Diversity of Canadian meningococcal serogroup B isolates and estimated coverage by an investigational meningococcal serogroup B vaccine (4CMenB) ☆☆

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ARTICLE INFO

ABSTRACT

Background: In collaboration with the Canadian Immunization Monitoring Program Active (IMPACT), the National Microbiology Laboratory, the UK Health Protection Agency and Novartis Vaccines, we tested the potential of an investigational 4-component meningococcal B vaccine (4CMenB) to cover Canadian strains circulating from 2006 to 2009.

Methods: IMPACT meningococcal surveillance is population based and includes over 50% of Canadian adults and children. All isolates were characterized by Meningococcal Antigen Typing System (MATS) and sequencing for factor H-binding protein (fHbp), *Neisseria* Heparin Binding Antigen (NHBA) and Neisserial adhesin A (NadA).

Results: In total, 157 isolates were tested. Overall, 4CMenB MATS predicted strain coverage was 66% (95% CI: 46–78%), with 26%, 29% and 11% of strains covered by one, two and three vaccine antigens, respectively. The coverage of each antigen was as follows: 13% PorA, 1% NadA, 52% fHbp and 51% NHBA. The majority of strains for clonal complex (cc) 41/44 and cc60 were covered by NHBA; the majority of strains for cc269 and cc32 were covered by fHbp and NHBA.

Coverage for two prevalent strains (sequence type (ST)-269 and ST-154) was 95% and 100%, respectively.

Conclusions: 4CMenB has the potential to protect against a significant proportion of Canadian invasive MenB strains.

1. Introduction

Serogroup B meningococci (MenB) account for 50–80% of invasive meningococcal disease (IMD) in Canada, with the highest incidence seen in children <5 years of age [1,2]. Despite the need for prevention, efforts to develop a vaccine against MenB disease have been hampered by the similarity of the polysaccharide capsule of the bacterium to human fetal neural tissue [3,4] and the inability to identify common protective surface antigens among MenB strains. However, reverse vaccinology has enabled the identification of several conserved non-capsular protein surface antigens, overcoming limitations of past epidemic-specific outer-membrane vesicle (OMV) MenB vaccines [5–7]. Three antigens (Neisserial adhesin A (NadA) allele 3, *Neisseria* Heparin Binding Antigen (NHBA), factor H-binding protein (fHbp) variant 1 along with OMV of the epidemic strain (PorA P1.4) from New Zealand have been combined into a recently approved vaccine against MenB disease (4CMenB) [8,9]. Two variants of fHbp have also been used to create an investigational bivalent MenB vaccine (rLP2086) [10].

To date, three OMV-based vaccines against invasive MenB disease have successfully contained clonal outbreaks in various countries [11–13]. However, immunogenicity of these vaccines was primarily based on the PorA outer membrane protein contained in the OMV and did not provide protection against strains carrying different PorA subtypes [14]. Antigens included in the newer MenB vaccines have the potential to provide broad cross-protection against MenB strains and potentially other serogroups. The predicted protection afforded by these newer vaccines is not known and will be highly dependent on both the quantity of vaccine antigens expressed by strains causing disease in a given geographic area and on the extent of their immunologic cross reactivity with the corresponding antigen in the vaccine. To this end, the Meningococcal Antigen Typing System (MATS) was developed to predict which individual MenB strains are likely to be covered by the 4CMenB vaccine [15]. To understand the potential coverage, a detailed epidemiologic, microbiologic and genetic characterization of the antigens found in MenB disease isolates is required.

In collaboration with the Canadian Immunization Monitoring Program Active (IMPACT) surveillance network, the National Microbiology Laboratory (NML), the UK Health Protection Agency (HPA) and Novartis Vaccines & Diagnostics, we tested the potential strain coverage of the 4CMenB vaccine against invasive MenB strains isolated in Canada from 2006 to 2009. During this time the incidence rate of MenB infection was stable at 0.25 per 100,000, but a higher rate occurred in Québec as a result of the circulation of clonal complex (cc) 269, [2,16,17] one of two hyper-endemic ccs in Canada.

