Genetic and antigenic characterization of Canadian invasive *Neisseria meningitidis* serogroup C (MenC) case isolates in the post-MenC conjugate vaccine era, 2009–2013

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We previously reported a shift in the electrophoretic type (ET) of invasive MenC in Canada from predominantly ET-15 to ET-37 in the post-MenC conjugate vaccine period. This study sought to confirm this trend by examining all culture-confirmed invasive MenC case isolates in Canada in the period from 1 January 2009 to 31 December 2013. Of the 50 MenC isolates, 18 belonged to ET-15, 28 belonged to ET-37 (but not ET-15), and four belonged to other clonal types. Analysis of the serotype and serosubtype antigens, *porA* and *fetA* gene sequences provided data to show that invasive MenC belonging to ET-15 and ET-37 were two very different subpopulations within the ST-11 clonal complex. Sequence analysis of the *fHbp* genes suggested that 12 different types of factor H-binding protein were found among the ET-15 isolates while 86% of ET-37 isolates were found to have *fHbp* genes predicted to encode peptide 22. The *nadA* gene in 12 MenC isolates was disrupted due to IS1301 insertion and 11 of these 12 isolates belonged to ET-15. Ten per cent of the invasive MenC were found to have a frame-shift mutation in their *fHbp* genes that predicted no fHbp produced. Significant diversity and frame-shift mutations of *fHbp* genes were found in invasive MenC strains in Canada.

INTRODUCTION

*Neisseria meningitidis* is an important invasive Gram-negative bacterium known to cause meningitis and sepsicaemia with a mean case fatality rate of 10% (Rosenstein et al., 2001). Laboratory surveillance and epidemiological studies of invasive meningococcal disease (IMD) involve characterization of the *N. meningitidis* serogroup, serotype, serosubtype antigens, and partial or complete gene sequences of a number of surface antigens such as PorA, PorB and FetA (Frasch et al., 1985; Harrison et al., 2011) as well as clonal analysis by multilocus enzyme electrophoresis (Caugant et al., 1986) or multilocus sequence typing (MLST) (Maiden et al., 1998).

Sergroup C *N. meningitidis* (MenC) has played a significant role in the epidemiology of IMD in Canada. It
began with the emergence of the electrophoretic type (ET)-15 clone in the late 1980s (Ashton et al., 1991), which then spread across North America and to various parts of the world (Jelfs et al., 2000). ET-15 is a genetic variant that belongs to the ET-37 or sequence type (ST)-11 clonal complex (Jelfs et al., 2000), and is characterized by a unique fumarase enzyme allele 2, confirmed by DNA sequencing of the \textit{fumC} gene (Vogel et al., 2000). In Canada, this strain has caused two waves of IMD outbreaks across the country. The most recent outbreaks, in 2000–2001, were widespread and led to the licensure of the MenC conjugate vaccine in 2001 (Pollard & Tam, 2011). Initially, only one province (Québec) implemented the MenC conjugate vaccine into their publicly funded immunization programme, but gradually other provinces and territories followed with their own programmes. By 2007–2011 all ten provinces and three territories had implemented MenC or MenA, C, W, Y conjugate vaccine programmes covering the infant and adolescent populations. The year of introduction, schedules and choice of the publicly funded MenC or MenA, C, W, Y conjugate vaccines employed by the different provinces and territories in Canada are described in a Public Health Agency of Canada’s online document (http://www.phac-aspc.gc.ca/im/ptimprog-progimpt/table-1-eng.php) and in an Advisory Committee Statement by the National Advisory Committee on Immunization (NACI, 2013).

Success of the MenC conjugate vaccine programmes has been evaluated (De Wals et al., 2011; Kinlin et al., 2009). The overall mean incidence rate of IMD in Canada for the surveillance period of 2006–2011 was 0.58 per 100 000 population and mean incidence rates according to serogroups were 0.33 for MenB, 0.07 for MenC, 0.03 for MenW, 0.1 for MenY, and 0.013 for other serogroups (Li et al., 2014). However, the percentage of culture-confirmed IMD cases caused by the different serogroups of meningococci varied according to the province. For example, the percentage of culture-confirmed IMD due to MenC varied from a low of 8.7 % in Saskatchewan to a high of 23.5 % in Ontario, while MenB was found in 71.7 % of culture-confirmed cases in Québec and 36.5 % in Alberta. The range of culture-confirmed IMD cases due to MenY ranged from a high of 43.5 % in Saskatchewan to a low of 6.2 % in Québec; and the figures for MenW were 17.3 % in Alberta and 4.5 % in British Columbia (Law et al., 2014; Jamieson et al., 2013; Zhou et al., 2012b).

