Analysis of phenotypic variants of the serogroup C ET-15 clone of *Neisseria meningitidis* by pulsed-field gel electrophoresis.

In Canada, two waves of hyperendemic meningococcal diseases were documented in the last 20 years. Both were due to a unique clone of serogroup C *N. meningitidis* designated by MLEE as ET-15 (1). When this clone first appeared, it was characterized by the antigenic formula C:2a:P1.5,2 (1). In 2001, antigenic variants of this ET-15 clone characterized as C:2a:P1.7,1 (2) or C:2a:P1.5 (3) emerged to cause outbreaks (4). In this study, we examined whether PFGE can be used as a discriminatory tool to differentiate between the antigenic variants C:2a:P1.7,1, C:2a:P1.5, and C:2a:P1.5,2 of the ET-15 clone of *N. meningitidis*.

Eleven serogroup C ET-15 *N. meningitidis* isolates from invasive meningococcal disease (IMD) cases were selected for this study. Ten of the eleven serogroup C isolates were identified as serotype 2a (1). DNA sequencing of the *porB* gene of the nontypeable isolate identified it as a serotype 2a mutant containing a previously described mutational hotspot (10). There were four different combinations of serosubtype antigens observed for these eleven isolates: five with the P1.5 antigen, three with the P1.7,1 antigens, two with the P1.5,2 antigens, and one with the P1.2 antigen. The *porA* genes of these strains were sequenced and their PorA VRs were summarized in Figure 1.

MLST was performed according to the established method by Maiden et al. (12) and isolates were assigned sequence types (STs) according to the Neisseria MLST
website (http://pubmlst.org/neisseria/). An additional region in the *fumC* gene was amplified to determine the presence of a G to A point mutation at position 360, characteristic of ET-15 strains (11). All eleven isolates contained this particular point mutation and were therefore classified as ET-15 strains.

PFGE analysis of the eleven isolates that represented the three antigenic variants, C:2a:P1.2,5, C:2a:P1.7,1 and C:2a:P1.5, was performed, as described by Tyler and Tsang (23). Restriction enzyme-digestion of genomic DNA with *NheI* (data not shown) and *SpeI* indicated that serogroup C ET-15 variants had overall similarity and were difficult to distinguish based on the banding patterns they exhibited (Figure 1). Using *SpeI*, PFGE pattern I was unique to isolates with the serosubtype P1.5 antigen. However, pattern II was common to all three ET-15 antigenic variants, while pattern III was common to C:2a:P1.2,5 and C:2a:P1.7,1 isolates, and pattern IV was common to C:2a:P1.2,5 and C:2a:P1.5 isolates.

Despite the widespread acceptance of the PFGE method (13, 24), the data presented in this study, showing an apparent lack of correlation between isolates’ DNA fingerprints and their antigenic profiles, serves to illustrate a potential limitation of PFGE in the analysis of *N. meningitidis* strains for molecular epidemiology studies of IMD. Nevertheless, for localized outbreak analysis, PFGE is still a very useful tool to identify strains linked to a common source (15, 20). In summary, a number of typing tools, including both phenotypic and genotypic methods, should be used in combination with
carefully documented epidemiological information for surveillance and analysis of meningococcal disease.

(480 words)

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References


Figure 1. Eleven PFGE profiles of SpeI-digested DNA (using Dice coefficient with a position tolerance 1.5% of and optimization of 2.0% on BioNumerics 3.5 software) representing the three Neisseria meningitidis serogroup C ET-15 antigenic variants C:2a:P1.5,2, C:2a:P1.7,1, and C:2a:P1.5.
The phenotype of this strain was C:NT:P1.2; antigens in brackets were deduced by DNA sequencing of the serotype and serosubtype antigen genes. This serotype 2a strain has a single non-synonymous point mutation that led to the non-serotypeable phenotype (2).

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