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Invasive Meningococcal Disease in Québec, Canada, Due to an Emerging Clone of ST-269 Serogroup B Meningococci with Serotype Antigen 17 and Serosubtype Antigen P1.19 (B:17:P1.19)

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During periods of endemic meningococcal disease, serogroup B *Neisseria meningitidis* is responsible for a significant percentage of invasive diseases, and no particular clone or strain predominates (F. E. Ashton and D. A. Caugant, Can. J. Microbiol. 47: 293–289, 2001), However, in the winter of 2004 to 2005, a cluster of serogroup B meningococcal disease occurred in one region in the province of Québec, Canada. The *N. meningitidis* strain responsible for this cluster of cases was identified as sequence type ST-269 with the antigenic formula B:17:P1.19. Retrospective analysis of isolates from 2000 onwards showed that this clone first emerged in the province of Québec in 2003. The emergence of this clone of serogroup B meningococci occurred after a mass vaccination against serogroup C *N. meningitidis*, suggesting possible capsule replacement.

Invasive meningococcal disease (IMD) is endemic in Canada, and rates of disease fluctuate from year to year without any definite pattern. However, increased incidences have been noted every 10 or 15 years in the past. Since the last serogroup A epidemic in 1941, IMD due to serogroup A strains has become rare, and most IMD in Canada is now due to serogroups B, C, Y, and W-135 (7, 27).

A major recent change in the epidemiology of IMD in Canada is the introduction of the new serogroup C clone of ET-15 *Neisseria meningitidis* (C:2a:P1.5,2) strains, which caused two waves of increased disease incidence throughout Canada in the last 15 years (2, 31). The first wave of disease caused by this C:2a:P1.5,2 ET-15 clone occurred in the early 1990s. As a response to the outbreaks caused by this new clone, targeted vaccination campaigns using the polysaccharide vaccines were carried out in several provinces (Ontario, Québec, Prince Edward Island, and British Columbia) (8, 13, 28, 29). In the province of Québec, a province-wide vaccination campaign was launched between December 1992 and March 1993 after initial vaccination of the target population did not curtail the spread of this clone in that province (8). These vaccination campaigns had been very successful in reducing the amount of serogroup C meningococcal disease activity in Canada from 1994 to 1999 (7, 26). The effectiveness and cost/benefit ratio of the province-wide immunization against serogroup C meningococci in Québec have been analyzed and reported (8, 9).

In 2000 and 2001, several provinces (British Columbia, Alberta, Manitoba, Ontario, and Québec) noted a resurgence in meningococcal disease activity due to serogroup C *N. meningitidis*. In Québec, the outbreak was due to a unique antigenic variant of the serogroup C ET-15 clone (32). To combat the spread of disease due to this second wave of IMD, the province of Québec initially launched local immunization programs using the plain polysaccharide vaccine to target high school students in the Québec City area where the initial outbreaks occurred. However, when the outbreak spread to other regions and also affected younger age groups, the province decided to launch another mass immunization campaign, and this time the newly licensed serogroup C glycoconjugated vaccine (Menjugate; Chiron, Emeryville, California) was used. Starting in May to June 2001, the conjugate vaccine was offered to all residents age 2 months to 20 years, initially in the Québec City area, and beginning in the summer of 2001, the immunization was extended to the same age group of residents in other regions of the province (10). The effectiveness of this glycoconjugate vaccine was reported to be 96.8% in reducing the number of cases of serogroup C meningococcal disease in Québec (10).

In Québec, IMD is a notifiable disease. Clinical and laboratory-confirmed cases are registered in a provincial notifiable disease database. Clinical isolates and culture-negative clinical specimens (for PCR diagnosis) must be submitted to the Laboratoire de Santé Publique du Québec (LSPQ) for further analysis and characterization. Between October 2004 and March 2005, local public health officials together with the LSPQ of the Institut Nationale de Santé Publique du Québec noticed a cluster of six IMD cases due to serogroup B meningococci in region 12 (Chaudière-Appalaches) and one documented contact case in adjacent region 03 (La Capitale Nationale, Québec

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TABLE 1. Cluster of serogroup B invasive meningococcal disease cases in South Shore of Québec City (region 12, Chaudière-Appalaches) and the adjacent region (region 03, La Capitale Nationale, Québec City), Québec, Canada, October 2004 to March 2005

