Meningococcal Infections in the Province of Québec, Canada, during the Period 1991 to 1992

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A total of 234 strains of *Neisseria meningitidis* obtained from hospitalized patients living in the province of Québec during the period 1991 to 1992 were characterized according to their serogroup, serotype, subtype, electrophoretic type, and antimicrobial susceptibility. All these strains were recovered from sterile body fluids, except for one strain that was isolated postmortem from a cutaneous lesion. For both years, serogroup C was the most prevalent (69.7%), followed by serogroup B (27.4%). Serotype 2a represented 80.3% of serogroup C isolates, and P1.2 was the most common subtype associated with this serotype. Clone ET 15 accounted for 76.5% of serogroup C isolates and 90.0% of serotype 2a strains. Although meningococcal disease occurred mostly in children under the age of 5 (9.7 cases per 100,000 children), with a peak incidence for children under 1 (20.3 cases per 100,000 children), most fatalities occurred among teenagers (12 to 19 years old). The total fatality rate was 11.5%, and serogroup C strains were responsible for 88.9% of these fatalities. Thirteen strains had a reduced susceptibility to penicillin G, and 28 strains were resistant to sulfadiazine. One strain was resistant to both rifampin and sulfadiazine and showed a reduced susceptibility to penicillin G.

Meningococcal disease is an important cause of morbidity and mortality across Canada (1). Incidence rates were generally low in the mid 1980s, but in the past few years, the number of cases of meningococcal infection, particularly infection due to serogroup C strains, has increased in Canada (1, 16, 33). By 1990, serogroup C strains belonging to enzyme electrophoretic type 15 (ET 15) were responsible for over 65% of the group C disease in the country (3).

In the province of Québec, various clusters of serogroup C meningococcal disease have been reported since the beginning of 1991 (7, 10, 13, 25). Between 24 December 1991 and 8 January 1992, seven patients died from a serogroup C *Neisseria meningitidis* infection. Because of the increased incidence of these infections and the increased fatality rate, health officials initiated mass immunization in addition to chemoprophylaxis of close contacts for those regions (Laurentides, l'Outaouais, and Lanaudière) most affected (16). Given the extensive use of chemoprophylaxis and the confirmation of our first rifampin- and sulfadiazine-resistant strain of *N. meningitidis* during 1991, the Laboratoire de Santé Publique du Québec (LSPQ) decided to establish a more active surveillance and to determine retrospectively the antimicrobial susceptibilities of all *N. meningitidis* strains isolated in the province since 1991.

On 2 December 1992, the immunization program was extended to all Québec regions for residents aged from 6 months to 20 years, inclusively. We report here the characterization of *N. meningitidis* strains isolated during the period 1991 to 1992.

MATERIALS AND METHODS

Bacterial strains. A total of 234 strains of *N. meningitidis* (1 strain per patient from 68 hospitals) isolated from hospitalized patients living in the province of

Québec were sent to the LSPQ between January 1991 and December 1992 for confirmation and further characterization. All these strains were recovered from sterile body fluids, except for one strain that was isolated postmortem from a cutaneous lesion. The identity of the strains was confirmed by standard bacteriological methods. Gram stains, oxidase tests, tests for growth on modified Thayer-Martin medium and nutrient and blood agar, and tests for reduction of nitrate and nitrite and for production of polysaccharide from sucrose were performed in accordance with the methods described by Morello et al. (22). Acid production from carbohydrates was studied by using the modified oxidation-fermentation medium developed by Knapp and Holmes (15), and gamma-glutamyl-transferase activity was detected according to the method of Riou et al. (28). Strains were kept frozen at -60°C in tryptic soy broth containing 10% glycerol (vol/vol) for further analysis.

Serogrouping. Serogrouping was performed by slide agglutination according to the procedure described by Ashton et al. (4), except that cells were suspended in formalinized saline at a concentration of 5.0% instead of 0.5%; this modification was introduced in order to obtain a suspension of killed organisms in less than 30 min without altering the quality of the results. Strains were tested with specific antisera against the seven major capsular serogroups of *N. meningitidis*.

