Characterization of serogroup A *Neisseria meningitidis* from invasive meningococcal disease cases in Canada between 1979 and 2006: Epidemiological links to returning travellers

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INTRODUCTION: Serogroup A *Neisseria meningitidis* has repeatedly caused epidemics of invasive meningococcal disease (IMD) in developing nations since the 1960s. The present study is the first detailed study of serogroup A bacteria isolated in Canada.

METHODS: Thirty-four serogroup A meningococcal isolates collected from individuals with IMD in Canada between 1979 and 2006 were characterized by serology and multilocus sequence typing of seven housekeeping enzyme genes and genes encoding three outer membrane protein antigens.

RESULTS: Isolates were assigned to either the sequence type (ST)-1 or the ST-5 clonal complex. Clones within the ST-1 complex were recovered between 1979 and 1992, while clones of the ST-5 complex were isolated between 1987 and 2006; respectively, they accounted for 70.6% and 29.4% of all isolates studied. Isolates of the ST-1 complex were characterized by serosubtype antigen P1.3 or P1.3,6 with PorB allele 60 (serotype 4) and FetA sequence F5-1, while isolates of the ST-5 complex were characterized by serosubtype antigen P1.9 with PorB allele 47 (also serotype 4) and FetA sequence F3-1.

CONCLUSIONS: The Canadian serogroup A IMD isolates likely originated in travellers returning from hyperendemic or epidemic areas of the globe where serogroup A bacteria circulate. Although the Canadian cases of serogroup A IMD were caused by clones known to have caused epidemics in developing countries, disease incidence remained low in Canada.

Key Words: Canada; Invasive meningococcal disease; *Neisseria meningitidis*; Serogroup A

Neisseria meningitidis is a Gram-negative diplococcus that naturally inhabits the upper respiratory tract of humans, most commonly colonizing the throat's epithelial lining without any symptoms (1). Occasionally, the bacterium gains entry to the bloodstream and/or penetrates through the meninges into the cerebrospinal fluid of the host, resulting in fevers, severe headache, stiffness of neck and possibly seizures. This form of the disease is termed 'invasive meningococcal disease' (IMD), and is usually fatal if left untreated. The development and administration of suitable antibiotics has lowered the case-fatality rate to approximately 10% (2).

La caractérisation de la *Neisseria meningitidis* de sérotype A parmi les cas de méningite à méningocoques envahissante entre 1976 et 2006 : Des liens épidémiologiques avec des voyageurs de retour au pays

INTRODUCTION : La *Neisseria meningitidis* de sérotype A a provoqué des épidémies de méningite à méningocoques envahissante (MMI) à répétition dans les pays en voie de développement depuis les années 1960. La présente étude est la première étude détaillée des bactéries du sérotype A isolées au Canada.

MÉTHODOLOGIE : Trente-quatre isolats de méningocoque de sérotype A prélevés chez des personnes atteintes de MMI au Canada entre 1979 et 2006 ont été caractérisés par sérologie et typage séquentiel multilocus de sept gènes enzymatiques domestiques et gènes codant trois antigènes protéiques de la membrane externe.

RÉSULTATS : Les isolats étaient divisés entre le typage séquentiel (TS)-1 ou le complexe clonal TS-5. Les clones du complexe TS-1 ont été récupérés entre 1979 et 1992, et ceux du complexe TS-5, entre 1987 et 2006. Ils représentaient, respectivement, 70,6 % et 29,4 % de tous les isolats à l'étude. Les isolats du complexe TS-1 se caractérisaient par les sous-sérotypes des antigènes P1.3 ou P1.3,6 avec l'allèle 60 PorB (sérotipe 4) et la séquence F5-1 FetA, tandis que les isolats du complexe TS-5 se caractérisaient par le sous-sérotipe de l'antigène P1.9 avec l'allèle 47 PorB (également le sérotipe 4) et la séquence F3-1 FetA.

