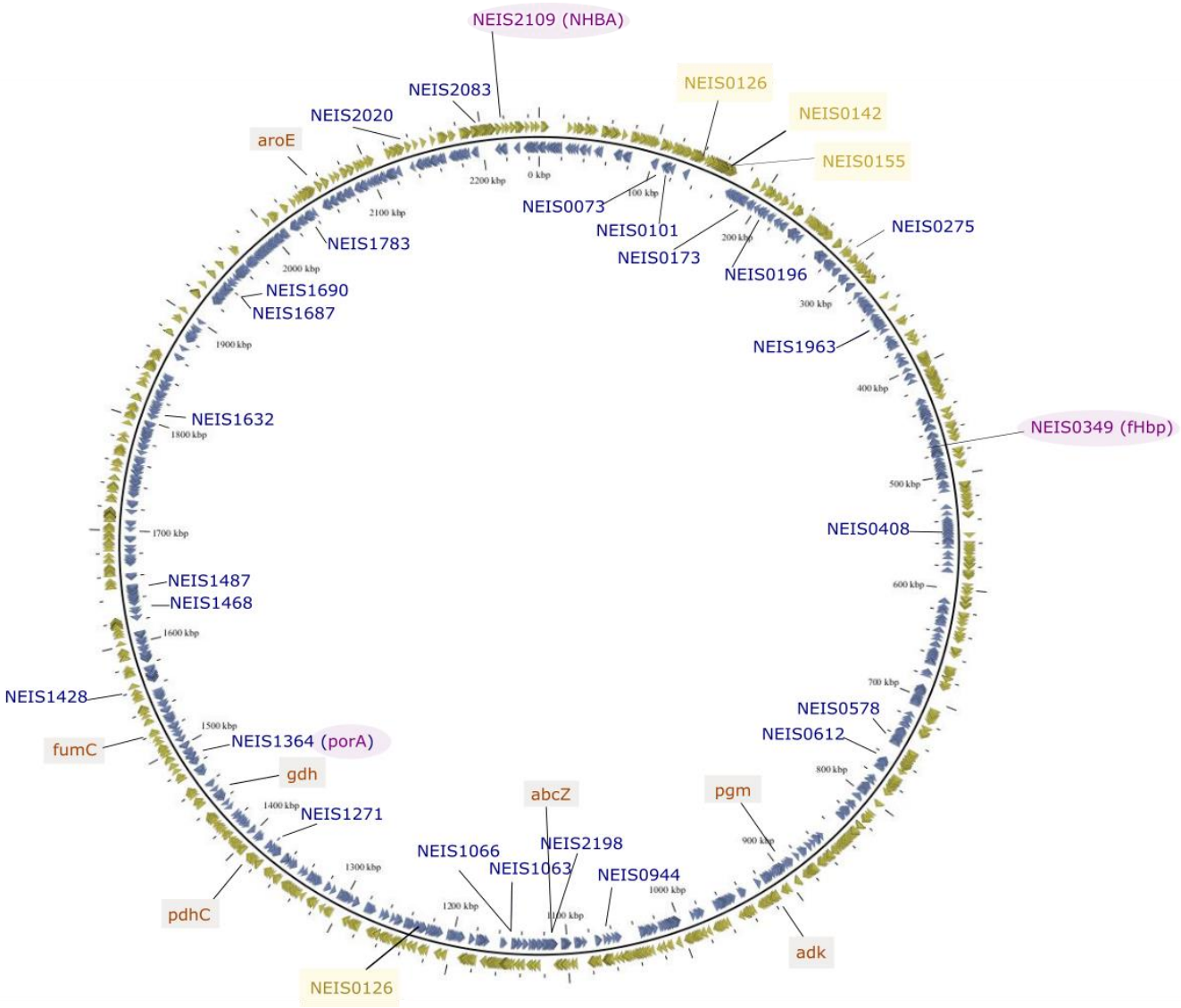


Figure 4-1 Representation of *Neisseria meningitidis* reference genome NZ05/33, drawn with Gview. NZ05/33 is an ST41/44 outbreak strain from New Zealand. The MeNZB™ vaccine was developed from NZ98/254, another ST41/44 isolate from the same New Zealand outbreak. Chromosomal location of the genes encoding the proteins included in the Outer Membrane Vesicle peptide Typing (OMVT) scheme are shown in blue. Genes encoding four cytoplasmic proteins are shown in yellow boxes. The multilocus sequence typing (MLST) loci are shown in brown boxes. The genes encoding the constituents of the Bexsero® Antigen Sequence Typing (BAST) scheme are shown in purple ellipses, note that *nadA* is absent in this strain.

Table 4-1 Details of the OMV proteins included in the Outer Membrane Vesicle peptide Typing (OMVT) scheme.
The corresponding NEISp number was assigned with respect to the NEIS nucleotide sequence on PubMLST.org, associated with the common reference NMB number, predicted cellular location and function. OM = outer membrane, EC = extracellular, IM = inner membrane, PP = periplasmic.

NEISp annotation	NEIS no. (Gene number)	Predicted cellular location	MC58 reference strain protein annotation	Isolates with peptide variant present n (%)	Isolates with null peptide variant (0) n (%)
0073	NEIS0073 (NMB0088)	EC, OM, PP	Outer membrane protein P1 (OmpP1)	3438 (98.06)	64 (1.83)
0101	NEIS0101 (NMB0109b)	OM	Peptidoglycan-binding LysM domain protein	3506 (100.00)	0 (0.00)
0173	NEIS0173 (NMB0182)	OM	Outer membrane protein (Omp85)	3501 (99.86)	0 (0.00)
0196	NEIS0196 (NMB0204)	IM, PP	Lipoprotein	3504 (99.94)	0 (0.00)
0275	NEIS 0275 (NMB0280)	OM	Organic solvent tolerance protein	3442 (98.17)	4 (0.11)
0408	NEIS0408 (NMB1812)	OM	Pilin (PilQ)	3429 (97.80)	36 (1.03)
0578	NEIS0578 (NMB0634)	PP	ABC transporter substrate-binding protein (FbpA)	3501 (99.86)	0 (0.00)
0612	NEIS0612 (NMB0663)	OM	Outer membrane protein (NspA)	3498 (99.77)	0 (0.00)
0944	NEIS0944 (NMB0964b)	EC, OM	TonB-dependent receptor (TBDR)	3203 (91.36)	64 (1.83)
1063	NEIS1063 (NMB1124/NMB1162)	EC, OM	Putative periplasmic protein	3451 (98.43)	6 (0.17)
1066	NEIS1066 (NMB1126/1164)	EC, OM, PP	CsgG family protein	3459 (98.66)	40 (1.14)
1271	NEIS1271 (NMB1333)	EC, IM, OM	M23 peptidase domain protein	3471 (99.00)	3 (0.09)
1364	NEIS1364 (NMB1429)	OM	Outer membrane protein PorA (PorA)	3400 (96.98)	52 (1.48)
1428	NEIS1428 (NMB1497)	OM	TonB-dependent receptor (TBDR)	3436 (98.00)	6 (0.17)

NEISp annotation	NEIS no. (Gene number)	Predicted cellular location	MC58 reference strain protein annotation	Isolates with peptide variant present n (%)	Isolates with null peptide variant (0) n (%)
1468	NEIS1468 (NMB1540)	OM	Lactoferrin-binding protein A (LbpA)	3240 (92.41)	43 (1.23)
1487	NEIS1487 (NMB1567)	OM	Macrophage infectivity potentiator	3504 (99.94)	0 (0.00)
1632	NEIS1632 (NMB1714)	OM	Multidrug efflux pump channel protein (MtrE)	3501 (99.86)	4 (0.11)
1687	NEIS1687 (NMB0464)	OM	Phospholipase A1 (PldA)	2732 (77.92)	729 (20.79)
1690	NEIS1690 (NMB0461)	OM	Transferrin-binding protein 1 (TbpA)	3268 (93.21)	2 (0.06)
1783	NEIS1783 (NMB0382)	OM	Outer membrane protein class 4 (RmpM)	3505 (99.97)	0 (0.00)
1963	NEIS1963 (NMB1988)	OM	Enterobactin receptor FetA (FetA)	3384 (96.52)	52 (1.48)
2020	NEIS2020 (NMB2039)	OM	Major outer membrane protein PIB (PorB)	3468 (98.92)	1 (0.03)
2083	NEIS2083 (NMB0375)	EC, OM	Putative lipoprotein MafA family (MafA)	3046 (86.88)	194 (5.53)
2198	NEIS2198 (NMB1053)	OM	Class 5 outer membrane protein (OpcA)	2217 (63.23)	1282 (36.57)

4.4.3. **Outer Membrane Vesicle peptide Types (OMVTs)**

For OMV profiles with all 24 loci present, each unique combination was assigned an integer, the OMVT. For example, OMVT-1 corresponded to the OMV peptide variants present in the NZ05/33 genome. All subsequent OMVTs were numbered in order of identification. Although OMVTs provided a rapid means of identifying isolates with identical profiles, there were related isolates that differed at ≤23 loci. To analyse these relationships between OMVTs, a clustering method was developed using eBURSTv3, which determined non-overlapping groups of closely-related strains, i.e. no OMVT was allocated to more than one OMVT cluster, and entry into a cluster required matching at ≥18 loci to the central OMVT (Feil *et al.* 2004). To ensure stability of OMVT clusters, a central OMVT was defined as the OMVT that differed from the largest number of other OMVTs at only a single peptide sequence. This was also used to name the cluster, for example OMVT-449 was the central OMVT of the OMVT-449 cluster. Bootstrapping for each group was assessed using the 23-peptide cut-off.

The OMVT scheme was used to catalogue the diversity of IMD isolates from the Meningitis Research Foundation Meningococcus Genome Library (MRF-MGL) and related isolate collections. This included 3506 WGS of all culture-confirmed meningococcal isolates in epidemiological years 2010/11 to 2016/17 from England, Wales, Scotland, and Northern Ireland, representing approximately 55% of all laboratory-confirmed IMD in these regions (Hill *et al.* 2015, Rodrigues *et al.* 2018). All isolates in the collection were automatically annotated for the OMV protein variants, with manual curation of new variants. OMVT and OMVT clusters were assigned accordingly.

4.4.4. **OMVT cluster analysis**

The GrapeTree software was used to cluster allelic profiles from large collections of WGS and visualise these relationships (Zhou *et al.* 2018). It provides an interactive, web-

based interface associated with relevant metadata including: year, serogroup, cc, and BAST. GrapeTree was run through the PubMLST.org plug-in.

4.4.5. Statistical analysis

All statistical analyses were performed using R version 3.2.4. Cramer's V coefficient was used to assess the association of peptide loci with cc and was calculated using the 'Cramer's V' function in the 'lsr' package in R. The diversity of each OMV protein was assessed using Shannon's index of diversity (H) and the "adjusted diversity" calculated from the natural log of the number of peptide variants/amino acid/isolate. H was calculated using the 'Vegan' package in R, H represents the uncertainty in predicting the peptide variant of a new isolate, given the number of peptide variants and the evenness in abundance of isolates possessing each variant. H increases as richness (number of variants) and evenness (distribution) in the population increases.

4.5. Results

4.5.1. Peptide variants in OMVT scheme

Peptide variants (including null, 0) were annotated in ≥98% of the WGS data of the 3506 UK isolates. As a consequence of incomplete gene sequence assembly, annotation was lower for: TonB-dependent receptor (NEISp0944), 3267/3506 isolates (93.2%); LbpA (NEISp1468), 3283/3506 (93.6%); TbpA (NEISp1690), 3270/3506 (93.3%); and MafA (NEISp2083), 3240/3506 (92.4%), (Table 4-1). H was used to assess the richness and evenness of variants, with TbpA the most diverse antigen (H=3.94) and putative periplasmic protein NEISp1063 the least diverse (H=1.04) (Figure 4-2, Table 4-2). The OMPs were more diverse than cytoplasmic proteins ($p<0.00001$). The adjusted diversity for each locus ranged from -10.3 for FbpA (NEISp0578) to -8.1 for TbpA (NEISp1690) and PorB (NEISp2020) (Figure 4-2, Table 4-2).

The distribution of OMVT protein variants within the UK IMD isolates was non-random. A cumulative frequency distribution of the peptide variants for each locus, for the 3506 IMD isolates, demonstrated the loci for which four variants are found in >90% of isolates including: FbpA (NEISp0578); putative periplasmic protein (NEISp1063); macrophage infectivity potentiator (NEISp1487); and PldA (NEISp1687) (Figure 4-3). Other loci, including TbpA, LbpA, FetA, PorA, and PorB, showed more diversity and variants were distributed more evenly across the 3506 isolates. The distribution of the variation within each protein was also non-random, i.e. the variation was not distributed evenly across the amino acid sequence (Figure 4-4, Figure 4-5). In many OMV proteins there were regions of high diversity concentrated in the so-called “variable regions”, representing surface-exposed loops under immune selection, previously well-characterised for TbpA, PorB, and PorA (van der Ley *et al.* 1991, Pajon *et al.* 1997, Russell *et al.* 2004).

Table 4-2 Outer Membrane Vesicle peptide Type (OMVT) scheme proteins association with clonal complex (Cramer's V) and diversity indices are given as Shannon's index of diversity and adjusted diversity.

OMV proteins in the OMVT scheme	Cramer's V	Shannon's index of diversity	Adjusted diversity
TonB-dependent receptor (NEISp0944)	0.92	2.84	-9.62
TonB-dependent receptor (NEISp1428)	0.92	2.62	-9.55
Tbp1 (NEISp1690)	0.90	3.94	-8.12
FetA (NEISp1963)	0.89	3.01	-8.80
LbpA (NEISp1468)	0.87	3.40	-8.72
PorA (NEISp1364)	0.83	3.19	-8.37
Organic solvent tolerance protein (NEISp0275)	0.80	3.11	-9.43
PorB (NEISp2020)	0.77	2.86	-8.12
Outer membrane protein P1 (NEISp0073)	0.74	2.33	-9.74
MtrE (NEISp1632)	0.73	2.46	-9.51
OMP85 (NEISp0173)	0.69	2.71	-9.89
PilQ (NEISp0408)	0.67	3.11	-9.43
MafA (NEISp2083)	0.63	2.16	-9.41
M23 peptidase domain protein (NEISp1271)	0.60	2.22	-10.08
OpcA (NEISp2198)	0.58	1.77	-9.81
NspA (NEISp0612)	0.57	2.17	-9.21
Lipoprotein (NEISp0196)	0.52	2.16	-9.02
FbpA (NEISp0578)	0.44	1.23	-10.28
Macrophage infectivity potentiator (NEISp1487)	0.41	1.65	-10.03
PldA (NEISp1687)	0.41	1.95	-9.59
RmpM (NEISp1783)	0.41	1.87	-9.57
Peptidoglycan-binding LysM domain protein (NEISp0101)	0.41	1.92	-10.16
CsgG family protein (NEISp1066)	0.41	1.62	-9.46
Putative periplasmic protein (NEISp1063)	0.34	1.04	-9.85

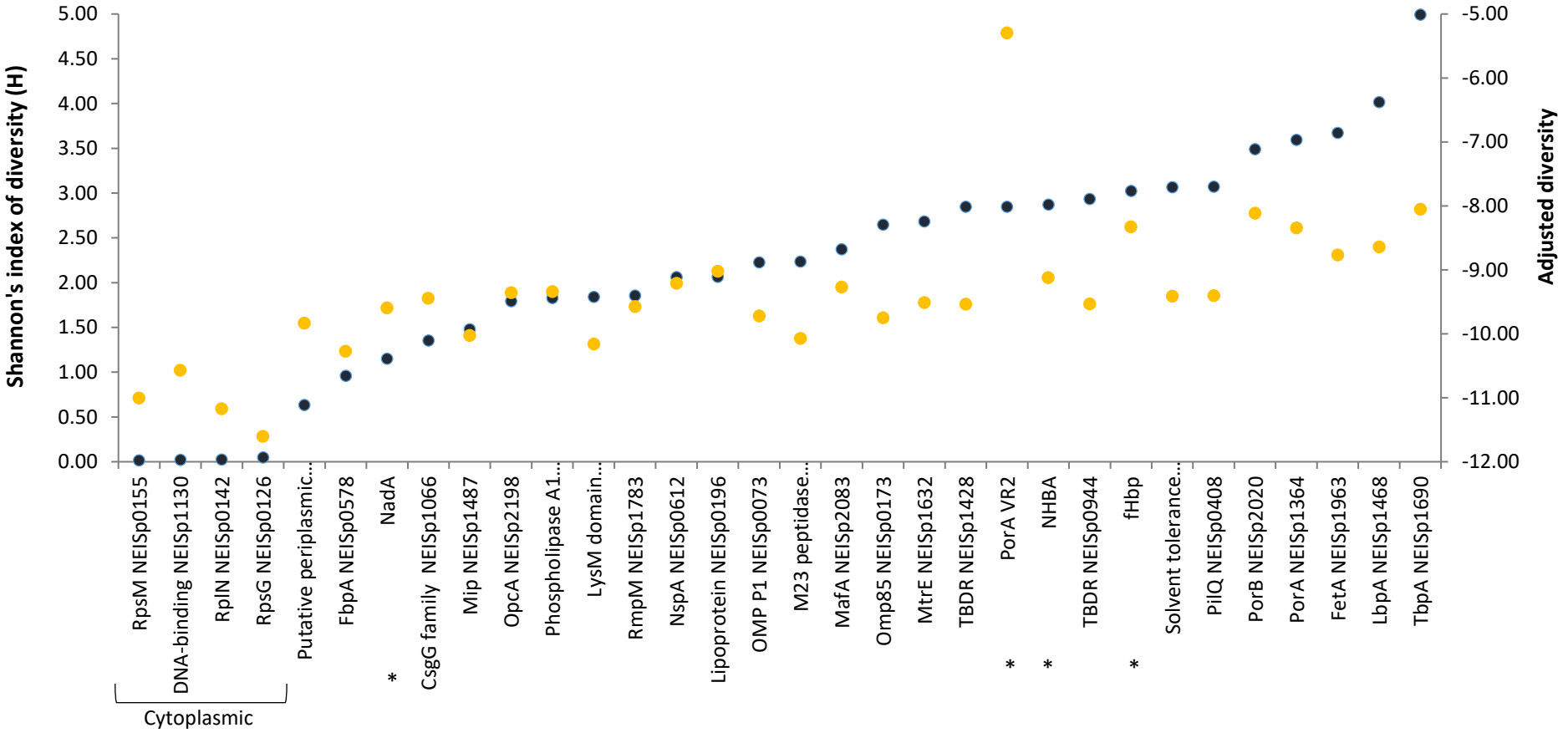


Figure 4-2 Diversity of each protein in the Outer Membrane Vesicle peptide Typing (OMVT) scheme.
Diversity is measured by Shannon's index of diversity (H) for each peptide locus (indicated by the dark blue dots and left axis) and adjusted diversity (AD), the natural log transformation of the number of variants per amino acid per isolate (indicated by yellow dots and right axis). All data were deduced from a dataset of 3506 UK invasive meningococcal disease isolates. Four cytoplasmic proteins are shown on the left of the x axis for reference, three of which are ribosomal proteins RpsM, RplN, RpsG known to be highly conserved in bacteria. The recombinant proteins in Bexsero® vaccine are marked with an *, factor-H binding proteins (fHbp), Neisserial Heparin binding antigen (NHBA), *Neisseria* adhesin A (NadA) and Porin A variable region 2 (PorA VR2) and are not included in the OMVT scheme.

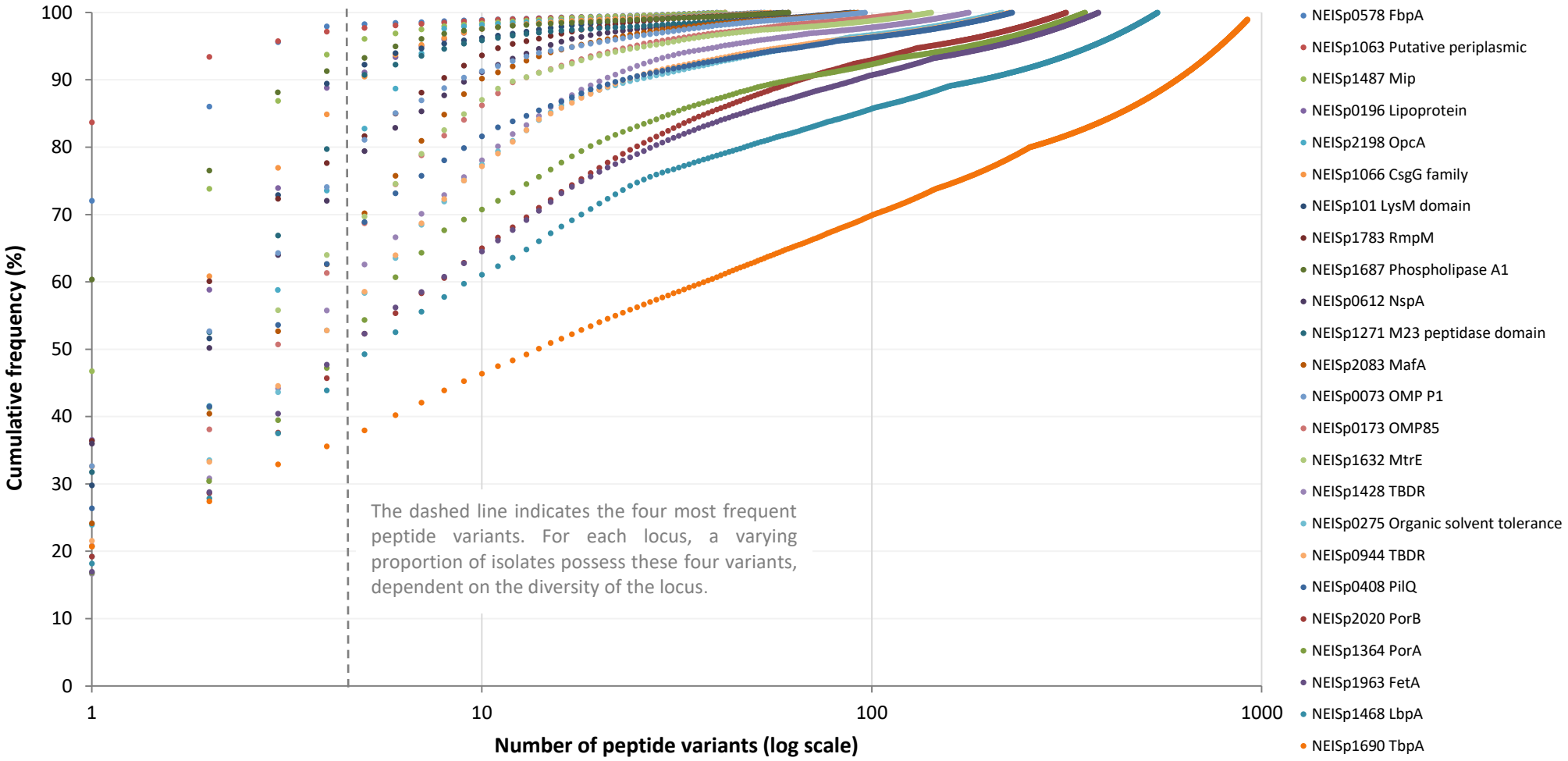


Figure 4-3 Distribution of Outer Membrane Vesicle peptide Type (OMVT) peptide variants in UK meningococcal disease isolates.
Cumulative frequency distribution shown for each locus in the OMVT scheme. For each peptide (represented by a different coloured dots), it shows the proportion of the 3506 isolates that were annotated with each successive peptide variant. The grey dashed line indicates the proportion of isolates within the dataset which possess the four most frequent variants. The distribution is highly skewed, as for FbpA (NEISp0578), Putative periplasmic protein (NEISp1063), Macrophage infectivity potentiator (NEISp1487) and PldA (NEISp1687) four variants annotated >90% of isolates, contrasting with TbpA for which only 35.6% of isolates possessed the four most frequent peptide variants.

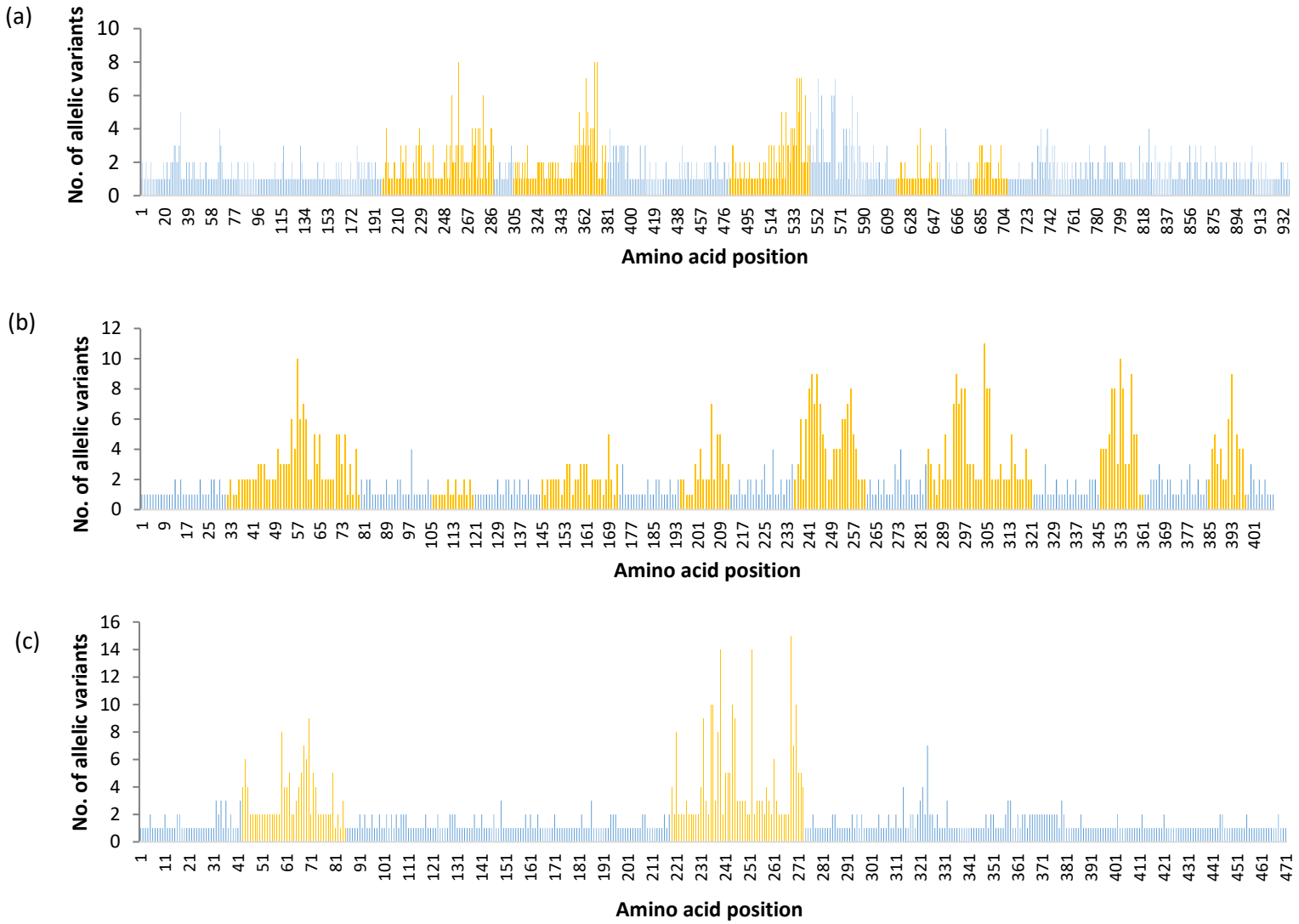


Figure 4-4 Variation in the diversity across the amino acid sequence for the three most diverse outer membrane proteins. The number of allelic variants for each position of the protein are shown for the three most diverse proteins a)TbpA (NEISp1690), b)PorB (NEISp2020), c)PorA (NEISp1364). Variable regions are shown in yellow for TbpA, PorB and PorA, as previously described (van der Ley *et al.* 1991, Pajon *et al.* 1997, Russell *et al.* 2004).

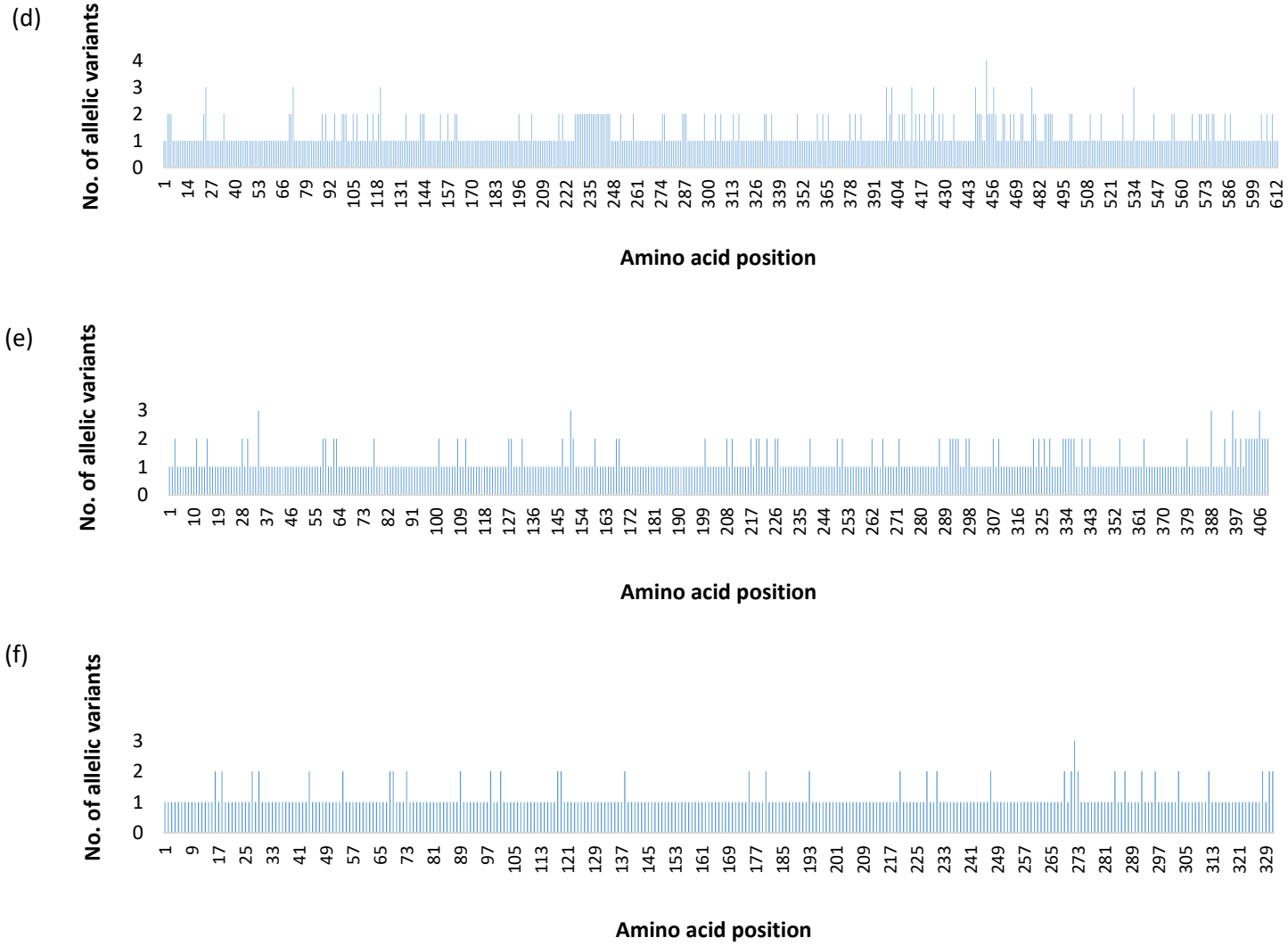


Figure 4-5 Variation in the diversity across the amino acid sequence for the three most conserved outer membrane proteins.
The number of allelic variants for each position of the protein are shown for the three most conserved proteins d) M23 peptidase domain protein (NEISp1271), e) Putative peptidoglycan-binding periplasmic protein, LysM domain-containing protein (NEISp0101), f) FbpA (NEISp0578).

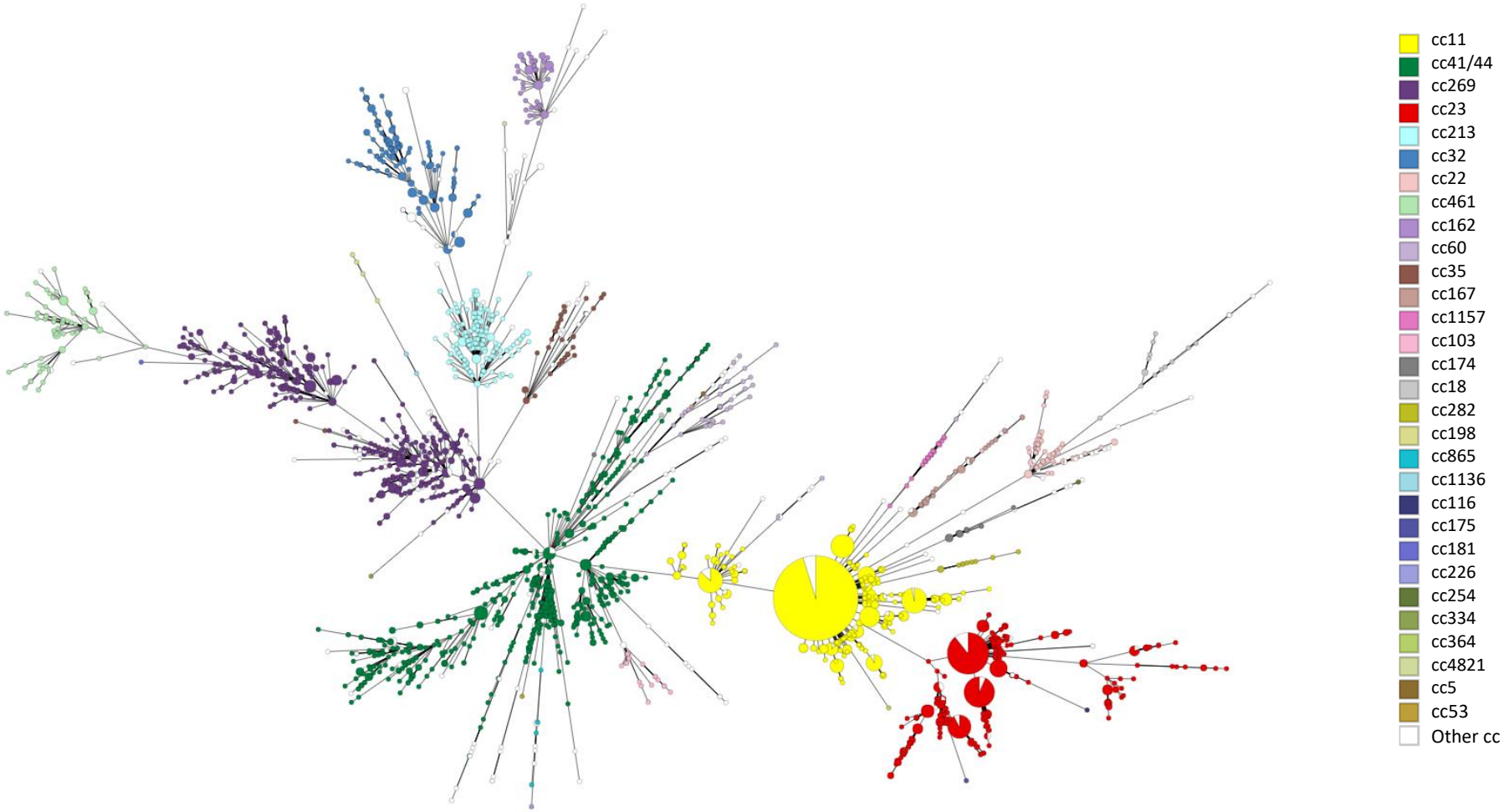


Figure 4-6 UK meningococcal disease isolates clustered by Outer Membrane Vesicle peptide Type (OMVT).
Invasive meningococcal disease isolates from epidemiological years 2010/11 to 2016/17 (n=3506) analysed using the 24 loci OMVT scheme and visualised using GrapeTree software. The OMVT cluster by clonal complex (cc), which are shown by different colours, with unfilled nodes representing isolates from the other ccs. The size of the circles is proportional to the number of isolates.