2. Materials and methods

Active, metropolitan area population-based surveillance for adult and pediatric hospital admissions related to infection with *Neisseria meningitidis* was conducted by the 12 centers of the IMPACT, in collaboration with local public health officials. IMPACT is a national surveillance initiative with centers located in 8 provinces [18]. Each center defined a population area and captured all IMD cases in children and adults. IMPACT meningococcal surveillance includes over 17 million Canadians, just over 50% of the population. Inclusion as a case required the isolation of *N. meningitidis* from a normally sterile site or a positive PCR test from blood or cerebrospinal fluid (CSF). Standardized case information was abstracted from the hospital record. Sequelae were defined as complications attributable to IMD still present at discharge. The surveillance methodology has been detailed elsewhere [19,20]. Ethics approval was obtained at all participating hospitals. All IMPACT MenB cases with a viable isolate that occurred from 2006 to 2009 and were identified as of August 2010 were included.

NML determined serogroup, serotype, sub-serotype and PorA sequencing of case isolates. The clonal identity of isolates (defined by Multilocus Sequence Typing (MLST) [21]) and PorA variants were determined following the guidelines included in the *Neisseria* pubMLST website [22]. The classification of fHbp followed the scheme available in the public fHbp database which divides peptide subvariants among three major variants, 1, 2 and 3 [22]. This peptide ID is similar to the Novartis classification, although in the Novartis classification it is preceded by the major variant number. NHBA and NadA classification followed Lucidarme et al. [23] and Bambini et al. [24].

HPA studied the levels of expression and cross-reactivity of NadA, fHbp, and NHBA in the MenB isolates using the MATS ELISA relative potency (RP) [15]. The MATS method established a minimum level of RP, named the positive bactericidal threshold (PBT) that predicts whether a given MenB isolate would be susceptible to killing in the human serum bactericidal antibody assay by antibodies induced by 4CMenB. Strain coverage was defined as the proportion of strains with RP above the PBT for at least one vaccine antigen in the MATS ELISA or matched to the PorA subtype P1.4 [15].

To account for inter-laboratory differences in the MATS, the 95% confidence intervals (CI) for vaccine strain coverage were calculated according to an inter-laboratory standardization study [25]. Chi-square and Fisher's exact tests were used to test for significant difference between groups. SAS version 9.3 (SAS Institute, Cary NC) was used for all analyses.

3. Results

A total of 157/200 (78.5%) MenB cases were tested. A viable isolate was not available for 2 cases and 41 cases were confirmed solely by PCR. No significant differences in PCR confirmation rates were found by age or center (data not shown).

The most frequent ccs among the 68 different STs identified were cc41/44 ($n = 51$), cc269 ($n = 51$), cc35 ($n = 11$), cc32 ($n = 8$) and cc60 ($n = 6$) cc213 ($n = 2$). Of the remaining 28 isolates, 21 were unassigned and 7 were singularly occurring ccs. Although cc41/44 and cc269 occurred with the same frequency, 25 different sequence types (ST) were identified among isolates in cc41/44 and only three of these contained multiple isolates (ST-154 ($n = 15$) and ST-571 ($n = 11$) and ST-340 ($n = 3$). In contrast, only 9 STs were found in cc269 and 90.1% of these isolates belonged to either ST-269 ($n = 37$), or single ($n = 6$) or double locus variants ($n = 3$) of ST-269 and the remaining 5 isolates showed three allelic differences from the ST-269 (ST-275 and ST-1161). The distribution of the most frequent cc and ST varied by province (Table 1).

The predicted strain coverage of the 4CMenB vaccine was 66% (95% CI: 46–78%); ranging, non-significantly, from a high of 72% (95% CI: 47–84%) in 2006 to a low of 58% (95% CI: 33–70%) in 2008. Overall, 26.1% of strains were covered by one vaccine antigen, 29.0% by two antigens and 11.5% by three. No isolates were covered by all four antigens. Coverage by each antigen was as follows: fHbp 52% (95% CI: 40–59%); NHBA 51% (95% CI: 21–71%); NadA 1% (95% CI: 0.6–3%); and PorA 13% (95% CI: 8–18%). Table 2 shows the frequency of antigen combinations sufficient for coverage. The coverage by age group, gender, ethnicity and province is shown in Table 3. Vaccine strain coverage did not differ significantly by any of these factors. Of the 6 isolates from fatal cases, 4 (67%) were predicted covered, as were 23 of the 34 (68%) isolates from cases that resulted in sequelae.

