Epidemiological typing of *Moraxella catarrhalis* by pulsed field gel electrophoresis

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SM DAVISON, DE LOW, RH CRUZ, D BEAULIEU, SR SCRIVER. Epidemiological typing of *Moraxella catarrhalis* by pulsed field gel electrophoresis. Can J Infect Dis 1995;6(3):141-144. Pulsed field gel electrophoresis (PFGE) was used to compare 59 strains of *Moraxella catarrhalis* to evaluate PFGE for the epidemiological typing of this organism. PFGE-generated patterns were compared with those obtained by small fragment restriction enzyme analysis (REA) and species-specific probe hybridization. The strains used in the study were isolated from various geographic locations and included proven epidemiologically related strains. PFGE yielded more unique patterns than DNA-DNA hybridization – 30 versus 18, respectively – but fewer than REA, which generated 45 unique patterns. Strains that demonstrated the same REA pattern or DNA-DNA hybridization pattern did not always demonstrate the same PFGE pattern. For example, in 23 epidemiologically unrelated strains that shared six REA patterns, PFGE differentiated the isolates into 12 patterns. Conversely, strains that demonstrated the same PFGE pattern did not always demonstrate the same REA pattern or hybridization pattern. For example, in 42 strains that shared 13 PFGE patterns, REA differentiated the isolates into 31 patterns and DNA-DNA hybridization differentiated them into 16 patterns. However, compared with REA, PFGE yielded less complex patterns that were more easily comparable, and compared with DNA-DNA hybridization, PFGE was technically easier.

Key Words: Gene probing, *Moraxella catarrhalis*, Pulsed field gel electrophoresis, Restriction enzyme analysis

Typage épidémiologique de *Moraxella catarrhalis* par électrophorèse en champ pulsé

RÉSUMÉ : L'électrophorèse en champ pulsé (ECP) a été utilisée afin de comparer 59 souches de *Moraxella catarrhalis* en vue de déterminer si l'ECP pouvait être utilisée pour le typage épidémiologique de cet organisme pathogène. Les modèles générés par ECP ont été comparés à ceux qu'avaient donné une analyse des enzymes de restriction de petits fragments (AER) et une hybridation par sonde spécifique à l'espèce. Les souches utilisées dans l'étude ont été isolées à partir de différents sites géographiques et comprenaient des souches épidémiologiquement apparentées. L'ECP a généré des modèles plus uniques que l'hybridation DNA-DNA, soit 30 contre 18, respectivement, mais moins que l'AER, qui a généré 45 modèles uniques. Les souches dont le modèle était semblable à l'AER ne se sont pas toujours exprimé semblablement à l'ECP. Par exemple, des 23 souches indépendantes sur le plan épidémiologique manifestant six modèles

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différents à l'AER, l'ECP a plutôt identifié 12 modèles. En revanche, les souches qui démontraient le même modèle à l'ECP n'ont pas toujours donné les mêmes résultats à l'AER ou à l'hybridation. Par exemple, des 42 souches qui se partageaient 13 modèles à l'ECP, l'AER a différencié les isolats entre 31 modèles, et l'hybridation DNA-DNA les a différenciés en 16 modèles. Toutefois, en comparaison avec l'AER, l'ECP a généré des modèles moins complexes, qui étaient plus facilement comparables et en comparaison à l'hybridation, elle s'est révélée plus facile à réaliser sur le plan technique.

MORAXELLA CATARRHALIS IS PART OF THE NORMAL ORAL AND nasopharyngeal flora and is a potential pathogen. The clinical importance of infection is compounded by the possible nosocomial transmission of this organism. A simple and reliable technique for the epidemiological typing of isolates is therefore necessary for defining outbreaks. Previously, typing of *M catarrhalis* was attempted via biochemical characterization, serotyping, electrophoretic typing of esterases and characterization of lipopolysaccharides (1-3). However, all of these phenotypic typing methods are limited in their practicality and/or discriminatory power. Conventional restriction enzyme analysis (REA), alone or in conjunction with DNA hybridization, have been used successfully to genotype *M catarrhalis* (4,5). However, these techniques are limited in the complexity of the patterns generated (REA) or by the required technical expertise (DNA probe analysis). Pulsed field gel electrophoresis (PFGE), which uses low frequency cleavage restriction enzymes to generate five to 15 well separated bands, has been used successfully for the typing of a wide variety of organisms including *Enterococcus faecium*, group B streptococcus and others (6,7). However, for genetically restricted subsets of clinically significant isolates such as methicillin-resistant *Staphylococcus aureus* or *Haemophilus influenzae* type b, strains with no apparent epidemiological connection may have the same PFGE pattern (8,9). In this study we evaluated PFGE for the epidemiological typing of *M catarrhalis*.