Patient	Sampling date	NML isolate no.	(yr)/sex[c]	Isolate source	Laboratory test results
1	24 Oct. 2004	2004-194	14/F	CSF	PCR diagnosis, positive for serogroup B *N. meningitidis*
	24 Oct. 2004	2004-195		Blood	PCR diagnosis, positive for serogroup B *N. meningitidis*; *porA* gene sequencing,[d] PorA VR1 = 19a and PorA VR2 = 15b
	28 Oct. 2004[a]	2004-200		Blood	Culture positive for *N. meningitidis*; B:17:P1.19 (ST-269)
2	24 Nov. 2004	2004-214	15/M	CSF	PCR diagnosis, positive for serogroup B *N. meningitidis*; *porA* gene sequencing, PorA VR1 = 19a and PorA VR2 = 15b
3	30 Nov. 2004	2004-237	20/M	Blood	Culture positive for *N. meningitidis*; B:17:P1.19 (ST-269)
4[b]	2 Dec. 2004	2004-229	75/M	CSF	PCR diagnosis, positive for serogroup B *N. meningitidis*; *porA* gene sequencing, PorA VR1 = 19a and PorA VR2 = 15b
5	23 Dec. 2004	2005-009	17/F	CSF	PCR diagnosis, positive for serogroup B *N. meningitidis*; *porA* gene sequencing, PorA VR1 = 19a and PorA VR2 = 15b
6	17 Mar. 2005	2005-075	6/F	Blood	PCR diagnosis, positive for serogroup B *N. meningitidis*; *porA* gene sequencing, PorA VR1 = 19a and PorA VR2 = 15b
7	25 Jan. 2005	2005-045	46/M	CSF	Culture positive for *N. meningitidis*; B:17:P1.19 (ST-269)

[a] Date specimen received at LSPQ.
[b] Resident of region 03 and grandfather of patient 3.
[c] F, female; M, male.
[d] PorA VR types according to the nomenclature of Sacchi et al. (23).

City). This cluster of cases included four cases identified by PCR diagnosis (detection of *N. meningitidis*-specific and serogroup B-specific DNA in normally sterile body fluids of patients), and the others were culture-confirmed cases. The National Microbiology Laboratory (NML) of the Public Health Agency of Canada was contacted to conduct a laboratory investigation of the causative agent in this cluster of IMD cases. This cluster of cases around the Québec City area prompted us to carry out an in-depth investigation, and here we present a report of the laboratory findings, which suggest that an emerging clone of serogroup B meningococci, belonging to sequence type 269 (ST-269), has been causing IMD in the province of Québec since 2003.

MATERIALS AND METHODS

PCR diagnosis and direct sequencing of antigen genes from clinical specimens. Extraction of DNA from cerebrospinal fluid (CSF) was accomplished by adding 1 ml of DNAzol (Molecular Research Center, Inc., Cincinnati, Ohio) to 0.1 ml of CSF in the presence of 0.01 ml of polyacryl carrier (Molecular Research Center, Inc., Cincinnati, Ohio) designed for isolation of small amounts of DNA, according to the manufacturer's instructions. For extraction of DNA from EDTA-treated whole-blood specimens, the QIAamp DNA minikit was used (QIAGEN Inc., Mississauga, Ontario, Canada).

PCR primers and thermal cycling conditions for the amplification of the serogroup-specific polysialyltransferase *siaD* genes as well as the *N. meningitidis*-specific *ctrA* gene were described by Borrow et al. (4, 5, 14). PCR products were confirmed by DNA hybridization using biotin labeled probes and digoxigenin-11-dUTP (Roche Diagnostic, Laval, Québec, Canada) in the PCR amplification step, followed by PCR-enzyme-linked immunosorbent assay (ELISA) as described by Borrow et al. (4, 5).