Serotyping and subtyping. Serotyping and subtyping with monoclonal antibodies were carried out with whole-cell meningococci as coating antigens and urease-conjugated rabbit anti-mouse immunoglobulin G (IgG) (Sigma Chemical Co., St. Louis, Mo.) in solid-phase enzyme-linked immunosorbent assays as described previously (2, 3). For preparation of antigens, organisms were grown on GC medium (13a) for 24 h at 36°C in 5 to 7% CO_2 . Monoclonal antibodies with specificities against serotypes 1 (MN3C6B), 2a (MN2D3F), 2b (MN2C3B), 4 (MN14G21.17), 14 (MN5C8C), and 15 (MN15A14H6) and subtypes P1.1 (MN14C2.3), P1.2 (MN16C13F4), P1.4 (MN20B34), P1.6 (MN19D6.13), P1.7 (MN14C11.6), P1.9 (MN5A10F), P1.10 (MN20F4.17), P1.12 (MN20A7.10), P1.14 (MN21G3.17), P1.15 (MN3C5C), and P1.16 (MN5C11G) were obtained from J. T. Poolman from the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Electrophoretic enzyme typing. Isolates were grown on GC medium for 18 h in the presence of 5% CO_2 and extracted as described previously (3). Electrophoretic analysis of enzymes and subsequent staining procedures were carried out as described by Selander et al. (31). Starch was purchased from Connaught Laboratories Ltd., Willowdale, Ontario, Canada. The following enzymes were assayed: malic enzyme, glucose-6-phosphate dehydrogenase, peptidase, isocitrate dehydrogenase, aconitase, NADP-linked glutamate dehydrogenase, NAD-linked glutamate dehydrogenase, fumarase, alkaline phosphatase, indophenol oxidase, adenylate kinase, phosphoglucosmutase, and glutamic oxaloacetic transaminase. Electromorphs (alloenzymes) of each enzyme were numbered in order of decreasing anodal mobility and were equated with alleles at the corresponding structural gene locus. Distinctive combinations of alleles over the 13-enzyme loci were designated ETs. ET designations are identical to those described previously by Ashton et al. (3).

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TABLE 1. Serogroup distribution according to isolation site

Serogroup	No. of strains/isolation site			No. of strains (%)
	Blood	CSF ^a	Other	
A	1	1	0	2 (0.9)
B	35	29	0	64 (27.4)
C	84	73	6	163 (69.7)
Y	2	1	0	3 (1.3)
W ₁₃₅	2	0	0	2 (0.9)
Total	124	104	6	234

^a CSF, cerebrospinal fluid.

Antimicrobial agents. The following antimicrobial agents were used: ceftriaxone (0.016 to 64 µg/ml), ciprofloxacin (0.002 to 4 µg/ml), penicillin G (0.032 to 16 µg/ml), rifampin (0.008 to 128 µg/ml), and sulfadiazine (1, 5, and 10 µg/ml). Antimicrobial powders were obtained from Sigma Chemical Co., except for penicillin, which was obtained from GIBCO Laboratories, Grand Island, N.Y.

Antimicrobial susceptibility testing. Susceptibility testing was performed by the agar dilution method in accordance with the procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (23, 24). Bacterial suspensions were prepared in Mueller-Hinton (MH) broth from an overnight culture on heart infusion sheep blood agar. A final inoculum of 10⁴ CFU was delivered onto the surface of MH agar medium (BBL Microbiology Systems, Cockeysville, Md.) by using a Cathra replicator. MH medium was supplemented with 5% lysed horse blood for rifampin and sulfadiazine testing. *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Staphylococcus aureus* ATCC 29213; *Enterococcus faecalis* ATCC 29212; and two clinical isolates, *N. meningitidis* LSPQ 3415 (resistant to rifampin and sulfadiazine, with a reduced susceptibility to penicillin G) and *N. meningitidis* LSPQ 3416 (susceptible to all antibiotics tested), were used as control strains. Plates were incubated in a 5 to 7% CO₂ atmosphere at 35°C for 24 h. For quality control purposes, NCCLS-recommended control strains were also incubated under aerobic conditions. Analyses were performed in triplicate.