4.5.2. Distribution of OMVTs in IMD and association with clonal complex

The OMVT scheme was applied to the WGS data from the 3506 UK meningococcal isolates. A total of 1752 OMVTs were assigned to 2492/3506 (71.1%) isolates. The remaining 1014 (28.9%) isolates had one or more incomplete OMV protein-encoding locus and it was not possible to assign an OMVT. The most frequently occurring type was OMVT-1149 in 246/2492 (9.9%) isolates, which was associated with cc11 genogroup W isolates (W:cc11). The most frequent OMVTs represented by 10 or more isolates were: OMVT-21, 64/2492 (2.6%); OMVT-50, 32/2492 (1.3%); OMVT-1275, 18/2492 (0.7%); OMVT-1161, 16/2492 (0.6%,); OMVT-1199 16/2492 (0.6%); and OMVT-1158, 12/2492 (0.5%). There were: 1547 OMVTs that were represented by only one isolate (1547/2492 (62.1%); 133 represented by 2 isolates (266/2492, 10.7%); and 65 represented by 3-10 isolates (275/2492, 11.0%). Analysis of all isolates for which an OMVT was available (71.1%, 2492/3506), demonstrated that OMVT was strongly associated with cc: Cramer's V=1, with values closer to 1 representing strong association (Figure 4-6) (Zhou *et al.* 2018). To determine the extent of association of individual proteins with cc, Cramer's V was calculated for each protein. The values ranged from 0.92 for two TonB-dependent receptors (NEISp0944 and NEISp1428) to 0.34 for the putative periplasmic protein (NEISp1063) (Table 4-2), suggesting that OMV proteins with lower Cramer's V were found across ccs.

4.5.3. OMVT clusters and association with BAST

Given the large number of possible OMVTs, they were grouped into OMVT clusters, which differed by up to six peptides from a defined central OMVT, i.e. isolates in OMVT-1149 cluster were identical to OMV-1149 for ≥18 peptides. There were 1587/3506 (45.3%) isolates assigned to 27 OMVT clusters. Each OMVT cluster was predominantly associated with a cc and a genogroup (Table 4-3). The recombinant Bexsero® antigens

had a strong association with the OMVTs, as measured by Cramer's V (fHbp=0.98; NHBA=0.99; NadA=0.96; PorA VR1=1; PorA VR2=1) and OMVT clusters were also associated with BAST (Cramer's V = 0.96) (Table 4-3) (Brehony *et al.* 2016). These associations were visualised with the GrapeTree clustering algorithm for OMVT clusters and BAST for cc11, cc269 and cc23 (Figure 4-7, Figure 4-8, Figure 4-9) (Zhou *et al.* 2018). Some large ccs exhibited a degree of subtracting, for example, cc11 exhibited three OMVT clusters (OMVT-1149 cluster, OMVT-218 cluster and OMVT-1158 cluster). Each OMVT cluster exhibited a non-overlapping repertoire of BASTs.

Table 4-3 Outer Membrane Vesicle peptide Type (OMVT) clusters and their association with clonal complex, genogroup and Bexsero® Antigen Sequence Type (BAST), comprised of the other recombinant proteins in the vaccine.

OMVT cluster	No. of isolates	Clonal complex	Genogroup	% with genogroup	BASTs
OMVT-104	10	32	Y	100	234, 539
OMVT-1142	14	23	B	100	4, 19
OMVT-1149	502	11	W	99.8	2, 3, 50, 59, 68, 116, 153, 179, 787, 820, 898, 913, 915, 917, 922, 928, 1402, 1491, 1762, 1780, 1880
OMVT-1152	26	32	B	100	5, 61, 62, 108, 110, 138, 155, 391, 535, 925, 1397, 1419, 1701, 1878
OMVT-1158	42	11	C	97.5	3, 8, 38
OMV T-1229	4	32	B	100	309
OMVT-1247	23	461	B	100	230, 296, 307, 407, 520, 525, 543, 571, 587, 1009, 1800
OMVT-1256	12	32	B	100	56, 88, 160, 195, 618, 918, 926, 1773, 2138
OMVT-1316	29	162	B	100	244, 246, 286, 326, 374, 502, 568, 807, 873
OMVT-1341	16	32	B	100	10, 178, 1764
OMVT-151	9	35	B	100	290, 445
OMVT-160	67	41/44	B	100	220, 223, 226, 229, 231, 236, 239, 280, 287, 312, 316, 347, 438, 448, 515
OMVT-186	10	269	B	100	238, 268, 313, 319, 591
OMVT-21	240	23	Y	99.1	221, 225, 228, 233, 357, 368, 387, 427, 444, 499, 579, 628, 964, 1473, 1792, 1824, 1954, 2498
OMVT-211	5	35	B	100	257, 293, 320
OMVT-218	53	11	C	84.3	126, 142, 235, 242, 521, 522, 546
OMVT-257	4	41/44	B	100	220
OMVT-27	91	213	B	100	224, 245, 258, 264,304, 310, 324, 328, 333, 335, 336, 346, 356, 369, 383, 395, 400, 403, 417, 471, 504, 509, 517, 518, 537, 552, 556, 608, 617, 623, 634, 2879, 2882

OMVT cluster	No. of isolates	Clonal complex	Genogroup	% with genogroup	BASTs
OMVT-368	91	269	B	100	222, 238, 249, 251, 252, 277, 358, 379, 455, 468, 480, 501, 550, 595, 602, 668, 973, 1413, 1505, 1766
OMVT-3782	196	269	B	100	219, 254, 267, 273, 291, 295, 431, 446, 452, 460, 473, 524, 536, 583, 598, 611, 616, 624, 633, 942, 946, 947, 953, 1273, 1424, 1816
OMVT-414	34	41/44	B	100	220, 223, 226, 240, 260, 278, 562, 589, 2874
OMVT-449	47	22	B	95.5	265, 300, 301, 330, 349, 386, 429, 440, 466, 1765, 1776
OMVT-627	17	269	B	62.5	222, 249, 360, 405, 423, 485, 491, 510, 604, 1414, 1821
OMVT-654	11	103	C	72.7	288, 311, 541, 544, 553, 566
OMVT-905	13	167	Y	100	261, 461, 577, 1406, 1805
OMVT-912	5	23	Y	100	228
OMVT-932	16	174	Y	80	11, 14, 72, 117, 127, 135, 146, 177

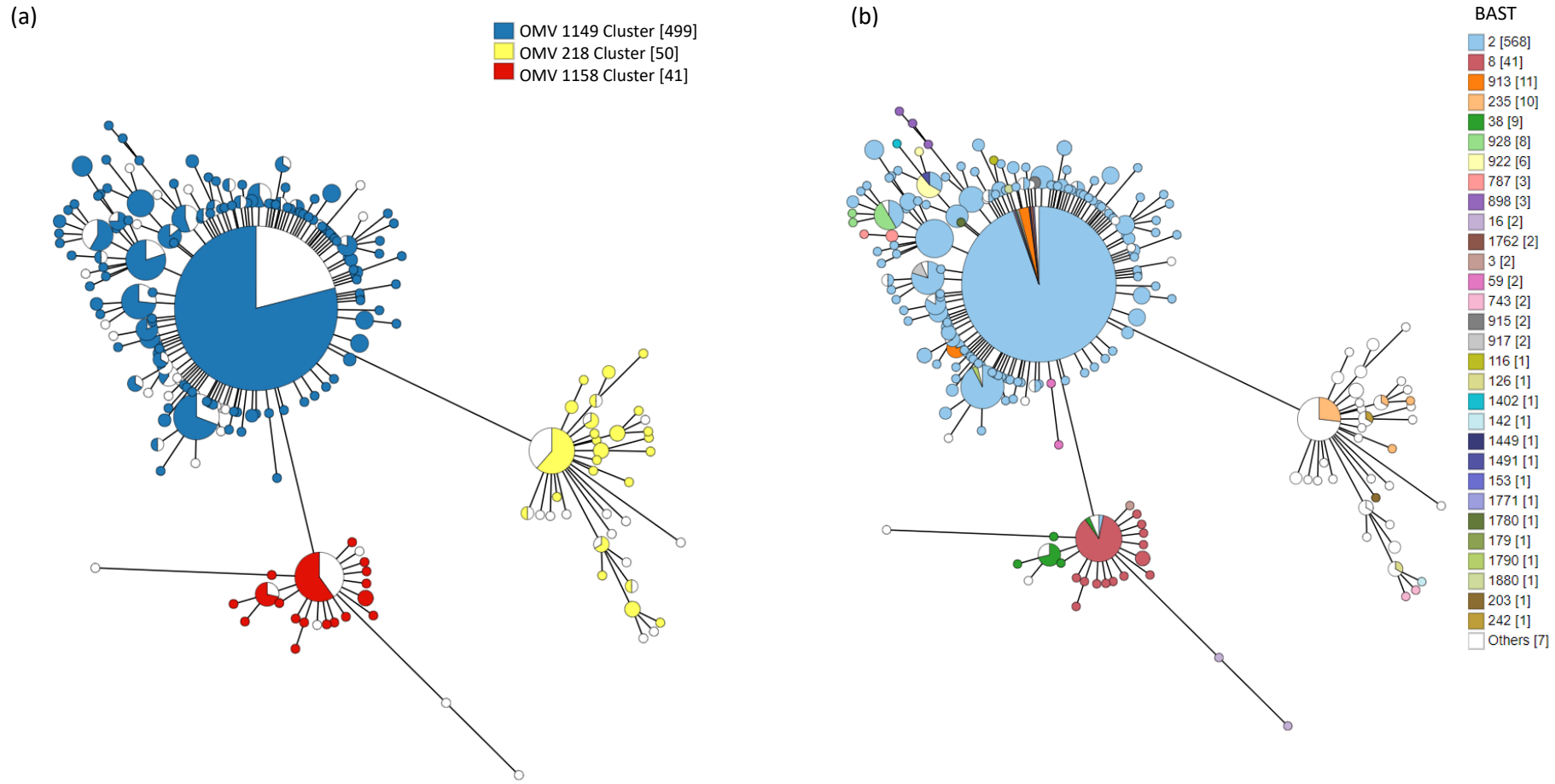


Figure 4-7 Outer Membrane Vesicle peptide Type (OMVT) clusters and association with Bexsero® Antigen Sequence Types (BASTs) for clonal complex 11 isolates. Clonal complex (cc) 11 isolates (n=777) were analysed using the OMVT scheme. (a) Clusters of OMVs that match 18 loci to the central OMVT are shown. Cc11 has three OMV clusters, 1149, 218 and 1158. OMVT 1149 cluster represents lineage 11.2, OMVT clusters 218 (yellow) and 1158 (red) represent lineage 11.1. (b) OMVT clusters are coloured by BASTs, demonstrating the strong non-overlapping association of BASTs with OMVT clusters. Unfilled nodes represent incomplete data, so a profile could not be assigned.

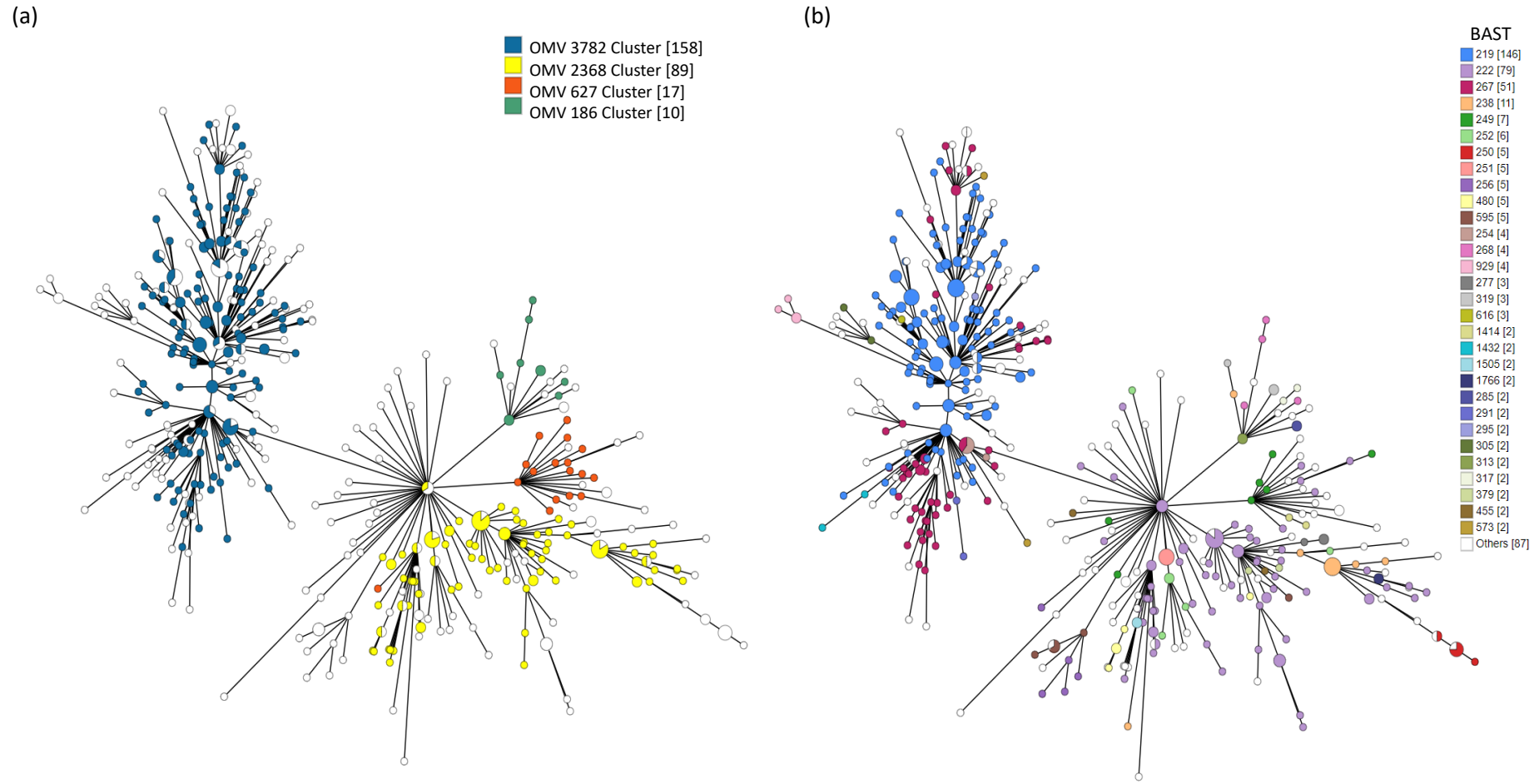


Figure 4-8 Outer Membrane Vesicle peptide Type (OMVT) clusters and association with Bexsero® Antigen Sequence Types (BASTs) for clonal complex 269 isolates. Clonal complex (cc) 269 isolates (n=500) were analysed using the OMVT scheme. (a) Clusters of OMVTs that match 18 loci to the central OMVT are shown. Cc269 has four OMVT clusters. (b) OMVT clusters are coloured by BAST, demonstrating the strong non-overlapping association of BASTs with OMVT clusters. Unfilled nodes represent incomplete data, so a profile could not be assigned.

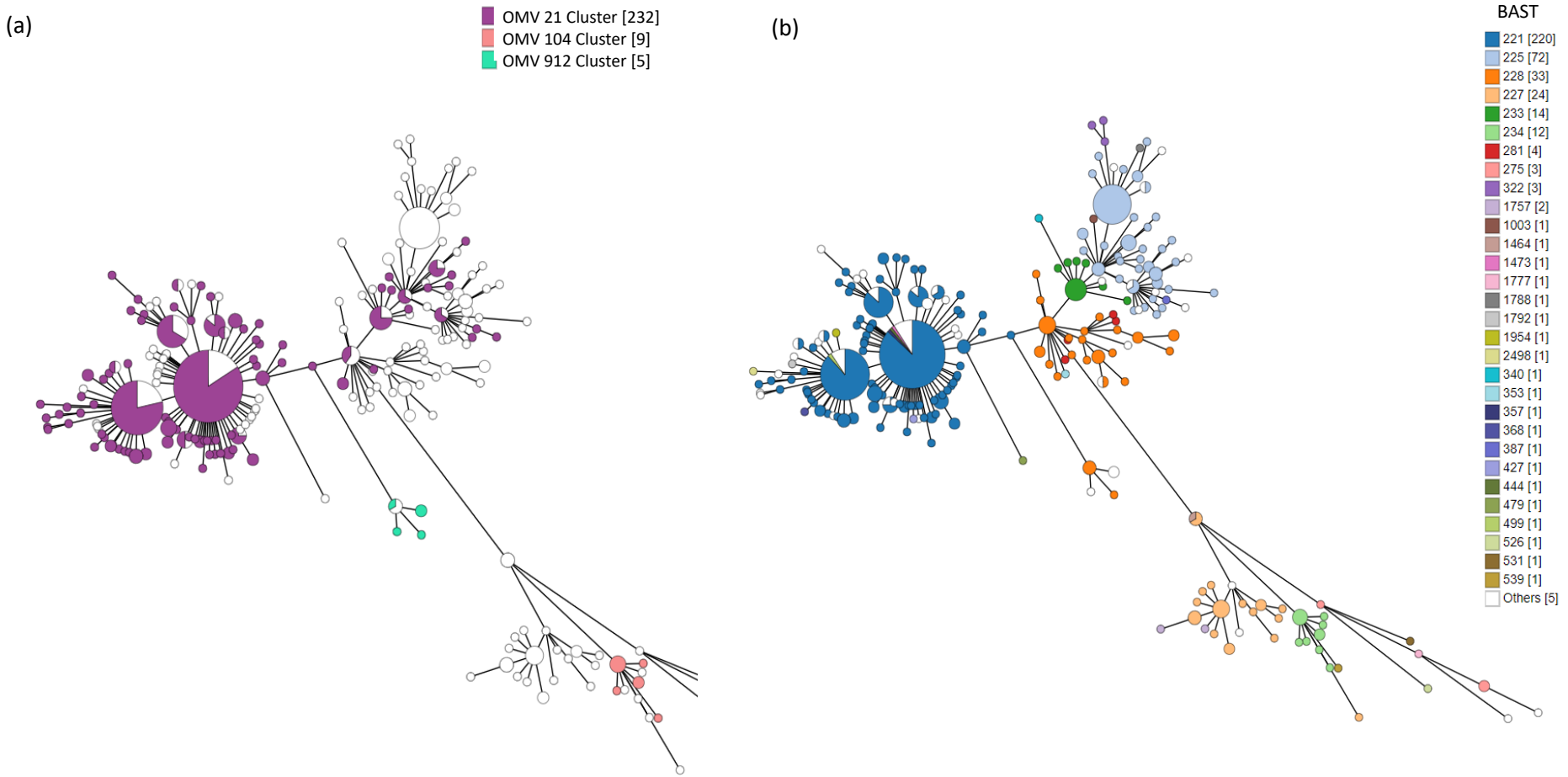


Figure 4-9 Outer Membrane Vesicle peptide Type (OMVT) clusters and association with Bexsero® Antigen Sequence Types (BASTs) for clonal complex 23 isolates. Clonal complex (cc) 23 isolates (n=459) were analysed using the OMVT scheme. (a) Clusters of OMVTs that match 18 loci to the central OMV are shown. Cc23 has three OMVT clusters. (b) OMVT clusters are coloured by BAST, demonstrating the strong non-overlapping association of BASTs with OMVT clusters. Unfilled nodes represent incomplete data, so a profile could not be assigned.

4.5.4. **Distribution of OMVT variants by OMV vaccine**

OMV vaccines can be described using OMVTs, for example MeNZB™ and Bexsero® vaccines are OMVT-1. Each OMV deduced protein from all 3506 isolates were compared to the variants present in OMVT-1, a perfect match giving a score of 24. Only 10/3506 (0.3%) of isolates did not share any peptide variants with MeNZB™, these belonged to cc23, cc213 and cc116, The only isolates with a defined cc that had 11 or more shared variants with MeNZB™ were from cc41/44 (Figure 4-10a). There were 34 isolates with undefined cc with ≥11 shared variants with MeNZB™, corresponding to STs that have not been assigned to a cc or isolates that had a partial MLST profile related to cc41/44. The cc41/44 isolates comprised 169 STs. Among these cc41/44 isolates, there was a bimodal distribution of OMVT variants shared with MeNZB™, suggesting two distinct populations: ST-41, ST-1194, ST-485, ST-340, ST-154 and ST-2314 isolates had ≥12 shared variants; whereas ST-1097, ST-409, ST-6508 and ST-571 isolates had ≤10 shared variants with MeNZB™. There were 2772/3506 (79%) isolates that shared 1-5 variants with MeNZB™, this was due to the presence of identical variants of: FbpA (NEISp0578); NspA (NEISp0612); putative periplasmic protein (NEISp1063); CsgG family protein (NEISp1066); and macrophage infectivity potentiator (NEISp1487). These were found in a high proportion of isolates, independent of cc: NEISp0578 2524/3506 (72.0%); NEISp0612, 1275/3506 (36.4%); NEISp1063 2932/3506 (83.6%); NEISp1066 2115/3506 (60.3%); and NEISp1487 1637/3506 (46.7%).

The same approach was used to analyse other OMV vaccines: MenBVac®; MENGOCOC-VA®; and the Chilean OMV vaccine, all of which were derived from cc32 isolates and optimised OMV formulations (Figure 4-10). By identifying variants that occurred at high frequency and across the most prevalent OMVT clusters, the composition of the 24 OMVT proteins was manipulated to improve the number of

antigenic matches amongst the set of UK disease isolates. Devising a formulation comprising the protein variants found in the most frequently occurring ccs, which differed at 14/24 loci from OMVT-1, exhibited the highest degree of shared variants to an OMV formulation (Figure 4-10b). The maximum number of shared variants was 14 (n=40) and all isolates shared ≥1 variant. The lower overall number of variant matches was because most isolates shared the loci that are independent of cc and additionally only a small number of cc-specific variants. Another way to broaden potential vaccine coverage, would be to create a vaccine containing multiple OMVTs, for example a formulation representing the five most prevalent hyperinvasive ccs (OMVT-160, -368, -1152, -27 and -21). With this formulation all isolates in the reference set shared ≥2 variants with the proposed vaccine, with 3310/3506 (94.4%) isolates having ≥8 variant matches, across all ccs, except cc11 (Figure 4-10c). All cc11 isolates had ≤8 shared variants with the multiple OMV vaccine; however, they are highly uniform and the development of a single-strain cc11 OMV vaccine, using for example OMVT-1149, would provide good coverage (Figure 4-10d), similar to that demonstrated by previously developed OMV vaccines in Cuba, Norway and New Zealand.

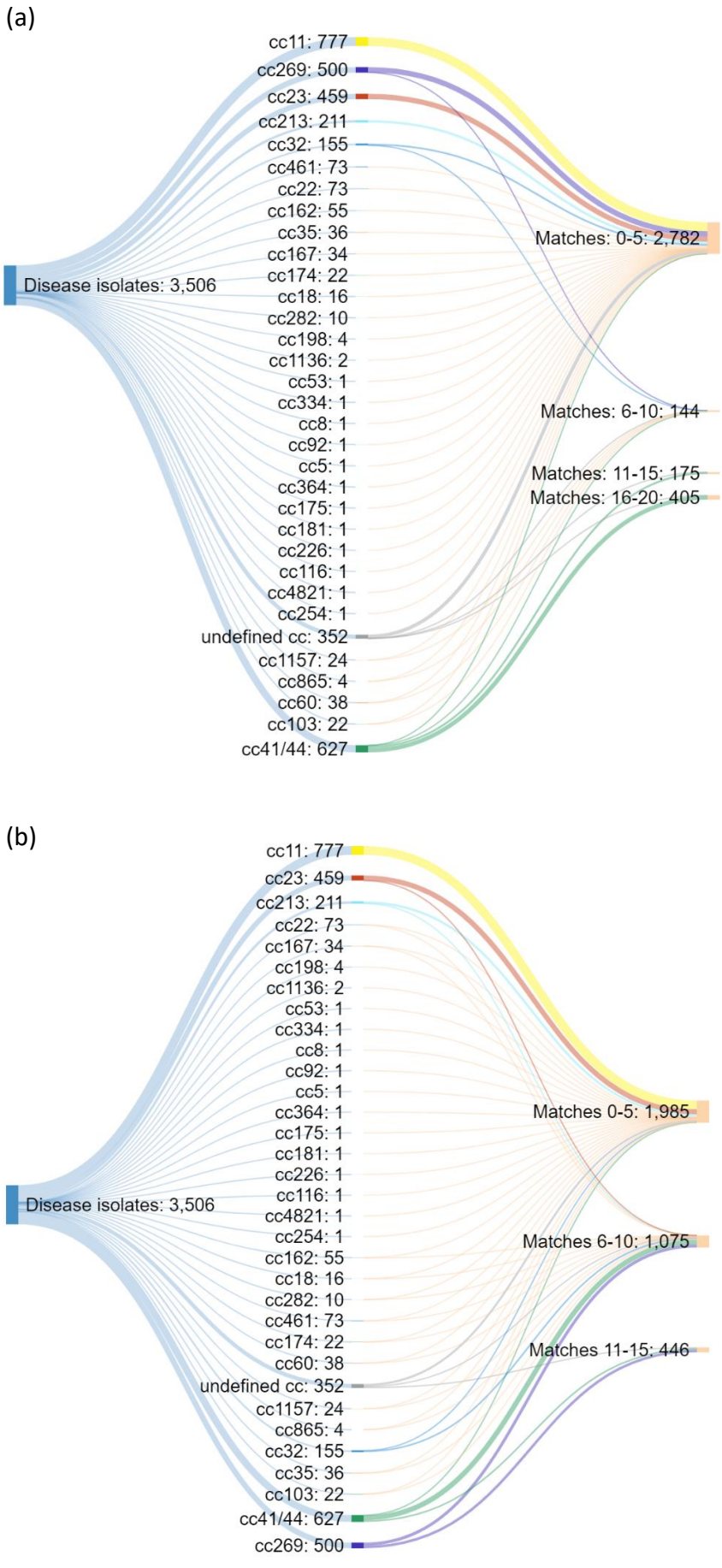
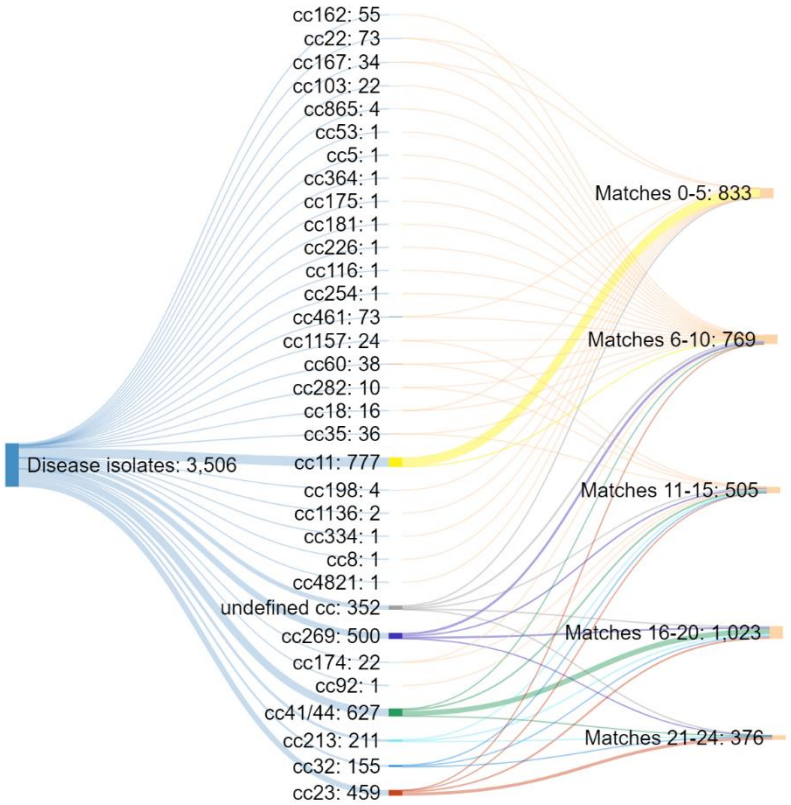
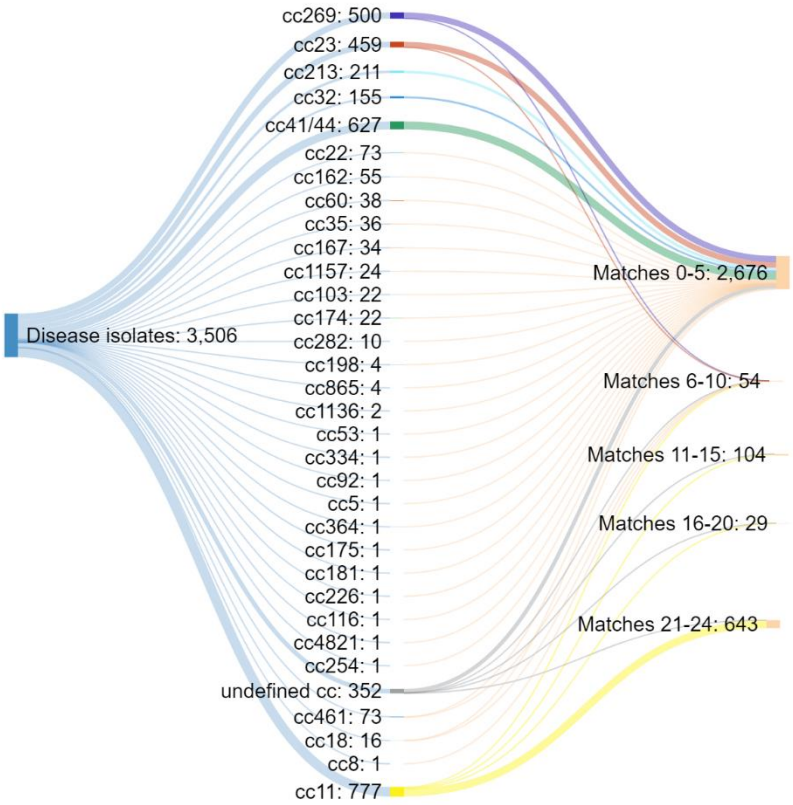


Figure 4-10 Distribution of UK meningococcal disease isolates that match the peptide variants in different vaccine formulations. Isolates were scored at each of the 24 loci that shared peptide sequence variants with real (a) and hypothetical vaccine formulations (b-d). (a) OMV vaccine MeNZB™ (cc41/44). (b) selected OMV protein variants that occur at high frequency in this dataset. (c) engineered OMV including multiple OMVTs (-160, -368, -1152, -27, -21). (d) OMVT-1149 based vaccine (cc11). If the locus matched the vaccine variant, it scored 1, with a maximum score of 24 if matching at all loci. The disease isolates (n=3506) were stratified by cc (n shown after each cc) and then the number of matches, 1-24, are displayed on the right (n shown after the number of matches). The thickness of the lines is proportional to the number of isolates. Cc=clonal complex, Undefined cc represents STs that have not been assigned to a cc or isolates that had a partial MLST profile.

(c)


Disease isolates: 3,506
cc162: 55
cc22: 73
cc167: 34
cc103: 22
cc865: 4
cc53: 1
cc5: 1
cc364: 1
cc175: 1
cc181: 1
cc226: 1
cc116: 1
cc254: 1
cc461: 73
cc1157: 24
cc60: 38
cc282: 10
cc18: 16
cc35: 36
cc11: 777
cc198: 4
cc1136: 2
cc334: 1
cc8: 1
cc4821: 1
undefined cc: 352
cc269: 500
cc174: 22
cc92: 1
cc41/44: 627
cc213: 211
cc32: 155
cc23: 459
Matches 0-5: 833
Matches 6-10: 769
Matches 11-15: 505
Matches 16-20: 1,023
Matches 21-24: 376

(d)


Disease isolates: 3,506
cc269: 500
cc23: 459
cc213: 211
cc32: 155
cc41/44: 627
cc22: 73
cc162: 55
cc60: 38
cc35: 36
cc167: 34
cc1157: 24
cc103: 22
cc174: 22
cc282: 10
cc198: 4
cc865: 4
cc1136: 2
cc53: 1
cc334: 1
cc92: 1
cc5: 1
cc364: 1
cc175: 1
cc181: 1
cc226: 1
cc116: 1
cc4821: 1
cc254: 1
undefined cc: 352
cc461: 73
cc18: 16
cc8: 1
cc11: 777
Matches 0-5: 2,676
Matches 6-10: 54
Matches 11-15: 104
Matches 16-20: 29
Matches 21-24: 643

4.6. Discussion

The diversity of the meningococcus and the complexity of its infection biology, in combination with fears of the possible harmful effects of vaccines containing the serogroup B capsular antigen, have hindered the development of a universal meningococcal childhood vaccine that comprises one or a few components (Finne *et al.* 1983, Caugant and Maiden 2009, Feavers and Maiden 2017). The meningococcus is an 'accidental' pathogen, as disease plays no part in onward transmission and, indeed, is inimical to it (Caugant and Maiden 2009). While disease rates are relatively low, asymptomatic carriage rates can be very high, especially in young adults in high-income settings and interruption of transmission is an important element of the efficacy of conjugate polysaccharide vaccines (Trotter and Maiden 2009, Christensen *et al.* 2010). OMV vaccines have been used a number of times, with evidence for efficacy against disease caused by the outbreak strain in Cuba (Sierra *et al.* 1991), Chile (Zollinger *et al.* 1991), Norway (Bjune *et al.* 1991), New Zealand (Galloway *et al.* 2009) and France (Caron *et al.* 2011). Evidence for the efficacy of OMV vaccines against carriage is less compelling. Although Bexsero® was designed as a four-component, the inclusion of the MeNZB™ OMV complicates assessment of its impact vaccine (Oster *et al.* 2005, Serruto *et al.* 2012). At the time of writing the vaccine was undergoing post-implementation investigation in the UK and evaluation of its impact on carriage in Australia (Ladhani *et al.* 2016, Parikh *et al.* 2016, Parikh *et al.* 2017, Marshall *et al.* 2018). We devised and evaluated the OMVT scheme to assist in the assessment of the diversity of OMV vaccine components.

The majority of the components of the OMVT scheme were OMPs, known to be present in the meningococcal outer membrane, and therefore potentially exposed to diversifying selection imposed by immune responses, although the most diverse components Pil and

Opa were too variable to be included in the scheme (Tani *et al.* 2014, Wachter and Hill 2016). Consistent with this view, the cytoplasmic proteins present in the OMV formulation, three of which were ribosomal subunit proteins, were the least diverse; however, among the OMPs, there was a wide range of diversity. The least diverse OMPs, a putative periplasmic protein (NEISp1063) and FbpA, (NEISp0578), may lack surface-exposed regions and might also be influenced by functional constraints. In the most diverse OMPs, TbpA (NEISp1690), PorA (NEISp1364) and PorB (NEISp2020), peptide sequence variability was localised in particular 'variable regions' (VRs), shown in a number of studies to represent surface-exposed parts of the sequence subject to immune attack by bactericidal antibodies (van der Ley *et al.* 1991, Pajon *et al.* 1997, Russell *et al.* 2004).