MATERIALS AND METHODS

Fifty-nine isolates of *M catarrhalis* from diverse geographic locations were used in this study. Twelve of these strains were used previously for an evaluation of REA by small fragment gel electrophoresis, and all were used in a previous study to evaluate DNA-DNA hybridization for the epidemiological typing of *M catarrhalis* (4,10,11). The 59 strains included 40 epidemiologically unrelated strains obtained from diverse geographic areas (Hôpital Laval, Québec [16 strains]; Sweden and Great Britain [two strains each]; Texas [three strains]; several centres across Canada [17 strains]) and the reference strain ATCC 25240. Also included were 12 strains isolated from patients in a pediatric intensive care unit during a two-month period in 1989 at the Hospital for Sick Children in Toronto, Ontario. These isolates were initially suspected to be epidemiologically related but were subsequently shown to be genotypically unrelated (11). Seven isolates were epidemiologically related strains that had been collected during an outbreak in a Veterans Administration Hospital in New Haven, Connecticut, USA (12). Five of these seven isolates were previously found by REA to be genotypically related (12).

REA by conventional gel electrophoresis and DNA probe analysis was performed as previously described (4,10,13,14). Isolates were considered genetically related by REA if their restriction patterns were identical in those areas of the gel where the bands were clearly resolvable. A difference in a single band between two patterns was sufficient to consider two isolates to be different and therefore unrelated. Hybridization patterns were considered identical if all bands were identical. PFGE was performed for all isolates of *M catarrhalis* by a modification of the method described by Miranda *et al* (6). Briefly, *M catarrhalis* were grown overnight in 10 mL brain heart infusion broth (Difco, Michigan) at 37°C and centrifuged. The packed cells were washed twice in PIV buffer (1.0 N sodium chloride, 0.01 M Tris-HCl, pH 7.6) and suspended in 1.5 mL of the same. A total of 500 µL of the bacterial suspension was mixed with an equal volume of 1.6% low melting point agarose (Biorad) to prepare agar plugs with a final agar concentration of 0.8%. Bacteria in agar plugs were lysed in 1.5 mL of 0.1 M Tris-HCl (pH 7.6), 0.5 M EDTA, 0.5 M sodium chloride, 0.1% Sarkosyl (Sigma Chemicals, Missouri), 2.0 µg/mL mutanolysin (Sigma) and 2.0 µg/mL of lysozyme (Sigma). After lysis for 5 h at 37°C, the lysis solution was replaced with 300 µg/mL proteinase K (Boehringer Mannheim) in 0.1 M Tris-HCl (pH 7.6), 0.1 M EDTA and 1.0 M sodium chloride and incubated with gentle agitation for 18 h at 42°C. The plugs were washed three times (30 mins each at 37°C) in TE buffer (10 mM Tris-HCl, 1 mM EDTA). The agar plugs were then digested with 50 units of *Sma*I (Boehringer Mannheim) at 25°C for 18 h. After incubation, the plugs and Lambda ladder pulsed field gel molecular size marker (New England Biolabs) were loaded into the wells of a 1.0% agarose gel. Electrophoresis was performed with the contour-clamped Homogenous Electric Field apparatus (CHEF DRII, Biorad) in 0.5 x TBE at 12°C at 150 V for 24 h using ramped pulse times beginning with 5 s and ending with 120 s. Subsequently, the gels were stained with 1 µg/mL ethidium bromide and photographed. A unique PFGE pattern was defined as any PFGE pattern that varied from another PFGE pattern by one or more clearly visible bands. There was an average of three to eight clearly resolvable bands in a PFGE pattern (Figure 1).

RESULTS

Among the 59 strains, PFGE generated 30 distinct patterns. REA and DNA-DNA hybridization generated 45 and 18 patterns, respectively. Of the 59 strains, 12 strains made up four groups of two or more isolates that shared identical patterns by all three techniques. One of these groups was made up of four strains that had previously been shown to be epidemiol-

ogically linked (5). The strains in the other three groups had no obvious epidemiological link.

In the remaining 47 strains, 14 strains made up seven groups of two isolates that shared patterns generated by two of the three techniques: eight strains in four groups shared identical REA and DNA-DNA hybridization patterns; six strains in three groups shared identical PFGE and DNA-DNA hybridization patterns; and none shared identical PFGE and REA patterns.