4CMenB coverage within the two most prevalent cc (cc269 and cc41/44) was 82% (95% CI: 47–90%) and 65% (95% CI: 55–80%), respectively. For the two most common STs (ST-269 and ST-154) this increased to 95% and 100%, respectively, while ST-571 was covered for only 1 isolate (9%). The occurrence of vaccine antigens in the most frequent cc is shown in Fig. 1.

3.1. Prevalence and diversity of PorA, fHbp, NadA and NHBA

The four most frequently detected PorA serosubtypes (P1.19 ($n = 34$), P1.14 ($n = 28$), P1.9 ($n = 22$), P1.4 ($n = 21$)) were found in 105 or 67% of isolates. Strains containing serosubtype P1.19 occurred predominantly in Québec ($n = 30/34$) and all strains were from cc269. P1.14 occurred primarily in Ontario ($n = 16$) and was found in a wide variety of cc. PorA P1.4 was present in 21 strains all from cc41/44. The majority of strains with P1.4 occurred in children 0–4 years of age ($n = 14$) and were distributed across Canada. Two antigen combinations occurred frequently among the PorA P1.4 strains: PorA P1.4 and NHBA peptide 2 ($n = 19$) and PorA P1.4 and fHbp 1.4 ($n = 16$).

Overall 44 different PorA variable region (VR) genosubtypes were identified, but only 12 genosubtypes occurred in more than one isolate. The seven most common PorA genosubtypes included

Table 1
Most frequent clonal complex and sequence type by province, 2006–2009 (IMPACT surveillance).

Clonal complex	Province						
	Atlantic	Quebec	Ontario	Central	Alberta	British Columbia	Total
cc41/44 (n = 51)							
ST154	5	0	7	0	3	0	15
ST571	0	10	1	0	0	0	11
ST340	2	0	0	0	0	1	3
Other[a]	1	8	5	3	2	3	22
cc269 (n = 51)							
ST269	0	33	2	0	1	1	37
ST1986	0	3	0	0	0	0	3
ST275	0	1	0	0	1	0	2
ST1161	2	0	0	0	0	0	2
ST13	0	0	2	0	0	0	2
Other[b]	0	2	0	1	0	2	5
cc35 (n = 11)							
ST35	0	2	1	0	0	0	3
ST570	0	1	2	1	0	0	4
ST790	0	0	0	0	2	0	2
Other[c]	0	1	1	0	0	0	2
cc32 (n = 8)							
ST32	0	1	2	0	0	1	4
Other[d]	1	2	0	0	0	1	4
cc60 (n = 6)							
ST60	0	3	0	0	0	0	3
Other[e]	1	1	0	0	1	0	3

[a] Other cc41/44 sequence types occurring once included the following: ST1433, ST146, ST1475, ST1578, ST2678, ST2820, ST2989, ST3752, ST41, ST43, ST46, ST5553, ST6465, ST6473, ST6541, ST6551, ST6591, ST6623, ST7702, ST7746, ST839, ST944.
[b] Other cc269 sequence types occurring once included the following: ST1284, ST283, ST6107, ST7812, ST7813.
[c] Other cc35 sequence types occurring once included the following: ST278 and ST6472.
[d] Other cc32 sequence types occurring once included the following: ST2017, ST2726, ST33, ST7814.
[e] Other cc60 sequence types occurring once included the following: ST1754, ST6546 and ST7877.

Table 2
Percentages of strains covered[a] by specific antigen combinations in Canada, 2006–2009 (IMPACT surveillance) (N = 157).