As reported previously for a number of meningococcal protein antigens, the variable components of the OMVT were non-randomly distributed among isolates, with the most diverse antigens exhibiting a non-overlapping structure (Gupta *et al.* 1996, Buckee *et al.* 2008). The persistence of particular non-random, non-overlapping combinations of PorA and FetA antigens, a few of which attain high frequency in the population, has been used as evidence to support the existence of stable stain types in the meningococcal population driven by immune and metabolic selection (Gupta *et al.* 1996, Buckee *et al.* 2008, Watkins and Maiden 2012). This structuring was especially exhibited by the more diverse proteins in the OMVT, with the exception of TbpA (NEISp1690) and Lbp (NEISp1468). Thus, although there were a very large number of variants of individual proteins and combinations of these variants, they grouped into a relatively small number of clusters, similar to those observed with FetA, PorA and the BAST scheme (Watkins and Maiden 2012, Brehony *et al.* 2016). In addition, as with PorA:FetA types and BASTs, OMVT clusters were strongly associated with ccs and capsular group (Yazdankhah *et al.* 2004,

Rodrigues *et al.* 2018). Ccs correspond to lineages within the meningococcal population, several of which have an increased propensity to cause invasive disease. These are referred to as the hyperinvasive lineages and they dominate collections of isolates from IMD, although they are rarer in isolates obtained from asymptomatic carriage (Yazdankhah *et al.* 2004, Caugant and Maiden 2009). A number of mechanisms have been proposed to explain the existence of persistence of these population structures in the highly recombinogenic meningococcus, but whatever the mechanism that generates them, these associations can be exploited in vaccine formulation, potentially simplifying it (Urwin *et al.* 2004, Watkins *et al.* 2016).

Indexing of the variation in the OMVT in the context of representative isolate collections, such as the MRF-MGL, along with the analysis tools embedded within the PubMLST.org website, enabled the *in silico* assessment of the likely performance of different OMV formulations (Hill *et al.* 2015). The OMVT from a given isolate could be rapidly determined and the likely content of an OMV made from the isolate deduced. The number of exact protein matches present in a given meningococcal population to any isolate could be readily established, indicating the likely degree of coverage that a given vaccine might be able to attain. Analysis of the UK reference dataset showed that the MeNZB™ OMV exhibited a similar number of exact matches to a putative cc11 OMV, with higher levels of exact matches attainable by alternative vaccine formulations or the inclusion of multiple OMVs in a single vaccine formulation. In the absence of evidence for very broad cross-protection, these comparisons suggest that multi-component or ‘designer’ OMVs that contained artificial repertoires of OMPs could have substantial advantages in terms of vaccine coverage.

Using a similar approach as the previously described MLST and BAST schemes, the OMVT scheme represents a portable and scalable means of cataloguing variation in the

OMV components of meningococcal vaccines (Maiden *et al.* 1998, Brehony *et al.* 2016, Feavers and Maiden 2017). This provides a stable basis for the comparison of the extensive variation of these molecules. OMVT has the potential to be enhanced with: (i) bioinformatic approaches that predict B- and T-cell epitopes (Christodoulides and Heckels 2017); and (ii) phenotypic expression and immunological data, to provide a more sophisticated prediction of likely cross-protection provided by these vaccines (Ladhani *et al.* 2012); however, while a peptide sequence catalogue can be comprehensive, this is unlikely to be the case for phenotypes and some deduction from sequence data is likely to remain essential for the foreseeable future. Correlation of the OMVT with immune responses in infants and those that affect carriage in older individuals is of particular importance in assessing the cost-effectiveness of novel meningococcal vaccines (Ladhani *et al.* 2016). The embedding of the OMVT scheme within the PubMLST.org/neisseria database enables the scheme to be implemented genus wide, permitting inter-species as well as intra-species diversity to be explored. (Jolley and Maiden 2013) This is of particular importance given the renewed interest in the development of novel vaccines against *Neisseria gonorrhoeae*, the gonococcus, and the possible impact of Bexsero® on gonococcal infection (Petousis-Harris *et al.* 2017). Intra-species diversity is also important when considering the possible impact of such vaccines on the oropharyngeal microbiota. Finally, this approach exemplifies how WGS data can be used to support the development and evaluation of complex multicomponent vaccines against highly variable pathogens.

4.7. Conclusion

The OMVT scheme facilitates systematic analysis of the diversity of abundant OMPs in all *Neisseria* species. It can be used to assess the impact of the multicomponent vaccines on disease-causing meningococci, carried meningococci (often acapsulate) and non-

pathogenic neisserial strains that inhabit the oropharynx and have a critical role in the host microbiota. Using this novel scheme, it has been demonstrated that discrete non-overlapping populations of meningococci exist, with combinations of OMPs including the Bexsero® antigens fHbp, NHBA and NadA strongly associated with genogroup and cc. This information, as well as individual protein diversity and distribution could inform future vaccine development.

Chapter 5

Longitudinal study of vaccine antigens in carried meningococci in Oxfordshire

5.1. Abstract

Meningococci exclusively colonise the human oropharynx. The biodiversity and complex interactions between microorganisms comprising the oropharyngeal microbiota contribute to the prevention of hyperinvasive organisms causing disease. Polysaccharide conjugate meningococcal vaccines, can selectively clear carriage of hyperinvasive variants providing indirect protection. Protein-based vaccines were developed to target serogroup B meningococci, but the impact of these vaccines on meningococcal carriage remains unclear. The aim of this study was to analyse the distribution and diversity of vaccine antigenic variants in whole genome sequences (WGS) of meningococcal carriage isolates from 1999 and 2014/15 with comparison to disease-causing isolates.

Novel genomic typing methods were used to analyse the vaccine antigens present in carried meningococci from Oxfordshire. Using retrospective carriage study collections from 1999 (n=498) and 2014/15 (n=149), which were whole genome sequenced, assembled, and annotated on PubMLST.org/neisseria, the Bexsero® Antigen Sequence Type (BAST) and Outer Membrane Vesicle peptide Types (OMVT) were catalogued. Seventy-four disease-causing meningococci from the South East of England (2014/15) were included for comparison to carriage isolates.

The carriage rate fell from 21.9% (498/2391) in 1999 to 7.0% (149/2138) in 2014/15 in Oxfordshire. Secular changes in clonal complexes (ccs) led to changes in genogroup distribution. Antigenic repertoires persisted through time for both BAST and OMVT but

overall diversity of these antigens was higher in carriage than disease isolates. Isolates with exact or putative cross-reactive fHbp peptide variants from Bexsero® (24.2% in 2014/15 Carriage) and Trumenba® (67.1% in 2014/15 Carriage) showed different distributions, with Trumenba® variants more likely to be found in meningococci with genogroups B, C, W, X, Y, or Z, and Bexsero® variants in genogroups E, L, or capsule null. There were 32.5-36.2% of carried meningococci that possessed exact and putatively cross-reactive antigenic variants contained in Bexsero®, which was lower than in disease isolates. When comparing the antigenic profile of carriage isolates to MeNZB™ (OMVT-1), most isolates had 0-4/24 matching loci, with no change over time.

The impact of protein-based vaccines is being examined in large-multicentre trials in Australia and the UK. To date, there is limited data on the effect of vaccine-induced antibodies on meningococcal isolates but data from mouse models suggest that the clearance of carriage is associated with a high number of antigenic targets and not correlated to antibody titre (Buckwalter *et al.* 2017). Overall, our finding is consistent with other studies of meningococcal carriage, that non-overlapping combinations of antigens persist through time for both disease-causing and harmless meningococci (Buckee *et al.* 2010).

5.1. Introduction

Understanding the transmission dynamics of meningococci is crucial to prevent disease by vaccination, through either direct or indirect mechanisms. *Neisseria meningitidis* is exclusively carried in the human oropharynx and transmitted by respiratory droplet spread to close contacts. Carriage rates range between 1-40%, with certain groups, such as adolescents, university students and military personnel having higher rates (MacLennan *et al.* 2006, Christensen *et al.* 2010). Carriage of meningococci is an asymptomatic, transient phenomenon. Once acquisition of meningococci has occurred, there are three potential events (i) colonisation of the oropharynx, (ii) transmission to another host, and/or (iii) clearance of carriage; however, the factors that control these events are not well defined. As a result of these processes, diverse communities of *Neisseria* species and other commensal bacteria form the human oropharyngeal microbiota. The beneficial functions of this microbiota remain incompletely understood but there are members of these bacterial communities that can cause invasive disease. Certain meningococci are inherently more invasive than others, as a function of the capsular expression or other virulence factors associated with particular genetic lineages, the so-called hyperinvasive lineages (Caugant 2008). Thus, the biodiversity and complex interactions between microbial species in the oropharynx, combined with the hosts physical barriers and immune responses generally inhibit hyperinvasive meningococci from causing disease. Perturbation of this equilibrium, by factors including temperature, humidity, smoking, or vaccination, could potentially lead to overgrowth of strains with propensity to cause invasive disease (de Steenhuijsen Piters *et al.* 2015).

Vaccinations aim to prevent disease in individuals or groups, though direct and/or indirect protection. Direct protection is obtained through the development of protective immune responses in the vaccinee, preventing invasive disease after further exposure to the same

pathogen. Indirect protection results from the reduced circulation of a pathogen by eradicating carriage amongst vaccinees and contributing to protection of non-immunised individuals. Polysaccharide conjugate meningococcal vaccines provide indirect protection by preventing colonisation with and transmission of hyperinvasive meningococci, without affecting non-pathogenic *Neisseria* species, maintaining their communities in the oropharynx. There is good evidence of the efficacy of this strategy from large multi-centre carriage studies in the UK (Maiden *et al.* 2008). Oropharyngeal carriage samples from 14,057 adolescents were taken before (1999) and 17,770 adolescents after (2001) the national conjugate meningococcal C vaccination campaign, with genotypic analysis demonstrating a dramatic reduction in the carriage of genogroup C and serogroup C ST-11 meningococci (Maiden *et al.* 2008). Other meningococci remained unchanged in the post-vaccination study (Maiden *et al.* 2008). Following implementation of the serogroup A polysaccharide conjugate vaccine (MenAfriVac®) in Chad, there was a significant reduction in carriage of serogroup A meningococci amongst vaccinees compared to unvaccinated individuals (Daugla *et al.* 2014).

Protein-based vaccines developed to target serogroup B meningococci, provide direct protection against certain meningococci that cause IMD. As these vaccines are being increasingly used worldwide, there is a growing need to understand their impact on carried meningococci. The two groups of vaccines that have been licensed for use against serogroup B disease include the outer membrane vesicle (OMV) vaccines and subcapsular protein vaccines. OMV vaccines were developed in the late 1980's and 1990's to target epidemics in Cuba, Norway and New Zealand (Holst *et al.* 2009). Epidemic strains formed the basis of these vaccines and they prevented disease caused by meningococci with homologous PorA types (Rosenqvist *et al.* 1995, Martin *et al.* 2006). The evidence for eradication of carriage with OMV vaccines remains limited, in

part due to the small study sizes and low carriage rates (Holmes *et al.* 2008, Delbos *et al.* 2013). The subcapsular protein vaccines were developed in the first decade of the 21st century. The 4CMenB (Bexsero®, GlaxoSmithKline, Siena) vaccine consists of three recombinant antigens; fHbp, NHBA and NadA all combined with the PorA-containing OMV from the MeNZB™ vaccine (Serruto *et al.* 2012). Bexsero® was introduced into the infant immunisation schedule in the UK in September 2015, with doses at two, four and 12 months. The rLP2086 vaccine (Trumenba®, Pfizer, New York) consists of two fHbp variants, from sub-families A and B, was licensed in the UK for individuals ≥10-year olds in May 2017 (Jiang *et al.* 2010). To date, the impact of these vaccines on meningococcal carriage have largely been assessed as part of outbreaks with limited randomised control trial data. One clinical trial comparing Bexsero® to meningococcal ACWY or control vaccines in UK university students demonstrated an 18.2% reduction in carriage of all meningococci, but did not show a reduction in serogroup B meningococci or disease-causing sequence types (STs) (Read *et al.* 2014).

Experience from US universities raises doubts whether either Bexsero® or Trumenba® can eliminate meningococcal carriage. During a university outbreak in 2013-14 in New Jersey, US, Bexsero® was administered to close contacts of meningococcal cases and induced direct protection (McNamara *et al.* 2015). Subsequently, another young adult who was in contact with the vaccinees was infected with the outbreak strain, suggesting that Bexsero® did not eradicate carriage in vaccinees (McNamara *et al.* 2015). Furthermore, larger cross-sectional surveys of students found persistent carriage despite multiple doses of Trumenba® vaccine (Soeters *et al.* 2017). An inherent limitation of these studies is the lack of power given the low meningococcal carriage rates and large numbers of participants required to see a significant effect of vaccination on carriage. To address this issue, at the time of writing large multi-centre trials were underway in South

Australia and the UK, studying the effect of Bexsero® and Trumenba® on carriage in adolescents, results are expected in mid-2019 (Marshall *et al.* 2018).

An alternative method of investigating the effect of vaccines on carriage isolates is to study the distribution and diversity of vaccine antigens in representative carriage collections. High-throughput whole genome sequencing (WGS) has enabled the study of bacterial diversity in a rapid and scalable manner, using bioinformatics techniques. By first identifying the presence or absence of relevant genes, one can then systematically type the protein sequence variants as demonstrated with Bexsero® antigens (BAST) and OMV peptides (OMVT) in over 3500 meningococcal disease isolates, using PubMLST.org *Neisseria* website (see Chapter 3 and Chapter 4) (Brehony *et al.* 2016, Rodrigues *et al.* 2018). These studies showed that despite the large diversity of antigenic variants of fHbp, NHBA and PorA amongst UK disease isolates, few occur at high frequency; *nadA* was found to be absent in two thirds of disease isolates. Twenty-four OMV peptide variants were catalogued using the OMVT scheme, demonstrating the variation in the degree of diversity of these OMPs and their association with cc. When grouped into OMVTs and OMVT clusters they were strongly associated with cc, genogroup, and BAST, suggesting that there were discrete populations of OMV and BAST combinations propagating in the oropharynx that have the potential to cause invasive disease. The aim of this study was to use these genomic typing methods to analyse the distribution and diversity of BAST and OMVT variants in the community of carried meningococci, within which disease-causing isolates exist; to identify changes over time, in relation to disease isolates and the implications for immunisation.

5.2. Materials and Methods

5.2.1. Isolate collections

N. meningitidis isolates from two historical carriage studies were used for the longitudinal analysis from 1999 and 2014/15. The carriage studies were performed in adolescents between 15-19 years old, the age group with the highest meningococcal carriage rate in industrialised countries (Christensen *et al.* 2010). UKMenCar1 was a national carriage study performed in 1999 prior to the introduction of the meningococccal C vaccine in November/December 1999. There were 2391 adolescents who had oropharyngeal swabs taken in Oxfordshire, which yielded 524 with meningococcal carriage (carriage rate 21.9%) (Maiden *et al.* 2008). UKMenCar4 was a national carriage study performed in 2014/15, prior to the introduction of both the ACWY vaccination programme for teenagers in August 2015 and 4CMenB (Bexsero®) for infants in September 2015. There were 2138 adolescents who had oropharyngeal swabs taken in Oxfordshire, which yielded 149 with meningococcal carriage (carriage rate 7.0%) (Maclennan *et al.* 2016). Both carriage studies were done in the same geographical areas, using the same methodology for culture and identification of *N. meningitidis*. UKMenCar1 and UKMenCar4 were herein referred to as "1999 Carriage" and "2014/15 Carriage", respectively.

Disease-causing meningococci tend to be from hyperinvasive lineages that can occur at low frequency in carriage. To compare the antigenic variants found in disease isolates to carriage isolates, a collection of 74 isolates from culture-confirmed IMD from the South East of England in 2014/15 were obtained from the Meningitis Research Foundation Meningococcal Genome Library (MRF-MGL). This geographical region included Oxfordshire and other areas of the South East as defined by Public Health England and was herein referred to as "IMD 14/15".

5.2.2. DNA extraction and sequencing

Isolates from 1999 Carriage were cultured from frozen (-80°C) archived stores using Columbia Blood Agar plates, at 37°C, 5% CO_2. Primary cultures of both 1999 and 2014/15 isolates were cultured for 18-24 hours. A single colony was sub-cultured for 18-24 hours at 37°C, 5% CO_2. DNA extraction from this single colony culture was performed using a Promega adapted protocol (Bratcher *et al.* 2014). DNA was quantified using the Qbit fluorometer (Quant-iT dsDNA BR Assay Kit, Invitrogen) and DNA integrity was confirmed by gel electrophoresis. Of the 524 1999 Carriage meningococci, 502 underwent WGS, two were contaminated on primary culture and despite subculture meningococci could not be isolated, 12 did not culture from archived frozen stores, seven isolates could not be located and one had DNA degradation after extraction.

5.2.3. Genomic analysis

WGS was performed for 502 1999 Carriage isolates and 149 2014/15 Carriage isolates using Illumina platform HiSeq4000, with paired-end reads. Short reads were assembled using Velvet/VelvetOptimiser and uploaded to the PubMLST *Neisseria* database. WGS were automatically annotated using the tools within the BIGSdb platform (which runs the PubMLST website) with manual curation of capsule, BAST, and OMV peptide loci for new allelic variants. Ribosomal MLST was used to confirm species for 1999 Carriage isolates: 498 were *N. meningitidi*s; two *N. lactamica*; one *N. subflava*; and one *N. cinerea* (Maiden *et al.* 2013). For 2014/15 carriage, all 149 were *N. meningitidi*s.

5.2.4. BAST and OMVT schemes

Vaccine antigen schemes were applied to WGS as previously described (Brehony *et al.* 2016). For the full description of the BAST scheme see Chapter 2 (section 2.4.2). For the full description of the OMVT scheme see Chapter 4 (section 4.4.1). Where there was a gene sequence with a frameshift mutation rendering the protein truncated, the isolate was

designated a null (0) variant. If the gene was confirmed to be absent, the isolate was designated a null (0) variant. New BAST or OMVTs were assigned to new allelic profiles.

5.2.5. **Statistical analysis**

Statistical analyses, including Fisher's exact tests, were performed using R software version 3.2.4 (https://www.r-project.org/). Simpson's index of diversity (*D*) was calculated using the Vegan package in R, to assess the diversity of individual proteins or allelic profiles, with values closer to one indicating greater diversity. Confidence intervals (CIs) for *D* were calculated using the online resource www.comparingpartitions.info.

5.3. Results

5.3.1. **Serogroup and genogroup distribution**

There were 478/498 (96.0%) 1999 Carriage isolates for which a serogroup result was determined, of these, 231 (48.3%) isolates were non-groupable (NG) and the remaining assigned to serogroups: B 124 (25.9%); C 13 (2.7%); W 23 (4.8%); Y 30 (6.3%); E 50 (10.5%); X 5 (1.0%); and Z 2 (0.4%) (Table 5-1). The presence of capsule genes provided further discrimination of the NG isolates, as meningococci possessing capsular genes may have regulated capsule production through phase variation. Of the 231 NG isolates, 64 (27.7%) were capsule null (*cnl*), and 157 (68.0%) had genes for capsules: B 46 (19.9%); C 13 (5.6%); E 41 (17.7%); L 4 (1.7%); W 21 (9.1%); Y 26 (11.3%); or Z 6 (2.6%). Seven (3.0%) isolates could not be determined due to missing capsule regions likely secondary to recombination events and three had sialic capsules of undetermined group due to missing genomic data, possibly due to gene sequencing or assembly issues (Harrison *et al.* 2013).

Table 5-1 Distribution of serogroup and genogroup for isolates in the two meningococcal carriage studies, 1999 Carriage and 2014/15 Carriage. NG=non-groupable by serotyping, *cnl*=capsule null on genotyping, p values calculated using Fisher's exact test.

	1999 Carriage serogroup (n)	%	2014/15 Carriage serogroup (n)	%	p value	1999 Carriage genogroup (n)	%	2014/15 Carriage genogroup (n)	%	p value
NG	231	48.3	90	60.4	0.01					
cnl						75	15.4	39	27.7	0.002
B	124	25.9	18	12.1	0.0003	170	35.0	32	22.7	0.006
C	13	2.7	-	-	-	29	6.0	-	-	-
W	23	4.8	9	6.0	0.83	50	10.3	12	8.5	0.63
Y	30	6.3	32	21.5	$4.68x10^{-7}$	57	11.7	40	28.4	$8.29x10^{-6}$
E	50	10.5	-	-	-	83	17.1	15	10.6	0.07
L	-	-	-	-	-	7	1.4	1	0.7	0.69
X	5	1.0	-	-	-	6	1.2	2	1.4	1.0
Z	2	0.4	-	-	-	9	1.9	-	-	-
Assigned	478	96.0	149	100.0		486	97.6	141	94.6	
Unassigned	20	4.0	-	-		12	2.4	8	5.4	
Total	**498**	**100**	**149**	**100**		**498**	**100**	**149**	**100**	

For 2014/15 Carriage isolates, by serogrouping there were; NG 90 (60.4%); B 18 (12.1%); W 9 (6.0%); and Y 32 (21.5%). Of the 90 NG isolates, 38 (42.2%) were *cnl*, 44 (48.8%) had genes for capsular group: B 14 (15.6%); E 14 (15.6%); L 1 (1.1%); W 4 (4.4%); Y 9 (10.0%); or X 2 (2.2%); with 8 (8.9%) indeterminate due to missing genomic data due to recombination or sequencing/assembly issues (Table 5-1). Immunological serogrouping for non-BCWY capsular types was not done in 2014/15.

There were significant reductions in serogroup B and genogroup B meningococci from 1999 to 2014/15 (p=0.0003, p=0.006 respectively) and genogroup E approached significance (p=0.07). There were increases in serogroup Y and genogroup Y meningococci ($p=4.890x10^{-7}$, $p=8.2920x10^{-6}$ respectively) and *cnl* genogroup (p=0.002) (Table 5-1).

5.3.1.1. Genogroups and clonal complexes

The changes in genogroup were secondary to the secular changes in cc. The cc distribution for genogroups representing ≥10 isolates were analysed (Figure 5-1). For each genogroup, the major cc persists from 1999 to 2014/15, except for genogroup W, which was predominantly caused by c22 in 1999 but was exclusively caused by cc11 in 2014/15. There were fewer genogroup B ccs in 2014/15 than 1999, in particular the absence of cc269. There were no genogroup C isolates for comparison in 2014/15. For genogroup E isolates, cc1157 became predominant in 2014/15 from cc60 in 1999

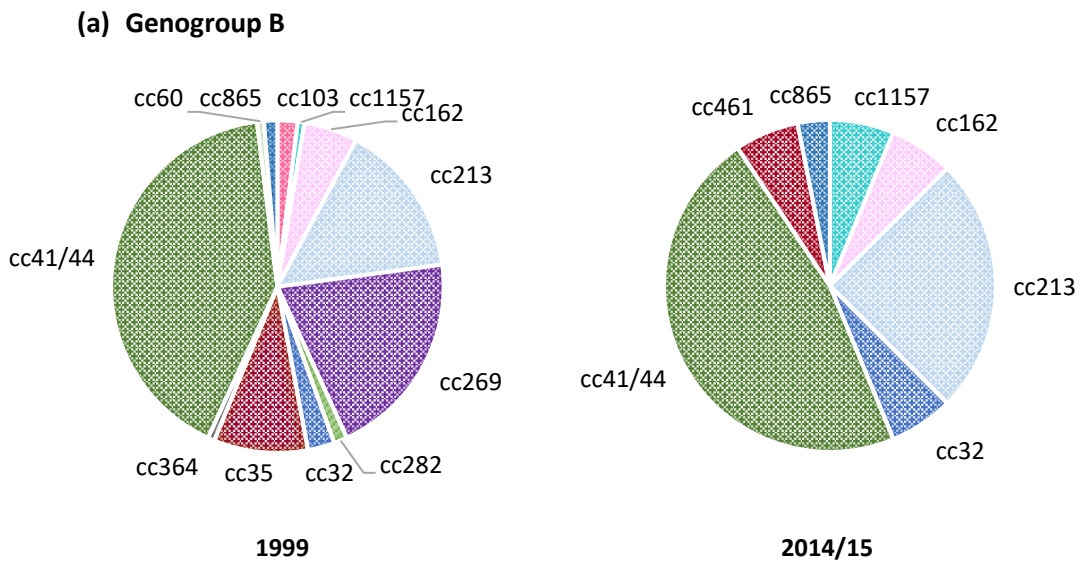
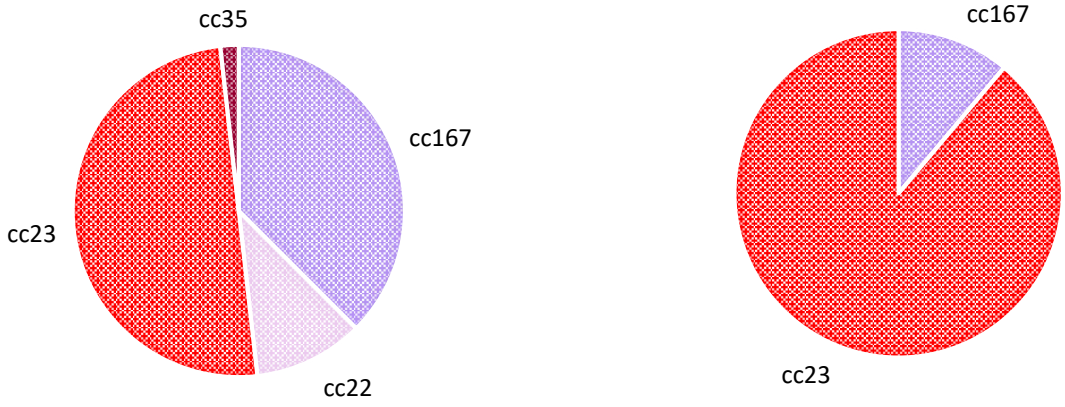


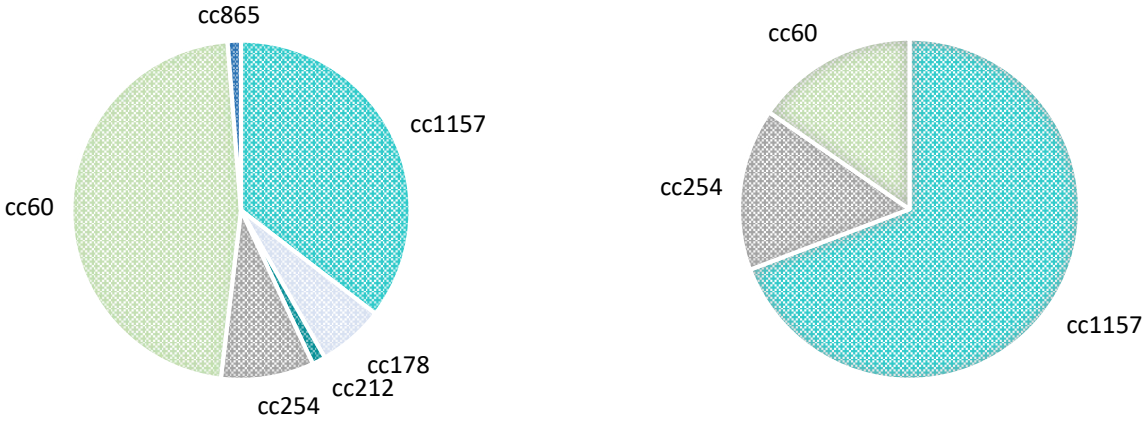
Figure 5-1 Clonal complex (cc) distribution for each genogroup with more than 10 isolates in either carriage collection.
The pie charts show the different cc composition of each genogroup in the two carriage collections of 1999 and 2014/15. (a) genogroup B, (b) genogroup W, (c) genogroup C, there were no genogroup or serogroup C isolates in 2014/15, (d) genogroup Y, (e) genogroup E, (f) genogroup *cnl*.

(d) Genogroup Y



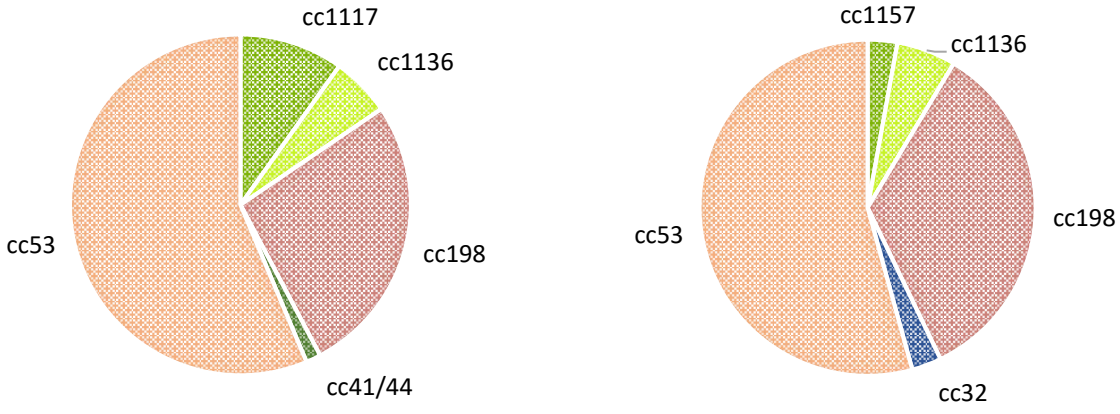
1999 **2014/15**

(e) Genogroup E



1999 **2014/15**

(f) Genogroup *cnl*



1999 **2014/15**

5.3.2. Clonal complex distribution

In 1999 Carriage there were 153 STs as defined by MLST, amongst 496/498 (99.6%) isolates and 26 ccs amongst 459/498 (92.2%) isolates. The five most frequently occurring ccs were: cc41/44 67 (14.6%), cc22 57 (12.4%), cc53 40 (8.7%), cc60 39 (8.5%), and cc269 34 (7.4%). In 2014/15 Carriage there were 53 STs amongst 148/149 (99.3%) isolates and 16 ccs amongst 136/149 (91.3%) isolates. The five most frequently occurring ccs were: cc23 32 (23.5%), cc41/44 21 (15.4%), cc53 19 (14.0%), cc11 13 (9.6%), and cc198 12 (8.8%). There were 15 ccs observed in 2014/15 that were also present in 1999, with incidence having decreased in all ccs except cc23 and cc11 (Figure 5-2). Isolates from cc461 were not identified in 1999 and those from cc22 were not identified in 2014/15. However, since not all STs were assigned to a cc or some isolates had incomplete MLST profiles, the population structure of carried meningococci was analysed using core genome MLST (cgMLST) analysis of the 647 isolates visualised with GrapeTree (Maiden *et al.* 2013, Zhou *et al.* 2018). This identified that most lineages were represented by isolates from both periods, except lineage 2 (cc269) and lineage 22 (cc22) which were not identified in 2014/15 (Figure 5-3).

5.3.3. Longitudinal changes in distribution of vaccine antigenic variants

5.3.3.1. Factor H binding protein (fHbp)

The fHbp antigen was annotated in all isolates in all three datasets. There were 51 variants in 1999 Carriage, 21 variants in 2014/15 Carriage and 17 variants in IMD 14/15. The distribution of these variants was different between carriage and IMD. The most frequent four variants represented 260/498 (52.2%) 1999 Carriage isolates, 80/149 (53.7%) 2014/15 Carriage isolates, and 55/74 (74.3%) IMD 14/15 isolates.

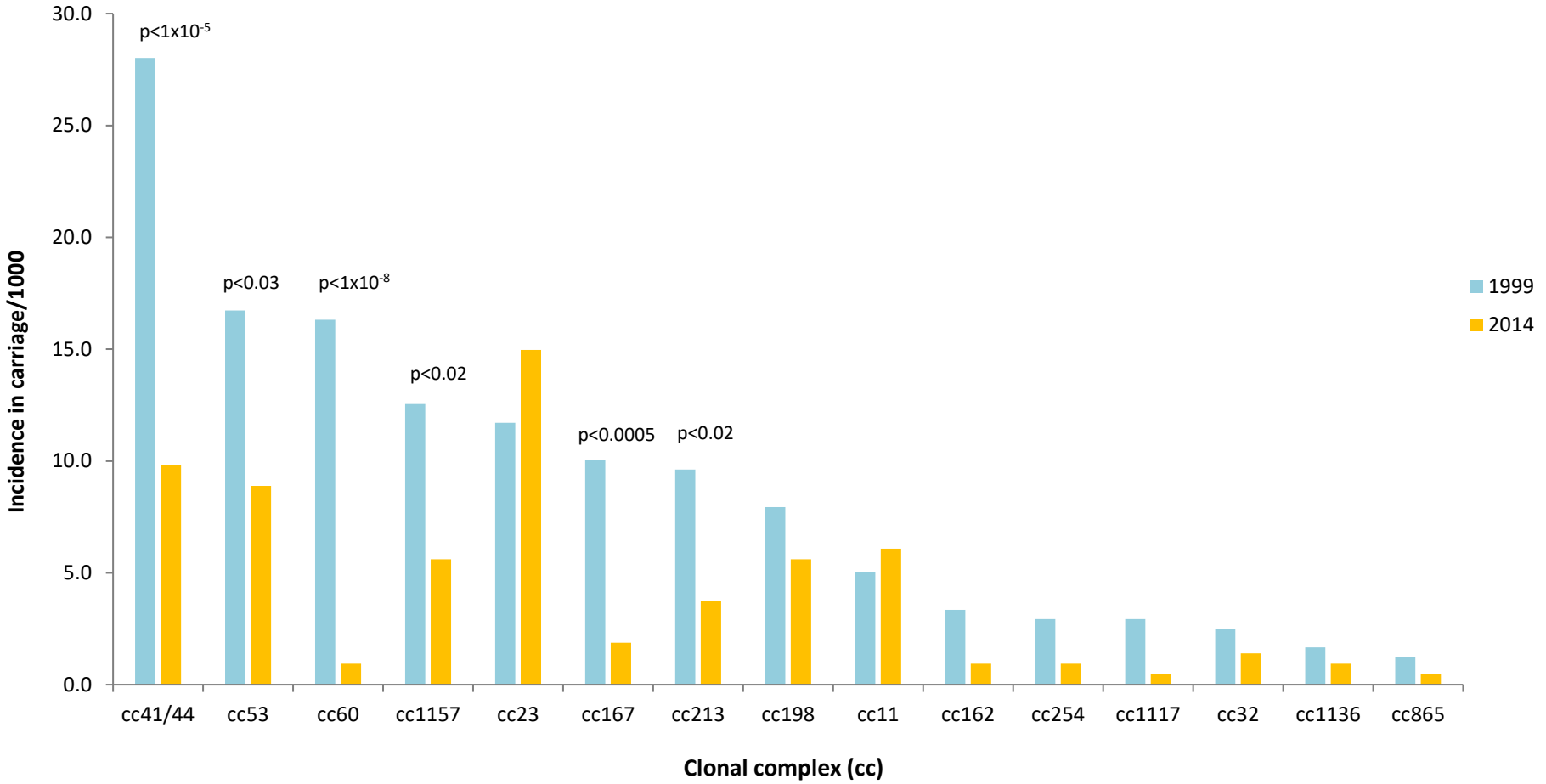


Figure 5-2 Incidence of clonal complexes that occurred in both 1999 Carriage and 2014/15 Carriage in Oxfordshire.
The incidence per 1000 adolescents who had oropharyngeal swabs is shown for the 15 clonal complexes (ccs) that occurred in both 1999 and 2014/15. Significant differences are shown by p value as calculated by Fisher's exact test. Only two ccs increased in frequency from 1999 to 2014/15, cc11 and cc23 but neither reached statistical significance (cc11 p=0.69, cc23p=0.36).

