Journal of Infection (2016) xx, 1–8

ELSEVIER

BIA British Infection Association

www.elsevierhealth.com/journals/jinf

Differences between culture & non-culture confirmed invasive meningococci with a focus on factor H-binding protein distribution

Stephen A. Clark [a,*], Aiswarya Lekshmi [a], Jay Lucidarme [a], Li Hao [b], How Tsao [b], Lisa Lee-Jones [c], Kathrin U. Jansen [b], Lynne S. Newbold [a], Annaliesa S. Anderson [b], Ray Borrow [a]

[a] *Vaccine Evaluation Unit, Public Health England, Clinical Sciences Building II, Manchester Royal Infirmary, Manchester M13 9WZ, United Kingdom*
[b] *Pfizer Vaccine Research, 401 N. Middletown Rd., Pearl River, NY 10965, United States*
[c] *Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester M1 5GD, United Kingdom*

Accepted 17 March 2016
Available online ■ ■ ■

KEYWORDS
Neisseria meningitidis;
Factor H-binding protein;
Meningococcal vaccines;
DNA sequencing

Summary *Objectives:* To compare the distribution of capsular groups and factor H-binding protein (fHBP) variants among meningococcal isolates and non-culture clinical specimens and to assess the representativeness of group B isolates amongst group B cases as a whole.
Methods: A PCR sequencing assay was used to characterise fHBP from non-culture cases confirmed from January 2011 to December 2013. These were compared to genotypic data derived from whole genome analysis of isolates received during the same period.
Results: Group W and Y strains were more common among isolates than non-culture strains. The distribution of fHBP variants among group B non-culture cases generally reflected that seen in the corresponding isolates. Nonetheless, the non-culture subset contained a greater proportion of fHBP variant 15/B44, associated with the ST-269 cluster sublineage.
Conclusions: Differences in capsular group and fHBP distribution among culture and non-culture cases may be indicative of variation in strain viability, diagnostic practice, disease severity and/or clinical presentation. Future analyses combining clinical case information with

* Corresponding author. Tel.: +44 (0) 161 276 8998.
E-mail addresses: stephen.clark@phe.gov.uk (S.A. Clark), aiswarya.lekshmi@phe.gov.uk (A. Lekshmi), jay.lucidarme@phe.gov.uk (J. Lucidarme), li.hao@pfizer.com (L. Hao), how.tsao@pfizer.com (H. Tsao), l.lee-jones@mmu.ac.uk (L. Lee-Jones), kathrin.jansen@pfizer.com (K.U. Jansen), lynne.newbold@phe.gov.uk (L.S. Newbold), annaliesa.anderson@pfizer.com (A.S. Anderson), ray.borrow@phe.gov.uk (R. Borrow).

http://dx.doi.org/10.1016/j.jinf.2016.03.012

laboratory data may help to further explore these differences. Group B isolates provide a good representation of group B disease in E&W and, therefore, can reliably be used in fHBP strain coverage predictions of recently-licensed vaccines.

Introduction

Hyper-virulent group B meningococci continue to cause a substantial proportion of meningococcal disease cases, particularly in Europe, North America and Australia.[1] The development and licensure of two novel protein-based vaccines offers the possibility of significantly reducing this disease burden.[2,3] An antigenic constituent shared by both of these vaccines is factor H-binding protein (fHBP). This outer membrane protein has been shown to elicit immune protection against a diverse array of group B *Neisseria meningitidis* strains.[4,5] As a ligand for human complement factor H (fH), the expression of fHBP by meningococci suppresses the alternative complement pathway and promotes bacterial survival *in vivo*.[6] Over 900 unique fHBP protein variants have thus far been identified and can be divided into two immunologically distinct and largely non-cross protective subfamilies (A and B)[7] or divided further into three variant groups.[8] The antigenic diversity exhibited by fHBP has greatly increased the complexity of vaccine strain coverage predictions as the breadth of protection provided by individual vaccine variants is largely dependent on the presence of cross-reactive epitopes among variants expressed by invasive strains.