Vaccine antigen	N	%
fHbp	20	12.7
NHBA	21	13.4
fHpb + NHBA	40	25.5
fHpb + PorA	3	1.9
fHbp + NadA	1	0.6
NHBA + NadA	1	0.6
PorA + fHbp + NHBA	18	11.5
Antigen not sufficient for coverage or not present[b]	53	33.8

[a] Strains were defined as covered by 4CMenB if they possessed PorA P1.4 or had a RP above the PBT for fHbp, NHBA or NadA.
[b] Included PorA alone, NadA alone, NHBA + PorA, fHbp + NHBA + NadA and PorA + fHbp + NHBA + NadA.

P1.19-1,15-11,36 (n = 34); P1.7-2,4,37 (n = 21); P1.22,14,36 (n = 16); P1.18-7,9,35-1 (n = 16); P1.22-1,14,38 (n = 12); P1.7,16,35 (n = 6); and P1.5,2,36-2 (n = 5). Together these represented 70.1% of the MenB isolates.

A total of 39 different fHbp peptides were identified, with 26 occurring only once. The majority (n = 100) were from variant 1; 46 (29.3%) were from variant 2; and 11 (7.0%) were from variant 3. Isolates from infants <1 year of age showed the greatest variability in their fHbp antigens: 34% (n = 14) of isolates in infants expressed fHbp variant 1; 56% (n = 23) expressed variant 2; and 10% (n = 4) expressed variant 3. In the remaining age groups fHbp variant 1 prevalence ranged from a low of 60% in adults to a high of 92% in children 5–14 years of age. All of the strains (n = 5) containing fHbp 1.1 (variant 1, peptide 1, included in 4CMenB) and 81% (n = 77) of those from variant 1 but with a different peptide (e.g. 4, 110,

Table 3
Potential coverage of 4CMenB vaccine in Canada, 2006–2009 (IMPACT surveillance) (n = 157).

Characteristic	Predicted covered according to MATS[a]			
	Number	Percent	95% CI	P-value
Age group				
0–4 years (n = 79)	48	60.8	44.3–79.7	0.14
5+ years (n = 78)	56	71.8	47.4–76.9	
Male (n = 80)	50	62.5	47.5–76.3	0.31
Female (n = 77)	54	70.1	44.2–80.5	
Ethnicity				
White (n = 99)	64	64.6	47.5–80.8	
Other (n = 19)	13	68.4	42.1–78.9	0.86
Unknown (n = 39)	27	69.2	43.6–71.8	
Province				
British Columbia (n = 14)	6	42.9	28.6–57.1	
Alberta (n = 13)	9	69.2	61.5–76.9	
Central Canada (Sasktchewan and Manitoba (n = 8)	8	100	75–100	0.08
Ontario (n = 38)	23	60.5	47.4–63.2	
Québec (n = 72)	48	66.7	38.9–84.7	
Atlantic (Nova Scotia and Newfoundland) (n = 12)	10	83.3	66.7–100	

[a] Strains were defined as covered by 4CMenB if they possessed PorA P1.4 or had a RP above the PBT for fHbp, NHBA or NadA.
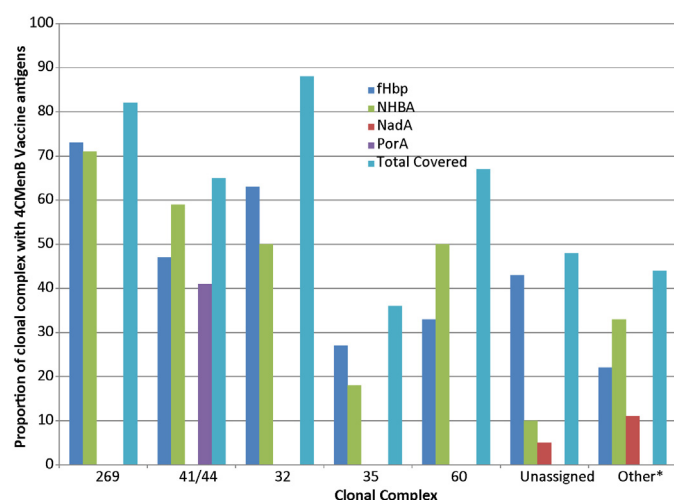
Fig. 1. Predicted 4CMenB vaccine antigen coverage (Strains were defined as predicted to be covered by 4CMenB if they possessed PorA P1.4 or had a RP above the PBT for fHbp, NHBA or NadA.) by serogroup B clonal complex in Canadian serogroup B strains, 2006–2009 (IMPACT surveillance). *Note*: Other includes 7 clonal complexes that were assigned only once and 1 clonal complex that was assigned twice. Unassigned represents isolates for which a clonal complex was unable to be determined (N=21).