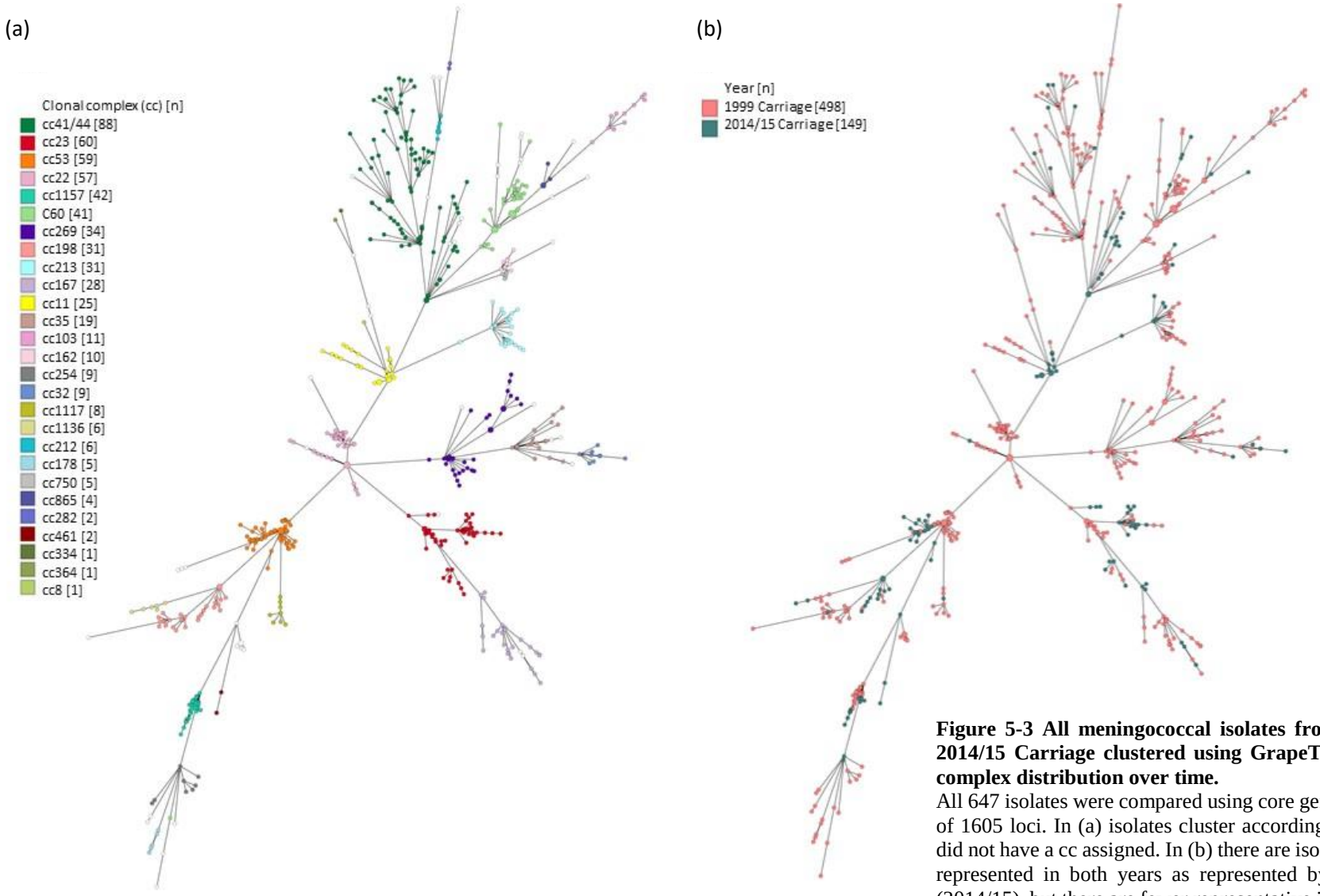


Figure 5-3 All meningococcal isolates from 1999 Carriage and 2014/15 Carriage clustered using GrapeTree to visualise clonal complex distribution over time.

All 647 isolates were compared using core genome MLST (cgMLST) of 1605 loci. In (a) isolates cluster according to cc, unfilled isolates did not have a cc assigned. In (b) there are isolates from almost all ccs represented in both years as represented by pink (1999) and teal (2014/15), but there are fewer representative isolates from 2014/15 as the carriage rate fell from 21.9% in 1999 to 7.0% in 2014/15 in Oxfordshire.

Table 5-2 Distribution of vaccine antigens amongst meningococcal whole genome sequences from two carriage studies from 1999 and 2014/15, with disease-causing isolates from 2014/15.
Antigenic variants considered putatively cross-reactive to Bexsero® were 4, 13, 14, 15, 37 and 232. *Antigenic variants considered putatively cross-reactive to Trumenba® were 4, 13, 14, 15, 16, 19, 21, 23, 24, 25, 30, 47, 76, 87, 180, 187, 252, 276 and 510. Hyperinvasive lineages as described in (Caugant 2008)**.** ***NadA* present in 22, 15 and 28 isolates in 1999 Carriage, 2014/15 Carriage and IMD 14/15 respectively, variants NadA1 and NadA2/3 considered putatively cross-reactive. NG=non-groupable by serotyping. *Cnl*=capsule null on genotyping.

	1999 Carriage (n=498) n (%)	**2014/15 Carriage (n=149) n (%)**	**IMD 14/15 (n=74) n (%)**	**p value 1999 Carriage vs 2014/15 Carriage**	**p value 2014/15 carriage vs IMD 14/15**
BAST-1 matches	35 (7.0)	5 (3.4)	11 (14.9)	0.12	0.0009
≥1 putative cross-reactive matches to BAST-1	162 (32.5)	54 (36.2)	47 (63.5)	0.43	0.0002
≥2 putative cross-reactive matches to BAST-1	18 (3.6)	2 (1.3)	9 (12.1)	0.21	0.001
fHbp match to Bexsero® (peptide 1)	2 (0.4)	0 (0.0)	3 (4.1)	1.0	0.04
fHbp exact or cross-reactive variants* to Bexsero®	127 (25.5)	36 (24.2)	21 (28.4)	0.35	0.52
• *fHbp exact or cross-reactive variants to Bexsero® in capsular groups B, C, W, X, Y, Z*	*44 (34.6)*	*7 (19.4)*	*20 (95.2)*	*0.10*	*$1.93x10^{-8}$*
• *fHbp exact or cross-reactive variants to Bexsero® in capsular groups E, L, cnl*	*82 (64.6)*	*28 (77.8)*	*1 (4.8)*	*0.16*	*$4.48x10^{-8}$*
• *fHbp exact or cross-reactive variants to Bexsero® in hyperinvasive lineages*	*37 (29.1)*	*5 (13.9)*	*14 (66.7)*	*0.08*	*$8.76x10^{-5}$*
fHbp exact match to Trumenba® (peptides 45, 55)	19 (3.8)	6 (4.0)	2 (2.7)	1.0	1.0

	1999 Carriage (n=498) n (%)	2014/15 Carriage (n=149) n (%)	IMD 14/15 (n=74) n (%)	p value 1999 Carriage vs 2014/15 Carriage	p value 2014/15 carriage vs IMD 14/15
fHbp exact or cross-reactive variants* to Trumenba®	363 (72.9)	100 (67.1)	41 (55.4)	0.18	0.11
• *fHbp exact or cross-reactive variants to Trumenba® in capsular groups B, C, W, X, Y, Z*	*265 (73.0)*	*65 (65.0)*	*40 (97.6)*	*0.13*	*$1.26x10^{-5}$*
• *fHbp exact or cross-reactive variants to Trumenba® in capsular groups E, L, cnl*	*92 (25.3)*	*29 (29.0)*	*1 (2.4)*	*0.52*	*0.0002*
• *fHbp exact or cross-reactive variants to Trumenba® in hyperinvasive lineages*	*136 (37.4)*	*52 (52.0)*	*32 (78.0)*	*0.01*	*0.005*
NHBA matches to Bexsero® (peptide 2)	23 (4.6)	4 (2.7)	6 (8.1)	0.36	0.09
NadA matches to Bexsero® (peptide 8)	0 (0.0)	0 (0.0)	0 (0.0)	-	-
NadA cross-reactive variants to Bexsero® **	16 (72.7)	15 (100.0)	27 (96.4)	0.06	1.0
PorA VR2 matches to Bexsero® (peptide 4)	18 (3.6)	1 (0.7)	5 (6.8)	0.09	0.02

The fHbp peptide 1, present in Bexsero®, was found in 2/498 (0.4%) 1999 Carriage isolates, 0/149 (0.0%) 2014/15 Carriage isolates, and 3/74 (4.1%) IMD 14/15 isolates only (1999 Carriage vs 2014/15 Carriage p=1.0, 2014/15 Carriage vs IMD 14/15 p=0.036). The fHbp peptides 45 and 55 are constituents of Trumenba®. Peptide 45 was present in 19/498 (3.8%) 1999 Carriage isolates, 6/149 (4.0%) 2014/15 Carriage isolates, and 2/74 (2.7%) IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=1.0, 2014/15 Carriage vs IMD 14/15 p=1.0). There were no isolates possessing fHbp peptide 55 in any dataset in this study (Table 5-2).

Given cross-reactive immune responses could be generated to variants similar but not identical to those in the vaccine, the presence of putatively cross-reactive variants was assessed. For Bexsero®, fHbp peptides 1, 4, 13, 14, 15, 37 and 232 (based on the Meningococcal Antigen Typing System (MATS) (Vogel et al. 2013)) were present in 127/498 (25.5%) in 1999 Carriage isolates, 36/149 (24.2%) in 2014/15 Carriage isolates, and 21/74 (28.4%) in IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.3497, 2014/15 Carriage vs IMD 14/15 p=0.517). For Trumenba®, fHbp peptides 1, 4, 13, 14, 15, 16, 19, 21, 23, 24, 25, 30, 45, 47, 55, 76, 87, 180, 187, 252, 276 and 510 (based on serum bactericidal assays with post-vaccination sera (Harris et al. 2017, Lujan et al. 2017, Ostergaard et al. 2017, Taha et al. 2017)) were present in 363/498 (72.9%) 1999 Carriage isolates, 100/149 (67.1%) in 2014/15 Carriage isolates, and 41/74 (55.4%) in IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.1792, 2014/15 Carriage vs IMD 14/15 p=0.105).

The genogroups of the isolates that possessed these variants, potentially impacted by the vaccines, included the capsular groups known to cause IMD, such as B, C, W, X, Y and Z but also those non-invasive strains that may be beneficial in the oropharyngeal microbiota such as capsular group E, L and *cnl*. For the 2014/15 Carriage isolates, 19.4%

of isolates possessing exact or putatively cross-reactive Bexsero® variants were found in IMD-causing capsular groups and 77.8% with capsular groups E, L, or *cnl*. The inverse was observed with Trumenba®, 65.0% of isolates possessing exact or putatively cross-reactive variants were found in IMD-causing capsular groups and 29.0% with capsular groups E, L, or *cnl*.

5.3.3.1. Neisserial heparin binding antigen (NHBA)

The NHBA peptide was annotated in all but two isolates amongst the three datasets, both had incompletely assembled *nhba* (2014/15 Carriage). There were 55 variants in 1999 Carriage, 33 variants in 2014/15 Carriage and 17 variants in IMD 14/15. The distribution was similar to fHbp peptides, whereby the most frequent four variants represented 184/498 (36.9%) 1999 Carriage isolates, 73/147 (49.7%) 2014/15 Carriage isolates, and 53/74 (71.6%) IMD 14/15 isolates. The NHBA peptide 2, the variant in Bexsero®, was present in 23/498 (4.6%) 1999 Carriage isolates, 4/147 (2.7%) 2014/15 Carriage isolates, and 6/74 (8.1%) IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.36, 2014/15 Carriage vs IMD 14/15 p=0.09) (Table 5-2). All but one (*cnl*) of these was capsular genogroup B.

5.3.3.2. Neisseria adhesin A (NadA)

In 1999 Carriage isolates, NadA peptide was present in 22/498 (4.4%), absent in 471/498 (94.6%) and incompletely assembled in 5/498 (1.0%). In 2014/15 Carriage isolates, NadA peptide was present in 15/149 (10.1%), absent in 133/149 (89.3%) and incompletely assembled in 1/149 (0.7%). In IMD 14/15 isolates, NadA peptide was present in 28/74 (37.8%), absent in 43/74 (58.1%), incompletely assembled in 3/74 (4.1%). There were 11 variants in 1999 Carriage, 2 variants in 2014/15 Carriage and 4 variants in IMD 14/15. There were no isolates that possessed peptide 8 found in Bexsero®. Of the isolates with peptides present, 16/22 (72.7%) 1999 Carriage isolates,

15/15 (100%) 2014/15 Carriage isolates, and 27/28 (96.4%) IMD 14/15 isolates had peptides from NadA 1 or 2/3 family, considered potentially cross-reactive variants (1999 Carriage vs 2014/15 Carriage p=0.06, 2014/15 Carriage vs IMD 14/15 p=1.0) (Table 5-2).

5.3.3.3. Porin A variable region 2 (PorA VR2)

The PorA VR2 was annotated in all isolates. There were 74 variants in 1999 Carriage, 45 variants in 2014/15 Carriage and 21 variants in IMD 14/15. The most frequent four variants represented 203/498 (40.8%) 1999 Carriage isolates, 51/149 (34.2%) 2014/15 Carriage isolates, and 49/74 (66.2%) IMD 14/15 isolates. The PorA VR2 peptide 4, present in Bexsero®, was found in 18/498 (3.6%) 1999 Carriage isolates, 1/149 (0.7%) 2014/15 Carriage isolates, and 5/74 (6.8%) IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.09, 2014/15 Carriage vs IMD 14/15 p=0.02) (Table 5-2). Of these, genogroup distribution was: B 20 (83.3%), E 1 (4.2%), Z 1 (4.2%) and 2 (8.3%) were indeterminate due to gene sequence assembly issues.

5.3.4. **Longitudinal changes in the distribution of antigenic repertoires – BASTs and OMVTs**

5.3.4.1. BASTs

A BAST was determined for 492/498 (98.9%) 1999 Carriage isolates, 146/149 (98.0%) 2014/15 Carriage isolates and 70/74 (94.6%) IMD 14/15 isolates. There were 217 unique BASTs in 1999 Carriage, 72 in 2014/15 Carriage and 36 in IMD 14/15 isolates. Twenty-one BASTs occurred in carriage in both time periods, eight of which had declined (BAST-349 ($p<1x10^{-9}$), BAST-271 ($p<0.05$), BAST-237 ($p<0.0001$), BAST-224, BAST-384, BAST-244, BAST-275, BAST-241) in incidence since 1999, 13 had increased (BAST-225, BAST-637, BAST-635, BAST-228, BAST-619, BAST-227, BAST-640,

BAST-741, BAST-315, BAST-657, BAST-1974, BAST-1978, BAST-246), and five also occurred in IMD 14/15 (Figure 5-4).

5.3.4.1. OMVTs and OMVT clusters

An OMVT was determined for 453/498 (91.0%) 1999 Carriage isolates, 143/149 (96.0%) 2014/15 Carriage isolates, and 46/74 (62.1%) IMD 14/15 isolates. There were 360 unique OMVTs in 1999 Carriage, 119 in 2014/15 Carriage, and 37 in IMD 14/15 isolates. There were only four OMVTs, OMVT-21, -50, -1149 and -1275, that occurred in more than one dataset (2014/15 Carriage and IMD 14/15). No OMVT had ≥10 isolates across any datasets in this study.

There were 23 OMVT clusters in 1999 Carriage isolates (212/498, 42.6%), 10 in 2014/15 Carriage isolates (52/149, 34.9%), and 13 in IMD 14/15 isolates (35/74, 47.3%). OMVT clusters were compared between the datasets, with four occurring in all three datasets: OMVT cluster-21 (Y:cc23), OMVT cluster-414 (B:cc41/44), OMVT cluster-1149 (W:cc11), OMVT cluster-4218 (B/E:cc1157) (Figure 5-5). As this distribution is likely to represent the sampling in this study, the PubMLST database, which holds 16,843 meningococcal WGS at the time of writing, was used to identify presence of these clusters more widely across the global isolate database. Of the clusters that occurred in 1999 Carriage only, OMVT clusters-104, -3948 and -3995 have only been observed in other carriage isolates to date. OMVT cluster-2435 present in 2014/15 Carriage has not appeared in any other isolate in the PubMLST database. Of the clusters seen only in IMD 14/15, none have been observed in carriage isolates in PubMLST except OMVT cluster-160, it may be the others are only carried transiently. Of the clusters seen in both 1999 Carriage and 2014/15 Carriage, all have been observed in IMD isolates in PubMLST except OMVT cluster-4003. As for the clusters seen in 1999 Carriage and IMD 14/15,

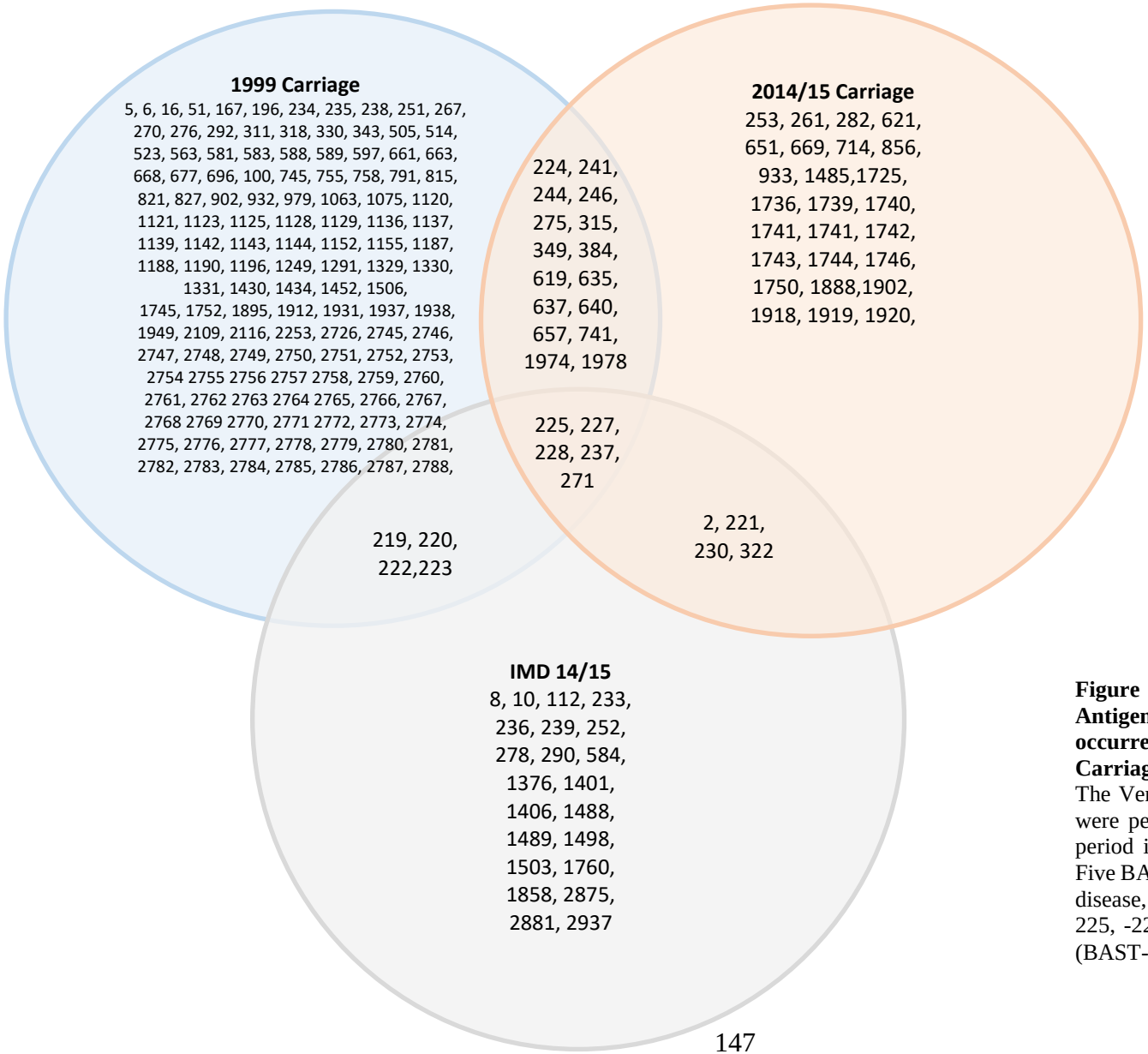


Figure 5-4 Representation of the Bexsero® Antigen Sequence Types (BASTs) that occurred in three isolate collections; 1999 Carriage, 2014/15 Carriage, and IMD 14/15. The Venn diagram demonstrates that 16 BASTs were persistently identified through the 15-year period in carriage, but not observed in disease. Five BASTs occurred in both carriage studies and disease, and were associated with cc23 (BAST-225, -227, -228), cc60 (BAST-237) and cc1157 (BAST-271).

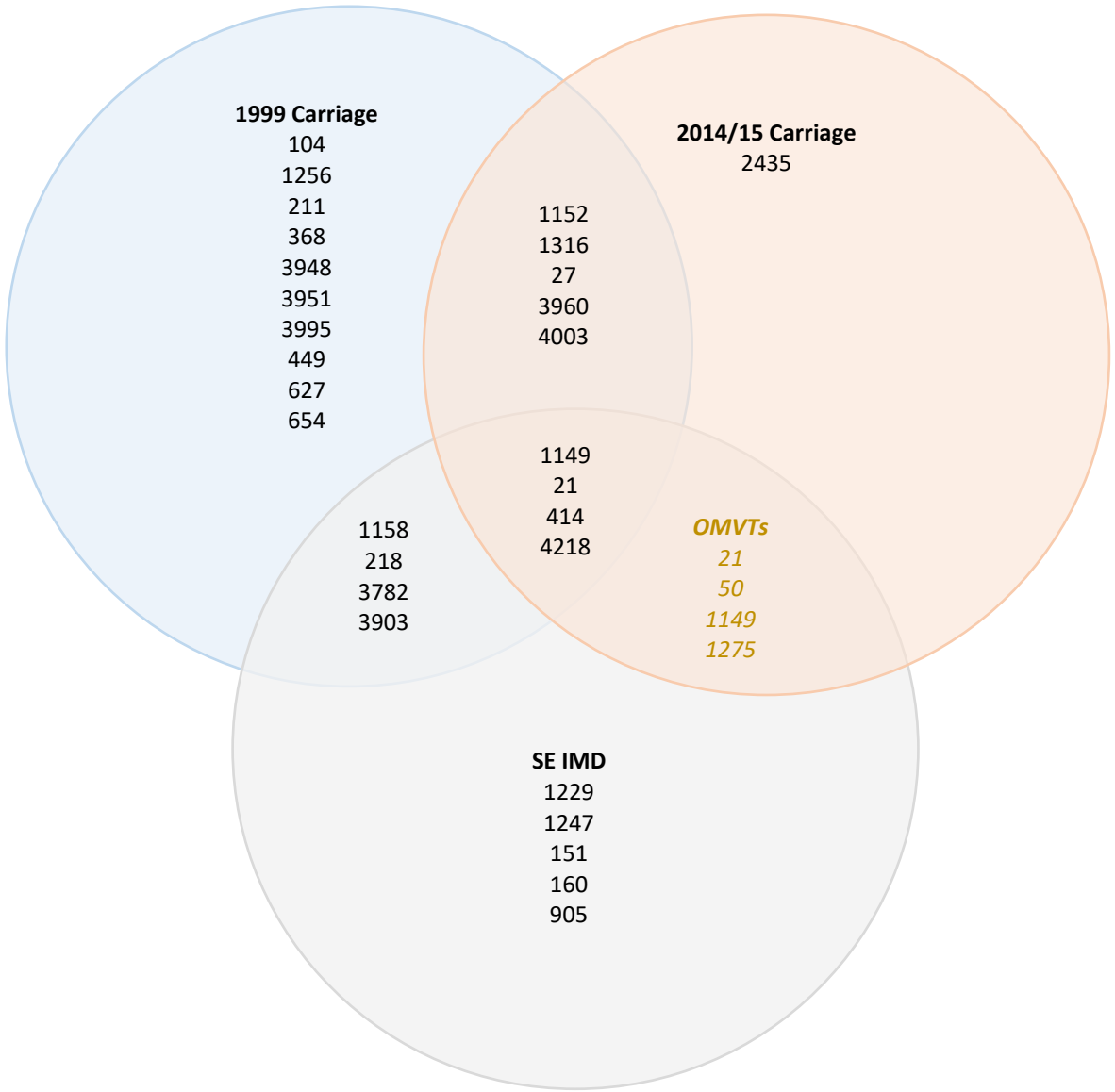


Figure 5-5 Representation of the Outer Membrane Vesicle peptide Typing (OMVT) clusters that occurred in three isolate collections; 1999 Carriage, 2014/15 Carriage, and IMD 14/15.
The Venn diagram demonstrates that five OMVT clusters were persistently identified through the 15-year period in carriage, but not observed in disease. For the four OMVT clusters in the centre, they were found in both carriage studies and disease, associated with cc11 (OMVT-cluster 1149), cc23 (OMVT-cluster 21), cc41/44 (OMVT-cluster 414) and cc1157 (OMVT-cluster 4218). The only OMVTs to be found across datasets were the four shown in gold italics.

but not 2014/15 Carriage, this is likely a sampling issue, as the isolates must still be in the transmission cycle to cause disease.

5.3.4.2. OMVT and OMVT cluster association with BAST

There were strong associations in the 1999 Carriage isolates between OMVT and BAST (Cramer's V=0.99), fHbp (Cramer's V=0.99), NHBA (Cramer's V=0.99), NadA (Cramer's V=0.96), PorA VR2 (Cramer's V=1) and OMVT clusters and BAST (Cramer's V=0.96). This was the same as associations in disease-causing isolates in the UK (see Chapter 4, section 4.5.3), but had not previously been shown for carriage isolates.

5.3.5. Diversity of antigenic repertoires – BAST and OMVTs in carriage and disease

5.3.5.1. BAST and individual antigen diversity

The diversity of BAST was measured by Simpson's index of diversity (*D*), representing the probability that two selected isolates will have different BASTs, with higher values representing higher diversity. For 1999 Carriage isolates, *D*=0.985 (95% CI 0.981-0.989), for 2014/15 Carriage isolates *D*=0.975 (95% CI 0.964-0.986), and for IMD 14/15 isolates *D*=0.880 (95% CI 0.817-0.943) (Table 5-3, Figure 5-6). For BAST, fHbp, NHBA and PorA VR2, there was comparable diversity in 1999 Carriage and 2014/15 Carriage, but lower diversity in IMD 14/15, as measured by Simpson's indices (Table 5-3, Figure 5-6).

5.3.5.2. OMVT diversity

The Simpson's indices were high for both OMVTs and OMVT clusters in both carriage and disease collections, with overlapping CIs and no discernible difference between carriage and disease as observed with BAST and the other antigens (Table 5-3, Figure 5-6).

5.3.5.3. OMV peptides

The diversity of the OMV profiles is driven by their constituent parts. To determine the longitudinal changes in variation of the individual proteins over the 15-year period, the diversity indices were calculated for each protein and each dataset. For the relatively more diverse proteins, where $D \geq 0.9$, 1999 Carriage and 2014/15 Carriage were equally diverse, whereas the IMD 14/15 isolates showed less diversity for each peptide. For those OMPs with D=0.5-0.9, the three datasets had overlapping indices and CIs, suggesting no difference in diversity (Figure 5-7). For the cytoplasmic proteins, NEISp0155, NEISp1130 and NEISp0142 diversity was very low D<0.1 and similar across carriage and disease. For NEISp0126, ribosomal protein 30S subunit S7, variants in carriage isolates were more diverse than disease.

Table 5-3 Diversity of vaccine antigens, Bexsero® Antigen Sequence Type (BASTs) and Outer Membrane Vesicle peptide Type (OMVT) amongst carriage and disease-causing meningococci.
Simpson's index of diversity (*D*) calculated with confidence intervals (CIs) for antigenic repertoires BAST, OMVT and OMVT-clusters and Bexsero® recombinant antigens fHbp, NHBA and PorA. NadA was absent in the majority of isolates so was not included.

	1999 Carriage		2014/15 Carriage		IMD 14/15	
	D	**95% CI**	***D***	**95% CI**	***D***	**95% CI**
BAST	0.985	0.981-0.989	0.975	0.964-0.986	0.880	0.817-0.943
fHbp	0.913	0.904-0.922	0.905	0.886-0.924	0.817	0.753-0.881
NHBA	0.944	0.936-0.952	0.924	0.904-0.944	0.799	0.729-0.869
PorA VR2	0.944	0.936-0.952	0.956	0.943-0.969	0.833	0.765-0.901
OMVT	0.998	0.997-0.999	0.994	0.990-0.998	0.971	0.937-1.000
OMVT clusters	0.883	0.862-0.904	0.822	0.770-0.874	0.761	0.633-0.889

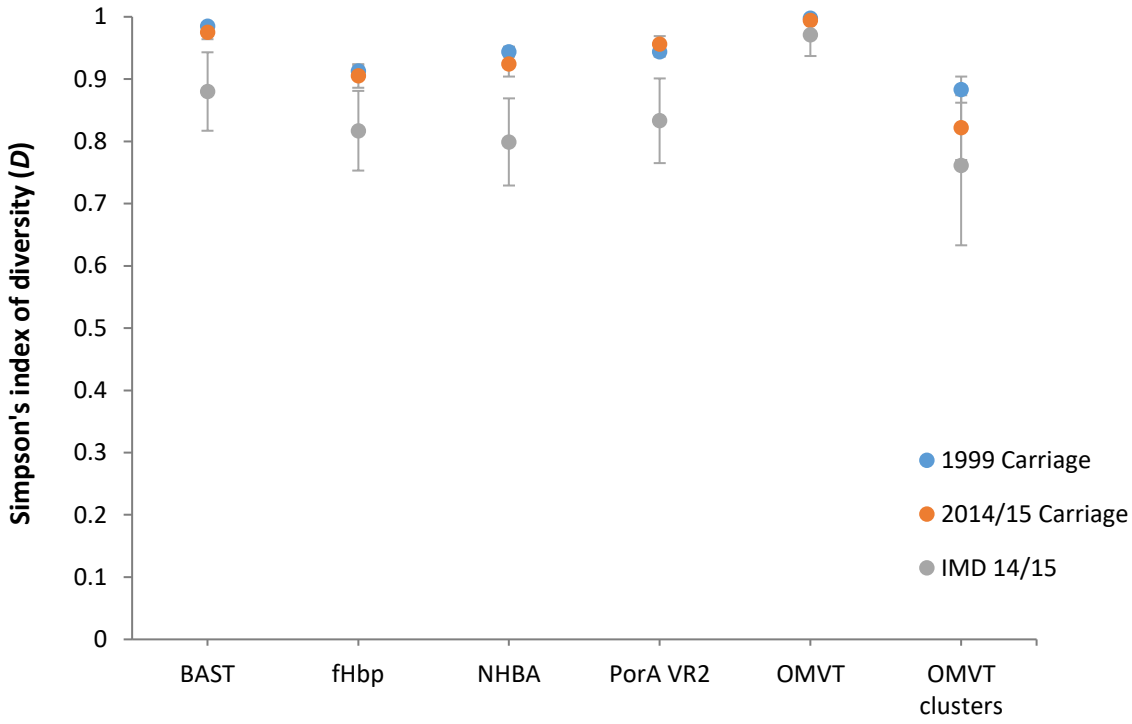


Figure 5-6 Diversity of vaccine antigens, Bexsero® Antigen Sequence Types BAST and Outer Membrane Vesicle peptide Types (OMVTs) amongst carriage and disease-causing meningococci. Simpson's index of diversity (*D*) with 95% confidence intervals for BASTs, factor H binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA), Porin A variable region 2 (PorA VR2) and OMVTs and OMVT clusters, shown for all three isolates collections; 1999 Carriage, 2014/15 Carriage and IMD 14/15. Simpson's index ranges from 0-1, with values closer to 1 indicating greater diversity.

5.3.1. Longitudinal changes in genomic coverage of vaccine antigenic variants

5.3.1.1. BAST

The prevalence of 4CMenB (Bexsero®) vaccine antigenic variants in meningococcal isolates can be estimated using genomic data, though no inference is made of protein expression or cross-reactivity (Brehony *et al.* 2016, Mowlaboccus *et al.* 2016, Rodrigues *et al.* 2018). The presence of BAST-1 antigenic variants in 1999 Carriage isolates was 7.0%, compared to 3.4% in 2014/15 Carriage isolates and 14.9% in IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.1220, 2014/15 Carriage vs IMD 14/15 p=0.0009) (Table 5-2). The presence of putatively cross-reactive variants (fHbp 4, 13, 14, 15, 37, 232, any NadA 1 or 2/3 family variants) in the 1999 Carriage isolates was

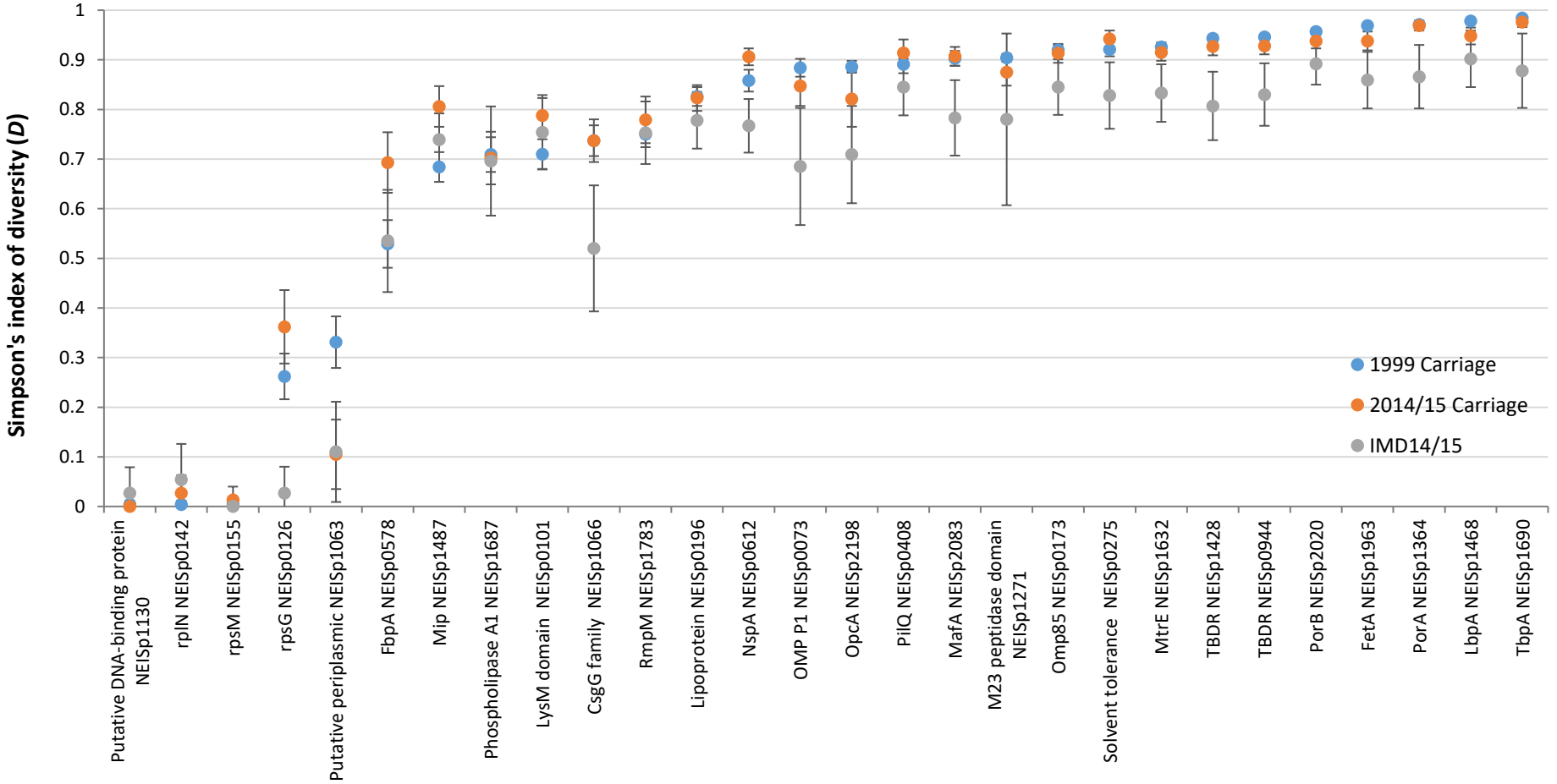


Figure 5-7 Diversity of Outer Membrane Vesicle peptide Typing (OMVT) scheme proteins amongst carriage and disease-causing meningococci.
Simpson's index of diversity (*D*) with 95% confidence intervals for the constituent proteins in the OMVT scheme and four cytoplasmic proteins; NEISp0126, NEISp0142, NEISp0155 and NEISp1130. Simpson's index is interpreted with values closer to 1 indicating greater diversity.