Traditionally, the distribution of such antigens among invasive strains has been determined by analysis of cultured isolates, the vast majority of which possess fHBP.[9–12] In many disease cases, however, the prompt administration of antibiotics prevents the isolation of a viable culture and laboratory confirmation can only be achieved through the detection of residual meningococcal DNA within 'non-culture' clinical specimens. In England and Wales (E&W), only ~50% of cases confirmed by the Meningococcal Reference Unit (MRU) yield a culturable isolate.[13] All clinical isolates subsequently undergo whole genome sequence analysis, from which the distribution of vaccine antigens such as fHBP can be ascertained. Indexed genomic information from invasive isolates collected over five epidemiological years (July–June inclusive): 2010/11 (n = 513), 2011/12 (n = 409), 2012/13 (n = 457), 2013/14 (n = 406) and 2014/15 (n = 521) (total n = 2306) is publicly available at the Meningitis Research Foundation Meningococcus Genome Library (MGL, http://www.meningitis.org/research/genome).[14] In addition to these genotypic analyses, the expression of fHBP by invasive isolates, a key prerequisite for immune protection, can be confirmed *in vitro*.[4,15]

In contrast, for non-culture cases, whole genome sequencing is yet to be established and, in the absence of a viable isolate, fHBP expression cannot be directly quantified. In order to tackle this significant epidemiological knowledge gap, a PCR sequencing assay was developed to sequence *fHBP* from non-culture clinical specimens.[16]

Here we present fHBP typing data from non-culture specimens submitted to the MRU over three calendar years (2011–2013). For the first time we can compare these data with those derived from isolates received during the same period to assess the representativeness of viable isolates among all invasive meningococcal strains in E&W.

Materials and methods

Clinical isolates

To determine the distribution of fHBP among isolates, fHBP peptide data representing E&W culture cases confirmed between January 2011 and December 2013 were downloaded from the MGL (n = 1336).

To assess the association between hyper-virulent clonal complexes and common fHBP variants, Multi-locus Sequence Typing (MLST) data and fHBP peptide data for E&W MGL isolates received from 2010/11 to 2014/15 (n = 2306) were downloaded. These were then combined with corresponding data from isolates received during the 2007/08 epidemiological year (n = 613) generated through previous PCR sequence analysis (total isolates n = 2919).

Clinical specimens

The MRU carries out disease confirmation from submitted culture-negative clinical specimens (e.g. blood and CSF specimens) using an in-house *ctrA*-directed TaqMan real-time PCR assay.[17] Specimens producing PCR cycle threshold (Ct) values of >45 are currently deemed negative. The assay also consists of capsular group-specific primers and probes complementary to sequences within the *siaD* or *mynA* capsular synthesis alleles to allow genogroup determination. DNA extraction and typing of fHBP from non-culture specimens was carried out as previously described.[16]

fHBP nomenclature

Differing nomenclatures are currently used for fHBP protein variants. In the system established by Fletcher et al., individual variants are given unique alphanumeric identifiers based upon the subfamily to which each variant belongs.[7] In an alternative nomenclature system, variants are assigned a unique number preceded by the variant group number, i.e. 1, 2 or 3.[8] In addition, the PubMLST database (hosted by the University of Oxford, UK) assigns arbitrary, sequential numbers to unique *fHBP* alleles and protein variants as they are submitted.[18] For clarity, fHBP protein variants are here referred to by both their assigned PubMLST peptide ID number and their alphanumeric subfamily ID (e.g. peptide 22/A10).

Please cite this article in press as: Clark SA, et al., Differences between culture & non-culture confirmed invasive meningococci with a focus on factor H-binding protein distribution, J Infect (2016), http://dx.doi.org/10.1016/j.jinf.2016.03.012