Random Amplified Polymorphic DNA Assay as a Rapid Tool in Screening for Neisseria meningitidis Serogroup C Isolates of Electrophoretic Type 24

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Neisseria meningitidis serogroup C (NMSC) isolates of electrophoretic type 24 (ET-24), as identified by multilocus enzyme electrophoresis, are the main cause of serogroup C meningococcal disease outbreaks and sporadic meningococcal disease in the United States. We evaluated a random amplified polymorphic DNA assay as a rapid tool to screen for isolates of ET-24 by testing 199 NMSC isolates of 51 different ETs. A sensitivity of 88% and a specificity of 87% was achieved in identification of ET-24 isolates when the patterns obtained by two primers, P1 and P5, were analyzed together.

Multilocus enzyme electrophoresis (MEE) is currently considered the “gold standard” for molecular subtyping of meningococci. By MEE, electrophoretic type 24 (ET-24) that belongs to the ET-37 complex, a virulent clone predominantly composed of Neisseria meningitidis serogroup C (NMSC) that contains over 50 different ETs, has been identified as the main cause of serogroup C meningococcal disease outbreaks, as well as being among the most common causes of sporadic meningococcal disease in the United States. Since MEE is labor-intensive, time-consuming, and difficult for interlaboratory comparison, very few laboratories worldwide currently maintain this expertise. New approaches, such as multilocus sequence typing, have been successfully used in population genetic analyses, but their applicability in epidemiologic investigations of meningococcal disease outbreaks and in active laboratory-based surveillance activities remain to be evaluated. Recent data suggest that random amplified polymorphic DNA (RAPD) assay can provide results similar to those obtained by MEE for analysis of N. meningitidis serogroup A (1) and might have distinguished outbreak-associated from sporadic N. meningitidis serogroup C isolates in a university outbreak (5). In this study, we specifically focused on the ability of RAPD assay to rapidly screen for isolates of ET-24 and to differentiate them from NMSC isolates of other ETs, both within and outside of the ET-37 complex.

All isolates were collected through active laboratory-based surveillance as part of the Emerging Infections Program, coordinated by Centers for Disease Control and Prevention. Between 1992 and 1998, 454 NMSC isolates were collected, and 199 (44%) were analyzed by MEE at the time of collection. We identified 51 distinct ETs; 77 isolates were ET-24, 38 were ET-27, 18 were ET-17, and the remaining 66 isolates were of 48 different ETs. This study comprehensively includes all isolates that were analyzed by MEE. N. meningitidis isolates from blood stocks were grown on sheep blood agar plates at 37°C overnight in a 5% CO2 atmosphere. A single colony from each isolate was streaked onto a sheep blood agar plate and incubated for 20 h in a 5% CO2 atmosphere. One loopful of bacterial growth from a 20-h plate was harvested with a 1-μl disposable loop, suspended in 300 μl of 10 mM Tris HCl (pH 8.0), incubated at 95°C for 20 min, and centrifuged at 6,000 × g for 5 min. Supernatant from each isolate was aliquoted and stored at −20°C. Before RAPD analysis was performed, isolate aliquots were diluted 1:10 with 10 mM Tris HCl (pH 8.0). Primers used for the RAPD assay were P1 (5′-GG TCGGGGAA-3′) and P5 (5′-AACCGGCAC-3′) from the commercially available Ready-to-Go RAPD Analysis Kit (Pharmacia Biotech, Piscataway, N.J.). The PCR mixture contained 25 pmol of primer, 200 μM concentrations of each of the nucleotides dATP, dCTP, dGTP, and dTTP (Clontech, Palo Alto, Calif.), 2.5 μl of 10X Advantage cDNA reaction buffer (Clontech), 1 U of Advantage cDNA polymerase mix (Clontech), and 1 μl (5 to 50 ng) of DNA in a final reaction volume of 25 μl. PCR cycling conditions consisted of 95°C for 1 min and 35 cycles at 94°C for 15 s, 36°C for 30 s, and 72°C for 3 min. Subsequently, 10 μl of each amplified product was electrophoresed on 0.7% SeaKem GTG agarose gel (FMC bioproducts, Rockland, Md.) with 0.9% Synergel (Diversified Biotech, Boston, Mass.) in 1× Tris-acetate buffer for 4 h at 100 V. Gels were stained with 0.4 mg of ethidium bromide per ml. RAPD patterns were visualized by UV illumination, and images were captured using a Gel Doc System (Bio-Rad, Hercules, Calif.). RAPD pattern designations were assigned by visual inspection. A difference of one band was the basis for recognition of individual RAPD pattern designations, using only bands of strong intensity and consistency. To ensure the reproducibility of the method within our laboratory, a 1.5-kb DNA ladder (Promega, Madison, Wis.) and four standard isolates with characteristic patterns were run on each of the 18 test gels when analysis was done with primer 1 and primer 5 as.

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follows: *N. meningitidis* M1951, P1-II, P5-II, and ET-24; *N. meningitidis* M0033, P1-II, P5-VII, and ET-24; *N. meningitidis* M0636, P1-III, P5-III, and ET-17; and *N. meningitidis* M1541, P1-III, P5-III, and ET-17. No deviation in band number and intensity was observed in any of the 144 lanes with both primers. Furthermore, only gels that contained distinctly clear images of bands were included in the analysis. MEE was performed as described previously testing for 24 enzymes (4). Numbers were assigned to enzyme alleles on the basis of enzyme mobilities, and each unique set of alleles was defined as an ET. An index of genetic relatedness was determined by weighting the degree of diversity at each of the 24 enzyme loci, and similarities among the ETs were assessed by dendrogram analysis (2).