Identification, serogrouping, and typing of meningococci. *N. meningitidis* was identified by biochemical tests, and serogrouping was determined by bacterial agglutination with rabbit serogrouping antisera at the LSPQ (19). Serotyping and serosubtyping were done at the NML by whole-cell ELISA (1), using monoclonal antibodies from a commercial kit (Rijksinstituut voor Volksgezondheid en Milieu, National Institute of Public Health, Bilthoven, The Netherlands). Monoclonal antibodies to serotype antigen 17 and serosubtype antigen P1.19 were provided by the Walter Reed Army Institute of Research. For strains that gave either no agglutination or nonspecific agglutination with the serogrouping antisera, detection of the serogroup B-, C-, Y-, and W135-specific *siaD* genes was carried out by PCR (4, 5). Multilocus sequence typing (MLST) was done according to method described by Maiden et al. (17), and isolates were assigned to STs according to the *Neisseria* MLST website (http://pubmlst.org/neisseria). Sequencing of *porB* and *porA* genes, which determine the serotype and serosubtype antigens, respectively, was done following the protocol and nomenclature of Sacchi et al. (22, 23). The PorA and PorB VR types were described according to the nomenclature of Sacchi (22, 23) as well as the nomenclature given in the *N. meningitidis* PorA variable-region (VR) database (http://neisseria.org/perl/agdbnet/agdbnet.pl?file = poravr.xml) and the *N. meningitidis* PorB variable-loop typing scheme (http://neisseria.org/nm/typing/porb/varloops.shtml). Pulsed-field gel electrophoresis (PFGE) of NheI restriction enzyme-digested meningococcal genomic DNA and data analysis of DNA fingerprints, including generation of dendrograms, were done as essentially described by Tyler and Tsang (34), using the Bionumerics software version 3.5 (Applied Maths, Kortrijk, Belgium).

RESULTS

Laboratory investigation of outbreak specimens. Between October 2004 and March 2005, seven epidemiologically related invasive meningococcal disease cases were identified in Québec's adjacent regions 03 and 12. Three cases were culture positive for serogroup B *N. meningitidis*, while four cases were identified by PCR diagnosis based on detection of both *N. meningitidis*-specific *ctrA* and the serogroup B-specific *siaD* genes (Table 1).

Conventional serotyping and serosubtyping with monoclonal antibodies failed to identify the serotype and serosubtype antigens in the three culture-positive serogroup B isolates, and they were reported to the LSPQ as B:NT:P1.- (serogroup B, nonserotypeable and nonserosubtypeable). In order to understand the serotype and serosubtype nature of the outbreak strain as well as to confirm that the PCR-diagnosed cases were

related to the culture-positive cases, we resorted to DNA sequencing of the *porB* and *porA* genes, which are responsible for synthesis of the class 2/3 PorB and class 1 PorA outer membrane proteins that bear serotype and serosubtype epitopes, respectively. In the three cases that yielded serogroup B meningococcal isolates, DNA was extracted from the bacterial strains, and the VRs of their *porA* genes were amplified and sequenced. All three isolates gave identical PorA VR1 and VR2 regions, identified as 19a and 15b, respectively, according to the nomenclature of Sacchi et al. (23) or as 19-1 and 15-11 according to the nomenclature of the *N. meningitidis* PorA variable-region database (http://neisseria.org/perl/agdbnet/agdbnet.pl?file = poravr.xml). For the PCR-diagnosed cases, DNA sequencing was performed directly on the clinical specimens (DNA extracted from blood or CSF specimens) after PCR amplification of the *porA* gene. In all four PCR-diagnosed cases, identical PorA VR1 and VR2 sequences or types (19a and 15b, respectively) which matched those found for the three positive cultures were obtained, thus suggesting that all seven epidemiologically linked cases were also related microbiologically. In one case the *porA* gene sequencing results from the bacterial culture and the whole-blood specimen were identical. Neither DNA sequencing of the *porB* genes nor MLST was done for the four PCR-diagnosed cases due to lack of adequate clinical specimens.

For the three bacterial cultures obtained, *porB* gene sequencing and MLST were performed to gain further knowledge about the nature of the outbreak strain. Results of *porB* gene sequencing suggested that the three isolates belong to serotype 17 based on the PorB VR types identified (VR1 = B, VR2 = C, VR3 = 7, and VR4 = 14b, according to the nomenclature of Sacchi et al. [22]). The equivalent PorB variable loops according to the nomenclature of the *N. meningitidis* PorB typing scheme were I.9, V.13, VI.9, and VII.12 (http://neisseria.org/nm/typing/porb/varloops.shtml).

