Characterization of invasive *Neisseria meningitidis* from Atlantic Canada, 2009 to 2013: With special reference to the nonpolysaccharide vaccine targets (PorA, factor H binding protein, *Neisseria* heparin-binding antigen and *Neisseria* adhesin A)

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BACKGROUND: Serogroup B *Neisseria meningitidis* (MenB) has always been a major cause of invasive meningococcal disease (IMD) in Canada. With the successful implementation of a meningitis C conjugate vaccine, the majority of IMD in Canada is now caused by MenB.
OBJECTIVE: To investigate IMD case isolates in Atlantic Canada from 2009 to 2013. Data were analyzed to determine the potential coverage of the newly licensed MenB vaccine.
METHODS: Serogroup, serotype and serosubtype antigens were determined from IMD case isolates. Clonal analysis was performed using multilocus sequence typing. The protein-based vaccine antigen genes were sequenced and the predicted peptides were investigated.
RESULTS: The majority of the IMD isolates were MenB (82.5%, 33 of 40) and, in particular, sequence type (ST)-154 B:4:P1.4 was responsible for 47.5% (19 of 40) of all IMD case isolates in Atlantic Canada. Isolates of this clone expressed the PorA antigen P1.4 and possessed the *nhba* genes encoding for *Neisseria* heparin-binding antigen peptide 2, which together matched exactly with two of the four components of the new four-component meningococcal B vaccine. Nineteen MenB isolates had two antigenic matches, another five MenB and one meningitis Y isolate had one antigenic match. This provided 75.8% (25 of 33) potential coverage for MenB, or a 62.5% (25 of 40) overall potential coverage for IMD.
CONCLUSION: From 2009 to 2013, IMD in Atlantic Canada was mainly caused by MenB and, in particular, the B:4:P1.4 ST-154 clone, which accounted for 47.5% of all IMD case isolates. The new four-component meningococcal B vaccine appeared to offer adequate coverage against MenB in Atlantic Canada.

Key Words: *Atlantic Canada; Invasive* Neisseria meningitidis*; MenB vaccine*

La caractérisation du *Neisseria meningitidis* invasif dans les Maritimes de 2009 à 2013, notamment les cibles du vaccin non polysaccharidique (porA, protéine de liaison au facteur H, antigène de liaison à l'héparine de *Neisseria* et adhésine A de *Neisseria*)

HISTORIQUE : Le *Neisseria meningitidis* du sérogroupe B (MenB) a toujours été une cause importante de méningococcie invasive (MI) au Canada. Depuis l'adoption d'un vaccin conjugué contre le méningocoque du groupe C, la majorité des MI au Canada sont désormais attribuables au MenB.
OBJECTIF : Examiner les isolats de cas de MI dans les Maritimes entre 2009 et 2013. Analyser les données pour déterminer la couverture potentielle du vaccin nouvellement homologué contre le MenB.
MÉTHODOLOGIE : Les chercheurs ont déterminé le sérogroupe, le sérotype et les antigènes des sous-types sérologiques des isolats de cas de MI. Ils ont effectué l'analyse clonale au moyen du typage génomique multilocus. Ils ont séquencé les gènes des antigènes du vaccin à base de protéines et examiné les peptides prédits.
RÉSULTATS : La majorité des isolats de MI étaient des MenB (82,5 %, 33 sur 40). Notamment, le type séquentiel (TS)-154 B:4:P1,4 était responsable de 47,5 % (19 sur 40) de tous les isolats de cas de MI dans les Maritimes. Les isolats de ce clone ont exprimé l'antigène porA P1.4 et étaient dotés des gènes *nhba* codant pour le peptide 2 de l'antigène de liaison à l'héparine de *Neisseria*. Ensemble, ces antigènes correspondaient exactement à deux des quatre composants du nouveau vaccin contre le méningocoque du groupe B à quatre composants. Dix-neuf isolats du MenB étaient dotés de deux correspondances antigéniques, tandis que cinq autres MenB et un isolat de la méningite Y étaient dotés d'une correspondance antigénique. Ces résultats assuraient une couverture potentielle du MenB de 72,7 % (24 sur 33) ou une couverture potentielle globale de la MI de 62,5 % (25 sur 40).
CONCLUSION : De 2009 à 2013, dans les Maritimes, la MI était surtout causée par le MenB, en particulier le clone B:4:P1.4 ST-154, responsable de 47,5 % de tous les isolats de cas de MI. Le nouveau vaccin contre le méningocoque du groupe B à quatre composants semble offrir une couverture pertinente contre le MenB dans cette région.