The MIC was defined as the lowest concentration of antibiotic that completely inhibited growth (23), except for sulfadiazine, for which an obvious 80 to 90% diminution of growth was interpreted as the endpoint (29). As suggested by Morello et al. (22), an isolate was defined as susceptible to penicillin G, sulfadiazine, and rifampin when MICs were ≤0.06, ≤10, and ≤0.25 µg/ml, respectively. For ceftriaxone and ciprofloxacin, results were interpreted according to the NCCLS criteria for microorganisms other than *Haemophilus* spp. and *Neisseria gonorrhoeae* (24).

Statistics. Statistical analysis of the data was performed by the chi-square test (30). Population figures were obtained from the Québec Ministry of Health and Social Services (21).

RESULTS

Most strains were isolated from blood or cerebrospinal fluid (97.4%). One strain was isolated postmortem from a cutaneous lesion, and five were isolated from articular fluid. Serogroup distribution according to isolation site is summarized in Table 1. For both years, serogroup C was the most prevalent serogroup (76.0% in 1991 and 65.2% in 1992), followed by serogroup B (20.8% in 1991 and 31.9% in 1992). Overall, 69.7% of strains belonged to serogroup C.

Serotype, subtype, and ET profile distribution for serogroup C strains is shown in Table 2. Of the 162 serogroup C isolates which were further characterized (one isolate did not grow upon subculture), 130 belonged to serotype 2a (80.3%), 14 belonged to serotype 2b (8.6%), and 18 were nontypeable (11.1%). Epitope P1.2 was the major subtype associated with serotype 2a isolates. ET 15 accounted for 76.5% (124 of 162) of group C isolates and 90% (117 of 130) of serotype 2a strains. A total of 121 C:2a:P1.2 or P1.- isolates (including 5 C:NT:P1.2) belonged to this clone. These isolates were responsible for 84.5% (49 of 58 cases) and 93.5% (72 of 77 cases) of C:2a disease for 1991 and 1992, respectively, and represented 68.1 and 80.0% of group C disease for those same years.

Serogroup distribution according to age group is presented in Table 3. Similar age group distribution patterns were

TABLE 2. Characterization of 162 serogroup C strains

Serotype	Subtype	ET	No. of strains (%)	
			1991	1992
2a	P1.- ^a	ET 15	5 (6.9)	16 (17.8)
	P1.-	ET 21	0	1 (1.1)
	P1.2	ET 1	0	1 (1.1)
	P1.2	ET 5	1 (1.4)	0
	P1.2	ET 9	1 (1.4)	0
	P1.2	ET 15	42 (58.3)	53 (58.9)
	P1.2	ET 16	0	2 (2.2)
	P1.2	ET 18	0	1 (1.1)
	P1.2	ET 21	5 (6.9)	0
	P1.2	non-ET 15	1 (1.4)	0
	P1.6	ET 15	1 (1.4)	0
2b	P1.-	ND ^b	1 (1.4)	1 (1.1)
	P1.2	ND	7 (9.7)	5 (5.6)
NT ^c	P1.-	ET 15	0	2 (2.2)
	P1.-	non-ET 15	0	2 (2.2)
	P1.2	ET 5	0	1 (1.1)
	P1.2	ET 9	2 (2.8)	0
	P1.2	ET 15	2 (2.8)	3 (3.3)
	P1.2	ET 21	4 (5.6)	0
	P1.9	ND	0	1 (1.1)
	P1.2	non-ET 15	0	1 (1.1)
Total			72	90

^a P1.-, nonreactive with subtyping antibodies.^b ND, not determined.^c NT, nontypeable.