413, etc.) were predicted to be covered by the vaccine. None of the fHbp variant 2 or 3 strains had RPs above the PBT for fHbp and would require expression of a different vaccine antigen (i.e. PorA, NHBA, NadA) to be covered. Table 4 shows the distribution of fHbp peptides by cc, and the relative coverage predicted by MATS specifically for this antigen. The most prevalent fHbp peptides were mostly associated with one cc and the fHbp-MATS phenotype was either covered (85% and 100% for 1.15 and 1.4, respectively) or not-covered (0% for 2.19). Of note, fHbp 1.15 occurred in isolates across Canada (e.g. Quebec, Ontario, British Columbia and Alberta) but was only found in cc269.

Table 5 shows the distribution of NHBA peptides by cc, and the relative coverage predicted by MATS specifically for this antigen. Thirty-three different NHBA peptides were identified with 18 occurring once. The most frequent peptides were 21 ($n=51$), 2 ($n=23$) 112 ($n=14$) and 6 ($n=14$). Peptides 21, 2 and 6 were distributed across all age groups, while peptide 112 was primarily from infants and young children. Peptides 21 and 112 were found primarily in Québec (peptide 21, $n=40$ and peptide 112, $n=12$) while peptide 6 was concentrated in Ontario ($n=13$). Peptide 2 was found everywhere except Québec. Of these 4 common peptides 71% ($n=36$) of peptide 21, and 96% ($n=22$) of peptide 2 had RPs over the NHBA PBT thus were predicted to be covered by the 4CMenB vaccine whilst only 7% of peptides 112 ($n=1$) and 6 ($n=1$) were predicted to be covered. NHBA peptide 2, the peptide contained within 4CMenB, was only found in cc41/44 where it constituted 41% (23/51) of the NHBA peptides in cc41/44 with MATS predicting coverage of 96% (22/23) (Table 5), whereas peptide 21 was found in two different ccs (cc269 $n=40$ and cc35 $n=11$) with a significantly different NHBA-MATS coverage phenotype (85% and 18%, respectively, $P<0.0001$), suggesting a consistently lower level of NHBA expression in cc35 compared to cc269.

The *nadA* gene was found in 12 isolates but only 2 isolates, bearing NadA alleles 2 and 3, expressed NadA with a RP over the PBT to be covered by the 4CMenB vaccine. The subvariant NadA-1.1, which accounted for half ($n=6$) of the isolates with a *nadA* gene, was not predicted to be covered.

Geographically, the prevalence of fHbp and NHBA antigen combinations were diverse except for two antigen combinations that were found primarily in Québec: NHBA 112 fHbp 2.19 in 15.3%

Table 4
Relationship among clonal complex, fHbp antigen genotype and MATS phenotype in Canada, 2006–2009 (IMPACT surveillance).

fHbp peptide[a]	Predicted Covered according to fHbp-MATS												Overall (N = 157)	
	cc269 (n = 51)		cc41/44 (n = 51)		cc35 (n = 11)		cc32 (n = 8)		cc60 (n = 6)		Other cc/unassigned (n = 30)			
	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered
1.1	–	–	1	1 (100%)	–	–	4	4 (100%)	–	–	–	–	5	5 (100%)
1.13	3	0 (0%)	–	–	–	–	–	–	6	2 (33%)	3	2 (67%)	12	4 (33%)
1.15	39	33 (85%)	–	–	–	–	–	–	–	–	–	–	39	33 (85%)
1.4	–	–	17	17 (100%)	–	–	–	–	–	–	3	2 (67%)	20	19 (95%)
2.16	–	–	–	–	6	0 (0%)	–	–	–	–	–	–	6	0 (0%)
2.19	2	0 (0%)	21	0 (0%)	–	–	–	–	–	–	1	0 (0%)	24	0 (0%)
2.24	–	–	3	0 (0%)	1	0 (0%)	–	–	–	–	1	0 (0%)	5	0 (0%)
Others[b]	7	4 (57%)	9	6 (67%)	4	3 (75%)	4	1 (25%)	–	–	22	7 (32%)	46	21 (46%)

