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Genomic Fingerprinting of *Neisseria meningitidis* Associated with Group C Meningococcal Disease in Canada

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A single electrophoretic type (ET15) of *Neisseria meningitidis* has been associated with an increased incidence of group C meningococcal disease in Canada. Genomic fingerprinting through pulsed-field gel electrophoresis of chromosomal DNA was used to characterize the clonal relationship among meningococcal isolates of different electrophoretic types and among isolates within ET15. The genomic fingerprints of the ET15 isolates, while similar as a group, were sufficiently distinct to confirm linkage for four pairs of strains from focal outbreaks and differed markedly from those of the other common electrophoretic types, ET5, ET9, and ET21.

The incidence of *Neisseria meningitidis* group C meningococcal disease has been increasing in Canada over several years (2, 6, 8). As part of the routine surveillance of disease-associated isolates, multilocus enzyme electrophoresis is used to monitor clonal spread and it is clear that a single clonal complex, in which the majority of disease isolates belong to a single electrophoretic type (ET15), is responsible for most of the group C disease in Canada (2). Furthermore, the emergence of the ET15 complex has been accompanied by a series of clustered outbreaks occurring in different geographical areas (2, 6). Discrimination among ET15 isolates by multilocus enzyme electrophoresis is difficult, however, abrogating further delineation of focal outbreaks or clusters of cases. As part of the present study, genomic fingerprinting through pulsed-field gel electrophoresis of chromosomal DNA was used to define clonal relationships among isolates from a recent outbreak in the National Capital region of Canada.

The *N. meningitidis* serogroup C isolates were cultured from the cerebrospinal fluid and blood of patients with meningococcal disease from across Canada between March 1991 and February 1992 (Table 1). Case reports for several of the strains have been described, and isolates 6, 7, 8, 10, and 11 correspond to patients 5, 1, 2, 6, and 7, respectively (5). Strains were grown overnight on GC agar at 37°C in 5% CO_2 (6a). Conventional serotyping and subtyping (1) were used in conjunction with multilocus enzyme electrophoresis (4) to characterize each isolate, and distinctive combinations of alleles over the various enzyme loci were designated electrophoretic types. As summarized in Table 1, the majority of isolates were serotype 2a, subtype P1.2; 13 were designated ET15, 2 were designated ET21, and 1 each was designated ET5 and ET9 according to an established electrophoretic typing scheme (2). These electrophoretic types differ at only 2 of 13 loci examined and are representative of a single clonal complex (2). To investigate the possible linkage of each isolate with a focal outbreak of meningococcal disease, and therefore further resolve the clonal relationship within the 17 strains, pulsed-field gel electrophoresis of chromosomal DNA was utilized.

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High-molecular-weight DNA from the growth of a single plate for each strain was extracted in agarose plugs by conventional methods and was digested with restriction endonucleases to generate a genomic fingerprint (7). In preliminary work, 18 restriction endonucleases, including *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Kpn*I, *Nar*I, *Nhe*I, *Not*I, *Pst*I, *Pvu*I, *Sfi*I, *Sma*I, *Spe*I, *Xba*I, and *Xho*I, were evaluated for their ability to generate genomic fingerprints of suitable complexity for analysis by pulsed-field gel electrophoresis (data not shown). Most of these proved inappropriate, since they are frequent cutters for *N. meningitidis* and each yielded more than 40 DNA fragments all under 100 kbp in size. Five endonucleases are relatively rare cutters; *Nhe*I and *Sfi*I each yielded ~10 DNA fragments, whereas *Bgl*II, *Not*I, and *Spe*I each yielded ~25 DNA fragments. Similar cutting frequencies have been described for serogroup A strains, confirming the general rarity of these recognition sites in the meningococcal genome (3). In addition, the genomic fingerprints of the latter strains were stable over at least eight passages in culture (3) and were not significantly affected by the relatively minor genetic rearrangements which commonly occur during subculture of these strains, for example, at the *pil* or *opa* loci.

Three endonucleases, *Bgl*II, *Not*I, and *Spe*I, were used to generate genomic fingerprints for each of the 17 isolates. All three endonucleases produced concordant fingerprints among these isolates, suggesting that the results are not adversely affected by site-specific DNA methylation. However, the discriminatory ability of the three endonucleases is somewhat strain dependent, with the genomic fingerprints generated by *Spe*I being less informative than those generated by *Bgl*II or *Not*I for the 17 isolates used in this study, and data are presented for the latter two enzymes only (Fig. 1). Initially, ET15 isolates with suspected outbreak linkage were examined. Isolates 6 through 11 were cultured from patients in the National Capital region of Canada within a 4-week period. Isolates 7 and 8 represent cultures from teenagers attending the same high school, and isolates 10 and 11 were cultured from patients with suspected common social contacts (5). In each case, the respective pairs of genomic fingerprints are identical for *Bgl*II and *Not*I (Fig. 1) and clearly confirm the suspected linkage of disease outbreak. Isolates 6 and 9 were cultured from patients with no