The National Microbiology Laboratory (NML) serves as a national reference centre for \textit{N. meningitidis}, and receives IMD isolates from all provinces and territories for antigenic and genetic characterization. Since the mid-2000s, the number of invasive MenC isolates received at the NML has decreased dramatically, from a high of 173 case isolates in 2001 (Law et al., 2005) to a low of only two in 2011 and six case isolates in 2013 (this study). Coincidentally to the decrease in invasive MenC cases, we noticed a shift in the ET of invasive MenC strains from ET-15 to predominantly ET-37 (not ET-15) type (Zhou et al., 2012a). In order to understand this phenomenon further, we extended our previous study to include more recent invasive MenC isolates (from 2009 to 2013) as well as to determine the genetic sequence of newer meningococcal vaccine targets, factor H-binding protein (fHbp) and \textit{Neisseria} adhesin A (NadA) (Richmond et al., 2012; Santolaya et al., 2012).

**METHODS**

This study examined all MenC isolates from culture-confirmed IMD cases in Canada from 1 January 2009 to 31 December 2013. Invasive MenC isolates were cultured and identified from blood, CSF or other normally sterile body sites of individual IMD patients using standard bacteriological culture methods. MenC isolates were characterized by indirect whole cell ELISA with murine monoclonal antibodies to determine their serotype and serosubtype antigens (Abdillahi & Poolman, 1987). Sequencing of their \textit{porB}, \textit{fetA} and MLST genes was done using methods previously described by others and adopted into our routine work (Zhou et al., 2012a, b; Sloan et al., 2008). Strains belonging to the ET-37 (ST-11) clonal complex were divided into those that belong to ET-15 and those that belong to ET-37 (but not ET-15) by extending the DNA sequencing of the \textit{fumC} gene to cover the fragment that contains the molecular signatures for differentiating these ET types (Vogel et al., 2000). The \textit{fHbp} and \textit{nada} genes were amplified by PCR from genomic DNA using PCR primers that flank the coding sequences, as described in the literature (Lucidarme et al., 2010). The \textit{fHbp} and \textit{nada} nucleotide sequences and peptide types were determined using the online sequence typing tool provided by the Neisseria.org website (http://pubmlst.org/neisseria/fHbp/ and http://pubmlst.org/neisseria/NadA/).

The ages of the invasive MenC cases were obtained from the specimen requisition forms, and statistical analysis to compare the age of MenC cases due to ET-15 with those due to ET-37 was done by the Student \textit{t}-test according to methods described by Hill (1977).

**RESULTS AND DISCUSSION**

The temporal and geographical distribution of the ET-15, ET-37 and other clonal types of invasive MenC isolates examined in this study is described in Table 1. Overall, 56 % of invasive MenC were ET-37 (not ET-15), 36 % were ET-15, and 8 % belonged to other clonal types. Strains of ET-15 were more common in central and eastern Canada (Ontario and Québec; \(n=17\)) than in western Canada (British Columbia, Alberta, Saskatchewan and Manitoba; \(n=1\)). In contrast, strains of ET-37 (not ET-15) type appeared to be more common in western Canada (\(n=18\)) than in central and eastern Canada (\(n=10\)) and there was no obvious temporal trend. The ages of the IMD cases caused by ET-15 versus those caused by ET-37 were not statistically different (Student \textit{t}-test, \(t=1.9798\); \(P=0.0543\)). The ages of the ET-15 cases ranged from 6 to 83 years, with a median age of 49 years. The age of the ET-37 cases ranged from 1 month to 82 years, with a median age of 33.5 years.

When the antigenic formula, PorA genotype, FetA, fHbp and NadA peptide types were compared between the ET-15 and the ET-37 (not ET-15) types, it was apparent that there were substantial differences between these two subpopulations of

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http://jmm.sgmjournals.org
ET-37 or ST-11 clonal complex of invasive MenC (Table 2). Strains of ET-15 invasive MenC expressed mainly the antigenic formula of either C:2a:P1.5 or C:2a:P1.7,1, supported by their PorA genotypes of P1.5-1,10-8, 36-2 and P1.7-1,1,35-1, respectively. The majority (13/18 or 72 %) of the ET-15 strains were found to have the FetA allele F3-6, two strains were found to have fetA gene deletion (Claus et al., 2007; Marsh et al., 2007), and the remaining three isolates were found to have two other FetA alleles (F1-7 or F1-18). Unlike the serotype, serosubtype antigens or FetA alleles, the fHbp peptide types found in the ET-15 isolates were rather diverse (12 peptide types found and four isolates with the genetic allele 669, which contained a frame-shift mutation). High sequence diversity in the fHbp genes was also reported among C:2a:P1.7,1 ST-11 isolates in France (Hong et al., 2012). The nadA genes in 11 (61 %) of the 18 ET-15 isolates belonged to allele 29 (except in two isolates, which contained a single nucleotide mutation) but were disrupted by an IS1301 insertion, and therefore, no peptide was predicted to be synthesized by these 11 strains. In a previous study, 68.6 % of ET-15 MenC examined were found to contain a disrupted nadA gene due to insertion by IS301 (Elias & Vogel, 2007). The nadA genes in the remaining seven isolates belonged to either allele 2, predicted to synthesize peptide 2 (n=3), or allele 3, predicted to synthesize peptide 3 (n=4).