[a] Strains were defined as predicted to be covered by 4CMenB if they possessed a RP above the PBT for fHbp.
[b] Other fHbp variants and peptides occurring in fewer than 5 isolates included the following: 1.8, 1.14, 1.54, 1.65, 1.89, 1.108, 1.110, 1.144, 1.226, 1.252, 1.407, 1.410, 1.413, 1.414, 1.415, 1.416, 2.23, 2.25, 2.101, 2.106, 2.118, 2.138, 2.411, 3.30, 3.31, 3.45, 3.47, 3.94, 3.406, 3.408, 3.409, 3.412.

Table 5
Relationship among clonal complex, NHBA antigen genotype and MATS phenotype in Canada, 2006–2009 (IMPACT surveillance).

NHBA peptide[a]	Predicted covered according to NHBA-MATS												Overall (N = 157)	
	cc269 (n = 51)		cc41/44 (n = 51)		cc35 (n = 11)		cc32 (n = 8)		cc60 (n = 6)		Other cc/unassigned (n = 30)			
	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered	Total	Covered
21	40	34 (85%)	–	–	11	2 (18%)	–	–	–	–	–	–	51	36 (71%)
2	–	–	23	22 (96%)	–	–	–	–	–	–	–	–	23	22 (96%)
6	3	0	–	–	–	–	–	–	–	–	11	1 (9%)	14	1 (7%)
112	–	–	14	1 (7%)	–	–	–	–	–	–	–	–	14	1 (7%)
24	–	–	–	–	–	–	–	–	6	3 (50%)	–	–	6	3 (50%)
20	–	–	–	–	–	–	1	0	–	–	4	2 (50%)	5	2 (40%)
Others[b]	8	2 (25%)	14	7 (50%)	–	–	7	4 (57%)	–	–	15	2 (13%)	44	15 (34%)

[a] Strains were defined as predicted to be covered by 4CMenB if they possessed a RP above the PBT for NHBA.
[b] Other NHBA peptides occurring in fewer than 5 isolates included the following: 1, 3, 5, 9, 10, 17, 18, 19, 29, 43, 44, 47, 122, 145, 197, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287.

(n = 11) of strains from Québec (and 1 from Ontario) and occurred primarily in infants (n = 9); and NHBA 21 fHbp 1.15 was found in 49.0% (n = 35) of Québec strains (and 2 Vancouver strains) across all age groups. Of these two common antigen combination 8.3% (n = 1) of NHBA 112 fHbp 2.19 were predicted to be covered and 95% (n = 35) of NHBA 21 fHbp 1.15 were covered. The two NHBA 21 fHbp 1.15 strains not predicted to be covered were from Québec.

4. Discussion

This study provides the first data on the potential coverage of Canadian MenB isolates by the investigational 4CMenB vaccine. Using a conservative predictor for coverage, 4CMenB appears to provide good strain coverage (65% for cc41/44 and 82% for cc269) for the most prevalent recent ccs, which include ST-269 and ST-154 predicted covered at 95% and 100%, respectively. Across all age groups, the majority of isolates are predicted to be covered by the 4CMenB vaccine. Of note the vaccine appears to provide coverage across a wide diversity of endemic strains and is not limited to protecting against one or two subtypes. At least 40% of isolates were covered by two or more vaccine antigens, with fHbp and NHBA contributing the most to vaccine coverage. The 4CMenB antigens are also found in non-MenB isolates thus protection against these other serogroups may be an added bonus, particularly in individuals not immunized with meningococcal conjugate vaccines. In terms of prevention, over two-thirds of the recent cases caused by MenB were potentially preventable with this vaccine.