32.5%, compared to 36.2% in 2014/15 Carriage isolates and 63.5% in IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.43, 2014/15 Carriage vs IMD 14/15 p=0.0002). The proportion of isolates with >1 antigens matching BAST-1 or putative cross-reactive variants in 1999 Carriage isolates was 3.6%, compared to 1.3% in 2014/15 Carriage isolates and 12.1% in IMD 14/15 isolates (1999 Carriage vs 2014/15 Carriage p=0.21, 2014/15 Carriage vs IMD 14/15 p=0.001).

5.3.1.2. OMV peptides

Vaccine-induced antibodies from the OMV components may interact with carried meningococci in the oropharynx, resulting in clearance of carriage. To estimate the extent of matching of OMVT peptides to the vaccine variants in carriage isolates, isolates were scored out of 24 for each locus that matched OMVT-1, which profiles MeNZB™ or Bexsero®. The majority of isolates in both 1999 Carriage and 2014/15 Carriage had 0-4 matches out of the 24 loci, 327/498 (65.7%) and 109/149 (73.2%) (Figure 5-8). The peptides that account for these matches include: outer membrane protein P1 (NEISp0073), peptidoglycan-binding LysM domain protein (NEISp0101), FbpA (NEISp0578), NspA (NEISp0612), putative periplasmic protein (NEISp1063), CsgG family protein (NEISp1066), macrophage infectivity potentiator (NEISp1487) and RmpM (NEISp1783). Only cc41/44 isolates had >9 matches, 30/498 (6.0%) in 1999 Carriage and 5/149 (3.4%) in 2014/15 Carriage.

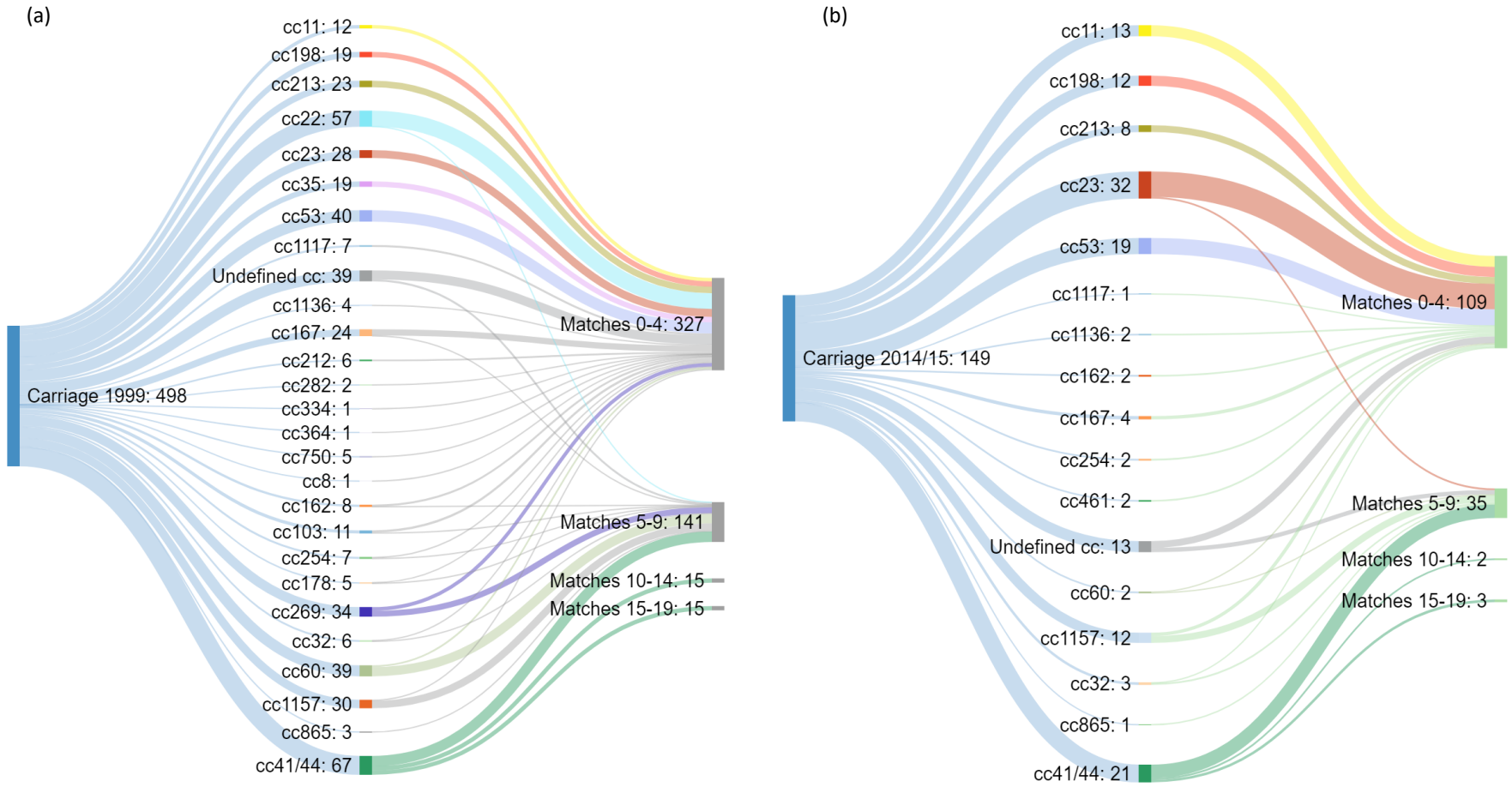


Figure 5-8 Distribution of Oxfordshire carriage isolates from 1999 and 2014/15 that match the peptide variants in the MeNZB™ vaccine.
The proportion of isolates that have protein variants matching to the MeNZB™ vaccine used in Bexsero® which has the OMVT-1 profile in (a) 1999 and (b) 2014/15. The total number of isolates in each collection is shown on the left, which are then stratified by clonal complex (cc). These then show the number of isolates that have 0-4 matches, 5-9 matches, 10-14 matches and 15-19 matches to MeNZB™. Undefined cc includes isolates with an incomplete multilocus sequence type (MLST) profile or a sequence type not assigned to a cc.

5.4. Discussion

As *N. meningitidis* is a key constituent of the human oropharyngeal microbiota, it is important to understand the distribution and diversity of the potential vaccine antigens in carriage isolates, as this has implications with the increasing use of OMV and peptide-based vaccines worldwide. Genomic typing methods were applied to meningococcal WGS to analyse the BAST and OMVT peptides over a 15-year period in Oxfordshire. To interpret these findings, it is necessary to consider the changes to vaccination policy in the UK over this time, the meningococcal C conjugate vaccine was introduced after the 1999 Carriage study for all ≤18 year olds. After the completion of the 2014/15 Carriage study, ACWY conjugate vaccine was introduced in August 2015 for adolescents and 4CMenB vaccine Bexsero® was introduced in September 2015 for infants (Ladhani *et al.* 2016). The overall carriage rate has fallen from 21.9% (1999) to 7.0% (2014/15) in Oxfordshire. This reflects an overall reduction in carriage in UK adolescents, who also reported less smoking, social interaction at public houses or clubs, and kissing than the cohort in 1999 (Maclennan *et al.* 2016). There have been major political and social changes over this period including; the national smoking ban introduced in the UK between 2004-06, increased use of mobile phones and social media, and more rigorous identity checks for admission to public houses and clubs which may have affected adolescent behaviour.

The epidemiology of IMD is dynamic, with secular changes in ccs even within this study timeframe (Rodrigues *et al.* 2018). It is notable that the prevalence of all ccs decreased over the 15-year period, except cc11 and cc23, but all major ccs were still represented, albeit by fewer isolates in 2014/15 (Figure 5-3). Thus, the changes in BASTs between 1999 and 2014/15 reflect the changes in ccs, with statistically significant reductions in BAST-349 (cc22), BAST-271 (cc60) and BAST-237 (cc1157). The antigenic variants in

Bexsero® came from cc32, cc8 and cc41/44, so with a decline in the incidence of these ccs, the resultant effect on BAST-1 matches and fHbp peptide 1, NHBA peptide 2 and PorA VR2 peptide 4 matches was not unexpected. The distribution of potential cross-reactive Bexsero® and Trumenba® antigenic variants in 1999 and 2014/15 has changed with potential implications for vaccinees. The fHbp peptide remains the most important when considering putative cross-reactivity, as PorA peptides have limited heterogeneic activity, *nadA* is absent in the majority of isolates and immunogenicity of NHBA antibodies remains unclear (Tappero *et al.* 1999, Partridge *et al.* 2017). For Bexsero®, 77.8% of cross-reactive peptides were in genogroup E, L or *cnl* isolates. Conversely, for Trumenba® 65.0% of cross-reactive peptides were in genogroup B, C, W, X, Y, or Z isolates, the capsular types that can cause invasive disease. If there were an impact on carried meningococci by vaccine-induced antibodies, then it would be preferable that strains with potential to cause disease were eliminated rather than those that may be providing a beneficial role within the oropharyngeal microbiota (Liu *et al.* 2015). To date, it remains unclear if meningococci possessing antigenic matches to the vaccine will be eliminated in vaccinated individuals, as protein expression was not assessed using genomic data in this study, nor the degree to which vaccine-induced antibodies will kill carried meningococci *in vivo*.

Expression of fHbp in 188 carriage strains from a French outbreak demonstrated that 30.3% did not have detectable levels of protein as measured by ELISA, despite presence of *fHbp* (Lemee *et al.* 2014). For those that expressed fHbp, the levels were noted to be lower than IMD isolates from the same cc. There is further evidence for fHbp and NHBA expression in carriage strains from adolescents in Spain, identified with the MATS assay, but the extent of bacterial killing with these levels of protein expression were not determined as the assay is not validated in unencapsulated or non-serogroup B isolates

(Abad *et al.* 2017). Owing to these limitations, a humanised hCEACAM1 mouse model has been used to study the effects of Bexsero® on carriage (Buckwalter *et al.* 2017). The vaccinated mice generated serum and nasal antibodies that prevented death in a disease challenge model but showed heterogeneous effects on carriage strains in a nasal challenge. Bexsero® prevented colonisation in nasal challenge with NZ98/254 (MeNZB™ strain, cc41/44), BuFa 02/03 (NadA matching strain), and three strains with MATS positivity to three antigens (fHbp, PorA, NHBA). However, for strains matching just one or two antigens to Bexsero®, murine immune responses were unable to prevent colonisation. Our study identified that, the proportion of carriage isolates with ≥1 exact or putatively cross-reactive matches to Bexsero® were 11.1% in 1999 and 3.7% in 2014/15, with the majority of carriage isolates only matching 0-4 OMV peptide variants with Bexsero® (65.7% in 1999, 73.2% in 2014/15). The only isolates that had 15-19 matches to OMV protein variants were cc41/44 (3.0% 1999, 2.0% 2014/15), perhaps a contributing factor in the eradication of carriage of NZ98/254. As antibody titres do not appear to correlate with clearance of carriage in the mouse model, alternative mechanisms involving synergistic activity or minor antigens may be important (Buckwalter *et al.* 2017). *In vitro* models of human meningococcal carriage proposed that protein-based vaccine prevention of carriage was not mediated through same mechanism as for polysaccharide conjugate vaccines, whereby antibodies prevented meningococcal capsular shedding, inhibiting colonisation on the epithelial surface (Vianzon *et al.* 2017).

It has previously been shown that combinations of antigenic variants, OMVTs and clusters, form non-overlapping association with BASTs that are likely to persist through time and exist due to host immune selection (see Chapter 4). Previous work has also shown that both PorA and FetA types form non-overlapping associations in carriage populations, with dominant combinations stable through time despite recombination

(Buckee *et al.* 2010). By studying two carriage collections, separated by 15 years, from the same geographic location, it is possible to observe the persistence of some BASTs and OMVT clusters. Of these OMVT cluster-4003 appears to be restricted to carriage populations as it has not been found in IMD isolates to date. Of the four OMVT clusters that persisted in both carriage and IMD since 1999, they were associated with cc11, cc41/44, cc23 and cc1157. OMVTs are less temporally stable than OMVT clusters. There were only four OMVTs found in more than one dataset, 2014/15 Carriage and IMD 2014/15 which is unsurprising given the number of loci and potential for any non-synonymous single nucleotide change over 15 years. The reduction in carriage rate, not expected prior to the study, meant that there were fewer meningococci identified in the later study. This may have impacted on our ability to detect rare variants and types, which occur at low frequency in carriage. This could be addressed by returning to the archived stores and performing WGS on the national collection from 1999 for comparison to national collection of carried meningococcal WGS from 2014/15.

Carriage populations have been described as more diverse than disease (Caugant and Maiden 2009). With respect to BASTs, fHbp, NHBA, PorA VR2, and OMP variants with Simpson's index >0.9, there was no overall difference in diversity between carriage collections from comparable groups in 1999 and 2014/15, but their diversity was greater than in IMD isolates from 2014/15. This is because the disease isolates are a subset of the entire carriage population. It is understood that not all carried meningococci have the propensity to cause disease, only those possessing virulence factors that constitute the hyperinvasive lineages (Caugant and Maiden 2009). Therefore, the carriage population contains many meningococcal lineages that have adapted to live symbiotically in the human, tolerated by the host immune system and other members of the oropharyngeal microbiota.

This study was limited to Oxfordshire, one county in the UK, due to the practical constraints of WGS of isolates. However, the two carriage studies were performed using the same methodology, sampling sites and sizes. Further, non-meningococcal *Neisseria* species were not analysed and it is yet to be determined whether other *Neisseria* species will be affected by protein-based vaccines.

5.1. Conclusion

The use of WGS and bioinformatics platforms allow large-scale, systematic analysis of representative isolate collections. In order to make informed decisions about vaccine implementation to disrupt transmission and generate herd immunity, strong evidence will be required, likely from multiple *in vivo*, *in vitro*, and *in silico* sources. Here, the changes in distribution and diversity of vaccine antigenic variants and repertoires amongst carried meningococci have been described between 1999 and 2014/15 with comparison of carriage and disease isolates, with potentially important implications for vaccination.

Chapter 6

Discussion

6.1. General discussion of research

From the perspective of a paediatrician specialising in infectious diseases, the development of safe and efficacious vaccines and their widespread use, has been one of the foremost scientific and public health successes of the last century. As medical physicians, we strive to use the best evidence to inform practice for the individual patient and for the wider public, despite the constraints of national health systems funded through the public purse. It is to this end that I embarked upon this area of research, at a time when the 4CMenB vaccine, Bexsero®, was introduced into the UK immunisation schedule. There were a number of outstanding questions about the genetic diversity of the proteins contained in the vaccine, the methods by which to analyse these, the relevance and implications of diversity for those being vaccinated and those unvaccinated. The work in this thesis begins to address these points.

The increasing availability and affordability of NGS technology means that it is possible to use genomic epidemiology to address population-based questions from outbreak tracking to disease surveillance (Gardy and Loman 2017). The challenges ahead involve developing the bioinformatic tools to support the analysis of this data and translate the findings into meaningful, interpretable results for those on the frontline of public health, microbiology, or clinical medicine. In order to develop genomic analysis tools that have longevity, it was necessary to use infrastructure and methodology that has a sound basis. The BAST scheme, in principle, was based on the same methodology as MLST, whereby every unique sequence receives a new variant identifier (Maiden *et al.* 1998). Each unique

allelic profile receives a different BAST number, akin to ST in MLST. This stable and portable nomenclature described in Chapter 2, is publicly-available on the PubMLST.org *Neisseria* website, available globally for users to quickly and reproducibly analyse regional isolates. To date, it has been used to assess Australian, European and Chinese meningococcal isolate collections, identifying region-specific diversity and contributing valuable information to assist in public health decisions about vaccine use (Mowlaboccus *et al.* 2016, Mowlaboccus *et al.* 2017, Bratcher *et al.* 2018, Chen *et al.* 2018).

In the UK, using a comprehensive, national dataset of meningococcal WGS, the non-random distribution of BASTs has been demonstrated, with few BASTs occurring at high frequency and a large number occurring only once. The strong, non-overlapping association between BAST and cc identified in this study, began to refute the view that MLST was unable to predict the genotype of vaccine antigens in individual isolates (Donnelly *et al.* 2010, Vogel *et al.* 2013). Although individual variants of the vaccine antigens were found across ccs (Cramer's V=0.489-0.716), the combination of the four antigens, BASTs, were strongly associated with cc (Cramer's V=0.946). This structuring of meningococci became clearer following work completed in Chapter 4. Here, 24 OMPs were typed using the same principles applied to BAST, but on a larger scale. Clustering the OMVTs at ≥18 loci to produce OMVT clusters, revealed discrete antigenic/protein repertoires which were preserved and propagated, in association with BASTs, ccs, and genogroups. The likely influences on this structuring include interstrain competition and host immune selection, which have previously been explored and mathematically modelled for other important surface proteins, PorA and FetA (Gupta *et al.* 1996, Buckee *et al.* 2008, Buckee *et al.* 2011). The stability of these antigenic/protein repertoires through time remains as yet undetermined, with insufficient sample size in the longitudinal carriage study to robustly address this point. Understanding the population

structure of these subcapsular proteins and OMPs has important implications when selecting antigens and variants for inclusion in existing or novel protein-based vaccines, for both meningococci and other *Neisseria* species, including gonococci. Further, once potential antigens have been identified, the OMVT scheme can be used to analyse amino acid sequence variability, delineating potential novel epitopes by accessing the PubMLST database of alleleic variants present amongst the >16,800 *Neisseria* WGS. After a retrospective case-control study in New Zealand demonstrated 31% (95% CI 21-39) efficacy of the OMV vaccine MeNZB™ against gonorrhoea, there was renewed interest in studying the OMV of *Neisseria gonorrhoeae* (Petousis-Harris et al. 2017). This was the first time the beneficial effects of an OMV vaccine had been seen on a mucosal infection, gonorrhoea, and the mechanism of protection and suitable gonococcal vaccine candidates were being investigated at the time of writing, applying the novel OMVT scheme to *N. gonorrhoeae*.

The potential coverage of protein-based meningococcal vaccine antigens was a timely and pertinent issue when the Bexsero® programme was implemented nationally in 2015. The MATS estimates of 73% were from an isolate collection from 2007-08 but as seen in the surveillance study from Chapter 3, there were secular changes in cc even within a period of six years, 2010-2016 (Vogel *et al.* 2013). The revised MATS estimate of 66% coverage for isolates from 2014/15 was much more closely aligned with both the genomic estimates of 58.8-60.8% presence of exact and putative cross-reactive antigen matches, but also the genotype-phenotype modelling estimate of 66.1% of UK isolates from 2010/11-2013/14 (see Chapter 2) (Parikh *et al.* 2017). As the Bexsero® vaccine antigens are not exclusive to serogroup B isolates, non-serogroup B meningococci from the UK were studied. There are exact and putatively cross-reactive antigens present in these isolates, in particular W:cc11, suggesting some protection could be afforded to these

strains, with a small study providing serological support for this finding (Ladhani *et al.* 2016). The interpretation of BAST for individual meningococcal isolates in public health laboratories was being developed at the time of writing, with Health Protection Scotland incorporating BAST into its laboratory meningococcal database to help inform public health decisions.

When using genomic coverage estimates, it is important to emphasise the limitations of this method, which establishes the presence of exact and putative cross-reactive gene variants, with no inference of protein expression or cross-protection. To date, the most extensively used method for assessing Bexsero® antigen cross-reactivity was the MATS assay, and therefore this was used to determine which fHbp variants were considered putatively cross-reactive. For NadA, variants 1 and 2/3 were considered putatively cross-reactive due to evidence that *in vitro* conditions may result in reduced protein expression, thereby underestimating its effect *in vivo* (Fagnocchi *et al.* 2013). There was insufficient data to support inclusion of any other NHBA or PorA variants. As for the assessment of protein expression, typing methods were being developed to correlate genome sequences of intergenic regions with the degree of protein expression, though to date, this has only been performed for fHbp with serogroup B, disease isolates (Biagini *et al.* 2016, Cayrou *et al.* 2018).

It was evident from the pre-vaccine surveillance study of the individual antigens (see Chapter 3), that most of the potential coverage is not based on exact antigenic matches to BAST-1. This suggests that the protection being observed in infants is either (i) through their ability to generate cross-protective antibodies, against broadly similar antigenic variants to those contained in the vaccine, or (ii) through other mechanisms such as minor antigens or synergistic effects that are not being measured at present. The evidence for infants' ability to generate antibodies to heterologous vaccine antigens, fHbp and PorA

at least, is extremely limited (Tappero *et al.* 1999, Brunelli *et al.* 2011). The investigation of alternative mechanisms of protection led to the development of the OMVT scheme to better understand the variation of the OMPs in Bexsero® to which the vaccinee is exposed (see Chapter 4). There was variable diversity in the OMPs catalogued, with some OMPs highly conserved across ccs, and others comparatively more diverse. Many of these more diverse proteins have been investigated a potential vaccine candidates such as FetA, TbpB, LbpA, and NspA, but none have been developed into a marketed vaccine. However, given the success of the OMV vaccines against their respective epidemics, and the increased immunogenicity observed with the OMV component added to the recombinant antigens in Bexsero®, it may be that the interaction between proteins is more immunogenic than a single vaccine antigen (Findlow *et al.* 2010). Therefore, theoretical vaccine formulations were proposed, using OMVTs that occur in the most frequently occurring ccs, based on regional surveillance. A dynamic vaccine strategy, such as that for the influenza vaccine, could be advantageous to address the degree of diversity in OMPs, though clearly the practicalities of manufacture and regulation would need careful consideration, as well as the institution of robust, ongoing surveillance systems to identify novel variants and secular changes.

For a comprehensive study of meningococcal vaccine antigens, it was important to study carriage isolates. The impact of protein-based vaccines on eradication of oropharyngeal carriage remains unclear, but as the vaccine antigens are found across all studied meningococci, there is potential for the vaccine-induced antibodies to interact with both pathogenic and non-pathogenic meningococci. This longitudinal analysis shows that carriage has reduced from 21.9% to 7.0% in Oxfordshire, reflective of the UK as a whole, but the diversity of the overall 2014/15 carriage population was not different to the 1999 carriage population, for fHbp, NHBA, PorA, or BAST. However, carriage isolates were

more diverse than disease isolates in keeping with previous studies (Jolley *et al.* 2000). There were secular changes in the frequency distribution of ccs in carriage isolates, but the population of meningococci remained similar in both years, with fewer isolates representing major ccs in 2014/15. There were BASTs and OMVT clusters that persisted through the 15 years, some of which were only associated with carriage, but others that were likely not identified due to the limitations of sampling. Carriage isolates universally possessed fHbp, NHBA and PorA, with 36.2% matching Bexsero® and 67.1% matching Trumenba® variants (exact or putatively cross-reactive). More reassuringly, most carriage isolates 65.7% in 1999 and 73.2% in 2014/15 had ≤4 matches to OMVT-1. However, the effect of vaccine-induced antibodies on these isolates is yet to be established *in vivo* or *in vitro*, but carriage studies investigating the impact of both protein-based vaccines on carriage in adolescents were in progress at the time of writing.

This body of work reflects a multi-disciplinary approach to addressing a public health question. The intellectual input of microbiologists, geneticists, bioinformaticians, statisticians, and clinicians has led to the development of genomic analysis platforms that are accessible to those developing, regulating, and delivering vaccines globally.

References

Abad, R., Medina, V., Farinas, M. D. C., Martinez-Martinez, L., Bambini, S., Dari, A., Medini, D., Pizza, M. and Vazquez, J. A. (2017). Potential impact of the 4CMenB vaccine on oropharyngeal carriage of *Neisseria meningitidis*. *Journal of Infection* 75(6): 511-520.

Abad, R., Medina, V., Stella, M., Boccadifuoco, G., Comanducci, M., Bambini, S., Muzzi, A. and Vazquez, J. A. (2016). Predicted Strain Coverage of a New Meningococcal Multicomponent Vaccine (4CMenB) in Spain: Analysis of the Differences with Other European Countries. *PLoS ONE* 11(3): e0150721.

Achtman, M. (1995). Epidemic spread and antigenic variability of *Neisseria meningitidis*. *Trends in Microbiology* 3(5): 186-191.

Achtman, M. (2004). Population structure of pathogenic bacteria revisited. *International Journal of Medical Microbiology* 294(2-3): 67-73.

Ali, A., Jafri, R. Z., Messonnier, N., Tevi-Benissan, C., Durrheim, D., Eskola, J., Fermon, F., Klugman, K. P., *et al.* (2014). Global practices of meningococcal vaccine use and impact on invasive disease. *Pathogens and Global Health* 108(1): 11-20.

Artenstein, M. S. (1975). Control of Meningococcal Meningitis with Meningococcal Vaccines. *Yale Journal of Biology and Medicine* 48(3): 197-200.

Bambini, S., De Chiara, M., Muzzi, A., Mora, M., Lucidarme, J., Brehony, C., Borrow, R., Masignani, V., *et al.* (2014). *Neisseria* adhesin A variation and revised nomenclature scheme. *Clinical Vaccine Immunology* 21(7): 966-971.

Bennett, J. S., Watkins, E. R., Jolley, K. A., Harrison, O. B. and Maiden, M. C. (2014). Identifying *Neisseria* species using the 50S ribosomal protein L6 (*rplF*) gene. *Journal of Clinical Microbiology* 52(5): 1375-1381.

Biagini, M., Spinsanti, M., De Angelis, G., Tomei, S., Ferlenghi, I., Scarselli, M., Rigat, F., Messuti, N., *et al.* (2016). Expression of factor H binding protein in meningococcal strains can vary at least 15-fold and is genetically determined. *Proceedings of the National Academy of Sciences of the United States of America* 113(10): 2714-2719.

Bjune, G., Høiby, E. A., Grønnesby, J. K., Arnesen, O., Fredriksen, J. H., Halstensen, A., Holten, E., Lindbak, A. K., *et al.* (1991). Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 338(8775): 1093-1096.

Borrow, R., Caugant, D. A., Ceyhan, M., Christensen, H., Dinleyici, E. C., Findlow, J., Glennie, L., Von Gottberg, A., *et al.* (2017). Meningococcal disease in the Middle East and Africa: Findings and updates from the Global Meningococcal Initiative. *Journal of Infection* 75(1): 1-11.

Borrow, R., Lee, J. S., Vazquez, J. A., Enwere, G., Taha, M. K., Kamiya, H., Kim, H. M., Jo, D. S. and Global Meningococcal, I. (2016). Meningococcal disease in the Asia-Pacific region: Findings and recommendations from the Global Meningococcal Initiative. *Vaccine* 34(48): 5855-5862.

Bratcher, H. B., Bennett, J. S. and Maiden, M. C. J. (2012). Evolutionary and genomic insights into meningococcal biology. *Future Microbiology* 7(7): 873-885.

Bratcher, H. B., Brehony, C., Heuberger, S., Pieridou-Bagatzouni, D., Krizova, P., Hoffmann, S., Toropainen, M., Taha, M. K., *et al.* (2018). Establishment of the European meningococcal strain collection genome library (EMSC-GL) for the 2011 to 2012 epidemiological year. *Eurosurveillance* 23(20).

Bratcher, H. B., Corton, C., Jolley, K. A., Parkhill, J. and Maiden, M. C. (2014). A gene-by-gene population genomics platform: *de novo* assembly, annotation and genealogical analysis of 108 representative *Neisseria meningitidis* genomes. *BMC Genomics* 15: 1138.

Bredius, R. G., Derkx, B. H., Fijen, C. A., de Wit, T. P., de Haas, M., Weening, R. S., van de Winkel, J. G. and Out, T. A. (1994). Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. *Journal of Infectious Diseases* 170(4): 848-853.

Brehony, C., Jolley, K. A. and Maiden, M. C. (2007). Multilocus sequence typing for global surveillance of meningococcal disease. *FEMS Microbiology Reviews* 31(1): 15-26.

Brehony, C., Rodrigues, C. M., Borrow, R., Smith, A., Cunney, R., Moxon, E. R. and Maiden, M. C. (2016). Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation. *Vaccine* 34(39): 4690-4697.

Brehony, C., Wilson, D. J. and Maiden, M. C. (2009). Variation of the factor H-binding protein of *Neisseria meningitidis*. *Microbiology* 155: 4155-4169.

Brunelli, B., Del Tordello, E., Palumbo, E., Biolchi, A., Bambini, S., Comanducci, M., Muzzi, A., Pizza, M., *et al.* (2011). Influence of sequence variability on bactericidal activity sera induced by Factor H binding protein variant 1.1. *Vaccine* 29(5): 1072-1081.

Buckee, C. O., Gupta, S., Kriz, P., Maiden, M. C. J. and Jolley, K. A. (2010). Long-term evolution of antigen repertoires among carried meningococci. *Proceedings of the Royal Society B: Biological Sciences* 277(1688): 1635-1641.

Buckee, C. O., Jolley, K., Recker, M., Penman, B., Kriz, P., Gupta, S. and Maiden, M. C. (2008). Role of selection in the emergence of lineages and the evolution of virulence in *Neisseria meningitidis*. *Proceedings of the National Academy of Sciences of the United States of America* 105(39): 15082-15087.

Buckee, C. O., Recker, M., Watkins, E. R. and Gupta, S. (2011). Role of stochastic processes in maintaining discrete strain structure in antigenically diverse pathogen populations. *Proceedings of the National Academy of Sciences of the United States of America* 108(37): 15504-15509.

Buckwalter, C. M., Currie, E. G., Tsang, R. S. W. and Gray-Owen, S. D. (2017). Discordant Effects of Licensed Meningococcal Serogroup B Vaccination on Invasive Disease and Nasal Colonization in a Humanized Mouse Model. *Journal of Infectious Diseases* 215(10): 1590-1598.

Campbell, H., Parikh, S. R., Borrow, R., Kaczmarski, E., Ramsay, M. E. and Ladhani, S. N. (2016). Presentation with gastrointestinal symptoms and high case fatality associated with group W meningococcal disease (MenW) in teenagers, England, July 2015 to January 2016. *Eurosurveillance* 21(12).

Campbell, H., Saliba, V., Borrow, R., Ramsay, M. and Ladhani, S. N. (2015). Targeted vaccination of teenagers following continued rapid endemic expansion of a single meningococcal group W clone (sequence type 11 clonal complex), United Kingdom 2015. *Eurosurveillance* 20(28).

Caron, F., du Chatelet, I. P., Leroy, J. P., Ruckly, C., Blanchard, M., Bohic, N., Massy, N., Morer, I., *et al.* (2011). From tailor-made to ready-to-wear meningococcal B vaccines: longitudinal study of a clonal meningococcal B outbreak. *Lancet Infectious Diseases* 11(6): 455-463.

Cartwright, K. A. V., Stuart, J. M., Jones, D. M. and Noah, N. D. (1987). The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidemiology and Infection* 99(3): 591-601.

Caugant, D. A. (2008). Genetics and evolution of *Neisseria meningitidis*: Importance for the epidemiology of meningococcal disease. *Infection Genetetics and Evolution* 8(5): 558-565.

Caugant, D. A., Høiby, E. A., Rosenqvist, E., Frøholm, L. O. and Selander, R. K. (1992). Transmission of *Neisseria meningitidis* among asymptomatic military recruits and antibody analysis. *Epidemiology and Infection* 109(2): 241-253.

Caugant, D. A. and Maiden, M. C. (2009). Meningococcal carriage and disease - population biology and evolution. *Vaccine* 27(Suppl 2): B64-70.

Cayrou, C., Akinduko, A. A., Mirkes, E. M., Lucidarme, J., Clark, S. A., Green, L. R., Cooper, H. J., Morrissey, J., *et al.* (2018). Clustered intergenic region sequences as predictors of factor H Binding Protein expression patterns and for assessing *Neisseria meningitidis* strain coverage by meningococcal vaccines. *PLoS ONE* 13(5): e0197186.

Centers for Disease Control and Prevention (2012). Comparison of meningococcal disease surveillance systems - United States, 2005-2008. *Morbidity and mortality weekly report* 61(17): 306-308.

Centers for Disease Control and Prevention (2015). *Epidemiology and Prevention of Vaccine-Preventable Diseases*, Washington D.C. Public Health Foundation.

Chen, M., Rodrigues, C. M. C., Harrison, O. B., Zhang, C., Tan, T., Chen, J., Zhang, X., Chen, M. and Maiden, M. C. J. (2018). Invasive meningococcal disease in Shanghai, China from 1950 to 2016: implications for serogroup B vaccine implementation. *Scientific Reports* 8(1): 12334.

Chokshi, D. A., Parker, M. and Kwiatkowski, D. P. (2006). Data sharing and intellectual property in a genomic epidemiology network: policies for large-scale research collaboration. *Bulletin of the World Health Organization* 84(5): 382-387.

Christensen, H., May, M., Bowen, L., Hickman, M. and Trotter, C. L. (2010). Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infectious Diseases* 10(12): 853-861.

Christodoulides, M. and Heckels, J. (2017). Novel approaches to *Neisseria meningitidis* vaccine design. *Pathogens and Disease* 75(3).

Coen, P. G., Cartwright, K. and Stuart, J. (2000). Mathematical modelling of infection and disease due to *Neisseria meningitidis* and *Neisseria lactamica*. *International Journal of Epidemiology* 29(1): 180-188.

Croucher, N. J., Finkelstein, J. A., Pelton, S. I., Mitchell, P. K., Lee, G. M., Parkhill, J., Bentley, S. D., Hanage, W. P. and Lipsitch, M. (2013). Population genomics of post-vaccine changes in pneumococcal epidemiology. *Nature Genetics* 45(6): 656-663.

Daugla, D. M., Gami, J. P., Gamougam, K., Naibei, N., Mbainadji, L., Narbe, M., Toralta, J., Kodbesse, B., *et al.* (2014). Effect of a serogroup A meningococcal conjugate vaccine (PsA-TT) on serogroup A meningococcal meningitis and carriage in Chad: a community study [corrected]. *Lancet* 383(9911): 40-47.