observed for serogroup B and C strains for both years. Meningococcal disease occurred mostly in children under 5 years of age (36.8%), and a higher average annual incidence rate was observed among this age group (Fig. 1), with 9.7 cases per 100,000 children under 5 compared to 1.7 per 100,000 for the entire population. Among these children, serogroup C and B strains were respectively responsible for 51.2 and 45.4% of infections. However, serogroup C was significantly more prevalent in children between the ages of 1 and 5 years, while children under 1 year of age were more likely to be infected by a serogroup B strain ($P < 0.05$). In fact, serogroup C strains were responsible for 70.2% of infections in children between the ages of 1 and 5 years, while serogroup B strains were responsible for 71.8% of infections occurring in children under the age of 1. A high proportion (26.5%) of meningococcal disease was also observed among teenagers (12 to 19 years old), with 88.7% of these infections caused by serogroup C strains. Overall, the median ages for patients infected with serogroup B and C strains were 2 and 13 years, respectively.

Table 4 summarizes the characterization of fatality-related strains. Meningococcal infection occurred in 124 female patients and 110 male patients. The total fatality rate was 11.5%, with 17 fatalities among women and 10 among men. Twenty-four of these deaths (88.9%) were caused by serogroup C strains, 20 of which (83.3%) belonged to ET 15. Most fatalities occurred among teenagers (33.3%) and children under the age of 5 years (25.9%).

Results of antimicrobial susceptibility testing for penicillin G, sulfadiazine, and rifampin are shown in Fig. 2. Six strains were unable to grow on MH medium. Thirteen strains (5.7%) had a reduced susceptibility to penicillin G (MIC, 0.12 to 0.25 µg/ml); nine of these strains belonged to serogroup C, three belonged to serogroup B, and one belonged to serogroup Y.

TABLE 3. Serogroup distribution according to age group

Age group (yrs)	No. of cases caused by serogroup(s):						Total no. of cases	
	B		C		A, Y, and W ₁₃₅		1991	1992
	1991	1992	1991	1992	1991	1992		
<1	7	21 (2) ^a	4 (1)	7			11 (1)	28 (2)
1<5	5	6	12 (2)	21 (2)	1 W ₁₃₅	1 A, 1 Y	18 (2)	29 (2)
5<12	0	1	10	12 (1)			10	13 (1)
12<19	3	3	26 (4)	29 (5)	1 Y		30 (4)	32 (5)
19<30	1	6	8 (2)	11 (1)			9 (2)	17 (1)
30<40	2	2	3 (2)	5 (1)			5 (2)	7 (1)
40<50	0	2 (1)	4	2			4	4 (1)
50<60	0	1	3 (1)	1		1 A	3 (1)	3
≥60	2	2	3 (2)	2	1 W ₁₃₅	1 Y	6 (2)	5
Total	20	44 (3)	73 (14)	90 (10)	3	4	96 (14)	138 (13)

^a Numbers in parentheses indicate fatalities included in the number of cases.

Sulfadiazine resistance (MIC > 10 µg/ml) was observed in 9 serogroup B isolates and 19 serogroup C isolates, including all strains belonging to ETs 1, 5, 9, and 21 ($n = 16$). None of the ET 15 isolates showed any resistance to this antibiotic. Only one strain was resistant to rifampin (MIC, 128 µg/ml). This strain was also resistant to sulfadiazine and presented a reduced susceptibility to penicillin G. All strains were susceptible to ceftriaxone and ciprofloxacin (MIC ≤ 0.016 µg/ml).

DISCUSSION

Meningococcal infections are notifiable in all Canadian provinces and territories, and surveillance data are collected nationally by the Laboratory Centre for Disease Control in Ottawa. In the province of Québec, the LSPQ maintains the provincial database for all notifiable diseases and is responsible for the confirmation and serogrouping of *N. meningitidis* strains isolated in this province.

According to the computerized surveillance data, 158 meningococcal infections were reported in 1991, an incidence of 2.3 cases per 100,000 people. For 1992, the incidence increased to 2.4 per 100,000, with 162 reported cases. In all, 291 of these 320 cases were laboratory confirmed; the remaining 29 cases reported were based on clinical diagnosis only. Isolates of *N.*

meningitidis were forwarded to the LSPQ in 234 of 291 (80.4%) instances, yielding an annualized incidence of 1.7 per 100,000.