In contrast, most (25/28 or 89 %) of the ET-37 (not ET-15) strains were found to have the PorA genotype of P1.5,2, 36-2; 18 isolates expressed the antigenic formula C:2a:P1.5,2. Also, most of the ET-37 strains were found to have the FetA F3-3 allele (23 strains or 82 %) and fHbp peptide 22 (24 strains or 86 %). Only four ET-37 isolates were found to have either other fHbp peptide types (201 or 483) or no fHbp due to genetic frame-shift in its fHbp gene (allele 669). Similar to the PorA genotype, 24 (86 %) of 28 ET-37 isolates were found to have the nadA gene allele 3, predicted to synthesize NadA peptide 3, and one isolate with a nadA gene allele 3 contained a frame-shift mutation that likely led to no NadA protein produced. In another isolate, the nadA gene allele 29 contained an IS1301 insertion that disrupted the gene. In the remaining two isolates, the nadA gene sequences were either identical to allele 117 (predicted to synthesize peptide 121) or related
Table 2. Antigenic and genetic profile of invasive MenC case isolates in Canada, 2009–2013

<table>
<thead>
<tr>
<th>Antigens</th>
<th>PorA genotype</th>
<th>MLST</th>
<th>fHbp peptide</th>
<th>FetA variant</th>
<th>Allele</th>
<th>Peptide</th>
<th>No. isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-11 or ET-37 clonal complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C : 2a : P1.5,- PorA P1.5-1,10-8,36-2 ET-15</td>
<td>F3-7</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 2a : P1.5,- PorA P1.5-1,10-8,36-2 ET-15</td>
<td>Deletion</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 2a : P1.5,- PorA P1.5-1,10-8,36-2 ET-15</td>
<td>Allele 669 Deletion</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 2a : P1.5,- PorA P1.5-1,10-8,36-2 ET-15</td>
<td>Allele 669 F1-7</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 2a : P1.5,- PorA P1.5-1,10-8,36-2 ET-15</td>
<td>Allele 669 F3-6</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Non-ST-11/ET-37 clonal complex</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C : NT : P1.5 PorA P1.5-1,10-4,36-2 ST-2006 (103 cc)</td>
<td>F3-9</td>
<td>Gene absent</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 15, 19 : P1.13 PorA P1.7-2,13,35-1 ST-278 (35 cc)</td>
<td>F1-7</td>
<td>Gene absent</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 19 : P1.14 PorA P1.22,14,36 ST-5571 (336 cc)</td>
<td>F5-1</td>
<td>Gene absent</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : NT : P1.14 PorA P1.7-2,14,36 ST-4821 (4821 cc)</td>
<td>F3-3</td>
<td>Gene absent</td>
<td>1</td>
<td></td>
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</tr>
</tbody>
</table>

* nadA allele 3 with 1 nt insertion leading to a frame-shift.
† Different from nadA allele 81 by 7 nt.
‡ Different from NadA peptide 91 by 4 aa.
§ Premature stop codon found in VR1.
|| VR1 deleted.
¶ No NadA peptide due to nadA gene disruption by insertion of IS1301.
# Different from nadA allele 29 by 1 nt insertion.
** Different from nadA allele 29 by 1 nt deletion.
to allele 81 (differing by 7 nt) and deduced to produce a peptide related to peptide 91 (with 4 aa difference).

Among the 50 invasive MenC strains, 17 different fHbp peptide types were found and five isolates were predicted not to express fHbp due to a frame-shift mutation that led to a premature stop codon (Lucidarme et al., 2011). Twelve of the MenC isolates were not predicted to produce NadA peptides due to the presence of an IS1301 insertion in their nadA genes. Most of the ET-37 invasive MenC isolates were from western Canada and predicted to synthesize fHbp peptide 22 and contained the FetA allele F3-3. In contrast, ET-15 is more often found in central and eastern Canada with FetA allele F3-6 but predicted to synthesize a wide variety of fHbp peptide types. Furthermore, the fHbp peptide types predicted to be produced by ET-15 MenC strains were different from the peptide types predicted to be produced by ET-37 MenC strains.

In conclusion, this study confirmed our previous observation of a shift in invasive MenC strains from predominantly ET-15 to ET-37, and the percentage of MenC being ET-37 was 1.56 times the percentage of ET-15 isolates. Furthermore, the fHbp peptide types predicted to be produced by ET-15 MenC strains were different from the peptide types predicted to be produced by ET-37 MenC strains.

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REFERENCES