By MLST the three isolates were identified as ST-269, since they all had the following housekeeping gene alleles: *abcZ*, 4; *adk*, 10; *aroE*, 15; *fumC*, 9; *gdh*, 8; *pdkc*, 11; and *pgm*, 9) (http://pubmlst.org/neisseria).

Based on the *porA* and *porB* gene sequencing results, the outbreak strain is identified as serotype 17 with the serosubtype antigens of P1.19,15. The lack of reaction of the outbreak strain with the anti-P1.15 monoclonal antibody may be related to the fact that in the outbreak strain, mutations involving two nucleotides in the VR2 region of its *porA* gene had resulted in changes in the amino acid composition of its VR2 epitope. Testing of the outbreak strains isolated from the three cases confirmed that they all reacted with the monoclonal antibody to serotype 17 and with the monoclonal antibody to the serosubtype antigen P1.19.

Retrospective analysis of B:NT:P1.- isolates with monoclonal antibodies that identified the serotype 17 and serosubtype P1.19 epitopes. Retrospective analysis of our records at NML revealed that the percentage of IMD isolates received from the province of Québec that typed as serogroup B in the last 4 years increased from 29% in 2001 to 46%, 58%, and 65% in 2002, 2003, and 2004, respectively. At the same time, the number and percentage of serogroup B IMD isolates that were typed as B:NT:P1.- rose from 3 or 4 isolates, or 13%, in both 2001 and 2002 to 10 isolates, or 37%, in 2003 and 20 isolates, or 53%, in 2004. In the first half of 2005, there were already 12 B:NT:P1.- isolates among the 21 serogroup B IMD isolates received from the province of Québec.

TABLE 2. Identification of the B:17:P1.19 clone from B:NT:P1.- *Neisseria meningitidis* invasive infection cases confirmed by culture in the province of Québec, Canada, January 2001 to June 2005

NML no.[a]	Yr/mo of isolation	Isolate source	Region	Reaction with monoclonal antibody to:		MLST type
				Serotype 17	Serosubtype P1.19	
2001-145	2001/03	CSF	06	−	−	ST-1473
2001-325	2001/06	Blood	06	−	−	ST-5554
2001-383	2001/08	Blood	03	−	+	ST-565
2002-189	2002/06	CSF	16	−	−	ST-1473
2002-191	2002/06	Blood	03	+	−	ST-269
2002-198	2002/07	CSF	16	−	−	ST-44
2002-266	2002/11	CSF	12	−	−	ST-5553
2003-065	2003/03	Synovial fluid	05	−	−	ST-2820
2003-069	2003/03	CSF	03	+	+	ST-269
2003-089	2003/03	Blood	06	+	+	ST-269
2003-093	2003/04	CSF	12	+	+	ST-269
2003-149	2003/06	CSF	03	+	+	ST-269
2003-167	2003/06	Blood	12	+	+	ST-269
2003-172	2003/08	Blood	03	+	+	ST-269
2003-179	2003/08	Blood	03	+	+	ST-269
2003-208	2003/10	Blood	13	+	+	ST-269
2003-250	2003/12	CSF	16	+	+	ST-269
2004-010	2003/12	CSF	06	−	−	ST-568
2004-011	2003/12	CSF	09	+	+	ST-269
2004-017	2004/01	CSF	16	+	+	ST-269
2004-021	2004/01	CSF	02	+	+	ST-269
2004-026	2004/01	CSF	12	+	+	ST-269
2004-032	2004/01	CSF	09	+	+	ST-269
2004-051	2004/02	CSF	04	+	+	ST-269
2004-064	2004/03	CSF	15	+	+	ST-269
2004-065	2004/03	CSF	16	+	+	ST-269
2004-066	2004/03	CSF	02	+	+	ST-269
2004-070	2004/03	CSF	04	+	+	ST-269
2004-104	2004/04	Blood	06	+	+	ST-269
2004-106	2004/04	Blood	02	+	+	ST-269
2004-119	2004/06	CSF	02	+	+	ST-269
2004-158	2004/07	CSF	05	−	−	ST-5552
2004-164	2004/08	Blood	06	−	−	ST-1473
2004-179	2004/09	Blood	06	+	+	ST-269
2004-200	2004/10	Blood	12	+	+	ST-269
2004-208	2004/11	CSF	14	+	+	ST-269
2004-237	2004/11	Blood	12	+	+	ST-269
2005-006	2004/12	CSF	03	+	+	ST-1986
2005-020	2005/01	Blood	03	+	+	ST-269
2005-022	2005/01	Blood	13	+	+	ST-269
2005-039	2005/01	Blood	03	+	+	ST-269
2005-045	2005/01	CSF	12	+	+	ST-269
2005-055	2005/02	Blood	11	+	+	ST-269
2005-061	2005/02	Blood	03	+	+	ST-269
2005-068	2005/02	CSF	03	+	+	ST-269
2005-110	2005/04	CSF	02	+	+	ST-269
2005-134	2005/05	CSF	02	+	+	ST-269
2005-143	2005/05	Blood	03	+	+	ST-269
2005-150	2005/06	CSF	02	+	+	ST-269