CONCLUSIONS : Selon toute probabilité, les isolats canadiens de MMI de sérotype A provenaient de voyageurs de retour d'une région hyperendémique ou épidémique du monde, où circulent les bactéries de sérotype A. Même si les cas canadiens de MMI de sérotype A étaient causés par des clones responsables d'épidémies dans les pays en voie de développement, l'incidence de la maladie demeurait faible au Canada.

Despite modern medical advances, however, *N meningitidis* continues to cause epidemics of meningitis and septicemia throughout the world (3).

Almost all meningococci express an antigenic polysaccharide capsule that serves to protect the bacterium during invasion into the host (4,5). Biochemical and immunological differences in meningococcal capsular polysaccharides allow classification of isolates into serogroups (6). Of the 13 known capsular serogroups, only five (A, B, C, Y and W135) have been shown to frequently cause disease, with serogroups A, B and C causing the majority of IMD cases worldwide (2).

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Although IMD epidemics are rare in developed countries, outbreaks do occur, which are usually caused by serogroups B, C and, more recently, Y and W135 (2). These serogroups are also largely responsible for the endemic incidence rates (ranging from 0.5 to three individuals per 100,000 population) in these regions, seen most often in young children and infants (7,8). Serogroup A epidemics have not occurred in any industrialized nation since World War II when the IMD incidence rate peaked at approximately 13 per 100,000 population per year (9). However, epidemics caused by serogroup A still frequently arise in developing nations, such as Africa and China, where serogroups B and C are associated with a smaller proportion of disease (2,10). In the developing nations, morbidity rates of IMD are several times higher than in industrialized countries, and have reached 1000 individuals per 100,000 population during peak epidemic periods in sub-Saharan Africa (11). Although several studies (12,13) have attempted to explain the reasons behind this variation in disease incidence, clear conclusions have yet to be drawn.

Investigations into the epidemiology of IMD rely on classification schemes based on genetic differences and phenotypic variation between the bacteria (14,15). Meningococci possess many immunogenic outer membrane proteins (OMPs) (16), two of which, PorB and PorA, form the basis for serotyping and serosubtyping, respectively (14). PorB is designated class 1 or 2 (16) based on its peptide sequence and possesses four variable regions ([VRs] 1 through 4, corresponding to loops I, V, VI and VII, respectively) (17), with serotype ascribed to the bacterial isolate as the monoclonal antibody(s) reacting with the immunodominant VR (18). PorA is a class 1 protein (19) that possesses three VRs, with serosubtypes determined by antibody(s) reacting with at least one of the VR epitopes (20,21). An iron-repressible OMP, FetA, has also been shown to invoke an immune response in humans (22) and, consequently, its VR has also been compared with meningococci (23). The bacterial population has been further subdivided into electrophoretic types and subgroups using multilocus enzyme electrophoresis (MLEE), which compares electrophoretic mobilities of a dozen or more cellular enzymes encoded by the bacterial genome (24). Multilocus sequence typing (MLST) has been used to replace MLEE studies due to the unambiguous nature and portability of MLST results among laboratories (25). The MLST technique identifies genetically similar bacteria by comparing the nucleotide sequences of seven housekeeping gene segments located within the core genome (25). Isolates possessing the same combination of alleles are grouped into a distinct sequence type (ST), with STs sharing identity at four or more loci belonging to the same clonal complex (25,26). The asymptotically carried meningococcal population is generally very diverse, while isolates causing epidemic disease often belong to a limited number of clonal groups known as 'hyperinvasive' lineages (25,27-29).