Davenne, T. and McShane, H. (2016). Why don't we have an effective tuberculosis vaccine yet? *Expert review of vaccines* 15(8): 1009-1013.

Davila, S., Wright, V. J., Khor, C. C., Sim, K. S., Binder, A., Breunis, W. B., Inwald, D., Nadel, S., *et al.* (2010). Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nature Genetics* 42(9): 772-776.

de Steenhuijsen Piters, W. A., Sanders, E. A. and Bogaert, D. (2015). The role of the local microbial ecosystem in respiratory health and disease. *Philosophical Transactions of the Royal Society B Biological Sciences* 370(1675): 20140294.

Deasy, A. M., Guccione, E., Dale, A. P., Andrews, N., Evans, C. M., Bennett, J. S., Bratcher, H. B., Maiden, M. C., *et al.* (2015). Nasal Inoculation of the Commensal *Neisseria lactamica* Inhibits Carriage of *Neisseria meningitidis* by Young Adults: A Controlled Human Infection Study. *Clinical Infectious Diseases* 60(10): 1512-1520.

Delbos, V., Lemee, L., Benichou, J., Berthelot, G., Deghmane, A. E., Leroy, J. P., Houivet, E., Hong, E., *et al.* (2013). Impact of MenBvac, an outer membrane vesicle (OMV) vaccine, on the meningococcal carriage. *Vaccine* 31(40): 4416-4420.

Diallo, K., Trotter, C., Timbine, Y., Tamboura, B., Sow, S. O., Issaka, B., Dano, I. D., Collard, J. M., *et al.* (2016). Pharyngeal carriage of *Neisseria* species in the African meningitis belt. *Journal of Infection* 72(6): 667-677.

Didelot, X. and Wilson, D. J. (2015). ClonalFrameML: Efficient Inference of Recombination in Whole Bacterial Genomes. *PLoS Computational Biology* 11(2): e1004041.

Diomande, F. V. K., Djingarey, M. H., Daugla, D. M., Novak, R. T., Kristiansen, P. A., Collard, J. M., Gamougam, K., Kandolo, D., *et al.* (2015). Public Health Impact After the Introduction of PsA-TT: The First 4 Years. *Clinical Infectious Diseases* 61: S467-S472.

Donald, R. G., Hawkins, J. C., Hao, L., Liberator, P., Jones, T. R., Harris, S. L., Perez, J. L., Eiden, J. J., *et al.* (2017). Meningococcal serogroup B vaccines: Estimating breadth of coverage. *Human Vaccines & Immunotherapeutics* 13(2): 255-265.

Donnelly, J., Medini, D., Boccadifuoco, G., Biolchi, A., Ward, J., Frasch, C., Moxon, E. R., Stella, M., *et al.* (2010). Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proceedings of the National Academy of Sciences of the United States of America* 107(45): 19490-19495.

Drew, R. J., Ó Maoldomhnaigh, C., Gavin, P. J., O'Sullivan, N., Butler, K. M. and Cafferkey, M. (2012). The impact of meningococcal polymerase chain reaction testing on laboratory confirmation of invasive meningococcal disease. *Pediatric Infectious Diseases Journal* 31(3): 316-318.

Enright, M. C. and Spratt, B. G. (1999). Multilocus sequence typing. *Trends in Microbiology* 7(12): 482-487.

Excoffier, L. and Lischer, H. E. L. (2010). ARLEQUIN (version 3.5): Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

Excoffier, L., Smouse, P. E. and Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131(2): 479-491.

Fagnocchi, L., Biolchi, A., Ferlicca, F., Boccadifuoco, G., Brunelli, B., Brier, S., Norais, N., Chiarot, E., *et al.* (2013). Transcriptional regulation of the nadA gene in Neisseria meningitidis impacts the prediction of coverage of a multicomponent meningococcal serogroup B vaccine. *Infection and Immunity* 81(2): 560-569.

Faria, N. R., Sabino, E. C., Nunes, M. R., Alcantara, L. C., Loman, N. J. and Pybus, O. G. (2016). Mobile real-time surveillance of Zika virus in Brazil. *Genome Medicine* 8(1): 97.

Feavers, I. M. and Maiden, M. C. (2017). Recent progress in the prevention of serogroup B meningococcal disease. *Clinical and Vaccine Immunology* 24(5): e00566-00516.

Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P. and Spratt, B. G. (2004). eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology* 186(5): 1518-1530.

Findlow, J., Borrow, R., Snape, M. D., Dawson, T., Holland, A., John, T. M., Evans, A., Telford, K. L., *et al.* (2010). Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant Meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clinical Infectious Diseases.* 51(10): 1127-1137.

Finne, J., Leinonen, M. and Makela, P. H. (1983). Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* 2(8346): 355-357.

Fletcher, L. D., Bernfield, L., Barniak, V., Farley, J. E., Howell, A., Knauf, M., Ooi, P., Smith, R. P., *et al.* (2004). Vaccine potential of the *Neisseria meningitidis* 2086 lipoprotein. *Infection and Immunity* 72(4): 2088-2100.

Fournier, P. E., Dubourg, G. and Raoult, D. (2014). Clinical detection and characterization of bacterial pathogens in the genomics era. *Genome Medicine* 6(11): 114.

Francisco, A. P., Vaz, C., Monteiro, P. T., Melo-Cristino, J., Ramirez, M. and Carrico, J. A. (2012). PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. *BMC Bioinformatics* 13.

Galloway, Y., Stehr-Green, P., McNicholas, A. and O'Hallahan, J. (2009). Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. *International Journal of Epidemiology* 38(2): 413-418.

Gardy, J. L. and Loman, N. J. (2017). Towards a genomics-informed, real-time, global pathogen surveillance system. *Nature Reviews Genetics* 19(1): 9-20.

Gaschignard, J., Levy, C., Romain, O., Cohen, R., Bingen, E., Aujard, Y. and Boileau, P. (2011). Neonatal Bacterial Meningitis: 444 Cases in 7 Years. *Pediatric Infectious Diseases Journal* 30(3): 212-217.

Giuliani, M. M., Adu-Bobie, J., Comanducci, M., Arico, B., Savino, S., Santini, L., Brunelli, B., Bambini, S., *et al.* (2006). A universal vaccine for serogroup B meningococcus. *Proceedings of the National Academy of Sciences of the United States of America* 103(29): 10834-10839.

Glover, J. A. (1918). Observations on the Meningococcus Carrier-Rate in relation to density of population in Sleeping Quarters. *The Journal of Hygiene* 17(4): 367-379.

Gold, R. and Artenstein, M. S. (1971). Meningococcal infections. 2. Field trial of group C meningococcal polysaccharide vaccine in 1969-70. *Bulletin of the World Health Organisation* 45(3): 279-282.

Gold, R., Goldschneider, I., Lepow, M. L., Draper, T. F. and Randolph, M. (1978). Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. *Journal of Infectious Diseases* 137(2): 112-121.

Goldschneider, I., Gotschlich, E. C. and Artenstein, M. S. (1969). Human Immunity to the Meningococcus. I. The Role of Humoral Antibodies. *Journal of Experimental Medicine* 129: 1307-1326.

Goldschneider, I., Gotschlich, E. C. and Artenstein, M. S. (1969). Human Immunity to the Meningococcus. II. Development of Natural Immunity. *Journal of Experimental Medicine* 129: 1327-1348.

Gordon, D. M., Mcgovern, T. W., Krzych, U., Cohen, J. C., Schneider, I., Lachance, R., Heppner, D. G., Yuan, G., *et al.* (1995). Safety, Immunogenicity, and Efficacy of a Recombinantly Produced Plasmodium-Falciparum Circumsporozoite-Protein Hepatitis-B Surface-Antigen Subunit Vaccine. *Journal of Infectious Diseases* 171(6): 1576-1585.

Gossger, N., Snape, M. D., Yu, L. M., Finn, A., Bona, G., Esposito, S., Principi, N., Diez-Domingo, J., *et al.* (2012). Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA* 307(6): 573-582.

Gotschlich, E. C., Goldschneider, I. and Artenstein, M. S. (1969). Human immunity to the meningococcus IV. Immunogenicity of group A and group C meningococcal polysaccharides. *Journal of Experimental Medicine* 129: 1367-1384.

Greenwood, B. (2007). The changing face of meningococcal disease in West Africa. *Epidemiology and Infection* 135(5): 703-705.

Grundmann, H., Hori, S. and Tanner, G. (2001). Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *Journal of Clinical Microbiology* 39(11): 4190-4192.

Gupta, S. and Anderson, R. M. (1999). Population structure of pathogens: the role of immune selection. *Parasitology Today* 15(12): 497-501.

Gupta, S. and Maiden, M. C. J. (2001). Exploring the evolution of diversity in pathogen populations. *Trends in Microbiology* 9(4): 181-192.

Gupta, S., Maiden, M. C. J., Feavers, I. M., Nee, S., May, R. M. and Anderson, R. M. (1996). The maintenance of strain structure in populations of recombining infectious agents. *Nature Medicine* 2(4): 437-442.

Harris, S. L., Donald, R. G., Hawkins, J. C., Tan, C., O'Neill, R., McNeil, L. K., Perez, J. L., Anderson, A. S., *et al.* (2017). Neisseria meningitidis Serogroup B Vaccine, Bivalent rLP2086, Induces Broad Serum Bactericidal Activity Against Diverse Invasive Disease Strains Including Outbreak Strains. *Pediatric Infectious Diseases Journal* 36(2): 216-223.

Harrison, O. B., Claus, H., Jiang, Y., Bennett, J. S., Bratcher, H. B., Jolley, K. A., Corton, C., Care, R., *et al.* (2013). Description and Nomenclature of Neisseria meningitidis Capsule Locus. *Emerging Infectious Diseases* 19(4): 566-573.

Heinsbroek, E., Ladhani, S., Gray, S., Guiver, M., Kaczmarski, E., Borrow, R. and Ramsay, M. (2013). Added value of PCR-testing for confirmation of invasive meningococcal disease in England. *Journal of Infection* 67(5): 385-390.

Hill, D. M. C., Lucidarme, J., Gray, S. J., Newbold, L. S., Ure, R., Brehony, C., Harrison, O. B., Bray, J. E., *et al.* (2015). Genomic epidemiology of age-associated meningococcal lineages in national surveillance: an observational cohort study. *Lancet Infectious Diseases* 15(12): 1420-1428.

Holmes, J. D., Martin, D., Ramsay, C., Ypma, E. and Oster, P. (2008). Combined administration of serogroup B meningococcal vaccine and conjugated serogroup C meningococcal vaccine is safe and immunogenic in college students. *Epidemiology and Infection* 136(6): 790-799.

Holst, J., Aaberge, I., Oster, P. and Lennon, D. (2003). A 'tailor made' vaccine trialled as part of public health response to group B meningococcal epidemic in New Zealand. *Eurosurveillance* 7(30).

Holst, J., Martin, D., Arnold, R., Huergo, C. C., Oster, P., O'Hallahan, J. and Rosenqvist, E. (2009). Properties and clinical performance of vaccines containing outer membrane vesicles from *Neisseria meningitidis*. *Vaccine* 27 Suppl 2: B3-12.

Hudson, R. R. (1994). Analytical results concerning linkage disequilibrium in models with genetic transformation and conjugation. *Journal of Evolutionary Biology* 7: 535-548.

Hunter, P. R. and Gaston, M. A. (1988). Numerical index of discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology* 26: 2465-2466.

Jiang, H.-Q., Hoiseth, S. K., Harris, S. L., McNeil, L. K., Zhu, D., Tan, C., Scott, A. A., Alexander, K., *et al.* (2010). Broad vaccine coverage predicted for a bivalent recombinant factor H binding protein based vaccine to prevent serogroup B meningococcal disease. *Vaccine* 28(37): 6086-6093.

Jolley, K. A., Brehony, C. and Maiden, M. C. (2007). Molecular typing of meningococci: recommendations for target choice and nomenclature. *FEMS Microbiology Reviews* 31(1): 89-96.

Jolley, K. A., Feil, E. J., Chan, M. S. and Maiden, M. C. (2001). Sequence type analysis and recombinational tests (START). *Bioinformatics* 17(12): 1230-1231.

Jolley, K. A., Kalmusova, J., Feil, E. J., Gupta, S., Musilek, M., Kriz, P. and Maiden, M. C. (2000). Carried meningococci in the Czech Republic: a diverse recombining population. *Journal of Clinical Microbiology* 38(12): 4492-4498.

Jolley, K. A. and Maiden, M. C. (2010). BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 11(1): 595.

Jolley, K. A. and Maiden, M. C. (2013). Automated extraction of typing information for bacterial pathogens from whole genome sequence data: *Neisseria meningitidis* as an exemplar. *Eurosurveillance* 18(4): 20379.

Jolley, K. A. and Maiden, M. C. (2014). Using MLST to study bacterial variation: prospects in the genomic era. *Future Microbiology* 9: 623-630.

Kaaijk, P., van Straaten, I., van de Waterbeemd, B., Boot, E. P., Levels, L. M., van Dijken, H. H. and van den Dobbelsteen, G. P. (2013). Preclinical safety and immunogenicity evaluation of a nonavalent PorA native outer membrane vesicle vaccine against serogroup B meningococcal disease. *Vaccine* 31(7): 1065-1071.

Kaczmarski, E. (2015) Public Health England Meningococcal Reference Unit User Manual. from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/472708/MRU_User_Manual_FINAL.pdf

Kaczmarski, E. B., Ragunathan, P. L., Marsh, J., Gray, S. J. and Guiver, M. (1998). Creating a national service for the diagnosis of meningococcal disease by polymerase chain reaction. *Communicable Disease and Public Health* 1(1): 54-56.

Kasanmoentalib, E. S., Brouwer, M. C. and van de Beek, D. (2013). Update on bacterial meningitis: epidemiology, trials and genetic association studies. *Current Opinions in Neurology* 26(3): 282-288.

Kimura, A., Toneatto, D., Kleinschmidt, A., Wang, H. and Dull, P. (2011). Immunogenicity and safety of a multicomponent meningococcal serogroup B vaccine and a quadrivalent meningococcal CRM197 conjugate vaccine against serogroups A, C, W-135, and Y in adults who are at increased risk for occupational exposure to meningococcal isolates. *Clinical Vaccine Immunology* 18(3): 483-486.

Kristiansen, P. A., Ba, A. K., Ouedraogo, A. S., Sanou, I., Ouedraogo, R., Sangare, L., Diomande, F., Kandolo, D., *et al.* (2014). Persistent low carriage of serogroup A

Neisseria meningitidis two years after mass vaccination with the meningococcal conjugate vaccine, MenAfriVac. *BMC Infectious Diseases* 14: 663.

Ladhani, S. N., Beebeejaun, K., Lucidarme, J., Campbell, H., Gray, S., Kaczmarski, E., Ramsay, M. E. and Borrow, R. (2015). Increase in Endemic *Neisseria meningitidis* Capsular Group W Sequence Type 11 Complex Associated With Severe Invasive Disease in England and Wales. *Clinical Infectious Diseases* 60(4): 578-585.

Ladhani, S. N., Campbell, H., Parikh, S. R., Saliba, V., Borrow, R. and Ramsay, M. (2015). The introduction of the meningococcal B (MenB) vaccine (Bexsero®) into the national infant immunisation programme-New challenges for public health. *Journal of Infection* 71(6): 611-614.

Ladhani, S. N., Flood, J. S., Ramsay, M. E., Campbell, H., Gray, S. J., Kaczmarski, E. B., Mallard, R. H., Guiver, M., *et al.* (2012). Invasive meningococcal disease in England and Wales: implications for the introduction of new vaccines. *Vaccine* 30(24): 3710-3716.

Ladhani, S. N., Giuliani, M. M., Biolchi, A., Pizza, M., Beebeejaun, K., Lucidarme, J., Findlow, J., Ramsay, M. E. and Borrow, R. (2016). Effectiveness of Meningococcal B Vaccine against Endemic Hypervirulent Neisseria meningitidis W Strain, England. *Emerging Infectious Diseases* 22(2): 309-311.

Ladhani, S. N., Ramsay, M., Borrow, R., Riordan, A., Watson, J. M. and Pollard, A. J. (2016). Enter B and W: two new meningococcal vaccine programmes launched. *Archives of Disease in Childhood* 101(1): 91-95.

Lemee, L., Hong, E., Etienne, M., Deghmane, A. E., Delbos, V., Terrade, A., Berthelot, G., Caron, F. and Taha, M. K. (2014). Genetic diversity and levels of expression of factor H binding protein among carriage isolates of *Neisseria meningitidis*. *PLoS ONE* 9(9): e107240.

Letunic, I. and Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research* 44(W1): W242-245.

Lewis, L. A. and Ram, S. (2014). Meningococcal disease and the complement system. *Virulence* 5(1): 98-126.

Li, J., Shao, Z., Liu, G., Bai, X., Borrow, R., Chen, M., Guo, Q., Han, Y., *et al.* (2018). Meningococcal disease and control in China: Findings and updates from the Global Meningococcal Initiative (GMI). *Journal of Infection* 76(5): 429-437.

Liu, G., Tang, C. M. and Exley, R. M. (2015). Non-pathogenic *Neisseria*: members of an abundant, multi-habitat, diverse genus. *Microbiology* 161(7): 1297-1312.

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., Cousens, S., Mathers, C. and Black, R. E. (2015). Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 385(9966): 430-440.

Lucidarme, J., Hill, D. M., Bratcher, H. B., Gray, S. J., du Plessis, M., Tsang, R. S., Vazquez, J. A., Taha, M. K., *et al.* (2015). Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. *Journal of Infection* 71(5): 544-552.

Lucidarme, J., Scott, K. J., Ure, R., Smith, A., Lindsay, D., Stenmark, B., Jacobsson, S., Fredlund, H., *et al.* (2016). An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015. *Eurosurveillance* 21(45).

Lujan, E., Partridge, E., Giuntini, S., Ram, S. and Granoff, D. M. (2017). Breadth and Duration of Meningococcal Serum Bactericidal Activity in Health Care Workers and Microbiologists Immunized with the MenB-FHbp Vaccine. *Clinical and Vaccine Immunology* 24(8).

MacLennan, J., Kafatos, G., Neal, K., Andrews, N., Cameron, J. C., Roberts, R., Evans, M. R., Cann, K., *et al.* (2006). Social behavior and meningococcal carriage in British teenagers. *Emerging Infectious Diseases* 12(6): 950-957.

Maclennan, J. M., Maiden, M. C. J. and UK Meningococcal Carriage Group (2016). UKMENCAR4: A meningococcal carriage study in 21,000 teenagers to understand changing meningococcal epidemiology and evaluate National vaccination policy. *International Pathogenic Neisseria Conference*, Manchester, UK.

Maiden, M. C. (2006). Multilocus Sequence Typing of Bacteria. *Annual Review of Microbiology* 60: 561-588.

Maiden, M. C. (2013). The impact of protein-conjugate polysaccharide vaccines: an endgame for meningitis? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 368(1623): 20120147.

Maiden, M. C. and Harrison, O. B. (2016). The population and functional genomics of the *Neisseria* revealed with gene-by-gene approaches. *Journal of Clinical Microbiology* 54(8): 1949-1955.

Maiden, M. C., Ibarz-Pavon, A. B., Urwin, R., Gray, S. J., Andrews, N. J., Clarke, S. C., Walker, A. M., Evans, M. R., *et al.* (2008). Impact of Meningococcal Serogroup C Conjugate Vaccines on Carriage and Herd Immunity. *Journal of Infectious Diseases* 197(5): 737-743.

Maiden, M. C., Jansen van Rensburg, M. J., Bray, J. E., Earle, S. G., Ford, S. A., Jolley, K. A. and McCarthy, N. D. (2013). MLST revisited: the gene-by-gene approach to bacterial genomics. *Nature Reviews Microbiology* 11(10): 728-736.

Maiden, M. C. J. (2006). Multilocus sequence typing of bacteria. *Annual Review of Microbiology* 60: 561-588.

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., *et al.* (1998). Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America* 95(6): 3140-3145.

Marshall, H. S., McMillan, M., Koehler, A., Lawrence, A., MacLennan, J. M., Maiden, M. C. J., Ramsay, M., Ladhani, S. N., *et al.* (2018). B Part of It protocol: a cluster randomised controlled trial to assess the impact of 4CMenB vaccine on pharyngeal carriage of Neisseria meningitidis in adolescents. *BMJ Open* 8(7): e020988.

Martin, D. R., Ruijne, N., McCallum, L., O'Hallahan, J. and Oster, P. (2006). The VR2 epitope on the PorA P1.7-2,4 protein is the major target for the immune response elicited by the strain-specific group B meningococcal vaccine MeNZB. *Clinical Vaccine Immunology* 13(4): 486-491.

Masignani, V., Comanducci, M., Giuliani, M. M., Bambini, S., Adu-Bobie, J., Arico, B., Brunelli, B., Pieri, A., *et al.* (2003). Vaccination against *Neisseria meningitidis* using three variants of the lipoprotein GNA1870. *Journal of Experimental Medicine* 197(6): 789-799.

Maynard Smith, J., Smith, N. H., O'Rourke, M. and Spratt, B. G. (1993). How clonal are bacteria? *Proceedings of the National Academy of Sciences of the United States of America* 90(10): 4384-4388.

McNamara, L. A., Shumate, A. M., Johnsen, P., MacNeil, J. R., Patel, M., Bhavsar, T., Cohn, A. C., Dinitz-Sklar, J., *et al.* (2015). First Use of a Serogroup B Meningococcal Vaccine in the US in Response to a University Outbreak. *Pediatrics* 135(5): 798-804.

Medini, D., Stella, M. and Wassil, J. (2015). MATS: Global coverage estimates for 4CMenB, a novel multicomponent meningococcal B vaccine. *Vaccine* 33(23): 2629-2636.

MenAfriCar Consortium (2015). The Diversity of Meningococcal Carriage Across the African Meningitis Belt and the Impact of Vaccination With a Group A Meningococcal Conjugate Vaccine. *Journal of Infectious Diseases* 212(8): 1298-1307.

Millar, B. C., Moore, P. J. A. and Moore, J. E. (2017). Meningococcal disease: has the battle been won? *Journal of the Royal Army Medical Corps* 163(4): 235-241.

Miller, E., Salisbury, D. and Ramsay, M. (2001). Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. *Vaccine* 20(Suppl 1): S58-67.

Mowlaboccus, S., Mullally, C. A., Richmond, P. C., Howden, B. P., Stevens, K., Speers, D. J., Keil, A. D., Bjornstad, O. N., *et al.* (2017). Differences in the population structure of *Neisseria meningitidis* in two Australian states: Victoria and Western Australia. *PLoS ONE* 12(10): e0186839.

Mowlaboccus, S., Perkins, T. T., Smith, H., Sloots, T., Tozer, S., Prempeh, L. J., Tay, C. Y., Peters, F., *et al.* (2016). Temporal Changes in BEXSERO® Antigen Sequence Type Associated with Genetic Lineages of *Neisseria meningitidis* over a 15-Year Period in Western Australia. *PLoS ONE* 11(6): e0158315.

Muros-Le Rouzic, E., Doemland, M., Gilmet, G. and Teyssou, R. (2004). Worldwide epidemiology of meningococcal disease: Examining a global risk. *Poster presented at International Pathogenic Neisseria Conference, 2004; Milwaukee, WN, USA*.

Naghavi, M., Wang, H. D., Lozano, R., Davis, A., Liang, X. F., Zhou, M. G., Vollset, S. E., Ozgoren, A. A., *et al.* (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 385(9963): 117-171.

Neal, K. R., Nguyen-Van-Tam, J., Jeffrey, N., Slack, R. C., Madeley, R. J., Ait-Tahar, K., Job, K., Wale, M. C. and Ala'Aldeen, D. A. (2000). Changing carriage rate of *Neisseria meningitidis* among university students during the first week of term: cross sectional study. *British Medical Journal* 320(7238): 846-849.

O'Hallahan, J., McNicholas, A., Galloway, Y., O'Leary, E. and Roseveare, C. (2009). Delivering a safe and effective strain-specific vaccine to control an epidemic of group B meningococcal disease. *The New Zealand Medical Journal* 122(1291): 48-59.

Okike, I. O., Johnson, A. P., Henderson, K. L., Blackburn, R. M., Muller-Pebody, B., Ladhani, S. N., Anthony, M., Ninis, N., *et al.* (2014). Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. *Clinical Infectious Diseases* 59(10): e150-157.

Okwo-Bele, J. M. and Cherian, T. (2011). The expanded programme on immunization: A lasting legacy of smallpox eradication. *Vaccine* 29: D74-D79.

Olsen, S. F., Djurhuus, B., Rasmussen, K., Joensen, H. D., Larsen, S. O., Zoffman, H. and Lind, I. (1991). Pharyngeal carriage of *Neisseria meningitidis* and *Neisseria lactamica* in households with infants within areas with high and low incidences of meningococcal disease. *Epidemiology and Infection* 106: 445-457.

Oster, P., Lennon, D., O'Hallahan, J., Mulholland, K., Reid, S. and Martin, D. (2005). MeNZB: a safe and highly immunogenic tailor-made vaccine against the New Zealand *Neisseria meningitidis* serogroup B disease epidemic strain. *Vaccine* 23(17-18): 2191-2196.

Ostergaard, L., Vesikari, T., Absalon, J., Beeslaar, J., Ward, B. J., Senders, S., Eiden, J. J., Jansen, K. U., *et al.* (2017). A Bivalent Meningococcal B Vaccine in Adolescents and Young Adults. *New England Journal of Medicine* 377(24): 2349-2362.

Pajon, R., Chinea, G., Marrero, E., Gonzalez, D. and Guillen, G. (1997). Sequence analysis of the structural *tbpA* gene: protein topology and variable regions within neisserial receptors for transferrin iron acquisition. *Microbial Pathogenesis* 23(2): 71-84.

Parikh, S. R., Andrews, N. J., Beebeejaun, K., Campbell, H., Ribeiro, S., Ward, C., White, J. M., Borrow, R., *et al.* (2016). Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. *Lancet* 388(10061): 2775-2782.

Parikh, S. R., Newbold, L., Slater, S., Stella, M., Moschioni, M., Lucidarme, J., De Paola, R., Giuliani, M., *et al.* (2017). Meningococcal serogroup B strain coverage of the multicomponent 4CMenB vaccine with corresponding regional distribution and clinical characteristics in England, Wales, and Northern Ireland, 2007-08 and 2014-15: a qualitative and quantitative assessment. *Lancet Infectious Diseases* 17(7): 754-762.

Partridge, E., Lujan, E., Giuntini, S., Vu, D. M. and Granoff, D. M. (2017). The role of anti-NHba antibody in bactericidal activity elicited by the meningococcal serogroup B vaccine, MenB-4C. *Vaccine* 35(33): 4236-4244.

Petousis-Harris, H., Paynter, J., Morgan, J., Saxton, P., McArdle, B., Goodyear-Smith, F. and Black, S. (2017). Effectiveness of a group B outer membrane vesicle meningococcal vaccine against gonorrhoea in New Zealand: a retrospective case-control study. *Lancet* 390(10102): 1603-1610.

Pollard, A. J., Perrett, K. P. and Beverley, P. C. (2009). Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. *Nature Reviews Immunology* 9(3): 212-220.

Public Health England (2013). Chapter 22 Meningococcal. *Immunisation against infectious disease*. M. Ramsay**:** 24.

Public Health England (2015) Invasive meningococcal disease (laboratory reports in England): 2014/2015 annual data by epidemiological year. *Health Protection Report* **9**, 14-19 from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/476989/hpr38-3915.pdf

Public Health England (2016) Invasive meningococcal disease (laboratory reports in England): 2015/2016 annual data by epidemiological year. *Health Protection Report* **10**, 7-10 from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/569408/hpr3716.pdf

Public Health England (2017) Invasive meningococcal disease (laboratory reports in England):2015/2016 annual data by epidemiological year. *Health Protection Report* **11**, 1-6 from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/655626/hrp3817_IMD.pdf

Public Health England (2017) Invasive meningococcal infections laboratory reports in England by age group and epidemiological year, 1998/1999-2016/2017. from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/663784/Table2_invasive_meningococcal_infections_lab_reports_England_by_agegroup_epiyear.pdf

Quick, J., Loman, N. J., Duraffour, S., Simpson, J. T., Severi, E., Cowley, L., Bore, J. A., Koundouno, R., *et al.* (2016). Real-time, portable genome sequencing for Ebola surveillance. *Nature* 530(7589): 228-232.

R Core Team (2015). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing, Vienna, Austria**.:** R Foundation for Statistical Computing.

Read, R. C., Baxter, D., Chadwick, D. R., Faust, S. N., Finn, A., Gordon, S. B., Heath, P. T., Lewis, D. J., *et al.* (2014). 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* 384(9960): 2123-2131.

Rodrigues, C. M. C., Lucidarme, J., Borrow, R., Smith, A., Cameron, J. C., Moxon, E. R. and Maiden, M. C. J. (2018). Genomic Surveillance of 4CMenB Vaccine Antigenic Variants among Disease-Causing *Neisseria meningitidis* Isolates, United Kingdom, 2010-2016. *Emerging Infectious Diseases* 24(4): 673-682.

Rodrigues, C. M. C. and Maiden, M. C. J. (2018). A world without bacterial meningitis: how genomic epidemiology can inform vaccination strategy [version 1; referees: 2 approved]. *F1000Research* 7(F1000 Faculty Rev): 401.

Rosenqvist, E., Arne Høiby, E., Wedege, E., Bryn, K., Kolberg, J., Klem, A., Rønnild, E., Bjune, G. and Nøkleby, H. (1995). Human antibody responses to meningococcal outer membrane antigens after three doses of the Norwegian group B meningococcal vaccine. *Infection and Immunity* 63: 4642-4652.

Russell, J. E., Jolley, K. A., Feavers, I. M., Maiden, M. C. and Suker, J. (2004). PorA variable regions of *Neisseria meningitidis*. *Emerging Infectious Diseases* 10(4): 674-678.

Sabat, A. J., Budimir, A., Nashev, D., Sa-Leao, R., van Dijl, J. M., Laurent, F., Grundmann, H., Friedrich, A. W. and Esgem (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance* 18(4): 17-30.

Salisbury, D., Ramsay, M. and Noakes, K. (2006). *Immunisation against infectious disease. Department of Health*. London, The Stationery Office.

Santolaya, M. E., O'Ryan, M. L., Valenzuela, M. T., Prado, V., Vergara, R., Munoz, A., Toneatto, D., Grana, G., *et al.* (2012). Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet* 379(9816): 617-624.

Serruto, D., Bottomley, M. J., Ram, S., Giuliani, M. M. and Rappuoli, R. (2012). The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: Immunological, functional and structural characterization of the antigens. *Vaccine* 30(Supplement 2): B87-B97.

Sierra, G. V. G., Campa, H. C., Varcacel, N. M., Garcia, I. L., Izquierdo, P. L., Sotolongo, P. F., Casanueva, G. V., Rico, C. O., *et al.* (1991). Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. *NIPH Annals* 14(2): 195-207.

Soeters, H. M., Whaley, M., Alexander-Scott, N., Kanadanian, K. V., MacNeil, J. R., Martin, S. W., McNamara, L. A., Sicard, K., *et al.* (2017). Meningococcal Carriage Evaluation in Response to a Serogroup B Meningococcal Disease Outbreak and Mass Vaccination Campaign at a College-Rhode Island, 2015-2016. *Clinical Infectious Diseases* 64(8): 1115-1122.

Sow, S. O., Okoko, B. J., Diallo, A., Viviani, S., Borrow, R., Carlone, G., Tapia, M., Akinsola, A. K., *et al.* (2011). Immunogenicity and Safety of a Meningococcal A Conjugate Vaccine in Africans. *New England Journal of Medicine* 364(24): 2293-2304.

Sridhar, S., Greenwood, B., Head, C., Plotkin, S. A., Safadi, M. A., Saha, S., Taha, M. K., Tomori, O. and Gessner, B. D. (2015). Global incidence of serogroup B invasive meningococcal disease: a systematic review. *Lancet Infectious Diseases* 15(11): 1334-1346.

Stephens, D. S., Greenwood, B. and Brandtzaeg, P. (2007). Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet* 369(9580): 2196-2210.

Stuart, J. M., Cartwright, K. A., Robinson, P. M. and Noah, N. D. (1989). Effect of smoking on meningococcal carriage. *Lancet* 2(8665): 723-725.

Taha, M. K., Hawkins, J. C., Liberator, P., Deghmane, A. E., Andrew, L., Hao, L., Jones, T. R., McNeil, L. K., *et al.* (2017). Bactericidal activity of sera from adolescents vaccinated with bivalent rLP2086 against meningococcal serogroup B outbreak strains from France. *Vaccine* 35(11): 1530-1537.

Tan, L. K. K., Carlone, G. M. and Borrow, R. (2010). Advances in the Development of Vaccines against *Neisseria meningitidis*. *New England Journal of Medicine* 362(16): 1511-1520.

Tani, C., Stella, M., Donnarumma, D., Biagini, M., Parente, P., Vadi, A., Magagnoli, C., Costantino, P., *et al.* (2014). Quantification by LC-MS(E) of outer membrane vesicle proteins of the Bexsero® vaccine. *Vaccine* 32(11): 1273-1279.

Tappero, J. W., Lagos, R., Ballesteros, A. M., Plikaytis, B., Williams, D., Dykes, J., Gheesling, L. L., Carlone, G. M., *et al.* (1999). Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. *JAMA* 281(16): 1520-1527.

Thigpen, M. C., Whitney, C. G., Messonnier, N. E., Zell, E. R., Lynfield, R., Hadler, J. L., Harrison, L. H., Farley, M. M., *et al.* (2011). Bacterial meningitis in the United States, 1998-2007. *New England Journal of Medicine* 364(21): 2016-2025.

Thompson, M. J., Ninis, N., Perera, R., Mayon-White, R., Phillips, C., Bailey, L., Harnden, A., Mant, D. and Levin, M. (2006). Clinical recognition of meningococcal disease in children and adolescents. *Lancet* 367(9508): 397-403.

Trotter, C. L., Andrews, N. J., Kaczmarski, E. B., Miller, E. and Ramsay, M. E. (2004). Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet* 364(9431): 365-367.

Trotter, C. L., Chandra, M., Cano, R., Larrauri, A., Ramsay, M. E., Brehony, C., Jolley, K. A., Maiden, M. C., *et al.* (2007). A surveillance network for meningococcal disease in Europe. *FEMS Microbiology Reviews* 31(1): 27-36.

Trotter, C. L., Lingani, C., Fernandez, K., Cooper, L. V., Bita, A., Tevi-Benissan, C., Ronveaux, O., Preziosi, M. P. and Stuart, J. M. (2017). Impact of MenAfriVac in nine countries of the African meningitis belt, 2010-15: an analysis of surveillance data. *Lancet Infectious Diseases* 17(8): 867-872.

Trotter, C. L. and Maiden, M. C. (2009). Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. *Expert Review Vaccines* 8(7): 851-861.

Tyski, S., Grzybowska, W., Dulny, G., Berthelsen, L. and Lind, I. (2001). Phenotypical and genotypical characterization of *Neisseria meningitidis* carrier strains isolated from Polish recruits in 1998. *European Journal of Clinical Microbiology and Infectious Diseases* 20(5): 350-353.