Invasive meningococcal disease (IMD) is a serious, nationally notifiable disease caused by the Gram-negative diplococcal bacterium *Neisseria meningitidis*. Invasive diseases caused by *N meningitidis* include meningitis, septicemia, pneumonia, septic arthritis and, occasionally, endocarditis, myocarditis and pericarditis, with an average case fatality rate of 10% (1). Six (A, B, C, W, X and Y) of the 12 recognized serogroups are

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TABLE 1
Distribution of serogroups* of invasive *Neisseria meningitidis* in Atlantic Canada from 2009 to 2013

Year	Province				
	New Brunswick	Nova Scotia	Prince Edward Island	Newfoundland and Labrador	Atlantic Canada
2009	6 (4B, 1Y, 1W)	2 (1B, 1C)	1 (B)	4 (4B)	13 (10B, 1C, 1Y, 1W)
2010	6 (4B, 1Y, 1E)	2 (1B, 1Y)	0	2 (2B)	10 (7B, 2Y, 1E)
2011	3 (3B)	3 (2B, 1Y)	0	1 (1B)	7 (6B, 1Y)
2012	6 (6B)	1 (1B)	1 (1B)	0	8 (8B)
2013	2 (2B)	0	0	0	2 (2B)
2009 to 2013	23 (19B, 2Y, 1W, 1E)	8 (5B, 1C, 2Y)	2 (2B)	7 (7B)	40 (33B, 1C, 4Y, 1W, 1E)

*Data presented as n (serogroup[s]). *At least 12 different serogroups are recognized by specific antisera: A, B, C, E, H, I, K, L, W, X, Y and Z*

responsible for most of the invasive diseases, which occur globally (2,3). However, geographical differences in serogroup distribution is well known (4); and in Canada, most IMD in the past 20 years have been caused mainly by serogroups B (MenB) and C (MenC) and, to a lesser degree, by serogroups W (MenW) and Y (MenY) (5-10). Aside from the serogroup antigen, the *N meningitidis* major outer membrane proteins, PorB (serotype antigen) and PorA (serosubtype antigen) are also important surface markers for typing and characterization of strains. Traditionally, clonal analysis of *N meningitidis* strains were performed using multilocus enzyme electrophoresis, which grouped isolates into electrophoretic types. However, it is not a user-friendly method, and the results generated are not portable for comparison between different laboratories. Therefore, it has been replaced by the more objective method of multilocus sequence typing (MLST), with isolates grouped together into sequence types (STs). Related STs are grouped together into clonal complexes (CCs). Most IMDs, especially those found in teenagers, adolescents and young adults, as well as those occurring in clusters or in epidemic form, are caused by a few major CCs known as hypervirulent clones (11).

After the introduction of meningococcal A, C, W and Y conjugate vaccine programs in Canada (12), the incidence of MenC disease has decreased substantially, with only six culture-confirmed MenC cases identified in Canada in 2013 (13). MenB is now the only major serogroup of meningococci left to be controlled by the newer MenB vaccines, which have either been licensed recently in Canada (14), or are in advanced clinical trials and awaiting regulatory approval for licensure in Canada (15).