The largest proportion of IMD pandemics and epidemics in developing nations have been caused by hyperinvasive isolates belonging to serogroup A (24,30). Serogroup A meningococci represent a unique group of *N meningitidis*. Unlike the other serogroups that are highly heterogeneous in their genetic make-up, serogroup A bacteria are relatively clonal (31). Serogroup A isolates within the same clonal subgroup (as defined by MLEE) share fairly uniform OMP types, with only occasional recombination events producing genetic variants that display 'mosaic' cell surface antigens (12). In addition, the

polysaccharide capsule expressed by serogroup A bacteria does not contain sialic acid, which is found in the other common serogroups (ie, B,C,Y and W135), but is instead composed of a homopolymer of α -1,6-linked N-acetyl-D-mannosamine-1-phosphate (32). Based on MLEE studies of *N meningitidis* isolates collected from IMD outbreaks and epidemics in different parts of the world since the 1960s, eight genetic subgroups have been identified (I and II [ST-1 complex], III [ST-5 complex], IV, V, VI, VII and VIII) within the serogroup A bacterial population (13). Of these, only subgroups I (ST-1 complex) and III (ST-5 complex) have worldwide distributions as a result of global spread. To gain insight into the epidemiology of the meningococcus, most serogroup A isolates have been collected and characterized from developing nations where serogroup A epidemics are frequent (13). Conversely, because serogroup A epidemics are rare in developed nations, data on these meningococci in these countries are scarce.

The present study examines serogroup A *N meningitidis* isolates collected from individuals with IMD in Canada over a 28-year time span. These data will not only lead to a greater overall understanding of the global epidemiology of serogroup A meningococci, but will allow us to build a database of serogroup A isolate characteristics for education and outbreak preparedness, should disease caused by serogroup A bacteria re-emerge in Canada.

METHODS

Bacterial isolates

Meningococci isolated from sterile sites (ie, cerebrospinal fluid and blood) of IMD patients were sent to the National Microbiology Laboratory (NML) of the Public Health Agency of Canada by provincial public health laboratories. For most cases, demographic information of the patient (such as age and sex) was provided on the provincial public health laboratory requisition form. Bacteria were grown on Columbia blood agar at 37°C in an atmosphere of 5% CO₂ for 18 h to 24 h, after which one loopful of cells were suspended in sterilized distilled water and boiled for 10 min to provide DNA for genetic analysis. DNA samples were stored at -20°C until the time of testing.

Serogrouping and typing of meningococci

Isolates were identified as serogroup A using bacterial agglutination with rabbit antisera against the different serogroups of *N meningitidis* (33,34). Polymerase chain reactions (PCRs) using serogroup A capsule-specific primers (35) were conducted on isolates whose agglutination results proved inconclusive. Serotypes and serosubtypes were determined by indirect whole-cell ELISA (36) using the following monoclonal antibodies – 1, 2a, 2b, 4, 14, 15, 17 and 19, and P1.1, P1.2, P1.3, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P1.14, P1.15, P1.16 and P1.19.

PCR and sequence analyses

Isolates were characterized by MLST (25) and their PorA and PorB VR types; their FetA VRs were determined by established methods (18,20,21,23). All MLST allele numbers, PorA, PorB and FetA VR sequence variants were assigned using existing nomenclature systems (18,20) and by interrogating the relevant electronic databases – <<http://pubmlst.org/neisseria/>> and <<http://neisseria.org/nm/typing>> – to which new MLST alleles, allelic profiles, OMP alleles and VR sequences were also submitted.

RESULTS

Case characteristics of serogroup A meningococci collected in Canada between 1979 and 2006

Of the 70 invasive serogroup A meningococcal isolates submitted to the NML by provincial laboratories between 1979 and 2006, most were from the year 1979, with much lower numbers seen in later years (Figure 1). Only 34 of these 70 isolates could be examined for the present study, either because some isolates were not preserved or because certain preserved cultures could not be revived. The majority of the isolates (28 of 34) came from eastern Canada (14 from Ontario, seven from Quebec and seven from the Maritime provinces [four from Nova Scotia, two from Newfoundland and one from New Brunswick]), while five isolates came from British Columbia, and one isolate came from Alberta. Approximately one-third of the isolates (13 of 34) were from individuals between 20 and 39 years of age, with the remaining isolates recovered from cases with a broad age distribution (ranging from five months to 87 years of age).