Urwin, R., Russell, J. E., Thompson, E. A., Holmes, E. C., Feavers, I. M. and Maiden, M. C. (2004). Distribution of Surface Protein Variants among Hyperinvasive Meningococci: Implications for Vaccine Design. *Infection and Immunity* 72(10): 5955-5962.

van de Beek, D., Brouwer, M., Hasbun, R., Koedel, U., Whitney, C. G. and Wijdicks, E. (2016). Community-acquired bacterial meningitis. *Nature Reviews Disease Primers* 2: 16074.

van de Beek, D., Cabellos, C., Dzupova, O., Esposito, S., Klein, M., Kloek, A. T., Leib, S. L., Mourvillier, B., *et al.* (2016). ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clinical Microbiology and Infectious Diseases* 22 Suppl 3: S37-62.

van der Ley, P., Heckels, J. E., Virji, M., Hoogerhout, P. and Poolman, J. T. (1991). Topology of outer membrane porins in pathogenic *Neisseria* species. *Infection and Immunity* 59(9): 2963-2971.

Vernikos, G. and Medini, D. (2014). Bexsero® chronicle. *Pathogens and Global Health* 108(7): 305-316.

Vianzon, V., Illek, B. and Moe, G. R. (2017). Effect of vaccine-elicited antibodies on colonization of *Neisseria meningitidis* serogroup B and C strains in a human bronchial epithelial cell culture model. *Clinical and Vaccine Immunology* 24(10): e00188-00117.

Viner, R. M., Booy, R., Johnson, H., Edmunds, W. J., Hudson, L., Bedford, H., Kaczmarski, E., Rajput, K., *et al.* (2012). Outcomes of invasive meningococcal serogroup B disease in children and adolescents (MOSAIC): a case-control study. *Lancet Neurology* 11(9): 774-783.

Vogel, U., Taha, M. K., Vazquez, J. A., Findlow, J., Claus, H., Stefanelli, P., Caugant, D. A., Kriz, P., *et al.* (2013). Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. *Lancet Infectious Diseases* 13(5): 416-425.

Wachter, J. and Hill, S. (2016). Positive Selection Pressure Drives Variation on the Surface-Exposed Variable Proteins of the Pathogenic *Neisseria*. *PLoS ONE* 11(8): e0161348.

Watkins, E. R. and Maiden, M. C. (2012). Persistence of hyperinvasive meningococcal strain types during global spread as recorded in the PubMLST database. *PLoS ONE* 7(9): e45349.

Watkins, E. R., Maiden, M. C. and Gupta, S. (2016). Metabolic competition as a driver of bacterial population structure. *Future Microbiology* 11: 1339-1357.

Yazdankhah, S. P., Kriz, P., Tzanakaki, G., Kremastinou, J., Kalmusova, J., Musilek, M., Alvestad, T., Jolley, K. A., *et al.* (2004). Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *Journal of Clinical Microbiology* 42(11): 5146-5153.

Zhou, Z., Alikhan, N. F., Sergeant, M. J., Luhmann, N., Vaz, C., Francisco, A. P., Carrico, J. A. and Achtman, M. (2018). GrapeTree: Visualization of core genomic relationships among 100,000 bacterial pathogens. *Genome Research*: In Press.

Zlotnick, G. W., Jones, T. R., Liberator, P., Hao, L., Harris, S., McNeil, L. K., Zhu, D., Perez, J., *et al.* (2015). The discovery and development of a novel vaccine to protect against *Neisseria meningitidis* Serogroup B Disease. *Human Vaccines & Immunotherapeutics* 11(1): 5-13.

Zollinger, W. D., Boslego, J., Moran, E., Garcia, J., Cruz, C., Ruiz, S., Brandt, B., Martinez, M., *et al.* (1991). Meningococcal serogroup B vaccine protection trial and follow-up studies in Chile. The Chilean National Committee for Meningococcal Disease. *National Institute of Public Health Annals* 14: 211-212.

Appendix

Appendix I – Supplementary figures

These figures were prepared by Dr Carina Brehony as part of the following publication, they are included here for completion of the work presented in Chapter 2.

Brehony C, Rodrigues CMC, Borrow R, Smith A, Cunney R, Moxon ER, Maiden MCJ. Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation. *Vaccine*. 2016 Sep 7;34(39):4690-4697

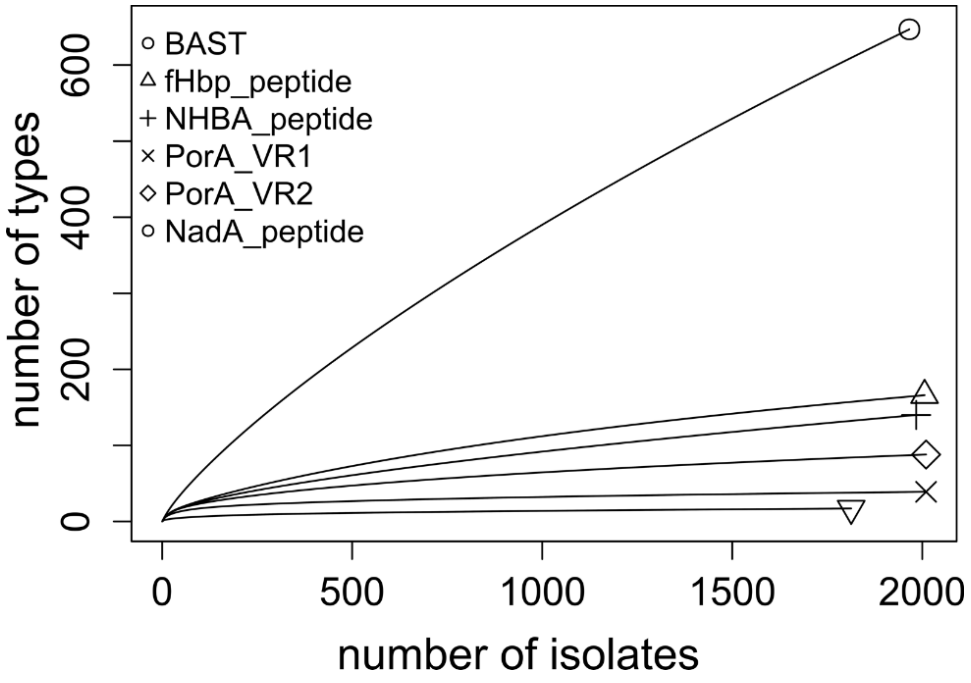


Figure S1: Rarefaction curve of increase in types (BAST, fHbp peptide, NadA peptide, NHBA peptide and PorA type) with number of isolates.

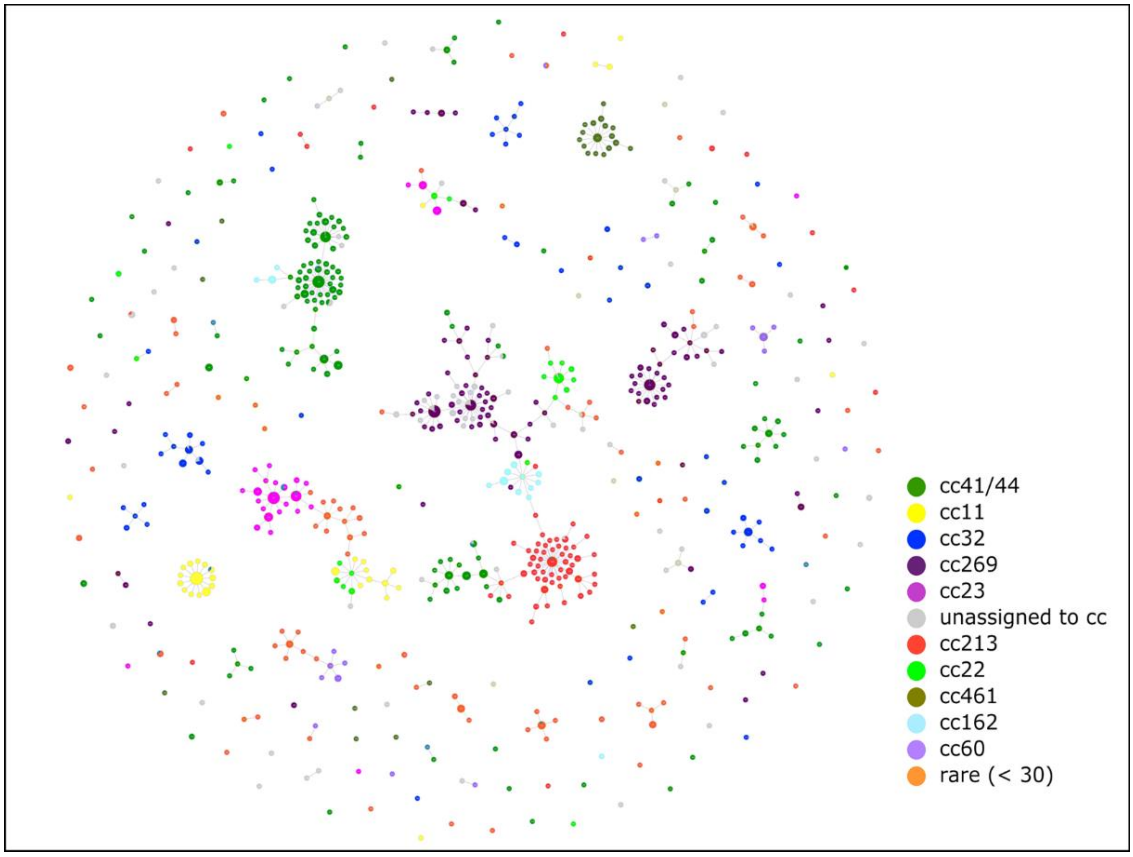


Figure S2: Predominant BAST clusters defined using the goeBURST algorithm in PHYLOViZ (Francisco *et al.* 2012) and coloured by clonal complex.
Clusters were defined at the single locus variant level (SLV) i.e. varying at one of the five peptide variants NHBA, fHbp, NadA, PorA VR1 or VR2 with colouring for each BAST based on clonal complex association. Each circle represents one of the BASTs found in the dataset and the size of the circle corresponds to the number of isolates with that particular BAST.

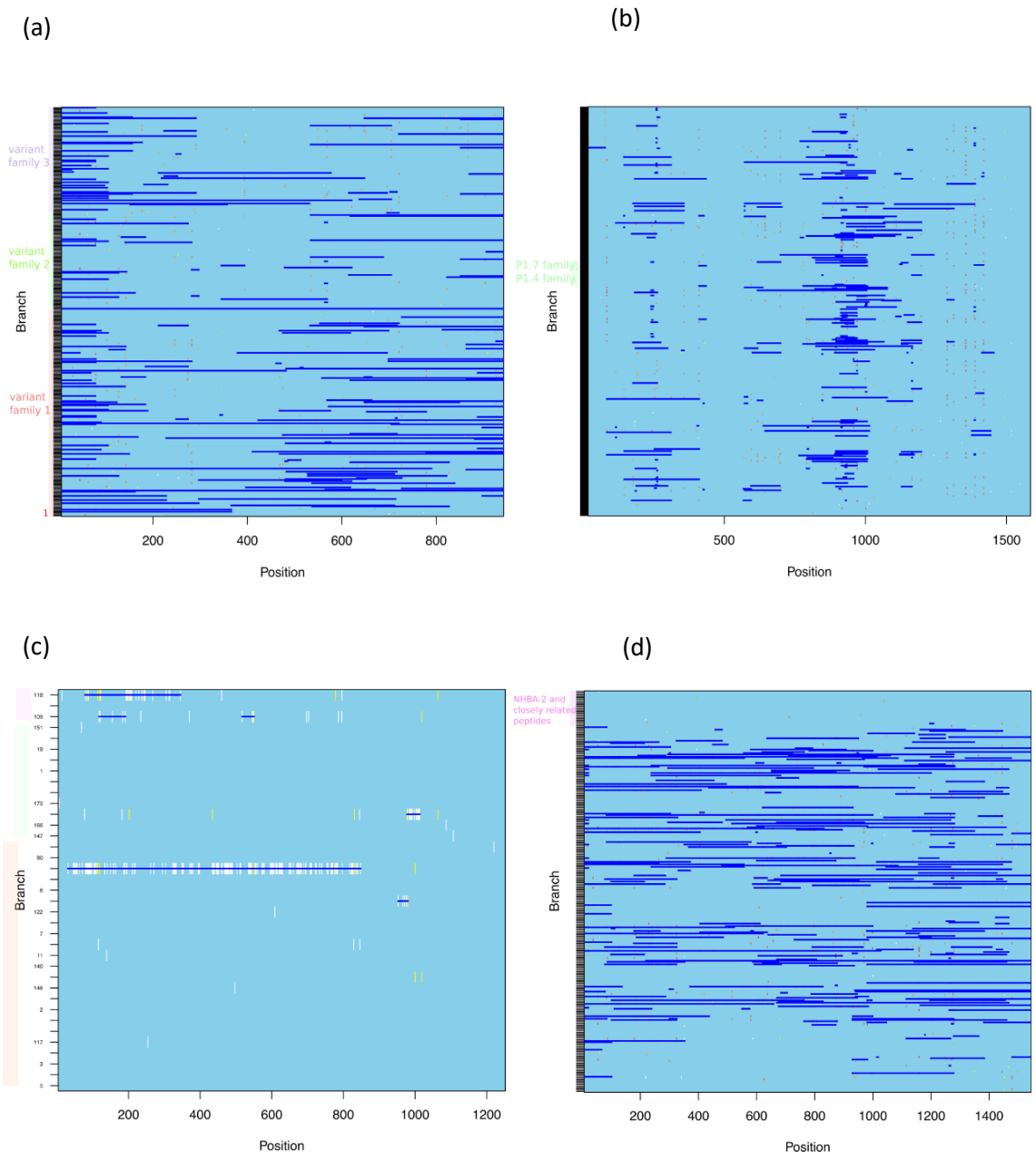


Figure S3: Detection of recombination in Bexsero® antigen alleles; (a) *fHbp*, (b) *porA*, (c) *nadA*, (d) *nhba* using ClonalFrameML (Didelot and Wilson 2015).
ClonalFrameML uses a maximum likelihood approach for phylogenetic reconstruction while taking into account recombination. For any branch of the genealogy and any position along the sequence, inferred recombination is indicated by blue horizontal bars.

Appendix II – Mass spectrometry data for OMVT development

Table S1 Complete list of protein content in the proteome of the OMV for the Bexsero® Vaccine Identified using multi-dimensional LC-MS/MS peptide sequencing and database searching (*N. meningitidis* MC58, Swiss-Pro FASTA release 2014_02). Following identification, protein FASTA sequences were submitted to a web server for protein subCELular LOcalisation prediction (http://cello.life.nctu.edu.tw/cello2go/). Peak area of the top three most abundant peptides were used to represent the quantity of the protein identified. OM=Outer membrane, EC=Extracellular, PP=Periplasmic, CP=Cytoplasmic, IM=Inner membrane. Protein number marked with * were selected as part of the OMV peptide Typing (OMVT) scheme.

Protein No.	UniProt Accession No.	NMB No.	Protein Name, Gene Name (GN=)	Predicted Location	Peak Area
1*	P30690	NMB2039	Major outer membrane protein P.IB GN=porB	OM	2.745E11
2*	P0A0V3	NMB0382	Outer membrane protein class 4 GN=rmpM	OM	1.325E11
3*	P0DH58	NMB1429	Major outer membrane protein P.IA GN=porA	OM	9.031E10
4*	Q7DDH4	NMB1126/ NMB1164	Lipoprotein NMB1126/NMB1164 GN=NMB1126	OM. EC, PP	3.275E10
5*	Q7DDI3	NMB1053	Class 5 outer membrane protein GN=opc	OM	2.584E10
6*	Q7DDM2	NMB0663	Outer membrane protein NspA GN=nspA	OM	2.377E10
7*	Q7DDB6	NMB1497	TonB-dependent receptor NMB1497 GN=NMB1497	OM	1.880E10
8*	Q9JXL3	NMB1988	Iron-regulated outer membrane protein FrpB GN=frpB	OM	1.546E10
9	Q7DDT2	NMB0152	50S ribosomal protein L14 GN=rplN	CP, IM	1.523E10
10*	P0A0Y4	NMB0634	Major ferric iron-binding protein GN=fbpA	PP	1.480E10
11	P64027	NMB0124/ NMB0139	Elongation factor Tu GN=tufA	CP	1.469E10
12*	Q9JZN9	NMB0964	TonB-dependent receptor NMB0964 GN=NMB0964	OM, EC,	1.152E10
13	Q9K1I2	NMB0161	50S ribosomal protein L15 GN=rplO	CP, IM	9.638E9
14	P66355	NMB0166	30S ribosomal protein S11 GN=rpsK	CP	8.959E9
15	Q9JY71	NMB1710	Glutamate dehydrogenase GN=gdhA	CP	8.171E9
16*	Q70M91	NMB1812	Type IV pilus biogenesis and competence protein PilQ GN=pilQ	OM	8.153E9
17	P05431	NMB0018	Fimbrial protein GN=pilE	EC, OM, PP	8.130E9
18	P66540	NMB2101	30S ribosomal protein S2 GN=rpsB	CP	8.114E9
19	Q9K1I4	NMB0154	50S ribosomal protein L5 GN=rplE	CP	7.524E9
20	P66577	NMB0159	30S ribosomal protein S5 GN=rpsE	CP	7.358E9
21	P66508	NMB0463	30S ribosomal protein S20 GN=rpsT	CP IM	6.653E9
22	Q9K1I1	NMB0169	50S ribosomal protein L17 GN=rplQ	CP, IM	6.454E9
23*	Q9K0U9	NMB0461	Transferrin-binding protein 1 GN=tbp1	OM	6.424E9
24	P66333	NMB0140	30S ribosomal protein S10 GN=rpsJ	CP	6.380E9
25	P66519	NMB1950	30S ribosomal protein S21 GN=rpsU	CP. IM	6.259E9
26	P66408	NMB0155	30S ribosomal protein S14 GN=rpsN	CP. IM	6.104E9
27	Q9K0K5	NMB0589	50S ribosomal protein L19 GN=rplS	CP	6.011E9
28	P66551	NMB0148	30S ribosomal protein S3 GN=rpsC	CP, IM	5.855E9
29	P66375	NMB0136	30S ribosomal protein S12 GN=rpsL	CP	5.836E9

30	Q9K1I3	NMB0157	50S ribosomal protein L6 GN=rplF	CP, IM	5.793E9
31*	Q9JS44	NMB0375/ NMB0652	Adhesin MafA GN=mafA1	OM, EP	5.713E9
32	Q7DDT4	NMB0149	50S ribosomal protein L16 GN=rplP	CP	5.666E9
33	Q9K0N4	NMB0554	Chaperone protein DnaK GN=dnaK	CP	5.561E9
34*	Q9K1F0	NMB0204	Outer membrane protein assembly factor BamE GN=bamE	IM, PP	5.348E9
35*	Q7DDE8	NMB1124 /NMB1162	Lipoprotein NMB1124/NMB1162 GN=NMB1124	OM, EP	5.343E9
36	P66642	NMB2056	30S ribosomal protein S9 GN=rpsI	CP	5.273E9
37	P0A0X7	NMB1321	30S ribosomal protein S18 GN=rpsR	CP, IM	5.066E9
38	Q7DDK4	NMB0946	Peroxiredoxin 2 family protein/glutaredoxin GN=NMB0946	CP, IM	4.902E9
39	Q9K1I8	NMB0138	Elongation factor G GN=fusA	CP	4.888E9
40	Q9K093	NMB0723	50S ribosomal protein L20 GN=rplT	CP	4.867E9
41	Q9JYI2	NMB1574	Ketol-acid reductoisomerase (NADP(+)) GN=ilvC	CP	4.764E9
42	Q7DDQ8	NMB0378	Phosphate transporter GN=NMB0378	IM	4.700E9
43	Q7DDR4	NMB0325	50S ribosomal protein L21 GN=rplU	CP	4.527E9
44	Q7DDT0	NMB0158	50S ribosomal protein L18 GN=rplR	CP	4.514E9
45	P61057	NMB0143	50S ribosomal protein L4 GN=rplD	CP, IM, PP	4.474E9
46	Q9K1I6	NMB0144	50S ribosomal protein L23 GN=rplW	CP, IM	4.433E9
47*	Q9K1H0	NMB0182	Outer membrane protein assembly factor BamA GN=bamA	OM	4.294E9
48	Q7DDT5	NMB0147	50S ribosomal protein L22 GN=rplV	CP	4.235E9
49*	Q9JZ20	NMB1333	Uncharacterized protein NMB1333 GN=NMB1333	OM, IM, EP	4.073E9
50	Q7DDH5	NMB1125/ NMB1163	Uncharacterized protein GN=NMB1125	CP, IM, PP	4.072E9
51	P42385	NMB1972	60 kDa chaperonin GN=groL	CP	3.721E9
52	P66386	NMB0165	30S ribosomal protein S13 GN=rpsM	CP, PP	3.613E9
53*	Q9K1M2	NMB0088	Outer membrane protein NMB0088 GN=NMB0088	OM, EP, PP	3.498E9
54	Q7DDS7	NMB0171	Septum site-determining protein MinD GN=minD	CP	3.489E9
55*	Q9K1K7	NMB0109	Uncharacterized protein GN=NMB0109	IM, CP, EP	3.331E9
56*	Q9JY68	NMB1714	Multidrug efflux pump channel protein GN=mtrE	OM	3.247E9
57	Q7DDS9	NMB0160	50S ribosomal protein L30 GN=rpmD	CP, IM	3.169E9
58	Q9JYD2	NMB1643	Translation initiation factor IF-2 GN=infB	CP	3.148E9
59	Q7DDL5	NMB0763	Cysteine synthase GN=cysK	CP	3.134E9
60	Q9JZN0	NMB0980	NAD(P) transhydrogenase subunit alpha GN=pntA	IM	3.087E9
61	Q7DD49	NMB2057	50S ribosomal protein L13 GN=rplM	CP	3.045E9
62	P66561	NMB0167	30S ribosomal protein S4 GN=rpsD	CP	2.929E9
63*	Q06379	NMB1540	Lactoferrin-binding protein A GN=lbpA	OM	2.928E9
64	P65235	NMB0875	Ribose-phosphate pyrophosphokinase GN=prs	CP, IM	2.789E9
65	P66628	NMB0156	30S ribosomal protein S8 GN=rpsH	CP	2.726E9
66	Q9K1I5	NMB0145	50S ribosomal protein L2 GN=rplB	CP, IM	2.689E9
67	P64371	NMB1583	Imidazoleglycerol-phosphate dehydratase GN=hisB	CP	2.550E9
68	Q9JZQ2	NMB0944	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase GN=metE	CP	2.525E9
69	Q9K1J3	NMB0127	50S ribosomal protein L11 GN=rplK [RL11_NEIMB]	CP, IM, PP	2.525E9

70	Q9K0K8	NMB0586	Adhesin GN=NMB0586	PP	2.494E9
71	Q7DDD7	NMB1220	Stomatin/Mec-2 family protein GN=NMB1220	CP, IM	2.435E9
72	P0A0S6	NMB0427	Cell division protein FtsZ GN=ftsZ	CP	2.426E9
73	P66047	NMB0130	50S ribosomal protein L10 GN=rplJ	CP, IM	2.414E9
74*	Q9JYI8	NMB1567	Probable FKBP-type peptidyl-prolyl cis-trans isomerase FkpA	OM, PP	2.367E9
75	Q9K0B1	NMB0703	Outer membrane protein assembly factor BamD GN=bamD	OM, PP	2.353E9
76	P64389	NMB1230	DNA-binding protein HU-beta GN=hupB	CP, IM	2.273E9
77	Q9K0A4	NMB0711	Uncharacterized protein GN=NMB0711	OM, PP	2.224E9
78	Q9JZ44	NMB1301	30S ribosomal protein S1 GN=rpsA	CP	2.135E9
79	Q7DD63	NMB1946	Lipoprotein GN=NMB1946	IM, PP	2.120E9
80	P66088	NMB0128	50S ribosomal protein L1 GN=rplA	CP	2.103E9
81	P66614	NMB0137	30S ribosomal protein S7 GN=rpsG	CP	2.081E9
82	Q9JY02	NMB1808	PilM protein GN=pilM	CP, EP, OM	2.013E9
83	P0DH59	NMB1445	Protein RecA GN=recA	CP	1.994E9
84	Q9K0C2	NMB0692	Tpc protein GN=tpc	CP	1.905E9
85*	Q9K0U7	NMB0464	Phospholipase A1 GN=NMB0464	OM, PP	1.869E9
86	Q9JXQ0	NMB1936	ATP synthase subunit alpha GN=atpA	CP, IM	1.842E9
87	Q9JZW3	NMB0876	50S ribosomal protein L25 GN=rplY	CP, IM, PP	1.835E9
88	Q9JZ37	NMB1313	Trigger factor GN=tig	CP	1.783E9
89	Q7DDK0	NMB0954	Citrate synthase GN=gltA	CP	1.763E9
90	Q9JZ12	NMB1341	Pyruvate dehydrogenase E1 component GN=pdhA	CP	1.687E9
91	Q7DDU1	NMB0052	Twitching motility protein PilT GN=pilT-1	CP, IM	1.655E9
92	Q9JZP6	NMB0956	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex GN=sucB	IM	1.647E9
93	Q9JYA8	NMB1668	Hemoglobin receptor GN=hmbR	OM	1.639E9
94	Q9K0D4	NMB0678	Tryptophan synthase alpha chain GN=trpA	CP	1.625E9
95	Q9JZR5	NMB0928	Uncharacterized protein NMB0928 GN=NMB0928	CP, IM, PP	1.611E9
96	P60733	NMB0153	50S ribosomal protein L24 GN=rplX	CP	1.588E9
97	Q9JXL6	NMB1985	Adhesion and penetration protein GN=hap	EP, OM	1.581E9
98*	Q9K187	NMB0280	LPS-assembly protein LptD GN=lptD	OM	1.540E9
99	P66487	NMB0146	30S ribosomal protein S19 GN=rpsS	CP, IM	1.523E9
100	Q9JZH2	NMB1057	Gamma-glutamyltranspeptidase GN=ggt	EP, IM, OM, PP	1.522E9
101	Q9K0J1	NMB0606	Uncharacterized protein GN=NMB0606	CP, IM, PP	1.508E9
102	Q9JZ29	NMB1323	30S ribosomal protein S6 GN=rpsF	IM	1.494E9
103	Q9K134	NMB0359	Glutamine synthetase GN=glnA	CP	1.485E9
104	Q9K0V0	NMB0460	Transferrin-binding protein 2 GN=tbpB	EP, OM	1.395E9
105	Q9K0X8	NMB0426	Cell division protein FtsA GN=ftsA	CP	1.379E9
106	Q7DDA0	NMB1576	Acetolactate synthase III, small subunit GN=ilvH	EP, PP	1.319E9
107	Q9JXN8	NMB1953	Stringent starvation protein A GN=sspA	CP, IM	1.296E9
108	P0A0X1	NMB0131	50S ribosomal protein L7/L12 GN=rplL	CP	1.294E9
109	Q9JY66	NMB1716	Membrane fusion protein GN=mtrC	CP, IM, PP	1.230E9
110	Q9K0I2	NMB0618	Phosphoenolpyruvate synthase GN=ppsA	CP	1.229E9
111	Q9JY54	NMB1735	GTP pyrophosphokinase GN=relA	CP	1.218E9

112	Q9JZP4	NMB0959	Succinate--CoA ligase [ADP-forming] subunit beta GN=sucC	CP, IM	1.215E9
113	P66704	NMB0168	DNA-directed RNA polymerase subunit alpha GN=rpoA	CP, IM	1.215E9
114	Q7DDM4	NMB0609	30S ribosomal protein S15 GN=rpsO	CP	1.207E9
115	Q9K165	NMB0313	TPR repeat-containing protein NMB0313 GN=NMB0313	CP, IM, OM, PP	1.185E9
116	Q9JXW3	NMB1861	Acetyl-CoA carboxylase, biotin carboxylase GN=accC	CP	1.174E9
117	Q9K027	NMB0798	ATP-dependent zinc metalloprotease FtsH GN=ftsH	IM	1.160E9
118	P60444	NMB0142	50S ribosomal protein L3 GN=rplC	CP	1.155E9
119	Q9JXB7	NMB2134	Uncharacterized protein GN=NMB2134	OM, PP	1.150E9
120	P66295	NMB0941	50S ribosomal protein L36 2 GN=rpmJ2	CP, IM	1.149E9
121	Q9K0H1	NMB0631	Phosphate acetyltransferase Pta GN=NMB0631	CP, IM	1.118E9
122	Q9K0U8	NMB0462	Spermidine/putrescine ABC transporter, PP spermidine/putrescine-binding protein GN=potD-1	CP	1.108E9
123	Q7DDQ0	NMB0476	Uncharacterized protein GN=NMB0476	CP	1.107E9
124	Q9JXQ2	NMB1934	ATP synthase subunit beta GN=atpD	CP	1.090E9
125	Q9JZN2	NMB0978	NAD(P) transhydrogenase subunit beta GN=pntB	CP	1.090E9
126	Q9K1R3	NMB0007	Cell division ATP-binding protein FtsE GN=ftsE	CP, IM	1.085E9
127	P66151	NMB0321	50S ribosomal protein L28 GN=rpmB	IM	1.081E9
128	Q9JYY0	NMB1379	Cysteine desulfurase IscS GN=iscS	CP	1.042E9
129	Q9JZP3	NMB0960	Succinate--CoA ligase [ADP-forming] subunit alpha GN=sucD	CP	1.036E9
130	Q59622	NMB0132	DNA-directed RNA polymerase subunit beta GN=rpoB	CP	1.010E9
131	Q9JXB6	NMB2135	Uncharacterized protein GN=NMB2135	CP, PP	1.010E9
132	Q9JXD8	NMB2095	Adhesin complex protein GN=NMB2095	CP, IM	1.007E9
133	Q9JZG1	NMB1070	2-isopropylmalate synthase GN=leuA	IM, CP	9.997E8
134	Q9JZQ3	NMB0943	5,10-methylenetetrahydrofolate reductase GN=metF	CP	9.923E8
135	P66438	NMB0592	30S ribosomal protein S16 GN=rpsP	CP	9.852E8
136	Q9K1J1	NMB0133	DNA-directed RNA polymerase subunit beta' GN=rpoC	CP, IM	9.441E8
137	Q9JX95	NMB2159	Glyceraldehyde-3-phosphate dehydrogenase GN=gapA-2	CP, IM	9.268E8
138	Q9K062	NMB0758	Polyribonucleotide nucleotidyltransferase GN=pnp	CP	9.190E8
139	P66130	NMB0324	50S ribosomal protein L27 GN=rpmA	CP	8.614E8
140	Q9JXK3	NMB1998	Serine-type peptidase GN=NMB1998	CP, PP	8.576E8
141	Q9JXD7	NMB2096	Probable malate:quinone oxidoreductase GN=mqo	OM, PP	8.471E8
142	Q7DDC7	NMB1306	Uncharacterized protein GN=NMB1306	CP	8.421E8
143	Q9JYM1	NMB1518	Acetate kinase 1 GN=ackA1	OM, PP	8.409E8
144	Q9K0J3	NMB0604	Alcohol dehydrogenase, zinc-containing GN=NMB0604	CP, IM	8.334E8
145	Q9JY09	NMB1799	S-adenosylmethionine synthase GN=metK	CP, IM, PP	8.111E8
146	Q9K0H4	NMB0628	Imidazole glycerol phosphate synthase subunit HisF GN=hisF	CP, IM	7.918E8
147	Q9JXE1	NMB2091	Hemolysin GN=NMB2091	CP, PP	7.910E8
148	Q9K0A7	NMB0707	LPS-assembly lipoprotein LptE GN=lptE	CP, IM	7.850E8
149	Q9JY11	NMB1796	Uncharacterized protein GN=NMB1796	CP	7.809E8

150	P56990	NMB1055	Serine hydroxymethyltransferase GN=glyA	CP, EP, IM	7.786E8
151	Q7DDJ9	NMB0955	2-oxoglutarate dehydrogenase, E1 component GN=sucA	CP, EP	7.770E8
152	Q9JZ21	NMB1332	Carboxy-terminal peptidase GN=prc	CP, EP, IM	7.730E8
153	P64347	NMB1579	ATP phosphoribosyltransferase GN=hisG	PP	7.711E8
154	Q9JXR1	NMB1921	3-oxoacyl-(Acyl-carrier-protein) reductase GN=fabG-2	CP, PP	7.651E8
155	Q9K0Z1	NMB0410	Transcriptional regulator MraZ GN=mraZ	CP	7.635E8
156	Q7DDT3	NMB0151	30S ribosomal protein S17 GN=rpsQ	CP, IM	7.556E8
157	Q9JZ11	NMB1342	Acetyltransferase component of pyruvate dehydrogenase complex GN=aceF	CP	7.518E8
158	Q9JZ80	NMB1252	Phosphoribosylformylglycinamidine cyclo-ligase GN=purM	CP	7.501E8
159	Q9JYI0	NMB1577	Acetolactate synthase GN=ilvI	OM, PP	7.372E8
160	P0A0U2	NMB1302	Integration host factor subunit beta GN=ihfB	CP	7.300E8
161	Q9JZP5	NMB0957	Dihydrolipoyl dehydrogenase GN=lpdA1	CP	7.263E8
162	Q9K1I0	NMB0170	Probable septum site-determining protein MinC GN=minC	CP	7.213E8
163	P0A0R1	NMB1207	Bacterioferritin A GN=bfrA	CP, EP	7.174E8
164	P65592	NMB0126	Transcription termination/antitermination protein NusG GN=nusG	CP	7.135E8
165	Q9K0D3	NMB0679	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta GN=accD	CP	7.104E8
166	Q9JXZ0	NMB1829	TonB-dependent receptor GN=NMB1829	CP	7.018E8
167	Q9K063	NMB0757	Phosphoribosylaminoimidazole-succinocarboxamide synthase GN=purC	IM	6.985E8
168	Q9K1B7	NMB0247	Uncharacterized protein GN=NMB0247	CP	6.976E8
169	Q9JZ18	NMB1335	CreA protein GN=creA	CP, EP, PP	6.960E8
170	Q7DD78	NMB1810	PilO protein GN=pilO	IM, PP	6.907E8
171	Q9JZ89	NMB1240	ABC transporter, ATP-binding protein GN=NMB1240	CP	6.624E8
172	Q9JYH8	NMB1581	Histidinol dehydrogenase GN=hisD	CP, IM	6.520E8
173	Q9K1P2	NMB0039	Uncharacterized protein GN=NMB0039	IM	6.471E8
174	Q9K151	NMB0336	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI GN=fabI [FABI_NEIMB]	CP, EP	6.352E8
175	Q9K112	NMB0387	ABC transporter, ATP-binding protein GN=NMB0387	CP	6.250E8
176	Q9K0L8	NMB0574	Aminomethyltransferase GN=gcvT	IM, CP	6.236E8
177	Q9JXM3	NMB1975	Transporter GN=NMB1975	CP, IM	6.232E8
178	Q9K1F8	NMB0196	Ribonuclease E GN=rne	CP, IM, PP	6.091E8
179	Q9K064	NMB0756	Putative dTDP-L-rhamnose synthase GN=NMB0756	CP	5.990E8
180	Q7DDR1	NMB0333	Pilus assembly protein PilG GN=pilG	CP	5.843E8
181	Q9K0G7	NMB0638	UTP--glucose-1-phosphate uridylyltransferase GN=galU	CP, IM	5.836E8
182	Q7DDP9	NMB0477	Uncharacterized protein GN=NMB0477	CP	5.766E8
183	Q9JYY7	NMB1368	ATP-dependent RNA helicase GN=NMB1368	CP, PP	5.702E8
184	Q9K177	NMB0295	Signal recognition particle protein GN=ffh	CP, IM, PP	5.687E8
185	Q9JXV5	NMB1869	Fructose-bisphosphate aldolase GN=cbbA	CP	5.669E8
186	Q9JZ50	NMB1291	Ribonucleoside-diphosphate reductase GN=nrdA	CP, IM	5.612E8
187	Q9K1H1	NMB0181	Outer membrane protein OmpH GN=NMB0181	CP	5.597E8
188	Q9JYG7	NMB1594	Putrescine-binding PP protein GN=potD-3	CP	5.594E8