In the remaining 33 strains, 31 strains made up 16 groups of two or more isolates that shared patterns generated by only one of the three techniques: 16 strains shared six PFGE patterns; two strains shared one REA pattern; and 25 strains shared nine DNA-DNA hybridization patterns. Only two strains were found to have completely unique patterns by all three methods.

Of the 12 strains that had been isolated from patients in a pediatric intensive care unit during a two-month period, REA revealed 12 patterns, PFGE revealed 11 patterns and DNA-DNA hybridization revealed 10 patterns. Of the seven strains that had been isolated from patients during an outbreak in a veterans hospital, REA revealed two patterns, PFGE revealed three patterns and DNA-DNA hybridization revealed two patterns.

Of the remaining 40 strains that had no epidemiological link, REA revealed 29 patterns, PFGE revealed 25 patterns and DNA-DNA hybridization revealed 14 patterns. There were two pairs of strains and one set of four strains that had identical patterns by all three techniques.

When DNA-DNA hybridization and REA results were compared, isolates with the same REA pattern showed the same DNA-DNA hybridization pattern. However, isolates with the same DNA-DNA hybridization pattern did not always share the same REA pattern. For 50 isolates that shared 11 DNA-DNA hybridization patterns, REA differentiated the strains into 39 patterns.

When PFGE and REA results were compared, isolates with the same REA did not always share the same PFGE pattern and conversely, isolates with the same PFGE did not always share the same REA pattern. For 23 isolates that shared six REA patterns, PFGE differentiated the strains into 12 PFGE patterns. However, for 42 isolates that shared 13 PFGE patterns, REA further differentiated the strains into 31 REA patterns.

The same was true for PFGE and DNA-DNA hybridization results. Specifically, for 53 isolates that shared 12 hybridization patterns, PFGE differentiated the strains into 24 PFGE patterns. For 42 isolates that shared 13 PFGE patterns, hybridization further differentiated the strains into 16 hybridization patterns.

DISCUSSION

The ideal typing system must not only be highly discriminatory but should also be technically simple and the results easy to interpret. Limited information is available about the utility of epidemiological typing techniques to characterize *M catarrhalis* because of the paucity of nosocomial outbreaks that have been investigated. Previous methods used to type *M catarrhalis* have relied upon phenotypic characteristics. However, these methods lack reproducibility because of the unpredictable expression of the characteristic being evalu-

Figure 1) Pulsed field gel electrophoresis of *Sma*I cleaved genomic DNA of eight epidemiologically unrelated strains of *Moraxella catarrhalis* from diverse geographical areas including Sweden, Great Britain, Texas and five Canadian provinces. Lane 9 shows lambda ladder pulsed field gel molecular size marker

ated (9,15). Molecular techniques have been more successful. Both small fragment REA and DNA-DNA probe hybridization with a species-specific probe have been used successfully in both outbreak and nonoutbreak *M catarrhalis* infections (4,5,11).

In this study, although REA had the highest discriminatory power, the major limitation of conventional REA is the difficulty inherent in reading and interpreting complex patterns, especially when different gels are being compared. Probe hybridization, on the other hand, generates patterns that are easy to interpret and compare. However, it was shown to have the lowest discriminatory power. Also the technical expertise and precautions required in using this system with radioisotopes will limit its widespread use until a nonradioactive probe can be shown to have the same sensitivity. PFGE for *M catarrhalis* is a relatively simple procedure that generated highly resolvable fragments in a band pattern representing the entire bacterial chromosome. PFGE-generated band patterns were easily comparable both within and between gels.

The discriminatory power of each of these molecular techniques was directly related to the extent to which the genome was evaluated for restriction fragment length polymorphism. REA, which uses restriction enzymes that cut DNA frequently, had the best discriminatory power. PFGE, which uses restriction enzymes that cut DNA infrequently, was less discriminatory but generated chromosomal restriction patterns that made comparison between strains much easier. DNA-DNA hybridization was the least discriminatory since only a few regions of the genome were being examined. In this study, strains were maximally differentiated when all three typing systems were used in conjunction. Similar results were found by Tenover et al (16) in their evaluation of methods of typing isolates of *S aureus*. However, time and cost limit the practicality of routinely using all methods during an epidemiological investigation. Since PFGE takes several days to complete

and requires expensive, specialized equipment – whereas REA can be performed overnight – the best method depends on the type of investigation. PFGE would be the recommended typing technique in an epidemiological investigation where the number of organisms is too numerous to run on a single gel. However, if the number of *M catarrhalis* to be investigated can be run on the same gel, it may be more practical to perform REA.

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