Phenotypic characterization of meningococci

Thirty-two of 34 *N meningitidis* isolates were determined to be serogroup A by bacterial agglutination, while two were autoagglutinable (Table 1). Autoagglutinable isolates were confirmed to be serogroup A by PCR. Thirty-three isolates were found to be serotype 4, while one was nontypeable. The most common serosubtypes were P1.3 (10 isolates), P1.3,6 (10 isolates) and P1.9 (nine isolates). One isolate was P1.15,19 and four were nontypeable, denoted by P1.– (Table 1).

Genetic characterization by MLST and sequencing of *porA*, *porB* and *fetA* genes

Comparing the seven housekeeping gene segments to existing alleles in the database allowed the assignment of 24 isolates to the ST-1 complex and 10 isolates to the ST-5 complex (Table 1). Within the ST-1 complex, 10 isolates belonged to ST-1 (all from the years between 1979 and 1983), and five to ST-1468 (between 1988 and 1991), while nine isolates displayed allelic profiles that did not match any existing STs in the database. Therefore, the allele sequence data of these isolates were submitted to the database and new STs were assigned as ST-5898 (one isolate) and ST-5899 (eight isolates) (Table 1). Within the ST-5 complex, five isolates belonged to ST-5 (four between 1987 and 1989, and one from 1997), three to ST-4789 (all from 2006), one to ST-7 (from 2004), while one isolate (from 2004) that could not be assigned to any current ST was assigned to the novel sequence type, ST-5900 (Table 1). Within the same clonal complex, all observed STs shared between five and six MLST loci, while STs of different complexes shared only two or three alleles (Table 2).

Genetic analysis of the *porB* gene revealed a high degree of sequence identity among isolates. All isolates possessed identical *porB* gene sequences encoding the VRs 2 to 4, which were characterized as VR2-D, VR3-7b and VR4-21, respectively (Table 1). The *porB* sequences encoding VR1 were different according to the clonal complex, with ST-5 complex isolates possessing VR1-4 and ST-1 complex isolates possessing a VR1 type not previously described. For the present study, we denoted the VR1 of ST-1 complex isolates 'VR1-NM' (VR1 – no match), although notably, it was closest to VR1-4 of the Sacchi et al (18) scheme – the two VR1 sequences sharing six of 11 amino acids (Table 3). ST-1 complex isolates possessed *porB* allele 60 (encoding peptide variable loops I.6, IV.7, V.11, VI.10,

Figure 1) The number of serogroup A *Neisseria meningitidis* isolates sent to the National Microbiology Laboratory from invasive meningococcal disease cases in Canada between 1979 and 2006

VII.7 and VIII.6), while ST-5 complex isolates possessed allele 47 (encoding peptide loops I.4, IV.7, V.11, VI.10, VII.7 and VIII.6). All *PorB* proteins were classified as class 3.

The most common serosubtypes, P1.3 and P1.3,6, were associated with *PorA* VR peptide sequences described as VR1-18-1, VR2-3 and VR3-38, while serosubtype P1.9 was primarily linked with VR1-20, VR2-9 and VR3-35-1 (Tables 1 and 3). Different *PorA* profiles were observed for one nontypeable isolate (isolate 1990-294) and for an isolate of serosubtype P1.15,19 (isolate 1980-120). No *porA* PCR product could be produced for isolate 1992-349 and, consequently, VR types could not be designated to this bacterium. Isolates possessing the VR1-20, VR2-9 and VR3-35-1 profile belonged to the ST-5 complex, whereas isolates displaying other *PorA* VR types belonged to the ST-1 complex (Table 1).