Serogroup distribution of strains received at the LSPQ did not differ significantly from that obtained with all laboratory-confirmed cases reported in the surveillance system ($P > 0.05$). Among the 234 isolates received at the LSPQ during the period 1991 to 1992, serogroups C and B were responsible for 69.7 and 27.4% of infections, respectively. Similar rates of 69.6 and 26.7% were obtained from the serogroup data available for laboratory-confirmed cases reported in the notifiable disease surveillance system. Further data analysis is based on the 234 strains received at the LSPQ.

Since 1986, there has been a significant increase in the detection of serogroup C strains in Canada (34). In fact, while serogroup C isolates accounted for 25.8% of meningococcal infections reported across the country during the 5-year period from 1983 to 1987 (34), this percentage reached 63% by 1990 (8). In our study also, serogroup C is the most prevalent serogroup. ET 15 accounts for 89.6% (121 of 135 cases) of C:2a:P1.2 disease and for 76.5% (124 of 162) of group C isolates. This situation is similar to the one reported for the province of Ontario in 1990, when ET 15 strains accounted for 85.1% of serotype 2a disease strains and for 74.0% of all serogroup C strains isolated in that province (3).

The most distinguishing feature of the ET 15 isolates is the

FIG. 1. Incidence of serogroup B and C meningococcal disease by age group during the period 1991 to 1992.

TABLE 4. Characterization of *N. meningitidis* strains isolated in fatality cases

Serogroup	Serotype	Subtype	ET	No. of strains (%)	
				1991	1992
C	2a	P1.2	ET 15	7 (50.0)	7 (53.8)
		P1.- ^a	ET 15	3 (21.4)	1 (7.7)
		P1.2	ET 21	1 (7.1)	0
	2b	P1.2	ND ^b	1 (7.1)	0
		P1.2	ET 15	0	2 (15.4)
		P1.2	ET 21	2 (14.3)	0
B	4	P1.-	ND	0	1 (7.7)
	NT	P1.15	ND	0	1 (7.7)
		P1.-	ND	0	1 (7.7)
Total				14	13

^a P1.-, nonreactive with subtyping antibodies.

^b ND, not determined.

^c NT, nontypeable.

FIG. 2. Antimicrobial susceptibilities of 228 strains of *N. meningitidis* isolated during the period 1991 to 1992. Asterisks indicate susceptible strains.

presence for fumarase of an allele variant (FUM-2) which is rarely found in serogroup C isolates causing disease globally (3). According to Wang et al. (35), our ET 15 clone is part of the ET 37 complex, although it differs by at least one enzyme locus from all the ETs included in that study.

As observed previously in Canada (1, 34), meningococcal disease occurred mostly in children under the age of 5 years, with a peak incidence of 20.3 per 100,000 observed among children under 1 year of age. The disappearance of transplacental maternal IgG and the low level of natural antibodies developed during the first year of life (20) probably account for the higher incidence of meningococcal disease observed in this latter group.

Only in infants under 1 year of age was the incidence of group B disease significantly greater than that of group C disease ($P < 0.05$). This could hypothetically be explained by the combination of (i) a lesser humoral immunity to group B than to group C through transplacental and/or naturally acquired antibodies and (ii) a greater exposure to group B than to group C.