Among the 199 NMSC isolates assayed by using primer P1, 19 RAPD patterns were identified. The 77 ET-24 isolates had four different RAPD patterns (Fig. 1a and 2). Pattern P1-II was seen in 74 isolates (96%), while the 3 remaining isolates had three different P1 patterns. A total of 122 isolates of 50 other ETs had 15 P1 patterns. Pattern P1-II, characteristic for 96% of the ET-24 isolates, was shared by 22 of these 122 isolates (18%); 16 of these were different by only a single enzyme from ET-24. Among the 45 isolates in which ETs were different by two enzymes from ET-24, only four had pattern P1-II, while the remaining 41 isolates had five different P1 patterns. When the same 199 isolates were assayed with the primer P5, 20 different patterns were identified (Fig. 1b and 2). The 77 ET-24 isolates had six different RAPD patterns; 62 (81%) had RAPD pattern P5-II, 7 (9%) had RAPD pattern P5-VII, and four other P5 patterns were seen in the 8 remaining isolates. Among the 122 isolates of 50 other ETs, 16 P5 patterns were observed. Pattern P5-II, characteristic for the 81% of the ET-24 isolates, was also identified in 15 of these 122 isolates, and pattern P5-VII was identified in four isolates. The remaining 103 isolates had 14 different patterns. Of these 122 non-ET-24 isolates, 20 were only different by one enzyme from ET-24; 11 had pattern P5-II, and 4 had pattern P5-VII. Among the 45 non-ET-24 isolates whose ETs were different by two enzymes from ET-24, only 3 had pattern P5-II. When analyzed individually, P1 was able to correctly identify 96% of the ET-24 isolates resulting in a sensitivity of 96% (a 95% confidence interval of 89 to 99%) and a specificity of 82% (a 95% confidence interval of 74 to 88%). A positive predictive value (PPV) of 77% and a negative predictive value (NPV) of 97% were obtained. P5 was able to correctly identify 90% of the ET-24 isolates, providing a sensitivity of 90% (a 95% confidence interval of 81 to 95%) and a specificity of 84% (a 95% confidence interval of 77 to 90%). Similarly to the P1, a PPV of 78% and an NPV of 93% were obtained. A sensitivity of 88%, with a 95% confidence interval of 79 to 95%, and specificity of 87%,
FIG. 2. Dendrogram showing the genetic relatedness of 51 electrophoretic types of 199 N. meningitidis serogroup C isolates collected in the United States through active laboratory-based surveillance (1992 to 1998) and the RAPD pattern distribution. P1, RAPD primer 1; P5, RAPD primer 5; RAPD patterns types, P1-II, P5-II, and P5-VII; Other, other RAPD patterns types; Δ, number of enzymes by which an ET is different from ET-24.
with a 95% confidence interval of 79 to 92%, were obtained when analysis was done combining the P1 and P5 patterns (PPV of 81% and NPV of 92%). The addition of isolates with ETs different by one enzyme from ET-24 lowered the sensitivity to 82% but increased the specificity to 96%. Further addition of isolates that differed by two enzymes from ET-24 substantially lowered the sensitivity to 58% as only three of such 45 isolates had P1 and P5 patterns typically seen in ET-24 isolates. Thus, RAPD analysis is useful for rapid identification of ET-24 and closely related ETs (a single-enzyme difference). Furthermore, RAPD analysis also clearly differentiated ET-24 isolates from those of ET-17 and ET-27, two other major ETs within the ET-37 complex (Fig. 2). Moreover, only 1 of 22 isolates outside of the ET-37 complex produced the pattern (P1-II) typical for ET-24 isolates. Thus, RAPD analysis is useful for rapid identification of ET-24 and closely related ETs (a single-enzyme difference).

Given the technical disadvantages of using MEE, the current gold standard, including the fact that it still takes almost a week for the assay to be completed, we evaluated the role that RAPD analysis might have as a rapid and reliable tool for identifying NMSC isolates of ET-24. Isolates of ET-24 are the main cause of serogroup C meningococcal disease outbreaks and sporadic meningococcal disease in the United States. Management of meningococcal outbreaks differs from that of sporadic cases, since it frequently involves mass vaccination campaigns, and rapid identification of outbreak-associated isolates can be a significant aid in identifying an outbreak and its extent. Recently, we analyzed isolates from four well-described NMSC outbreaks that occurred in the United States from 1993 to 1995 by several molecular subtyping methods (3). Two of these outbreaks were associated with NMSC isolates of ET-24. With a single exception, all 43 ET-24 isolates, both those outbreak-associated and those identified as being from sporadic cases but collected during the outbreak investigations, were correctly identified as such using primers P1 and P5 (sensitivity of 98%) (3). Just like MEE, RAPD analysis does not allow for strict differentiation between outbreak-associated and sporadic isolates, but by being inclusive rather than exclusive it provides support in defining potential association of an isolate with the outbreak. Among our sporadic isolates collected though the active laboratory surveillance, a sensitivity of 88% and a specificity of 87% in the identification of NMSC ET-24 isolates were observed. Analysis of both outbreak-associated and sporadic isolates suggests that RAPD analysis could be very useful for rapid screening of NMSC isolates to identify those belonging to ET-24. Information obtained in such a rapid manner could be of initial assistance to public health officials in outbreak investigations and in monitoring of NMSC isolates for changes in their population structure that may have epidemiologic relevance.

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