Unlike the capsule vaccine used for serogroups A, C, W and Y meningococci, a MenB capsule would be nonimmunogenic due to the presence of a self-antigen (16) and, therefore, not feasible. Noncapsule meningococcal vaccines developed in the past were based on the *porA* outer membrane protein (OMP) vesicles; however, such vaccines are strain specific, effective against strains displaying homologous PorA antigens to the OMP vesicle (OMV) vaccine. Nevertheless, they have been used successfully in the past to control epidemics of MenB IMD (17-20). Genome sequencing of microbial pathogens have renewed interest in the development of universal MenB vaccines. One such newer MenB vaccine (Bexsero, Novartis, Canada) recently licensed in Canada, has been developed using reverse vaccinology based on whole genome sequence information of meningococci (21). This four-component meningococcal B (4CMenB) recombinant vaccine comprises: factor H binding protein (fHbp) subfamily B or variant 1, peptide 1; *Neisseria* heparin binding antigen (NHBA) peptide 2; *Neisseria* adhesion A (NadA)-3.8; and the OMV vaccine used in New Zealand to control the MenB epidemic caused by the strain B:4:P1.4 (20). Another investigational MenB vaccine is based on a bivalent recombinant fHbp or lipoprotein 2086 (22).

fHbp is classified into three variants (1, 2 and 3) or two subfamilies, with subfamily A equivalent to variants 2 and 3, and subfamily B equivalent to variant 1. Because of amino acid sequence similarities between proteins in each subfamily of fHbp, cross-protection between proteins within a subfamily is possible, but not between subfamilies (23). Similarly, cross-protection among multiple NHBA peptide types have been reported (24). With regard to NadA, cross-protection between peptides that belong to NadA-1 and NadA-2/3 have been reported (25).

In preparation for the introduction of the newer MenB vaccine in Canada, the National Microbiology Laboratory (NML; Winnipeg, Manitoba) in collaboration with provinces, has been expanding laboratory surveillance activities to include a more in-depth study of MenB strains (26,27), as well as characterization of the noncarbohydrate protein-based vaccine antigen genes in Canadian IMD isolates (28). The present study characterizes IMD case isolates submitted to the NML from Atlantic Canada over the period from January 1, 2009 to December 31, 2013. Characterization includes analysis of serogroup, serotype and serosubtype antigens, *porA* genotypes and nucleotide sequences of genes that encode for the protein-based vaccine targets, fHbp, NHBA and NadA.

METHODS

Individual IMD case isolates received at the NML between 2009 and 2013, from the Atlantic provinces of New Brunswick (NB), Nova Scotia (NS), Prince Edward Island (PEI), and Newfoundland and Labrador (NFLD) were included in the present study. All IMD case isolates were obtained from normally sterile body sites (such as blood, cerebrospinal fluid, synovial fluid, pleural fluid or pericardial fluid), as per the national case definition (http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/list-eng.php). In addition, the present study did not capture the polymerase chain reaction (PCR)-diagnosed culture-negative cases, including at least one such case identified in NB.

All isolates were tested for serogroup using bacterial agglutination and/or PCR methods using procedures previously described (26,27). Serotyping and serosubtyping were performed using a monoclonal antibody typing kit (Rijksinstitut voor Volksgezondheid en Milieu, Bilthoven, Netherlands) and indirect whole cell ELISA method (29). Monoclonal antibodies to serotypes 2c, 17, 19 and serosubtype P1.19 were provided by Dr Wendell Zollinger, Walter Reed Army Institute of Research, United States. Isolates were analyzed using multilocus sequence typing (MLST) according to Maiden et al (30) and ST/CC was assigned using tools available in the *Neisseria* MLST website (http://pubmlst.org/neisseria/). Variable regions of *porA* genes were determined using protocols described by Sacchi et al (31) and Clark et al (32), following the nomenclature given in the *N meningitidis porA* variable region database (http://pubmlst.org/neisseria/PorA/) and by de Filippis et al (33). The *fHbp*, *nhba* and *nadA* gene sequences were determined using PCR amplification and standard sequencing reactions, following the protocols described by Lucidarme et al (34); and their peptide types were determined using online tools available from the Neisseria.org website (http://pubmlst.org/neisseria/fHbp/ and http://pubmlst.org/neisseria/NHBA/ and http://pubmlst.org/neisseria/NadA/).