189	Q9JXS8	NMB1902	DNA polymerase III subunit beta GN=dnaN	CP	5.585E8
190	Q9K1D8	NMB0219	3-oxoacyl-[acyl-carrier-protein] synthase 2 GN=fabF-1	CP	5.550E8
191	Q9JRV8	NMB1139/ NMB1177	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha GN=accA1	CP, IM	5.476E8
192	Q9JYK4	NMB1541	Lactoferrin-binding protein B GN=lbpB	EP,IM, PP	5.233E8
193	P57026	NMB1533	Outer membrane protein H.8 GN=NMB1533	CP	5.222E8
194	Q9JXK6	NMB1995	Nitrogen regulatory protein P-II GN=glnB	CP	5.129E8
195	P63700	NMB1206	Putative bacterioferritin B GN=bfrB	CP, IM	5.069E8
196	Q9JYI4	NMB1572	Aconitate hydratase B GN=acnB	CP	5.048E8
197	Q9JY36	NMB1762	Hemolysin activation protein HecB GN=NMB1762	CP, IM	5.047E8
198	Q7DDC3	NMB1389	RpiR/YebK/YfhH family protein GN=NMB1389	CP	5.041E8
199	Q9JYY4	NMB1371	Acetylornithine aminotransferase GN=argD]	CP	5.017E8
200	Q9JYD3	NMB1642	Transcription termination/antitermination protein NusA GN=nusA	CP	4.896E8
201	Q9JXY2	NMB1839	Formate--tetrahydrofolate ligase GN=fhs	CP	4.867E8
202	Q7DDM6	NMB0595	DNA-binding response regulator GN=NMB0595	CP, IM	4.851E8
203	Q7DDD6	NMB1228	Homoserine dehydrogenase GN=metM	CP	4.840E8
204	Q7DDK9	NMB0861	Uncharacterized protein GN=NMB0861	CP	4.802E8
205	Q9K0D8	NMB0671	Malate oxidoreductase (NAD) GN=sfcA	CP	4.794E8
206	P64100	NMB1729	Biopolymer transport protein ExbB GN=exbB	CP, PP	4.785E8
207	P65533	NMB1307	Nucleoside diphosphate kinase GN=ndk	IM, OM, PP	4.781E8
208	P63916	NMB0110	Peptide deformylase GN=def	CP	4.766E8
209	Q7DDN3	NMB0543	L-lactate permease GN=NMB0543	CP	4.759E8
210	Q9K1M4	NMB0085	Sodium/glutamate symporter GN=gltS	CP, IM, PP	4.702E8
211	Q9JZV2	NMB0888	Uncharacterized protein GN=NMB0888	CP	4.656E8
212	Q9K169	NMB0307	Phospho-2-dehydro-3-deoxyheptonate aldolase GN=aroG	CP	4.531E8
213	Q9JYQ8	NMB1472	Chaperone protein ClpB GN=clpB	CP	4.528E8
214	Q9JZB5	NMB1201	Inosine-5'-monophosphate dehydrogenase GN=guaB	CP	4.519E8
215	P95379	NMB0178	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase GN=lpxA	CP	4.515E8
216	Q9K0P0	NMB0546	Alcohol dehydrogenase, propanol-preferring GN=adhP	CP	4.495E8
217	Q9JYR2	NMB1468	Uncharacterized protein GN=NMB1468	CP	4.438E8
218	Q9K152	NMB0335	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase GN=dapD	CP	4.433E8
219	Q9JXW8	NMB1855	Carbamoyl-phosphate synthase large chain GN=carB	CP	4.361E8
220	Q9JYJ8	NMB1554	CTP synthase GN=pyrG	EP, IM, OM	4.336E8
221	Q9JS34	NMB1154/ NMB1192	Sulfate adenylyltransferase GN=cysD-2	IM, PP	4.329E8
222	P64051	NMB2102	Elongation factor Ts GN=tsf	CP	4.327E8
223	Q7DDQ9	NMB0355	Lipopolysaccharide export system protein LptA GN=lptA	CP	4.280E8
224	Q9JYY2	NMB1373	Ribosome-binding factor A GN=rbfA [RBFA_NEIMB]	CP	4.215E8
225	Q9JZW4	NMB0874	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase GN=ispE	CP, IM	4.176E8
226	Q9JYN6	NMB1498	Aspartokinase GN=lysC	CP	4.147E8
227	Q9JYS0	NMB1457	Transketolase GN=tktA	CP	4.131E8

228	Q9JZ95	NMB1231	Lon protease GN=lon	CP	4.129E8
229	Q9JZV1	NMB0889	Uncharacterized protein GN=NMB0889	CP	4.089E8
230	P67029	NMB1932	Glycine--tRNA ligase alpha subunit GN=glyQ	CP, IM, PP	4.039E8
231	P65915	NMB1874	Orotate phosphoribosyltransferase GN=pyrE	CP, IM	3.887E8
232	Q9JZB7	NMB1199	GTP-binding protein TypA GN=typA	CP	3.864E8
233	P57004	NMB1730	Protein TonB GN=tonB	CP	3.834E8
234	Q9K0I3	NMB0617	Transcription termination factor Rho GN=rho	CP	3.818E8
235	Q4W563	NMB1809	PilN protein GN=pilN	CP	3.818E8
236	P65932	NMB2103	Uridylate kinase GN=pyrH	CP, IM	3.772E8
237	Q9JYY3	NMB1372	ATP-dependent Clp protease ATP-binding subunit ClpX GN=clpX	CP	3.766E8
238	Q9K135	NMB0356	ABC transporter, ATP-binding protein GN=NMB0356	CP	3.748E8
239	Q9JXT2	NMB1897	Leucine--tRNA ligase GN=leuS	CP	3.740E8
240	Q9K0B8	NMB0696	Amino acid ABC transporter, ATP-binding protein GN=NMB0696	CP, IM, OM, PP	3.730E8
241	Q9K022	NMB0804	NAD(P)H nitroreductase GN=NMB0804	CP	3.718E8
242	Q9JS61	NMB1150/ NMB1188	Dihydroxy-acid dehydratase GN=ilvD1	CP	3.685E8
243	Q9JYG6	NMB1595	Alanine--tRNA ligase GN=alaS	CP, IM	3.675E8
244	Q9JYT7	NMB1437	Uncharacterized protein NMB1437 GN=NMB1437	CP	3.671E8
245	Q9JZ14	NMB1595	Proline--tRNA ligase GN=proS	CP	3.613E8
246	Q9K1N4	NMB0051	Twitching motility protein GN=NMB0051	PP	3.490E8
247	Q9K1E8	NMB0207	Glyceraldehyde-3-phosphate dehydrogenase GN=gapA-1	IM	3.475E8
248	Q9K0I4	NMB0615	Ammonium transporter GN=NMB0615	CP	3.467E8
249	Q9K035	NMB787	Amino acid ABC transporter, PP amino acid-binding protein GN=NMB0787	CP	3.427E8
250	Q9JY67	NMB1715	Multiple transferable resistance system protein MtrD GN=mtrD	CP, IM, PP	3.383E8
251	Q9K0H2	NMB0630	Imidazole glycerol phosphate synthase subunit HisH GN=hisH	CP	3.380E8
252	Q9JZX9	NMB0854	Histidine--tRNA ligase GN=hisS	CP	3.367E8
253	Q9JZ31	NMB1320	50S ribosomal protein L9 GN=rplI	CP	3.290E8
254	Q9K1Q3	NMB0017	UDP-3-O-acyl-N-acetylglucosamine deacetylase GN=lpxC	CP	3.230E8
255	Q9JZW1	NMB0878	L-threonine dehydratase GN=ilvA	CP, IM	3.223E8
256	Q9K147	NMB0341	TspA protein GN=NMB0341	CP	3.215E8
257	Q9JXS9	NMB1900	Polyphosphate kinase GN=ppk	CP, IM, PP	3.162E8
258	P65113	NMB0163	Translation initiation factor IF-1 GN=infA	CP, IM	3.148E8
259	Q9JZF7	NMB1074	Acetylglutamate kinase GN=argB	CP, IM	3.138E8
260	Q9JYK7	NMB1538	RNA polymerase sigma factor RpoD GN=rpoD	CP, IM	3.104E8
261	Q7DD99	NMB1580	Uncharacterized protein GN=NMB1580	CP, IM	3.075E8
262	Q9K0Z9	NMB0401	Bifunctional protein PutA GN=putA	IM, PP	3.074E8
263	Q9K1H7	NMB0174	Valine--tRNA ligase GN=valS	CP, IM	3.057E8
264	Q9JYN9	NMB1493	Carbon starvation protein A GN=cstA	CP	3.045E8
265	Q9K103	NMB0396	Nicotinate-nucleotide pyrophosphorylase GN=nadC	CP, IM, PP	3.022E8
266	Q9K1M1	NMB0089	Pyruvate kinase GN=pykA	CP	3.021E8
267	Q9K0X7	NMB0428	Uncharacterized protein GN=NMB0428	CP	3.019E8
268	Q9K1P6	NMB0035	Efem/EfeO family lipoprotein NMB0035 GN=NMB0035	CP	3.019E8

269	Q9JZ53	NMB1285	Enolase GN=eno	IM	3.002E8
270	Q9JXC1	NMB2129	Argininosuccinate synthase GN=argG	CP, IM	2.987E8
271	Q9K0U3	NMB0468	Biosynthetic arginine decarboxylase GN=speA	IM, OM	2.965E8
272	Q9JY94	NMB1685	D-lactate dehydrogenase GN=ldhA	CP, EP	2.944E8
273	Q9JXS7	NMB1903	Chromosomal replication initiator protein DnaA GN=dnaA	CP	2.928E8
274	Q9JXB2	NMB2139	Uncharacterized protein GN=NMB2139	IM	2.924E8
275	Q9JXX4	NMB1849	Carbamoyl-phosphate synthase small chain GN=carA	CP, IM	2.902E8
276	Q9JXW9	NMB1854	Uncharacterized protein GN=NMB1854	CP, IM	2.869E8
277	Q9JY01	NMB1814	3-dehydroquinate synthase GN=aroB	CP, IM	2.785E8
278	Q9K157	NMB0323	UbiH family protein GN=NMB0323	CP	2.770E8
279	Q9JXY7	NMB1833	Isoleucine--tRNA ligase GN=ileS	CP	2.763E8
280	Q7DDA2	NMB1563	Transcriptional regulator, GntR family GN=NMB1563	CP	2.752E8
281	P66849	NMB1460	Single-stranded DNA-binding protein GN=ssb	CP	2.707E8
282	Q9JXM8	NMB1968	Aldehyde dehydrogenase A GN=aldA	CP	2.704E8
283	Q9JZV5	NMB0885	Replicative DNA helicase GN=dnaB	CP	2.701E8
284	Q9JZI8	NMB1032	Type II restriction enzyme NlaIV GN=nlaIVR	CP	2.697E8
285	Q9JYB6	NMB1659	Guanosine-3`,5`-bis(Diphosphate) 3`-pyrophosphohydrolase GN=spoT	IM	2.689E8
286	Q9JXK4	NMB1997	Hydroxyacylglutathione hydrolase GN=gloB	CP	2.687E8
287	Q9JYH0	NMB1590	Alkyl hydroperoxide reductase AhpD GN=NMB1590	CP, IM	2.686E8
288	Q9K0U5	NMB0466	Aspartate--tRNA(Asp/Asn) ligase GN=aspS	CP	2.682E8
289	Q9K048	NMB0774	Uracil phosphoribosyltransferase GN=upp	OM, PP	2.678E8
290	Q9K1Q1	NMB0029	Glycerate dehydrogenase GN=hprA	CP, IM	2.634E8
291	Q9JZ73	NMB1263	CobW-related protein GN=NMB1263]	CP	2.630E8
292	Q9JXI0	NMB2040	Phosphomethylpyrimidine synthase GN=thiC	CP, IM	2.628E8
293	Q9K0L9	NMB0573	Transcriptional regulator, AsnC family GN=NMB0573	CP	2.608E8
294	Q9JRU7	NMB1153/ NMB1191	Sulfate adenylyltransferase subunit 1 GN=cysN-2 [Q9JRU7_NEIMB]	CP	2.593E8
295	Q9JZI7	NMB1033	Cytosine-specific methyltransferase GN=NMB1033	CP	2.586E8
296	Q7DDL8	NMB0709	Uncharacterized protein GN=NMB0709	CP	2.582E8
297	Q9K1G8	NMB0184	1-deoxy-D-xylulose 5-phosphate reductoisomerase GN=dxr	CP	2.582E8
298	Q9JZM7	NMB0983	Bifunctional purine biosynthesis protein PurH GN=purH	CP	2.581E8
299	P57011	NMB0045	Signal recognition particle receptor FtsY GN=ftsY	CP	2.572E8
300	Q9JZS1	NMB0920	Isocitrate dehydrogenase, NADP-dependent, monomeric type GN=icd	CP, IM	2.568E8
301	P0C277	NMB2077	Bifunctional protein FolD GN=folD	CP	2.547E8
302	Q9JZU5	NMB0895	UPF0246 protein NMB0895 GN=NMB0895	CP	2.516E8
303	Q9JYK8	NMB1536	Protein translocase subunit SecA GN=secA	CP	2.506E8
304	Q9K013	NMB0814	ATP phosphoribosyltransferase regulatory subunit GN=hisZ	CP	2.503E8
305	Q9JY06	NMB1802	tRNA N6-adenosine threonylcarbamoyltransferase GN=tsaD	CP	2.500E8
306	Q9K0T7	NMB0475	Uncharacterized protein GN=NMB0475	CP	2.468E8
307	Q9K1Q5	NMB0015	6-phosphogluconate dehydrogenase, decarboxylating GN=gnd	CP, IM, PP	2.445E8
308	Q9JYF0	NMB1612	Amino acid ABC transporter, PP amino acid-binding protein GN=NMB1612	CP	2.433E8

309	Q4W575	NMB0632	Fe(3+) ions import ATP-binding protein FbpC GN=fbpC	CP, IM	2.428E8
310	P0A0Y8	NMB1526	SsrA-binding protein GN=smpB	CP	2.422E8
311	Q9JYZ1	NMB1364	NH(3)-dependent NAD(+) synthetase GN=NMB1364	CP, IM	2.411E8
312	Q9JYM0	NMB1519	Thiol:disulfide interchange protein DsbD GN=dsbD	CP	2.374E8
313	Q9K0N8	NMB0548	AcrA/AcrE family protein GN=NMB0548	CP	2.367E8
314	P0A0R9	NMB1519	Biopolymer transport protein ExbD GN=exbD	IM	2.351E8
315	Q9K089	NMB0728	Phenylalanine--tRNA ligase beta subunit GN=pheT	CP, IM	2.343E8
316	Q9JYZ4	NMB1361	Pseudouridine synthase GN=NMB1361	CP	2.340E8
317	Q9JY23	NMB1779	Hemagglutinin/hemolysin-related protein GN=NMB1779	CP, PP	2.278E8
318	Q9K0Q0	NMB0535	Glucose/galactose transporter GN=gluP	IM	2.277E8
319	Q9K095	NMB0720	Threonine--tRNA ligase GN=thrS	CP, IM, OM	2.259E8
320	P65773	NMB0807	NAD kinase GN=nadK	CP, IM, PP	2.246E8
321	Q9JYX5	NMB1384	DNA gyrase subunit A GN=gyrA	CP	2.220E8
322	Q9K0G2	NMB0643	MafB-related protein GN=NMB0643	CP, IM	2.219E8
323	Q7DDM9	NMB0561	Protein GrpE GN=grpE	CP	2.201E8
324	Q9JZY1	NMB0852	GTPase Der GN=der [DER_NEIMB]	CP	2.189E8
325	Q9JZ09	NMB1344	Dihydrolipoyl dehydrogenase GN=lpdA2	CP, IM	2.174E8
326	Q9JXF2	NMB2074	Uncharacterized protein GN=NMB2074	CP	2.132E8
327	Q9JXD2	NMB2105	MafB protein GN=mafB	CP	2.121E8
328	Q9JXJ1	NMB2017	ComEA-related protein GN=NMB2017	CP	2.096E8
329	Q7DDN2	NMB0550	Thiol:disulfide interchange protein DsbC GN=dsbC	CP	2.071E8
330	Q7DD45	NMB2094	Uncharacterized protein GN=NMB2094	CP	2.042E8
331	Q9JYQ6	NMB1475	Uncharacterized protein GN=NMB1475	CP, IM	2.041E8
332	Q9JRT0	NMB1127	Oxidoreductase, short chain dehydrogenase/reductase family GN=NMB1127	CP	2.031E8
333	Q9JYM8	NMB1506	Arginine--tRNA ligase GN=argS	CP, EP, IM, PP	1.999E8
334	Q9JZL6	NMB0998	Oxidoreductase GN=NMB0998	CP	1.970E8
335	Q9JXR6	NMB1916	3-oxoacyl-[acyl-carrier-protein] synthase 3 GN=fabH	CP	1.963E8
336	Q9JXA0	NMB2154	Electron transfer flavoprotein, alpha subunit GN=etfA	CP	1.962E8
337	Q9JS04	NMB116/ NMB1131	Chaperone protein HscA homolog GN=hscA	CP	1.960E8
338	Q9JYT6	NMB1438	Uncharacterized protein GN=NMB1438	CP	1.936E8
339	Q7DDI8	NMB1035	Uncharacterized protein GN=NMB1035	CP	1.924E8
340	Q9K1P0	NMB0041	ABC transporter, PP solute-binding protein GN=NMB0041	CP, IM	1.897E8
341	Q9JYH6	NMB1584	3-hydroxyacid dehydrogenase GN=NMB1584	CP, PP	1.897E8
342	Q9JZ03	NMB1351	fmu and fmv protein GN=NMB1351	CP	1.871E8
343	Q9K144	NMB0346	Uncharacterized protein GN=NMB0346	CP	1.860E8
344	Q9JZ58	NMB1280	Very long chain acyl-CoA dehydrogenase-related protein GN=NMB1280	CP	1.853E8
345	Q9JY16	NMB1789	Protein-export protein SecB GN=secB	CP, IM	1.848E8
346	Q9K0P5	NMB0540	Aminotransferase GN=aspC	CP	1.835E8
347	Q9JZI6	NMB1034	3-isopropylmalate dehydratase small subunit GN=leuD	CP, IM	1.828E8

348	Q9JYZ7	NMB1358	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B GN=gatB [GATB_NEIMB]	CP	1.806E8
349	Q9JYY8	NMB1367	Ribosomal RNA large subunit methyltransferase K GN=rlmK	CP, IM	1.802E8
350	Q9K186	NMB0281	Chaperone SurA GN=NMB0281	CP	1.737E8
351	Q9K1E4	NMB0212	DNA gyrase subunit B GN=gyrB	CP	1.727E8
352	Q9K1P9	NMB0031	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] GN=glmS	CP, IM	1.720E8
353	Q9JYC3	NMB1652	UPF0210 protein NMB1652 GN=NMB1652	CP	1.717E8
354	Q9K0G4	NMB0641	Inorganic pyrophosphatase GN=ppa	CP	1.697E8
355	Q9K0J0	NMB0607	Protein translocase subunit SecD GN=secD	CP	1.693E8
356	Q9JYE7	NMB1616	Phosphomethylpyrimidine kinase GN=thiD	CP	1.676E8
357	Q9JZY2	NMB0851	Recombination-associated protein RdgC GN=rdgC	CP	1.668E8
358	Q7DDK2	NMB0951	Succinate dehydrogenase iron-sulfur subunit GN=sdhB	CP, IM, OM	1.665E8
359	Q9JYU6	NMB1425	Lysine--tRNA ligase GN=lysS	CP	1.664E8
360	Q9JYH7	NMB1582	Histidinol-phosphate aminotransferase GN=hisC	CP	1.617E8
361	Q9K1N8	NMB0044	Peptide methionine sulfoxide reductase MsrA/MsrB GN=msrAB	CP, IM	1.617E8
362	Q9JZ40	NMB1310	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) GN=ispG	CP, IM	1.615E8
363	Q9K116	NMB0381	Cys regulon transcriptional activator GN=cysB	CP	1.604E8
364	Q51117	NMB1926	Lacto-N-neotetraose biosynthesis glycosyltransferase LgtE GN=lgtE	CP IM	1.573E8
365	Q9JXP1	NMB1949	Soluble lytic murein transglycosylase GN=NMB1949	CP	1.563E8
366	Q9JZ59	NMB1279	Membrane-bound lytic murein transglycosylase B GN=NMB1279	CP	1.562E8
367	Q9K041	NMB0781	Uroporphyrinogen decarboxylase GN=hemE	CP	1.556E8
368	Q9K0M3	NMB0569	Na(+)-translocating NADH-quinone reductase subunit A GN=nqrA	CP, EP, IM, PP	1.533E8
369	Q9K0H3	NMB0629	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase GN=hisA	CP	1.530E8
370	Q9JZL7	NMB0997	D-lactate dehydrogenase GN=dld	CP	1.518E8
371	Q7DD87	NMB1737	Secretion protein GN=NMB1737	CP	1.516E8
372	Q9JYQ7	NMB1473	Aminotransferase, class I GN=NMB1473	CP, PP	1.510E8
373	Q9JYZ9	NMB1356	Glutamyl-tRNA(Gln) amidotransferase subunit A GN=gatA [GATA_NEIMB]	CP, IM	1.507E8
374	Q9K053	NMB0768	Twitching motility protein PilT GN=pilT-2	CP	1.503E8
375	Q9K0B7	NMB0697	Ribosomal RNA small subunit methyltransferase A GN=rsmA	EP, OM, PP	1.500E8
376	Q9JZH7	NMB1046	Threonine synthase GN=thrC	CP	1.490E8
377	Q9K0I9	NMB0610	Polyamine-transporting ATPase GN=potA-1	CP	1.485E8
378	P0A0V8	NMB0071	Capsule polysaccharide export outer membrane protein CtrA GN=ctrA	CP	1.461E8
379	Q9JZI5	NMB1036	3-isopropylmalate dehydratase large subunit GN=leuC	CP, IM	1.444E8
380	Q9JX99	NMB2155	Electron transfer flavoprotein, beta subunit GN=etfB	OM, PP	1.438E8
381	Q9JZ77	NMB1256	3,4-dihydroxy-2-butanone 4-phosphate synthase GN=ribB	CP	1.431E8
382	Q9JXK5	NMB1996	Phosphoribosylformylglycinamidine synthase GN=purL	CP, IM, PP	1.411E8
383	Q7DD46	NMB2085	Uncharacterized protein GN=NMB2085	CP	1.409E8
384	Q9K1B0	NMB0257	NADH-quinone oxidoreductase subunit L GN=nuoL	CP	1.405E8

385	Q9K029	NMB0795	Peptidyl-tRNA hydrolase GN=pth	CP, IM	1.398E8
386	Q9JXN3	NMB1961	VacJ-related protein GN=NMB1961	CP	1.385E8
387	Q9JZ38	NMB1312	ATP-dependent Clp protease proteolytic subunit GN=clpP	CP	1.381E8
388	P0A0T5	NMB1621	Glutathione peroxidase homolog GN=gpxA	CP	1.374E8
389	Q7DD70	NMB1843	Transcriptional regulator, MarR family GN=NMB1843	CP	1.310E8
390	Q9K1B8	NMB0246	NADH dehydrogenase I, F subunit GN=nuoF	CP, IM	1.303E8
391	Q9JZI9	NMB1031	3-isopropylmalate dehydrogenase GN=leuB	CP, IM, PP	1.287E8
392	Q9JYR3	NMB1467	Exopolyphosphatase GN=ppx	CP	1.273E8
393	Q7DDR0	NMB0345	Peptidylprolyl isomerase GN=NMB0345	CP	1.267E8
394	Q9JXR2	NMB1920	GMP synthase [glutamine-hydrolyzing] GN=guaA	CP, EP, OM, PP	1.265E8
395	Q9JZP8	NMB0950	Succinate dehydrogenase flavoprotein subunit GN=sdhA	CP	1.260E8
396	Q9JY95	NMB1684	Serine--tRNA ligase GN=serS	CP, EP, OM	1.258E8
397	Q9K012	NMB0815	Adenylosuccinate synthetase GN=purA	CP	1.249E8
398	Q9JZQ0	NMB0947	2-oxoglutarate dehydrogenase, E3 component, lipoamide dehydrogenase GN=NMB0947	CP	1.246E8
399	Q9K055	NMB0766	Elongation factor 4 GN=lepA [LEPA_NEIMB]	EP, OM, PP	1.244E8
400	Q7DD71	NMB1838	Ribosome-binding ATPase YchF GN=ychF	CP	1.241E8
401	Q9JXQ5	NMB1930	Glycine--tRNA ligase beta subunit GN=glyS	IM, OM, PP	1.224E8
402	Q7DDP7	NMB0479	Uncharacterized protein GN=NMB0479	CP, IM, OM, PP	1.203E8
403	P56927	NMB1560	Glutamine--tRNA ligase GN=glnS	CP	1.194E8
404	Q9K002	NMB0828	ADP-L-glycero-D-manno-heptose-6-epimerase GN=hldD	CP	1.186E8
405	Q9JYU4	NMB1428	Aminopeptidase GN=NMB1428	CP	1.159E8
406	Q7DDR2	NMB0329	Type IV pilus assembly protein GN=pilF	CP	1.148E8
407	Q9JZ41	NMB1309	Fimbrial biogenesis and twitching motility protein GN=NMB1309	CP	1.147E8
408	Q7DDD1	NMB1290	Cytosine-specific methyltransferase GN=NMB1290	CP, IM	1.140E8
409	Q7DDC4	NMB1377	L-lactate dehydrogenase GN=lldD	CP	1.116E8
410	Q9JXN4	NMB1958	Putative thioredoxin GN=NMB1958	CP	1.113E8
411	Q9JXG5	NMB2061	Phosphoenolpyruvate carboxylase GN=ppc	OM	1.105E8
412	Q9JXX6	NMB1846	Iron-sulfur cluster carrier protein GN=NMB1846	CP	1.103E8
413	P0A0Z6	NMB1807	Penicillin-binding protein 1A GN=mrcA	CP	1.096E8
414	Q9JXG6	NMB2060	Glycerol-3-phosphate dehydrogenase [NAD(P)+] GN=gpsA	CP	1.091E8
415	Q9K0H6	NMB0626	Peptide chain release factor 3 GN=prfC	CP	1.050E8
416	Q9JRV5	NMB1128	Uncharacterized protein GN=NMB1128	PP	1.050E8
417	Q7DD96	NMB1614	Trk system potassium uptake protein TrkA GN=trkA	CP	1.039E8
418	Q9K183	NMB0284	Adenylosuccinate lyase GN=purB	CP, IM	1.037E8
419	Q9JYH3	NMB1587	Putative protease GN=NMB1587	CP	1.031E8
420	Q9JY30	NMB1768	Hemagglutinin/hemolysin-related protein GN=NMB1768	IM, PP	1.031E8
421	Q9K1L0	NMB0105	PhnO-related protein GN=NMB0105	CP	1.027E8
422	Q9K1E2	NMB0214	Oligopeptidase A GN=prlC	IM	1.008E8
423	Q9JXE9	NMB2078	Uncharacterized protein GN=NMB2078	CP	1.003E8
424	Q7DDQ7	NMB0380	Transcriptional regulator, Crp/Fnr family GN=NMB0380	CP	9.935E7

425	Q9JYA0	NMB1679	tRNA/tmRNA (uracil-C(5))-methyltransferase GN=trmA	CP	9.907E7
426	Q9JYC2	NMB1653	UPF0237 protein NMB1653 GN=NMB1653	CP	9.805E7
427	Q9JZI4	NMB1037	Glutamate--cysteine ligase GN=gshA	CP, IM	9.259E7
428	Q9K092	NMB0724	Phenylalanine--tRNA ligase alpha subunit GN=pheS	CP	9.160E7
429	Q9JX97	NMB2157	Putative pyrazinamidase/nicotinamidase PncA GN=NMB2157	CP, IM	8.934E7
430	Q9JYX2	NMB0724	Glucose-6-phosphate 1-dehydrogenase GN=zwf	CP	8.746E7
431	Q9JYN5	NMB1499	Ribonuclease PH GN=rph	CP	8.702E7
432	Q7DD58	NMB1983	Uncharacterized protein GN=NMB1983	CP	8.270E7
433	Q9K0M8	NMB0564	Na(+)-translocating NADH-quinone reductase subunit F GN=nqrF	CP	7.790E7
434	Q9K0E2	NMB0667	Cell division protein ZipA GN=NMB0667	CP, IM, PP	7.699E7
435	Q9JYX1	NMB1393	Phosphogluconate dehydratase GN=edd	CP, IM	7.631E7
436	Q9K0K6	NMB0588	ABC transporter, ATP-binding protein GN=NMB0588	CP	7.563E7
437	Q9K078	NMB0740	DNA repair protein RecN GN=recN	EP, IM, OM	7.389E7
438	Q9JX94	NMB2160	DNA mismatch repair protein MutS GN=mutS	CP	7.341E7
439	Q9JXQ1	NMB1935	ATP synthase gamma chain GN=atpG	CP	7.241E7
440	P65616	NMB0106	Aspartate carbamoyltransferase GN=pyrB	CP, IM	7.237E7
441	Q9K1Q0	NMB0030	Methionine--tRNA ligase GN=metG	CP	7.202E7
442	Q9K0V1	NMB0459	Uncharacterized protein NMB0459 GN=NMB0459	CP, PP	6.965E7
443	Q9JZ28	NMB1324	Thioredoxin reductase GN=trxB	CP	6.925E7
444	Q9K0Z0	NMB0411	Ribosomal RNA small subunit methyltransferase H GN=rsmH	CP	6.778E7
445	Q9K025	NMB0801	Delta-aminolevulinic acid dehydratase GN=hemB	CP	6.205E7
446	Q9JXS2	NMB1909	Maf-like protein NMB1909 GN=NMB1909	CP	6.019E7
447	Q9JXZ2	NMB1827	DNA polymerase III subunit alpha GN=dnaE	CP	5.968E7
448	Q9K0N7	NMB0549	Macrolide export ATP-binding/permease protein MacB GN=macB	CP	5.924E7
449	Q9K0G8	NMB0637	Argininosuccinate lyase GN=argH	CP	5.717E7
450	Q9JZB6	NMB1200	Ribonuclease R GN=rnr	CP	5.475E7
451	Q9K0C1	NMB0693	Folylpolyglutamate synthase/dihydrofolate synthase GN=folC	CP	5.378E7
452	Q9JYE9	NMB1613	Fumarate hydratase class I GN=fumB	CP	5.164E7
453	H2VFI5	NMB0070	UDP-N-acetylglucosamine 2-epimerase GN=siaA	PP	4.818E7
454	Q9JZW5	NMB0872	Uncharacterized protein GN=NMB0872	CP, IM	3.424E7
455	Q9K004	NMB0825	Putative ADP-heptose synthase GN=NMB0825	CP	3.413E7
456	Q9JZL5	NMB0999	tRNA-dihydrouridine(16) synthase GN=dusC	CP	2.904E7
457	Q9JZ02	NMB1353	Aldehyde dehydrogenase family protein GN=NMB1353	CP, IM	2.757E7
458	Q9K017	NMB0810	Transcriptional regulator, TetR family GN=NMB0810	CP	2.643E7
459	Q9JXP9	NMB1937	ATP synthase subunit delta GN=atpH	CP	2.601E7
460	Q9K0K1	NMB0594	Sensor histidine kinase GN=NMB0594	CP	1.687E7
461	Q9JXE6	NMB2083	Cysteine--tRNA ligase GN=cysS	IM, OM, PP	8.152E6

Appendix III – Genome coverage of meningococcal WGS isolate collections

Table S2 Genome coverage summary statistics.
The mean has been calculated for each statistic detailed in the table. Full details of each isolate record can be found on the PubMLST.org *Neisseria* website using the publications listed in the table. Quality of meningococcal genome sequences has been previously assessed, using some of the isolates below, to determine their use for such studies (Bratcher *et al.* 2014).

	No. of WGS	Origin of isolates	No. of contigs	Total genome length	Min length of contigs	Max length of contigs	Mean length of contigs	N50 contig number	N95 contig number	GC content (%)	Alleles designated (%)
Chapter 2	2016	Disease	224.6	2159685.4	203.6	115218.0	10321.8	21.3	94.1	51.6	99.0
	Isolate records can be found on PubMLST.org *Neisseria* isolate database by searching under the publication Brehony *et al*. 2016 Vaccine 34;4690-7										
Chapter 3	3073	Disease	264.7	2145713.6	203.0	103020.4	8971.4	27.0	121.5	51.7	98.8
	Isolate records can be found on PubMLST.org *Neisseria* isolate database by searching under the publication Rodrigues *et al.* 2018 Emerg Infect Dis 24;673-682										
Chapter 4	3506	Disease	254.8	2146475.6	208.0	111093.7	9442.0	25.8	116.2	51.7	98.2
	Isolate records can be found on PubMLST.org *Neisseria* isolate database and will be linked when the data are published										
Chapter 5	498	Carrier	165.5	2165210.1	249.0	152890.6	13886.2	15.5	66.7	51.6	99.6
	149	Carrier	151.3	2156233.0	267.9	159531.3	15179.4	15.2	63.1	51.8	99.8
	Isolate records can be found on PubMLST.org *Neisseria* isolate database and will be linked when the data are published										

Appendix IV – Co-author consent for re-use of published material in thesis

29th May 2018

To Whom It May Concern,

RE: PERMISSION FOR RE-USE OF PUBLISHED MATERIAL IN DPHIL THESIS.

I hereby confirm that Dr Charlene Rodrigues, DPhil student at University of Oxford (from October 2015) was the joint primary author of the manuscript and figures from the publication below:

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- Preparing figures 2, 4a and 4b.
- Submission and revision for publication with peer-reviewed journal Vaccine.

As a co-author on this publication, I give my permission for the accepted manuscript to be used as part of her DPhil thesis, stipulating her contribution as above.

Signed

Carine Brehony

Date

29th May 2018

Signed

CHARLENE RODRIGUES

Date 29th May 2018

30th May 2018

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Signed 

Ray Borrow

Date 30/05/2018

Signed 

CHARLENE RODRIGUES

Date 30th May 2018

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Signed 

Claire Cameron

Date 29th May 2018

Signed 

CHARLENE RODRIGUES

Date 29th May 2018

UNIVERSITY OF OXFORD

29th May 2018

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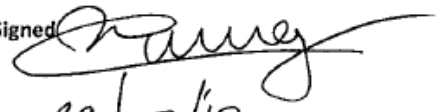
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Signed 

ROBERT CUNNEY

Date 29/5/18

Signed 

CHARLENE RODRIGUES

Date 29th May 2018

UNIVERSITY OF OXFORD

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Signed JM LUCIDARME

Date 29/05/18

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11th September 2018

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Signed		**Signed**	
	MARTIN MAIDEN		CHARLENE RODRIGUES
Date	11th September 2018	**Date**	11th September 2018

29th May 2018

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Signed

RICHARD MOXON

Date June 20 2018

Signed

CHARLENE RODRIGUES

Date 29th May 2018

29th May 2018

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Signed Andrew Smith

Date 29/5/18

Signed

CHARLENE RODRIGUES

Date 29th May 2018