The *FetA* VR peptide sequences of the isolates corresponded primarily to either F5-1 (24 isolates) or F3-1 (nine isolates) (Tables 1 and 3). F5-1 isolates were associated with serosubtypes P1.3 and P1.3,6 and belonged to the ST-1 complex, while F3-1 isolates were mainly of serosubtype P1.9 and belonged to the ST-5 complex. One other *FetA* VR peptide, F1-78, belonged to an isolate of serosubtype P1.9 (isolate 1997-088).

DISCUSSION

The Canadian serogroup A IMD cases occurring over the past 28 years were caused by either ST-1 complex or ST-5 complex bacteria, demonstrating that the worldwide dissemination of these meningococci also included Canada. Furthermore, the appearances of these bacterial groups in Canada seem to coincide with their temporal prevalence in the global epidemiology – first, the ST-1 complex, followed by the ST-5 complex.

Over the past 28 years, serogroup A ST-1 complex bacteria were found in Canada between 1979 and 1992, although these meningococci had existed in the nation since at least 1971 (24,37). The ST-1 complex isolates in our study were of four sequence types (ST-1, ST-1468, ST-5898 and ST-5899), which shared highly uniform OMP profiles (Table 1). Isolates of ST-1 were recovered in various parts of eastern Canada between 1977 and 1983. Because only five of the 14 serogroup A isolates reported to the NML in 1979 were available for analysis, we can only speculate this clone was responsible for the incidence spike that year. It appears that ST-1 organisms disappeared from Canada and were replaced by ST-1468 and ST-5899 organisms some time between 1984 and 1988.

TABLE 1

Phenotypic and genetic characterization of 34 serogroup A *Neisseria meningitidis* isolates collected in Canada and characterized at the National Microbiology Laboratory between 1979 and 2006

cc/sg and isolate	ST	Year	Province	Source	Phenotype*	FetA	PorA			PorB					
							VR1	VR2	VR3	Class	Allele	VR1	VR2	VR3	VR4
ST-1/sgII															
1979-012	1	1979	NS	Blood	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1979-247	1	1979	NS	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1979-250	1	1979	NS	Blood	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1979-833	1	1979	ON	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1980-120	1	1980	NFLD	CSF	A:4:P1.15,19	F5-1	19	15	36	3	60	NM†	D	7b	21
1980-277	1	1980	ON	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1981-018	1	1981	AB	Blood	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1981-221	1	1981	QC	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1981-233	1	1981	QC	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	b	21
1983-225	1	1983	NFLD	Blood	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1979-017	5898	1979	NS	CSF	A:4:P1.3,6	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1988-072	1468	1988	ON	CSF	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1989-083	1468	1989	BC	CSF	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1989-108	1468	1989	NB	Blood	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1989-169	1468	1989	ON	Blood	A:4:P1.—	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1991-056	1468	1991	BC	CSF	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1988-125	5899	1988	QC	CSF	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1988-185	5899	1988	QC	Blood	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1988-241	5899	1988	QC	Blood	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1988-252	5899	1988	ON	Blood	AA:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1989-554	5899	1989	ON	CSF	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1990-294	5899	1990	ON	CSF	A:4:P1.—	F5-1	18-1	10-2	36-2	3	60	NM†	D	7b	21
1992-301	5899	1992	QC	Blood	A:4:P1.3	F5-1	18-1	3	38	3	60	NM†	D	7b	21
1992-349	5899	1992	QC	CSF	A:4:P1.—	F5-1	?	?	?	3	60	NM†	D	7b	21
ST-5/sgIII															
1987-212‡	5	1987	ON	CSF	A:4:P1.—	F3-1	20	9	35-1	3	47	4	D	7b	21
1987-213‡	5	1987	ON	CSF	A:NT:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
1988-137	5	1988	ON	CSF	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
1989-521	5	1989	ON	CSF	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
1997-088§	5	1997	ON	Blood	AA:4:P1.9	F1-78	20	9	35-1	3	47	4	D	7b	21
2004-076	5900	2004	ON	Blood	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
2004-217	7	2004	ON	Blood	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
2006-037¶	4789	2006	BC	Blood	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
2006-045¶	4789	2006	BC	Blood	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21
2006-116	4789	2006	BC	Blood	A:4:P1.9	F3-1	20	9	35-1	3	47	4	D	7b	21