TABLE 1. *N. meningitidis* serogroup C isolates used in this study

Isolate(s)	Electrophoretic type	Serotype: subtype	Canadian region and date isolated	Outcome of patient(s)
1	15	2a:P1.2	Calgary, Alberta, December 1991	Died
2	15	NT:P1.2	Prince Edward Island, December 1991	Died
3	15	2a:P1.2	Prince Edward Island, January 1992	Survived
4, 5	15	2a:P1.2	Toronto, Ontario, February 1992	Both survived
6	15	NT:P1.-	Ottawa, Ontario, December 1991	Died
7, 8	15	2a:P1.2	Ottawa, Ontario, December 1991	Both died
9	15	NT:P1.2	Hull, Quebec, January 1992	Died
10, 11	15	2a:P1.2	Ottawa, Ontario, January 1992	Survived, died
12, 13	15	2a:P1.-	Quebec, January 1992	Died, survived
14	5	2a:P1.2	Kamloops, British Columbia, December 1991	Died
15	9	2a:P1.2	Trail, British Columbia, December 1991	Survived
16	21	NT:P1.2	Boucherville, Quebec, March 1991	Died
17	21	2a:P1.2	Knowlton, Quebec, December 1991	Died

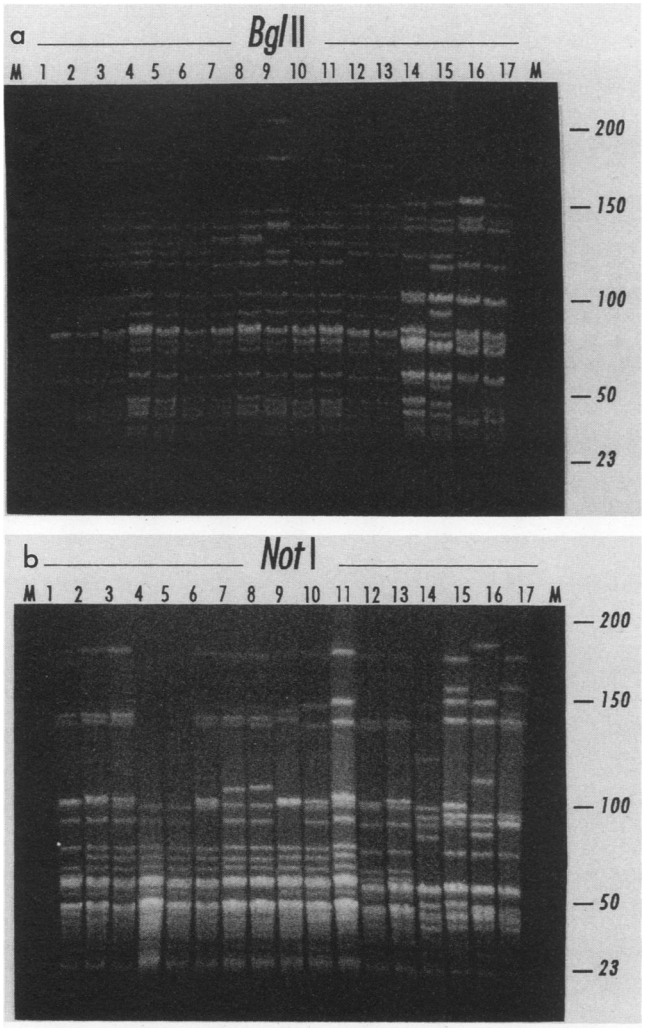

FIG. 1. Genomic fingerprints of *N. meningitidis* serogroup C isolates. Genomic DNA was digested with either *Bgl*II (a) or *Not*I (b), as indicated, and separated through a 1% agarose gel at 200 V for 24 h with total pulse times ramped from 5 to 30 s by a contour-clamped homogeneous electric field system (Pulsaphor Plus; Pharmacia LKB, Uppsala, Sweden). The isolates are indicated along the top of the figure. Lane M contains a size standard and is a mixture of λ *Hin*dIII and λ concatamers (Gibco/BRL), with the molecular sizes indicated in kilobase pairs down the side of the figure.

traceable social contacts to the previous four (5), and in each case the respective genomic fingerprints differ significantly for both endonucleases (Fig. 1), effectively ruling out linkage. Similar examples confirming linkage within other clusters of cases are illustrated by isolates 4 and 5, which were cultured from two children who attended a common day-care facility, and isolates 12 and 13, which were cultured from an adolescent and an adult with proven social contacts.

All of the ET15 isolates exhibit very similar genomic fingerprints regardless of the fact that some were cultured from patients living in geographically distant regions. For example, isolates 2 and 3 were cultured from patients living in a common region with no suspected outbreak linkage, yet their respective genomic fingerprints differ by only a single band for *Bgl*II and *Not*I (Fig. 1, ca. 50 and 100 kbp, respectively; two faint bands at 170 kbp are partial digests). A similar relationship exists between isolate 1 and isolates 12 and 13 (Fig. 1, ca. 175 and 100 kbp, respectively). Furthermore, the genomic fingerprints of ET15 isolates differ markedly from those of other common electrophoretic types (ET5, ET9, and ET21), as exemplified by isolates 14 through 17, respectively (Fig. 1).

Considered with the electrophoretic typing data, genomic fingerprinting confirms that the majority of group C disease in Canada can be traced to a discrete clonal population. Electrophoretic typing cannot effectively further resolve the ET15 complex, whereas genomic fingerprinting clearly demonstrates the presence of different subclones. The identification of such subclones should enhance epidemiological tracing of focal outbreaks or related clusters of cases. Finally, because their genomic fingerprints are distinct from those of the other clonal populations, these data strongly suggest a genetic basis for the increased spread of the ET15 complex through the general population.

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