Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation

Carina Brehony [a,1], Charlene M.C. Rodrigues [a,1,*], Ray Borrow [b], Andrew Smith [c,d], Robert Cunney [e], E. Richard Moxon [f], Martin C.J. Maiden [a]

[a] Department of Zoology, University of Oxford, South Parks Road, Oxford, United Kingdom
[b] Public Health England, Meningococcal Reference Unit, Manchester Royal Infirmary, Manchester, United Kingdom
[c] Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, Glasgow Royal Infirmary, Glasgow, United Kingdom
[d] College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, United Kingdom
[e] Irish Meningitis and Meningococcal Reference Laboratory, Temple Street Children's University Hospital, Dublin, Ireland
[f] Department of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form 1 August 2016
Accepted 3 August 2016
Available online xxxx

Keywords:
Neisseria meningitidis
Vaccine design
Molecular epidemiology
Genome
Surveillance
Bexsero® Antigen Sequence Type

ABSTRACT

Serogroup B is the only major disease-associated capsular group of *Neisseria 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. Whole genome sequencing (WGS), as realised in the Meningitis Research Foundation Meningococcus Genome Library (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 clonal complex (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.

1. Introduction

Invasive meningococcal disease (IMD), caused by Gram negative organism *Neisseria meningitidis*, remains an important cause of morbidity and mortality worldwide [1]. 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 [2]. Protein-polysaccharide conjugate vaccines have been successfully implemented against the major disease-associated serogroups in high and low income settings, with the

Abbreviations: BAST, Bexsero® Antigen Sequence Type; cc, clonal complex; fHbp, factor-H binding protein; IMD, invasive meningococcal disease; MATS, Meningococcal Antigen Typing System; MLST, multilocus sequence type; MRF-MGL, Meningitis Research Foundation Meningococcus Genome Library; NadA, neisserial adhesion A; NHBA, neisserial heparin-binding antigen.

* Corresponding author.
E-mail addresses: carina.brehony@nuigalway.ie (C. Brehony), charlene.rodrigues@gtc.ox.ac.uk (C.M.C. Rodrigues), ray.borrow@phe.gov.uk (R. Borrow), andrew.smith@glasgow.ac.uk (A. Smith), Robert.Cunney@cuh.ie (R. Cunney), richard.moxon@paediatrics.ox.ac.uk (E.R. Moxon), martin.maiden@zoo.ox.ac.uk (M.C.J. Maiden).
[1] These authors contributed equally and should be considered as joint first authors.

http://dx.doi.org/10.1016/j.vaccine.2016.08.015
0264-410X/

exception of serogroup B [3,4]. The immuno-chemical similarity of the serogroup B capsule and human cell surface polysaccharides, results in poor immune responses and safety concerns [5]. Consequently, development of 'group B' vaccines has concentrated on sub-capsular antigens, using either outer membrane vesicles, purified proteins, or both [6]. Bexsero® was licensed in Europe in 2014 and by the end of 2015, two vaccines, Bexsero® [7] and Trumenba® [8], were licensed in the USA. Bexsero®, included in the UK infant immunisation schedule in September 2015 [9], combines the protein antigens factor-H binding protein (fHbp), neisserial adhesion A (NadA), neisserial heparin-binding antigen (NHBA) and PorA with an outer membrane vesicle from the MeNZB™ vaccine [7,10].

Molecular typing approaches are important for IMD diagnosis in the absence of a confirmed culture [11,12]; as over half of IMD cases are diagnosed solely by PCR in the UK [13]. Nucleotide sequence-based typing of meningococci has many applications [14], including post-vaccination surveillance, where multilocus sequence typing (MLST) established that meningococcal C conjugate vaccine significantly reduced carriage of the outbreak strain in the UK population [15]. 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 [16]. Analysis of MRF-MGL data from 2010/11 onwards, publicly accessible on the PubMLST database [17], 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 [18,19].

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® [20]. Based on serogroup B IMD isolates, strain coverage of 73% in England and Wales was predicted [21]. Here, we characterised the diversity and structure among disease-causing *N. meningitidis* isolates from the UK and Ireland over four years (2010/14), defining a reproducible method for typing Bexsero® vaccine antigens. As a demonstration of the utility of WGS, we developed a model for predicting the likely MATS result from genotype.

2. Materials and methods

2.1. 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 *Neisseria* public database (http://pubmlst.org/neisseria/). 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), geographical and temporal spread.

2.2. 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 [22]. 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) [23–25] assigned a unique identification number. 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 [26]. 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.

2.3. 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 [27,28]. 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. [29]. 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 [30]. ClonalFrameML uses a maximum likelihood approach for phylogenetic reconstruction while taking into account recombination.

2.4. 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 [31]. The 'standardised index of association', I_A^S, detected linkage disequilibrium among the BAST loci using the START2 program 0.9.0 beta [32,33]. The f^* metric measured the amount of non-overlapping structure in the combinations of antigens [34]. 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 [35]. F_{ST} and AMOVA were calculated with Arlequin version 3.1 [36]. The chi squared test and chi squared test for trend detected changes in BAST over time and calculated with 'prop.test'

Please cite this article in press as: Brehony C et al. Distribution of Bexsero® Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: Implications for immunisation. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.08.015