Patient age and sex, as well as specimen sources of the IMD isolates were obtained from specimen requisition forms. Population estimates were obtained from Statistic Canada's website (www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/demo02a-eng.htm).

TABLE 2
Antigenic formula and *porA* genotypes of invasive *Neisseria meningitidis* in Atlantic Canada from 2009 to 2013

Antigenic formula	*PorA* genotype	Isolates, n
B:4:P1.4	P1.7-2,4,37	17
B:4:P1.4	P1.7-2,4,36	2
B:NT:P1.4	P1.7-2,4,37	1
B:4:P1.14	P1.22,14,36	2
B:4:P1.14	P1.22-1,14,38	1
B:4:P1.-	P1.12-6,13-4,35-1	1
B:4:P1.-	P1.22,14-6,36-2	1
B:15:P1.4	P1.7-2,4,37	1
B:15:P1.7,16	P1.7,16,35	1
B:17:P1.9	P1.22,9,35-1	1
B:17:P1.19	P1.19-1,15-11,36	2
B:1,19:P1.6	P1.18,25,38-1	1
B:1,19:P1.19	P1.19,13-1,36	1
B:1,19:P1.-	P1.18-1,34,38	1
C:2a:P1.-	P1.5,2,36-2	1
Y:2c:P1.5	P1.5-1,10-4,36-2	1
Y:15,19:P1.16	P1.21,16,37-1	1
Y:NT:P1.5	P1.5-1,10-4,36-2	1
Y:NT :P1.-	P1.18-1,3,38	1
W:NT:P1.-	P1.18-1,3,38	1
E:NT:P1.-	P1.22,14-6,36-2	1

TABLE 3
Predicted four-component meningococcal B vaccine antigens present in invasive *Neisseria meningitidis* strains in Atlantic Canada from 2009 to 2013

Vaccine antigen type	Isolates, n (serogroup[s])
PorA antigen P1.4	21 (B)
Factor H-binding protein peptide	
1	1 (B)
4	20 (B)
13	2 (B)
14	1 (B)
16	3 (B; E; Y)
19	3 (B)
21	1 (Y)
22	2 (C, W)
23	1 (Y)
24	1 (Y)
47	1 (B)
106	1 (B)
110	1 (B)
414	1 (B)
626	1 (B)
Neisseria heparin-binding antigen peptide	
2	21 (B)
3	2 (B)
6	3 (2B, 1Y)
7	1 (B)
9	2 (Y)
17	1 (B)
20	2 (W, Y)
21	3 (B)
29	2 (B, C)
43	1 (B)
197	1 (B)
336	1 (E)
Nesseria adhesion A peptide	
1	1 (B)
3	1 (C)
8	1 (Y)

RESULTS

Temporal and geographical distribution of serogroups

Table 1 describes the serogroup distribution of invasive *N meningitidis* collected in Atlantic Canada from 2009 to 2013. Of the 40 invasive isolates collected, 33 (82.5%) belonged to MenB and 19 were submitted from NB.

Population biology of invasive *N meningitidis* in Atlantic Canada

Clonal analysis of the 33 MenB isolates revealed that 73% belonged to ST-41/44 CC, 9% to ST-269 CC and 6% to ST-32 CC, and the remaining 12% comprised one isolate each of ST-35 CC and ST-461 CC; two strains were not assigned to any known CC by the Neisseria.org MLST website (http://pubmlst.org/neisseria/).

Of the 24 isolates that belonged to ST-41/44 CC, 19 were ST-154 and the other five STs (ST-207, ST-340, ST-409, ST-7612, ST-9411) were each made up of only one isolate. The three MenB isolates that belonged to ST-269 CC were from NB (two isolates both typed as ST-8924) and NFLD (ST-1161). The two MenB isolates that belonged to ST-32 CC were from NFLD and were typed as ST-290 and ST-7814. The remaining four MenB isolates included one each of ST-8770 (ST-35 CC) from NB, ST-461 (ST-461 CC) from PEI, and two isolates of ST-5571 from NS (identified to belong to a new CC of MenB common in central and eastern Canada) (35).

