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Neisseria meningitidis with Decreased Susceptibility to Penicillin in Saskatchewan, Canada

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Moderately penicillin-resistant *Neisseria meningitidis* is rare in North America. We report an outbreak of meningococcal disease in Saskatoon, Saskatchewan, Canada, with serogroup C *N. meningitidis*. The MICs of penicillin ranged from 0.12 to 0.25 μg/ml, and all isolates showing decreased susceptibility had identical genomic fingerprints when they were compared by pulsed-field gel electrophoresis. Our data indicate that *N. meningitidis* that is moderately resistant to penicillin is prevalent in Saskatchewan, Canada.

Neisseria meningitidis with decreased susceptibility to penicillin has been reported in several parts of the world, including North America. Such isolates have been described for Spain (15), the United Kingdom (21), South Africa (4), Romania (6), Sweden (23), Canada (14), and the United States (9). The MICs of penicillin that are associated with fully susceptible strains are <0.05 μg/ml, while the MICs for strains with decreased susceptibility are between 0.1 and 1.28 μg/ml. Serogroup distribution of isolates showing decreased susceptibility to penicillin includes group B, group C, and nongroupable isolates; however, serogroup C and nongroupable isolates are about three times more common among strains showing decreased susceptibility to penicillin (17).

Previously, Ashton et al. (2) reported the emergence and spread of a virulent clone of *N. meningitidis* in Canada. The clone was serogroup C, serotype 2a, and enzyme electrophoretic type 15 and was responsible for an initial outbreak in Ontario, Canada (7) and subsequently in other Canadian provinces. In 1993, this clone was recognized as the cause of a local outbreak of group C disease in Saskatchewan, Canada (3). Susceptibility testing measuring the MICs of penicillin revealed that the majority of isolates recovered in Saskatoon showed decreased susceptibility to penicillin, while isolates from outside this geographic region but in the province of Saskatchewan did not (3). Isolates demonstrating decreased susceptibility were of a common subclone or genetic variant of ET15, as determined by pulsed-field gel electrophoresis (PFGE). Contact tracing revealed no common feature linking the cases of patients infected with *N. meningitidis* serogroup C that showed decreased susceptibility to penicillin. Our data indicate that this microorganism is prevalent in Saskatoon, Saskatchewan, Canada and that it was responsible for an outbreak of meningococcal disease.

MATERIALS AND METHODS

Susceptibility testing. All isolates of *N. meningitidis* were tested for MICs to penicillin by using the Microscan (Baxter, Sacramento, Calif.) MIC plus gram-positive MIC panel. Testing was performed according to the manufacturer's instructions, and interpretation was in accordance with MIC interpretative standards in the National Committee for Clinical Laboratory Standards publication M7-A2 (13). Briefly, 4 to 5 large or 5 to 10 small well-isolated colonies from 18- to 24-h noninhibitory agar plates were transferred to 3 ml of inoculum water with a wooden applicator stick. The final concentration was equivalent to a McFarland barium sulfate turbidity standard of 0.5. Following vortexing, 0.1 ml (100 μl) of the standard suspension was added to 25 ml of inoculum water with pluronic-D. Following mixing, 0.1 ml of this final suspension was added to each well of the MIC plate, and the plates were incubated for 18 to 24 h at 35°C. The plates were read manually, and the MIC was defined as the lowest concentration showing no growth. For some isolates, MICs were determined by using the E test (AB Biodisk, Solna, Sweden), with testing performed according to the manufacturer's instructions. E test values were equivalent to those from the MIC plates.

Serotyping and subtyping. Typing was carried out with whole-cell meningococci as coating antigens in solid-phase enzyme-linked immunosorbent assays. Antigens were prepared as described by Abdillahi and Poolman (1). Monoclonal antibodies with serotype specificities 1, 2a, 2b, 2c, 4, 14, and 15 and subtype specificities P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15, and P1.16 were used to type strains with rabbit anti-mouse immunoglobulin G conjugated to urease (Sigma Chemical Co.) as described previously (2). The monoclonal antibodies were kindly supplied by J. T. Poolman, National Institute of Public Health and Environment, Bilthoven, The Netherlands, and W. D. Zollinger, Walter Reed Army Institute of Research, Washington, D.C.