[a] The NML number reflects the year when the specimen was received at NML and the laboratory number given for that year.

Prior to this study, monoclonal antibodies to the serotype antigen 17 and the serosubtype antigen P1.19 had not been used to characterize meningococcal strains in Canada, partly because these reagents were not commercially available. Table 2 lists the B:NT:P1.- isolates recovered from IMD cases in Québec from 2001 to the first half of 2005 and their typing results with the anti-serotype 17 and anti-serosubtype P1.19 monoclonal antibodies. All 12 B:NT:P1.- isolates from IMD

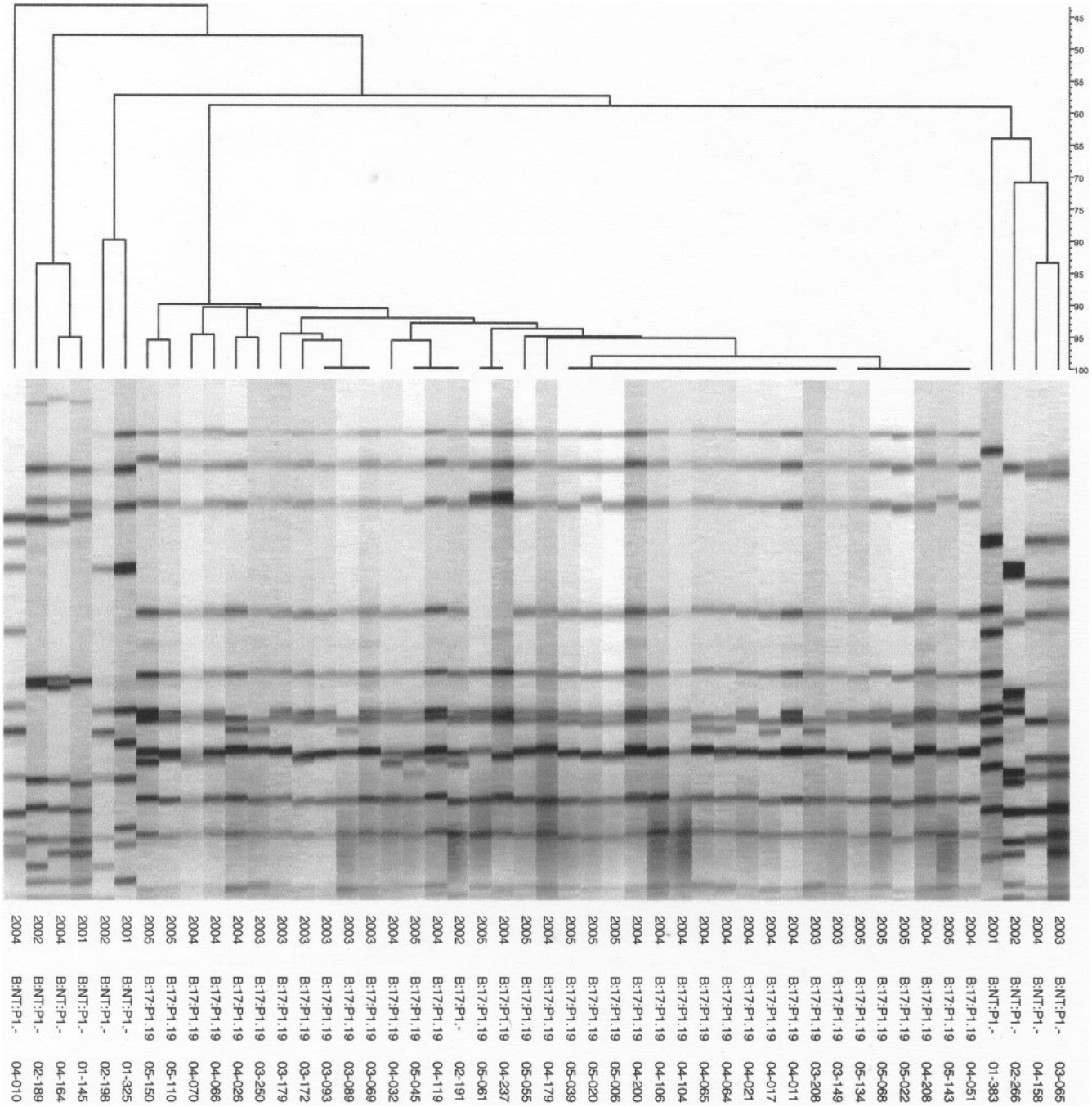

FIG. 1. Pulsed-field gel electrophoresis profiles of NheI-digested DNAs from the B:17:P1.19 and B:NT:P1.- strains. Strains 2004-200 and 2004-237 were isolates from index cases of the outbreak in region 12 of Québec. Strain 2002-191 (B:17:P1.-) belongs to ST-269.

cases in 2005 were typed as B:17:P1.19. Seventeen (85%) out of 20 and 9 (90%) out of 10 B:NT:P1.- IMD isolates from 2004 and 2003, respectively, were typed as B:17:P1.19. None of the B:NT:P1.- isolates from 2002 (four cases) and 2001 (three cases) belonged to the B:17:P1.19 strain. One isolate from 2002 (isolate 2002-191) reacted with the monoclonal antibody to serotype 17 but did not react with the monoclonal antibody to serosubtype P1.19. This isolate was found to belong to ST-269.

All 49 B:NT:P1.- isolates have been analyzed by MLST, and their STs are described in Table 2. The 38 B:17:P1.19 isolates were found to belong to the ST-269 clonal complex (37 were ST-269, and the remaining isolate was found to belong to ST-1986, which has an allelic profile identical to that of ST-269 with the exception of an allele 5 in its *adk* locus instead of an allele 10 in ST-269). One isolate typed as B:17:P1.