*Defined as serogroup:serotype:serosubtype; [†]Nearest match (NM) was variable region (VR)1–4, different by five base pairs from the typical VR1–4 gene sequence (69.7% similar), with VR amino acids DYQDGQVYSVE (54.5% similar to the VR1–4 prototype); [‡]With travel history to Mecca, Saudi Arabia; [§]With travel history to India; [¶]Both cases were admitted to hospital in Kamloops, British Columbia, one of which had recent travel history to India. ? Polymerase chain reaction unable to amplify gene region; AA Autoagglutinable (strains confirmed to be serogroup A by polymerase chain reaction; see text); AB Alberta; BC British Columbia; cc Clonal complex; CSF Cerebrospinal fluid; NB New Brunswick; NFLD Newfoundland; NS Nova Scotia; ON Ontario; P1.– Nonserosubtypable; QC Quebec; sg Subgroup; ST Sequence type

The serogroup A ST-5 complex meningococci isolated in Canada between 1987 and 2006 were likely due to the pandemic spread of this clonal group. The sequential emergence of ST-5 complex isolates belonging to ST-5 (1987 to 1997), ST-7 (2004) and ST-4789 (2006) in Canada appears to coincide with the temporal appearance of these STs in other parts of the world – ST-5 in China (1963), ST-7 in China (1992) and ST-4789 in Dhaka (2002) (data from <<http://pubmlst.org/neisseria/>> and as described here). According to the specimen requisition forms, the two ST-5 isolates recovered from Ontario in 1987 (isolates 1987-212 and 1987-213) were from individuals who had travelled to and

from Mecca, Saudi Arabia, that same year. Mecca was the site of a large outbreak caused by ST-5 complex serogroup A meningococci in August of 1987 during the annual Hajj pilgrimage, after which returning pilgrims introduced the bacteria to many countries throughout the world (38). After 1989, no ST-5 cases were documented until 1997 when it was isolated from a returning traveller from Punjab, India. It is unknown whether the solitary ST-7 isolate recovered in 2004 (isolate 2004-217) was associated with any overseas strain, although this ST had been present in Europe since at least 1992 (39) and had caused IMD epidemics in Africa in 2003 (40). In 2006, three more cases of IMD emerged in elderly individuals

TABLE 2

Allelic composition of multilocus sequence typing (MLST) corresponding to clonal complex (cc), subgroup (sg) and sequence type (ST) of serogroup A *Neisseria meningitidis* isolates examined in the present study

cc/sg	ST	MLST allele number						
		<i>abcZ</i>	<i>adk</i>	<i>aroE</i>	<i>fumC</i>	<i>gdh</i>	<i>pdhC</i>	<i>pgm</i>
cc1/sgII	1	1	3	1	1	1	1	3
	5898	1	3	1	32*	1	1	3
	5899	1	3	1	1	1	24*	3
	1468	1	3	1	168†	1	24*	3
cc5/sgIII	5	1	1	2	1	3	2	3
	7	1	1	2	1	3	2	19*
	4789	1	1	2	1	3	334*	19*
	5900	1	1	23*	1	3	2	19*

*Denotes allelic difference from the central ST, listed as the first ST according to the clonal complex it represents; †Denotes allelic difference from the central ST by single-point mutation

(men 62 to 87 years of age) residing in British Columbia due to isolates of ST-4789. These cases appear to be related to a serogroup A meningococcal disease outbreak in Delhi, India, in spring 2005 (41), because at least one of them had recent travel history to India (BC Centre for Disease Control, personal communication).