The single MenC strain from NS belonged to ST-11 (ST-11/ ET-37 CC). Two of the four MenY isolates were from NB, and typed as ST-1466 (ST-174 CC) or ST-3923 (ST-167 CC). The other two MenY strains were from NS, and typed as ST-184 (ST-22 CC) or ST-8522 (not assigned to any known CC). The single MenW isolate from NB was typed as ST-1221 (ST-22 CC), and the MenE isolate also from NB was typed as ST-9856 (not assigned to any known CC).

ST-154 MenB

There were 19 individual MenB case isolates typed as ST-154 (ST-41/44 CC), which included 47.5% of all the invasive *N meningitidis* found in Atlantic Canada during the study period. Fifteen of the case isolates were from NB, two from NS and two from NFLD. These 19 ST-154 MenB cases involved 12 male and seven female patients, with ages ranging from one month to 92 years (median 11 years of age). Ten of the isolates were recovered from blood cultures and the remaining nine were recovered from cerebrospinal fluid. Eighteen isolates were typed as B:4:P1.4 (serogroup B, serotype 4 and serosubtype P1.4), and one isolate was typed B:NT:P1.4 (nontypeable [NT]). The *porA* genotype for 17 isolates was P1.7-2,4,37 and two were typed as P1.7-2,4,36.

Characterization of noncarbohydrate protein-based meningococcal vaccine targets in MenB in Atlantic Canada

The antigenic formula (serotype and serosubtype antigens) expressed and the *porA* genotypes of the 40 invasive *N meningitidis* isolates are summarized in Table 2. Thirty-five isolates expressed seven different serotype antigens, and serotype 4 was the most commonly detected antigen in 24 isolates; five isolates did not express any detectable serotype antigens. Thirty-three isolates expressed eight different serosubtype antigens, including P1.4 detected in 21 isolates. Seven isolates did not express any detectable serosubtype antigens. There were 16 different *porA* genotypes found among the 40 isolates, and P1.7-2,4,37 was found in 19 of these (Table 2).

Aside from possessing the identical *porA* genotype of P1.7-2,4, all 19 ST-154 MenB isolates were found to have a *fHbp* gene allele predicted to encode for peptide 4 (variant 1 or subfamily B). Eighteen of

the 19 isolates were found to have *nhba* genes predicted to encode for NHBA peptide 2 and the remaining isolate was predicted to encode NHBA peptide 7. None of the isolates possessed the *nadA* gene.

The remaining MenB isolates were more diverse in terms of their *porA*, *fHbp* and *nhba* gene sequences. From the 14 non-ST-154 MenB isolates, 11 different *fHbp* gene alleles were identified that encoded for peptides 1, 4, 13, 14, 16, 19, 47, 106, 110, 414 and 626. Eight NHBA peptide types (2, 3, 6, 17, 21, 29, 43 and 197) were also predicted to be encoded by the non-ST-154 MenB isolates based on their *nhba* gene sequences. Only two MenB isolates that belonged to the ST-32 CC were found to have the *nadA* genes: in one isolate, the *nadA* gene allele 1 was found, which encoded for NadA peptide 1; and the other isolate was found to have the *nadA* gene allele 85, not predicted to produce a NadA protein, due to a frame-shift mutation.

Overall, 21 of 33 MenB isolates were found to express the PorA antigen P1.4 and display the *porA* genotype of P1.7-2,4, which represented 87.5% of the MenB isolates that belonged to the ST-41/44 CC (n=24). Eleven fHbp peptide types were predicted from the 33 MenB isolates, with seven of them (peptides 1, 4, 13, 14, 110, 414 and 626) belonging to variant 1 or subfamily B; three peptide types (peptides 16, 19 and 106) belonged to variant 2 (subfamily A); and one peptide type (peptide 47) belonged to the variant 3 (also subfamily A). Twenty-seven (81.8%) of the 33 MenB isolates were predicted to encode variant 1 fHbp peptides; five isolates (15.2%) were predicted to encode variant 2 peptides; and one isolate (3.0%) predicted to encode variant 3 peptide.