- (*porA* gene sequencing confirmed that this strain did not have the P1.19 epitope) was found to belong to ST-269, while another isolate typed as B:NT:P1.19 (*porB* gene sequencing confirmed that

TABLE 3. Number and percentage of cases of invasive B:17:P1.19 *Neisseria meningitidis* disease by age group and sex in the province of Québec, Canada, March 2003 to June 2005

Age group (yr)	No.		
	Male	Female	Total in age group (%)
<1	0	0	0 (0)
1–4	3	1	4 (10.5)
5–9	0	2	2 (5.3)
10–19	5	12	17 (44.7)
20–39	8	2	10 (26.3)
40–59	2	1	3 (7.9)
≥60	1	1	2 (5.3)
Total	19	19	38 (100)

this strain did not have the serotype 17 epitope) was found to belong to ST-565 (a member of the ST-269 complex that has an allelic profile with six of its seven loci identical to those of ST-269). The remaining nine B:NT:P1.- isolates were confirmed by DNA sequencing of their *porB* and *porA* genes to have serotype and serosubtype antigens unrelated to 17:P1.19 (data not shown). Seven different STs were found among these nine isolates, but they all belong to the lineage 3 or the ST-41/44 complex.

Forty-eight B:NT:P1.- isolates, including 37 isolates typed as B:17:P1.19, were analyzed by PFGE, and the results are presented in Fig. 1. Those isolates typed as B:17:P1.19 have either identical or highly similar (90% or higher relatedness) DNA fingerprints, while those strains that showed no reaction to the serotype 17 and serosubtype P1.19 monoclonal antibodies show different PFGE patterns (60% or less relatedness).