According to our study, over the past 28 years, the highest incidence of serogroup A IMD in Canada occurred between 1979 and 1989 (Figure 1). Because there was no change in the Canadian surveillance system for monitoring IMD in 1979, we speculated that the surge of isolates seen in that year did reflect higher disease activity. However, the lack of information on the date of disease onset for many of the cases could have resulted in isolates recovered in earlier years being submitted to the NML in 1979, thus creating an artificial peak for the year. On the other hand, the number of serogroup A IMD cases per year may be under-represented for several reasons – due to the surveillance system in Canada being passive, some IMD cases may have gone unreported; some serogroup A isolates may have been lost or not preserved for further testing at the local hospitals and, therefore, were not sent to the NML; any serogroup A isolates collected from nonsterile or unknown specimen sites were never confirmed as 'invasive' and, thus, could not be included in our study; and because detection of *N meningitidis* by nucleic acid analysis (ie, PCR) was not incorporated into the national case definition for IMD until 2006 (42), some serogroup A cases may have gone undetected by the less sensitive antigen detection and/or culture tests performed before this time. Another limitation of our study is the lack of detailed clinical and epidemiological data, which prevented us from understanding the complete picture of serogroup A meningococcal disease in Canada.

Despite low levels of serogroup A disease in Canada, recent trends of antibiotic resistance (43,44) and the appearance of a new strain of serogroup A meningococci in Africa (characterized as ST-2859), raises the possibility of another epidemic wave of IMD in the African meningitis belt (45), which may in turn result in further pandemic spread. The work carried out in the present report will aid in our understanding of the global epidemiology of meningococcal disease and, in particular, clonal complexes associated with serogroup A *N meningitidis*. Furthermore, these data will serve to increase nation-wide

TABLE 3

Deduced peptide sequences of PorA variable region (VR)-1, VR2, VR3, PorB VR1 and FetA VR of examined serogroup A meningococcal isolates collected in Canada between 1979 and 2006 and characterized at the National Microbiology Laboratory

cc/sg	VR	PorA type	Reactivity with MAb*	Protein sequence
ST-1/sgI/II	VR1	18-1	ND	PPSQGQTGNKVTKG
		19	+	PPSKSQPQVKVTKA
ST-5/sgIII		20	+	QPQTANTQQGGKVKVTKA
ST-1/sgI/II	VR2	3	+	TLANGANNTIIRVP
		10-2	H+	HFVQDKKQGPPTLVP
		15	+	HYTRQNNADVFPV
ST-5/sgIII		9	+	YVDEQSKYHA
ST-1/sgI/II	VR3	36	ND	LLGSTSDE
		36-2	ND	LLGSGSDE
		38	ND	LLGRIGDDDE
ST-5/sgIII		35-1	ND	LLGSGSDQ
PorB type				
ST-1/sgI/II	VR1	4	+	EHNGGQVVSVSE
ST-5/sgIII		NM	ND	DYQDGQVVSVE
FetA type				
ST-1/sgI/II	VR	F5-1	N/A	GEFEISGKKKDPKDPKKEID-DEEKAKDKKMDLVH-SYKLS
ST-5/sgIII		F3-1	N/A	GEFSIPTKEKK---NGKEVDKPMQEQKKDRADEATVHAYKLS
		F1-78	N/A	GEFAIKDEASA-----TTAEKQKNRDNKIVHAYKLT

*Reactivity with monoclonal antibody (MAb), as listed in Sacchi et al (18,21) with the exceptions of PorA VR1 18-1 and PorA VR2 10-2, which were listed in the relevant database at <<http://neisseria.org/nm/typing>> and in Suker et al (46), respectively. + Positive reaction; H+ Positive when a high concentration of MAb is used; cc Clonal complex; N/A Not applicable; ND Not described in the literature; NM Nearest match is VR1-4; sg Subgroup; ST Sequence type

preparedness should disease caused by serogroup A bacteria re-emerge in Canada in the future.

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