Noncarbohydrate protein-based meningococcal vaccine targets in MenC, -Y, -W and -E isolates in Atlantic Canada

Five fHbp peptide types (peptides 16, 21, 22, 23 and 24) were found among the seven non-B meningococcal isolates; and only peptide 16 was found among the Atlantic Canada MenB isolates. In addition, five NHBA peptide types were found: peptides 6, 9, 20, 29 and 336; peptides 6 and 29 were also found among the MenB isolates in Atlantic Canada. Only two isolates were found to possess the *nadA* gene: in the MenC isolate, the *nadA* gene allele 3 encodes a *nadA*-2/3 peptide 3, while a MenY isolate was found to have *nadA* allele 80, which encoded for a NadA-2/3 peptide 8.

Predicted 4CMenB (Bexsero) vaccine antigens present in invasive *Neisseria meningitidis* strains from Atlantic Canada

The variety of fHbp, NHBA and NadA peptide types found among invasive *N meningitidis* strains in Atlantic Canada is summarized in Table 3. No isolate was found to have all four antigens (PorA, fHbp, NHBA and NadA) displaying an exact match to the 4CMenB vaccine components. In addition, no isolate was found with three matching antigens to the 4CMenB vaccine. However, 19 isolates (all MenB) were found to have two antigens (PorA P1.4 and NHBA peptide 2) that displayed an exact match to the 4CMenB vaccine. An additional six isolates were found to have at least one antigen that matched with the 4CMenB vaccine (two MenB for PorA P1.4; two other MenB isolates with NHBA peptide 2; another MenB with fHbp peptide 1; and 1 MenY with a NadA peptide 8). Collectively, 25 isolates were found to have one or more antigens that matched the 4CMenB and, therefore, at least 62.5% of all invasive *N meningitidis* from Atlantic Canada in the period from 2009 to 2013, were predicted to be covered by the vaccine. However, the number of non-MenB strains studied was very small and, therefore, the prediction for these other serogroups would not be meaningful. The predicted coverage for MenB alone in Atlantic Canada was significantly higher (24 of 33 isolates [73%]) due to the predominant presence of the ST-154 clone, which shared two exact match antigens (PorA P1.4 and NHBA peptide 2) with the vaccine.

DISCUSSION

The present study provides a snap shot of the molecular epidemiology of IMD in Atlantic Canada from 2009 to 2013. A striking feature was the predominance of MenB (82.5%), as a cause of IMD in this region of Canada. For the same period in western Canada, the overall abundance of MenB among all invasive *N meningitidis* case isolates was 42.9%, including 48.5% in British Columbia, 36.5% in Alberta, 39.1% in Saskatchewan and 45.0% in Manitoba (28). In Ontario, between 2001 and 2010, 39.1% of all invasive meningococcal case isolates were MenB (27). In Quebec, the percentage of MenB for the period of 2003 to 2010 was higher (71.1%) due to an increase in the B:17:P1.19 ST-269 clone, which emerged in 2003 (36) and persisted until at least 2010 (26).