Our results are similar to those found in England and Wales where the overall proportion of strains estimated to be covered in 2007–2008 was 73% (57–87%) and the combinations of antigens with MATS RP above the PBT was similar to that observed in Canada [26]. The overall frequency of coverage by at least two antigens was lower (40% vs. 50%) in Canadian than in English and Welsh isolates [26], thus the chance for escape mutants to emerge with vaccine use could differ between the two countries.

The last national characterization of MenB isolates was from 1994 to 1996. In this earlier study the most commonly expressed PorA serosubtypes were P1.14 (13.3%), P1.16 (11.3%), P1.5 (7.9%), P1.7 (7.0%), P1.13 (7.0%), and P1.2 (4.3%); and the only hypervirulent clones were cc32 and cc11 [27]. The most noticeable differences in our current study were the emergence of the ST-269 clone in Québec and a change in the prevalence of other hypervirulent clones. CC32 decreased from 12.0% in 1994–1996 to 5.1% in 2006–2009 and cc41/44 became a predominant clone, accounting for about 33% of MenB isolates in 2006–2009. Besides these temporal changes, we noted geographical differences in the distribution of common hypervirulent clones from 2006 to 2009 as exemplified by the finding of ST-269 (cc269) and ST-571 (cc41/44) mainly in the province of Québec, and ST-154 (cc41/44) from Ontario and the Atlantic provinces. By province, the predicted coverage of 4CMenB ranged from 43% to 100% and reflected the strains circulating within each region and the level of antigen expression within each isolate. 4CMenB coverage of Canadian hyper-endemic strains (ST-269 and ST-154, 95% and 100%, respectively) was significantly higher than other STs in the same cc, indicating that cc cannot be used to determine if an isolate is potentially covered by 4CMenB. For both fHbp and NHBA, antigen peptides with high frequency in the sample were associated mostly with one or two ccs, the most diverse cc being cc41/44 for both antigens. In general each peptide had a similar proportion of coverage when found in strains belonging to different ccs, with the exception of the NHBA peptide 21 that was significantly more covered in cc269 than in cc35, suggesting a bias in the level of antigen expression associated with the genetic diversity between the two ccs. Albeit strains harboring specific combinations of MLST and antigen genotype were consistently

covered (e.g. cc32 and fHbp1.1; cc41/44 and fHbp1.4; cc41/44 and NHBA2) the majority of genetic profiles had both strains covered and not covered, confirming that antigen genotyping, neither alone nor in combination with MLST, would be sufficient to predict vaccine strain coverage for all isolates.

While our active population-based sentinel surveillance data provide the most comprehensive measurement of IMD in Canada, several limitations apply. MenB IMD is rare and the numbers in any given age group or province are small; therefore our ability to detect differences among subgroups is limited, and differences in strain coverage among age or geographic groups were not statistically significant. Approximately 20% of MenB cases in our data were confirmed by PCR only with no isolate available for testing. Additionally, IMPACT surveillance includes primarily urban areas of Canada and may not be representative of remote or rural regions. The MATS provides a conservative estimate of vaccine coverage, which may be an underestimate [15,28]. Finally, although the *nadA* gene was found in 12 isolates (7%) in our study, only two (1%) expressed NadA with a RP above the PBT. Since expression of NadA is repressed in vitro, but not in vivo, conditions, MATS may underestimate NadA's contribution to vaccine strain coverage [29,30].

5. Conclusions

Our study characterizes the current MenB molecular epidemiology and provides a good estimate of the potential coverage of 4CMenB. Accurate post-implementation surveillance and/or post-implementation effectiveness studies will be necessary to determine the true effectiveness of this new vaccine [31], taking into account the level of vaccine coverage in the population and any herd protection.