Of the 38 IMD cases caused by the B:17:P1.19 strain, 19 (50%) were in males and 19 were in females (Table 3). Their ages range from 20 months old (1.71 years) to 86 years old (median, 18 years). Approximately 45% (17 cases) of the patients were aged 10 to 19 years, 26% (10 cases) were 20 to 39 years, and 16% (6 cases) were 1 to 9 years. No patients were younger than 1 year old. Cases were located in 11 (61%) of the 18 regions of Québec, and 23 (61%) of the 38 patients lived in three adjacent regions.

Potential capsule replacement by the finding of C:17:P1.19 belonging to the ST-269 clonal complex. Coincidental to the finding of an increase in B:NT:P1.- isolates in Québec, three C:NT:P1.- isolates were found in 2003 (two cases) and 2004 (one case). All three C:NT:P1.- isolates were typed positive with the anti-serotype 17 and anti-serosubtype P1.19 monoclonal antibodies. Two of these C:17:P1.19 isolates were found by MLST to be ST-269, and the third C:17:P1.19 isolate was ST-1095 (a member of the ST-269 clonal complex which shared six out of seven alleles with ST-269). DNA fingerprinting confirmed their close identity with the B:17:P1.19 isolates, with the exception of their capsular polysaccharide antigen (Fig. 2).

DISCUSSION

Serogroup B *N. meningitidis* is responsible for causing endemic disease in Canada and has been described as genetically heterogeneous (3), with no single clone or any particular antigenic type predominating among the invasive disease isolates. For example, during a survey of serogroup B isolates obtained from disease cases from 1994 to1996, 177 multilocus enzyme electrophoretic types (ETs) were identified among the 301 isolates, with most of the 136 ETs represented by single isolates. The most common clone or clonal complex is the ET-5 complex, and there were 36 isolates identified in this clone. All 36 isolates that belong to the ET-5 complex had either serotype antigen 1, 4, or 15. The other common ETs identified were ET-98 (with 18 isolates), ET-169 (16 isolates), and ET-134 (8 isolates). ET-98 isolates were either typed as serotype 14 and/or serosubtyped as P1.14. Thirteen of the 16 ET-169 isolates were typed as NT:P1.13. These data suggests that the ST-269 clone identified in this study and represented by the antigenic formula B:17:P1.19 is unrelated to these previously identified clones. We do not have further information on the ET-134 clone, but it did not appear to be isolated solely from one province, nor did it appear to have any unique antigens. Strains of *N. meningitidis* with the serotype antigen 17 are common in neither Brazil (21) nor the United States (20, 30); however, serogroup B strains with the serosubtype antigens P1.19 and 15 were commonly found in both the United States (24, 30) and Brazil (25).

Serogroup B clones that have been described in the literature as causing epidemic disease include ET-5 (ST-32 complex), cluster A4 (ST-8 complex), and lineage III (ST-41/44 clonal complex) (6). Although sporadic cases due to these three well-characterized serogroup B clones have been found in Canada, they do not appear to share any properties (housekeeping enzyme alleles or serotype/serosubtype antigens) with the ST-269 clone identified in this report. Also, no outbreaks due to these clones have been reported in Canada, with the exception of a spatio-temporal cluster of five IMD cases due to ET-5 serogroup B meningococci that occurred in 1995 on the Island of Montréal (16). The occurrence of this cluster of cases coincided with the increase in the proportion of IMD due to

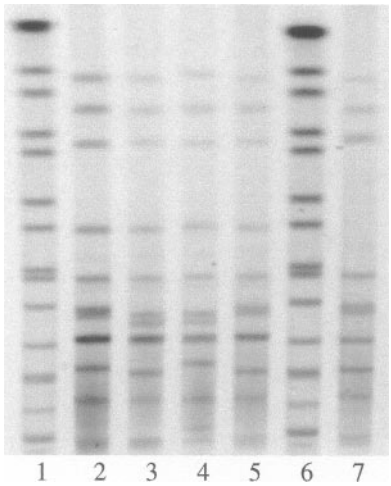

FIG. 2. Pulsed-field gel electrophoresis patterns of B:17:P1.19 and C:17:P1.19 strains. Lanes 1 and 6, molecular weight makers provided by *Salmonella enterica* serovar Barenderup digested with NheI. Lanes 5 and 7, B:17:P1.19 ST-269 isolates. Lane 2, isolate 2004-114 (C:17:P1.19, ST-269), Lane 3, isolate 2003-195 (C:17:P1.19, ST-269). Lane 4, isolate 2003-138 (C:17:P1.19, ST-1095/ST-269 clonal complex).