Preparation of enzyme extracts. Isolates were grown on GC medium (11) for 18 h at 36°C in the presence of 5% CO_2. The growth for one petri dish (150 by 15 mm) was harvested and suspended in 2.5 ml of 10 mM Tris–1 mM EDTA–0.5 mM NADP (pH 6.8) with 1.0 ml of powdered glass beads (75 to 100 μm; G-2381; Sigma). Each mixture was kept in an ice bath and removed immediately prior to vortexing. The mixture was vortexed vigorously for 3 min and centrifuged (4°C), and the supernatant was filtered through a Millex-GV filter (0.22-μm pore size; Millipore). The filtrates were dispensed into aliquots of 0.5 ml and stored at −70°C.

Electrophoretic enzyme typing. Electrophoretic analysis of enzymes and subsequent staining procedures were carried out as described by Selander et al. (19) and Caugant et al. (5). 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, phosphoglucomutase, and glutamic oxaloacetic transaminase.

Electromorphs 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 electrophoretic types (ETs). ET designations are the same as those described previously (2) and are independent of those described by Caugant et al. (5).

PFGE. Meningococci were grown on GC medium (11) for 18 h at 36°C in the presence of 5% CO_2. High-molecular-weight DNA was extracted from each strain and digested with either of the restriction endonucleases *Bgl*II, *Not*I, or *Spe*I overnight at 37°C in a water bath (20, 22). Digested genomic DNA was separated through a 1% agarose gel at 200 V for 24 h, with total pulse times ranging from 5 to 30 s, by a contour-changed homogeneous electric field system (Pulsaphor Plus; Pharmacia LKB, Uppsala, Sweden). PFGE profiles were assigned letters on the basis of examination of fingerprints generated by the three enzymes.

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TABLE 1. Demographic information for patients with *N. meningitidis* with decreased susceptibility to penicillin and PFGE profiles of strains

Age (yr)	Sex[a]	Residence	Culture	Mo and yr	MIC (μg/ml)[b]	PFGE profile
1	F	Saskatoon	Native	January 1993	0.25	A
4	F	Saskatoon	Native	January 1993	0.25	A
8 mo	M	Saskatoon	Native	January 1993	0.25	A
1	M	Saskatoon	Native	January 1993	0.12	A
2[c]	F	Saskatoon	Native	April 1993	0.25	A
6 mo	M	Saskatoon	Native	May 1993	0.25	A
5	M	Saskatoon	Native	June 1993	0.25	A
27	F	Saskatoon	Nonnative	September 1993	0.25	A
5	M	Saskatoon	Nonnative	September 1993	0.12	A
37	F	Vanscoy (south of Saskatoon)	Nonnative	November 1993	0.12	A
9 mo	F	Mosquito Reserve (northwest of Saskatoon)	Native	February 1994	0.25	A

[a] F, female; M, male.
[b] All isolates were serogroup C and ET15.
[c] Father was nasopharyngeal positive (MIC, 0.25 μg/ml).

RESULTS

Demographic information on patients infected with *N. meningitidis* showing decreased susceptibility to penicillin is presented in Table 1. All patients were from Saskatoon and the surrounding area. Field investigation revealed that only 3 of the 11 patients were possibly epidemiologically linked. Five patients were male, and six patients were female. Nine of the patients were 5 years of age or younger. Cases were detected from January 1993 to February 1994; however, 7 of 11 cases were detected in the first six months of 1993. All isolates were serogroup C, ET 15. The MICs of penicillin ranged from 0.12 to 0.25 μg/ml. A nasopharyngeal specimen collected from the father of one patient was positive for *N. meningitidis* serogroup C, with a MIC of penicillin of 0.25 μg/ml. This isolate was unavailable for further typing. All resistant isolates had identical genomic fingerprints when either *Bgl*II, *Not*I, or *Spe*I was used as the restriction enzyme.

Table 2 shows demographic information for two patients from Saskatoon who were infected with *N. meningitidis* that was fully susceptible to penicillin. Both patients were female, and one was of native ancestry. Both were infected with *N. meningitidis* serogroup C, ET15, and the MICs were <0.03 μg/ml. A distinct genomic fingerprint was obtained for each strain after PFGE regardless of the restriction enzyme used.