Here, and in our previous studies, we have shown that the epidemiology of IMD in different parts of Canada may reveal both geographical and temporal differences. Clonal analysis of MenB in western Canada and Ontario reflected an endemic disease picture where no single clone or ST predominated (27,28). This was in contrast to the data from Quebec, where almost one-half (49%) of all MenB was typed as a single clone, ST-269, which accounted for 35% of all culture-confirmed IMD case isolates from 2003 to 2010 (26). In Atlantic Canada, most (57.6%) of the MenB isolates belonged to a strain characterized as ST-154 with the antigenic formula of B:4:P1.4. This strain was responsible for 47.5% of all culture-confirmed IMD cases in the region; however, the majority (15 of 19 [79%]) of the case isolates were identified in NB, including three to four culture-confirmed cases per year between 2009 and 2012. In 2013, only one culture-confirmed case was due to this strain. In contrast, for the same period, only five ST-154 MenB isolates were found among the culture-confirmed IMD cases in western Canada (28). The combined population in western Canada in 2012 was estimated to be 10.77 million, while in Atlantic Canada the combined population in 2012 was estimated to be 2.37 million. This MenB strain was also rare in Quebec (population estimated to be 7.9 million in 2010), with only two isolates found among 334 invasive MenB strains between 2003 and 2010 (26). In Ontario (population estimated to be 13.1 million in 2010), 10 such MenB strains were detected among 193 invasive MenB isolates between 2001 and 2010 (27). Sequence type 154 belongs to the ST-41/44 CC or lineage 3, a well-known hypervirulent clone. A strain characterized as B:4:P1.4 ST-154 or ST-42 was the cause of the MenB epidemic in New Zealand in the 1990s (37,38), which prompted the New Zealand government to commission production of a strain-specific OMV vaccine based on the PorA P1.4 OMP (20).

The percentage of MenB involving *fHbp* genes that encoded for variant 1 or subfamily B peptides was significantly higher in Atlantic Canada (82%) (the present study) than in western Canada (38%) (28), or in the Immunization Monitoring Program Active (IMPACT) study examining MenB isolates from 12 pediatric tertiary care centres across the country (64%) (39). The strain differences in these three studies were probably the reason for the observed difference in fHbp types reported. In western Canada, ST-213 CC was the second most common CC found, and most (92%) of the isolates from this CC were predicted to have fHbp peptide 45 (variant 3). In addition, the strains involved in MenB disease in western Canada were very diverse in terms of their antigens and ST, which presented a picture of endemic disease (28). In contrast, the IMPACT study was in some way skewed by the elevated incidence of MenB disease in Quebec over the past several years due to the ST-269 clone (26,40). Most ST-269 MenB were found to have the *fHbp* gene allele 15, predicted to synthesize variant 1 or subfamily B fHbp protein. In Atlantic Canada, the predominance of the ST-154 MenB strain, which uniformly have the *fHbp* gene allele 4, was predicted to synthesize variant 1 or subfamily B peptide. This contributed to the highest percentage of MenB found in Canada, predicted to be covered by the fHbp component present in the 4CMenB or the bivalent recombinant fHbp vaccine. A similar study from the United States revealed that 59% of MenB isolates were found to encode variant 1 or subfamily B fHbp peptides (41), while in Europe the percentage of MenB encoding for variant 1 or subfamily B fHbp differed according to country, and ranged from 65.2% to 76.7% (42).

Similar to the observation in western Canada, the *fHbp* genes in all seven non-MenB strains in Atlantic Canada encoded variant 2 or subfamily A fHbp proteins. None of the strains had the *nhba* gene, which predicts the synthesis of NHBA peptide 2. Only two non-MenB strains were found to have *nadA* genes that enable synthesis of NadA peptides, likely covered by the NadA component of the 4CMenB vaccine.

Overall, 25 (62.5%) of the 40 invasive strains found in Atlantic Canada had at least one antigen that matched exactly to the 4CMenB vaccine components. Additional coverage may be possible by the NHBA component of 4CMenB as cross-protection against different NHBA peptides that have been reported (24). Furthermore, components other than the *porA* P1.4 that are present in the OMV may also provide further protective immunity.

One limitation of the present study was the relatively small number of non-MenB strains examined and, therefore, the prediction for these other serogroups would not be meaningful. The predicted coverage for MenB alone in Atlantic Canada was significantly higher (24 of 33 [73%] isolates) due to the predominant presence of the ST-154 clone, which shared two exact match antigens (PorA P1.4 and NHBA peptide 2) with the vaccine. Second, we did not perform the meningococcal antigen typing system assay (43) to study the degree of expression and cross-reactivity of antigens on clinical isolates with those in the 4CMenB vaccine. Regardless, true coverage may not be known unless the vaccine is used in a population because strains within a region may change over time. Furthermore, true vaccine coverage rates may change because the epidemiology of clones may shift over time and among regions (44-46).

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