(i) **A lesser humoral immunity to group B than to group C through transplacental and/or naturally acquired antibodies.** It has been established that only IgM can be produced against the group B capsule. However, such antibodies are rarely found in human sera because of our probable tolerance to the α 2,8 sialic acid homopolymer of the group B capsule, which structurally and immunologically resembles sialic acid epitopes of human fetal and neonatal brain tissues (9). Even when induced, IgM antibodies are short-lived, show low avidity at 37°C, are poorly bactericidal in the presence of human

complement, and are never passed on transplacentally (9, 12). The only immunity passed onto the fetus by the mother is therefore type specific (12). However, levels of maternal IgG decline rapidly (20), and the period of time required for the infant to achieve autonomous type-specific immunity to a variety of group B subcapsular antigens may be greater than that required to acquire immunity to other meningococcal serogroups, consequently putting the child at greater risk of contracting group B disease during this extended period of time. In fact, of the 13 different type-subtype combinations responsible for group B disease in Québec during the period 1991 to 1992, 10 have been encountered in infants. Finally, cross-reactive antibodies induced by the carriage of bacteria (such as nonencapsulated *N. meningitidis* and *Neisseria lactamica*), thought to be responsible for the development of natural immunity, are found at a low level during the first year of life (10, 11, 20). Should a seroprevalence study with infants support this hypothesis, determination of the source and the nature of the antibodies could be most helpful to the understanding of the natural history of the disease.

(ii) **A greater exposure to group B than to group C.** It has been suggested that strains responsible for outbreaks or epidemics may be more virulent and are rarely found among asymptomatic carriers (17, 18). Assuming that the number of group B carriers in the population is greater than that of group C carriers, as found in two studies (17, 18) conducted in Ontario (Québec's neighboring province), one might speculate that a greater risk of exposure to group B carriers combined with a low level of group B-specific antibodies developed during the first year of life may explain the predilection of serogroup B strains for these infants.

On the other hand, the high level of social activity among teenagers (12 to 19 years old) and the day care environment for most children between the ages of 1 and 5 years may explain why these two age groups were most affected by the group C disease following the introduction in 1988 of the new ET 15 clone (3) in a population with a low prevalence of natural antibodies against this strain.

The average annual incidence of meningococcal infection is 1.6 cases per 100,000 for males compared with 1.8 per 100,000 for females, with no difference in age- and sex-specific incidence. This observation differs from the situation previously described by Varughese and Carter (34), who found that females in all age groups under 20 had a lower attack rate than males. On the other hand, fatal cases occurred more frequently among female than among male subjects, with a 1.7:1.0 ratio.

There seems to be an increasing number of reports of *N. meningitidis* strains with a reduced susceptibility to penicillin G in Canada (6, 14, 26). In the present study, 13 such strains were found, none of which produced beta-lactamase with the nitrocefin disk. As observed by others (5, 19), these strains do not seem to arise from a single clone. Effectively, apart from the three strains related to the outbreak described by Perron and Laferrière (25), the strains belonged to serogroup B, C, or Y and had different serotypes, subtypes, and ET profiles. Finally, the MIC for one strain was 0.25 μg/ml. The context in which this strain was isolated has been described previously (27).

Sulfadiazine resistance was observed in 12.3% of the strains we studied. One of these strains was also resistant to rifampin and showed a reduced susceptibility to penicillin G. This strain (defined as the *N. meningitidis* LSPQ 3415 control strain) was also isolated during the outbreak reported by Perron and Laferrière (25), where all isolates ($n = 4$) were found to be resistant to sulfadiazine and were characterized as C:NT:P1.2:ET 21. Because of the ongoing pattern of sulfadiazine resistance in Canada (32), we agree with the recommendation

of the Advisory Committee on Epidemiology to the effect that drugs of the sulfa group should not be used as first-line prophylaxis unless the strain is known to be sensitive (1).

Laboratory results were also consistent with and supportive of the epidemiologic information provided by local health officials for two other clusters of cases that have been described previously (7, 13). All strains isolated during these two outbreaks were characterized as C:2a:P1.2:ET 15. All clusters were restricted in location, and the number of cases in each cluster was limited ($n < 5$).

Considering the widespread use of chemoprophylaxis and the mass immunization campaign initiated in the province of Québec during the period 1991 to 1992, it will be of the utmost importance for public health considerations to closely monitor serogroup distribution and antimicrobial resistance of *N. meningitidis* strains isolated in this province during the next few years. The occurrence of antimicrobial resistance also strengthens the need for the development of specific NCCLS susceptibility criteria for *N. meningitidis*.

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