serogroup B in the province of Québec, and this is possibly related to the mass immunization campaign with the tetravalent (A, C, Y, and W135) or bivalent (A and C) vaccines used in 1993 for controlling the spread of the ET-15 serogroup C meningococci in that province (16). Whether the current increase in the finding of the B:17:P1.19 ST-269 clone was also related to the province-wide vaccination campaign in 2001 to 2002 cannot be answered for sure. Nevertheless, it is interesting to note that no B:17:P1.19 ST-269 meningococcal strain was identified in Québec prior to 2003 (the massive use of the conjugated serogroup C meningococcal vaccine occurred in 2001).

Although ST-269 and the ST-269 clonal complex are not a well-known emerging clone, a substantial number of isolates that belong to the ST-269 clonal complex existed in the *Neisseria meningitidis* MLST database (http://pubmlst.org/neisseria). When accessed on 6 December 2005, this database showed that there were altogether 236 records describing strains belonging to the ST-269 clonal complex. A high degree of heterogeneity within this clonal complex was also observed, with over 200 different unique STs. Sixty-four percent of the isolates in the ST-269 clonal complex belong to serogroup B, 7% belong to serogroup C, and the rest are mostly either nongroupable or have serogroup information listed as unspecified. Slightly fewer than half of the isolates were recovered from carriers, and an equal number were from invasive disease cases, while the remaining small numbers were from unspecified sources. The earliest record of isolates belonging to the ST-269 clonal complex is from two invasive disease cases in The Netherlands in 1970, and both strains belonged to serogroup B. Also interesting is the recent report of an increase in the ST-269 clone among invasive disease isolates of *N. meningitidis* in Scotland, which may be related to the introduction of the meningococcal serogroup C polysaccharide conjugate vaccine in the United Kingdom in 2000 (11).

In contrast to what has been described for endemic serogroup B meningococcal disease, which has the highest incidence in the age group of under 2 in Canada (12, 18), IMD cases due to the current B:17:P1.19 strain affected mainly adolescents and young adults between the ages of 10 and 30 (25 out of 38 cases, or 65.8%). In more than half of the cases (22 cases, or 57.9%), the B:17:P1.19 isolates were obtained from CSF cultures, and in 16 cases (42.1%), the bacteria were isolated from blood cultures. Further clinical and epidemiological information on IMD cases caused by this emerging clone of serogroup B meningococci is pending and will subsequently be published.

Concurrent with the findings of an increase in the number of isolates belonging to the B:17:P1.19 ST-269 clone in Québec was the appearance of three serogroup C isolates (two isolated in 2003 and one in 2004) that were originally typed as C:NT:P1.- but were subsequently found to be C:17:P1.19. Two of these three isolates belong to ST-269, and the third isolate belongs to ST-1059, which is a member of the ST-269 complex. Although it is very difficult to know for sure whether the B:17:P1.19 changed into C:17:P1.19 or vice versa, the following evidence suggests that the capsule switch took place from serogroup B to serogroup C. First, most serogroup C isolates from IMD cases in Canada belong to the ET-15 clonal type (15), and serogroup C ST-269 isolates with the antigenic formula C:17:P1.19 had never been isolated before. Second, the C:17:P1.19 isolates were found in the later half of the year 2003, when B:17:P1.19 isolates were becoming common. The hypothesis that these C:17:P1.19 isolates arose by capsule switching from the B:17:P1.19 strain and the mechanism involved warrant further studies. Capsule switching from C:2a:P1.5,2 to B:2a:P1.5,2, as well as from C:2a:P1.7,1 to B:2a:P1.7,2 and from Y:2c:P1.5 to B:2c:P1.5, has been previously reported in Canada (33, 34; NML, unpublished data).

With the success in the control of serogroup C meningococci by using the conjugated serogroup C vaccine in Québec, it is important to examine whether mass vaccination against a single serogroup of meningococci would contribute to the emergence of a non-vaccine-preventable serogroup of meningococci, such as the potentially hypervirulent clone of ST-269 (B:17:P1.19). The only way to know is through enhanced surveillance of IMD by using both phenotypic and genotypic tools such as those described in this report.

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