Acknowledgements

Author contributions: J.A. Bettinger is the PI for the study and first author on the manuscript. She is Co-PI for IMPACT's Invasive Meningococcal Surveillance project. She was involved with conception and design of the invasive meningococcal surveillance project and the study reported here as well as data acquisition. She analyzed and interpreted the data and wrote and revised the submitted manuscript. D.W. Scheifele is the IMPACT Data Center Director and Co-PI for IMPACT's Invasive Meningococcal Surveillance project. He was involved with conception and design of the meningococcal surveillance project and the study reported here as well as data acquisition and interpretation of the data. He revised and approved the submitted manuscript. S.A. Halperin is one of two Co-PIs for the IMPACT surveillance network. He was involved with conception and design of the meningococcal surveillance project and the study reported here as well as data acquisition. He revised and approved the submitted manuscript. W. Vaudry is the second of two Co-PIs for the IMPACT surveillance network. She was involved with conception and design of the meningococcal surveillance project, the study reported here and data acquisition. She revised and approved the submitted manuscript. J. Findlow was responsible for characterizing the serogroup B isolates by MATS and sequencing fHbp, NHBA and NadA at the Health Protection Agency. He revised and approved

the submitted manuscript. R. Borrow was responsible for characterizing the serogroup B isolates by MATS and sequencing fHbp, NHBA and NadA at the Health Protection Agency and was involved with interpretation of the data. He revised and approved the submitted manuscript. D. Medini provided access to and explanation of the laboratory and statistical methods used in the Plikaytis et al. inter-laboratory study and the Donnelly et al. MATS manuscript. He revised and approved the submitted manuscript. R. Tsang is responsible for the maintenance of the IMPACT *N. meningitidis* isolate collection at the National Microbiology Laboratory. He was responsible for the serogroup and sequencing typing of the serogroup B isolates and was involved with interpretation of the data. He revised and approved the submitted manuscript. *Conflicts of interest:* JAB: ad-hoc Advisory Boards (Novartis Vaccines, Canada) and speaker honoraria (Novartis Vaccines, Pfizer Inc., Baxter Inc.). SAH: ad-hoc Advisory Board for Novartis Vaccines, Canada and speaker honoraria in the past year (Novartis Vaccines). DWS: ad hoc Advisory Board for Novartis Vaccines, Canada. WV: Data Safety and Monitoring Board, Novartis Vaccines. RB has performed contract research on behalf of the Health Protection Agency for Baxter Biosciences, GSK, Novartis, Merck, Pfizer and Sanofi Pasteur. JF has performed consultancies for Baxter, GSK, Novartis and Pfizer, received travel support from Baxter Biosciences, GSK, Novartis and Pfizer and performed contract research on behalf of the Health Protection Agency for Baxter Biosciences, GSK, Novartis, Merck, Pfizer and Sanofi Pasteur. DM: employee (Novartis Vaccines). RT: None. *Funding statement:* The Canadian Immunization Monitoring Program, Active (IMPACT) is a national surveillance initiative managed by the Canadian Paediatric Society and conducted by the IMPACT network of pediatric investigators. From 2002 to 2011, IMPACT meningococcal surveillance was supported by a grant from Sanofi-Pasteur. The additional typing and laboratory testing performed in this study was supported by a grant from Novartis Vaccines & Diagnostics. JAB is supported by a Career Investigator Award from the Michael Smith Foundation for Health Research.

Appendix A. IMPACT co-investigators who were responsible for data and isolate collection at each site from 2006 to 2009 include the following

Robert Morris, Janeway Children's Health and Rehabilitation Centre, St. John's, Canada. Scott Halperin, IWK Health Centre Halifax, Canada. Pierre Déry, Centre Mère-Enfant de Québec, Québec City, Canada. Dorothy Moore, Montreal Children's Hospital, Montreal, Canada. Marc Lebel, Hôpital Ste-Justine pour les enfants, Montreal, Canada. Nicole Le Saux, Children's Hospital of Eastern Ontario, Ottawa, Canada. Dat Tran, Lee Ford-Jones, The Hospital for Sick Children, Toronto, Canada. Joanne Embree, Winnipeg Children's Hospital Winnipeg, Canada. Raymond Tsang, National Microbiology Laboratory, Winnipeg, Canada. Ben Tan, Royal University Hospital, Saskatoon, Canada. Wendy Vaudry, Stollery Children's Hospital, Edmonton, Canada. Taj Jadavji, Alberta Children's Hospital, Calgary, Canada. David Scheifele, Laura Sauvé, Julie Bettinger, BC Children's Hospital, Vancouver, Canada.

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