Table 3 shows demographic information for patients from other geographical areas of Saskatchewan. Six of the eight patients were infected with serogroup C, ET 15 *N. meningitidis*. All patients were female, and ages ranged from 2 to 18 years. Isolates were recovered from January to October 1993, and six of eight isolates were recovered between July and October. The MICs were <0.03 μg/ml. Five distinct genomic fingerprints were found among the five isolates examined by PFGE.

DISCUSSION

N. meningitidis showing decreased susceptibility to penicillin is being reported with greater frequency worldwide (3, 4, 8–10, 14, 16, 21). Moderate resistance to penicillin in *N. meningitidis* was described from Spain in 1985 (16), and at that time, 0.4% of all meningococcal isolates were showing this trend. By 1990, more than 40% of all meningococcal isolates recovered in Spain were showing decreased susceptibility to penicillin (18). The MICs of penicillin ranged from 0.1 to 0.8 μg/ml. Subsequent reports have also come from Great Britain (21) and South Africa (4). In a further report by Jones and Sutcliff (10), 3% of meningococcal infections in Britain were due to strains that were moderately resistant to penicillin (MICs ≥0.16 μg/ml; the MICs for two isolates were 0.64 and 1.28 μg/ml, respectively). Both serogroup B and C meningococcal isolates with decreased susceptibility to penicillin were found; however, group C isolates were three times more common among isolates showing decreased susceptibility. Characterization of the mechanism of reduced susceptibility showed that only four isolates were producing the enzyme β-lactamase, while the remaining isolates were found to have altered penicillin-binding proteins (12, 17). Hughes et al. (8) found that the MICs for 90% of 102 meningococcal strains from Iowa and Illinois that were tested by the E test were 0.094 μg/ml. Their data and ours and those of Riley et al. (14) and Jackson et al. (9) demonstrate a need to further characterize the epidemiology of meningococcal susceptibility to penicillin in North America. Prior to this finding, the MICs for all isolates were less than or equal to 0.03 μg/ml. At this time, we believe that the mechanism of reduced susceptibility to penicillin in our isolates is altered penicillin-binding proteins, since β-lactamase activity was not detected in any of the strains recovered.

Ashton et al. (2) described the emergence and spread of a virulent clone of *N. meningitidis* (serotype C, 2a) that was associated with meningococcal group C disease in Canada. This strain was associated with an outbreak of meningococcal disease in Ontario (7) and subsequently in other regions of Canada. Saskatchewan was the last province in Canada to experience an outbreak of group C disease due to ET15 meningococci. The uniqueness of this outbreak is associated with the fact that the causative organisms showed decreased susceptibility to penicillin. PFGE of isolates in the Saskatoon (outbreak) area revealed the existence of identical genomic fingerprints, in contrast to different genomic fingerprints which

TABLE 2. Demographic information of patients with *N. meningitidis* fully sensitive to penicillin

Age (yr)	Sex	Residence	Culture	Mo (1993)	MIC (μg/ml)[a]	PFGE profile
6	Female	Saskatoon	Nonnative	June	<0.03	B
8	Female	Saskatoon	Native	July	<0.03	C

[a] All isolates were serogroup C and ET15.

TABLE 3. Demographic information for nonresident patients with *N. meningitidis* infection

Age (yr)	Sex	Residence	Mo	MIC (μg/ml)[a]	PFGE profile
8	Female	Black Lake	May	<0.03	ND[b]
2	Female	Hatchett Lake	August	<0.03	D
6	Female	Hatchett Lake	September	<0.03	E
18	Female	Neudorf	August	<0.03	F
2	Female	Regina	September	<0.03	ND
17	Female	Balgonie	October	<0.03	G
9	Female	Sandy Lake	January	<0.03	H
15	Female	Wadena	July	<0.03	ND

[a] Non-ET15 isolates.
[b] ND, not determined.

were exhibited by isolates associated with sporadic cases in the province. Thus, the outbreak was caused by the emergence within ET15 of a subclone or genetic variant which has developed decreased susceptibility to penicillin. This observation, along with the findings of others (8, 9, 14), demonstrates the need for comprehensive monitoring of the development of resistance to penicillin and other antibiotics by meningococci. Since the original outbreak, we have continued to isolate meningococcal strains showing decreased susceptibility to penicillin (data not shown), indicating that meningococci that are moderately resistant to penicillin remain in this geographical area.

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