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Characterization of *Neisseria meningitidis* Serogroup C by Multilocus Enzyme Electrophoresis and Ribosomal DNA Restriction Profiles (Ribotyping)

TONI C. WOODS,[1]* LETA O. HELSEL,[1] BALASUBRAMANIAN SWAMINATHAN,[1] WILLIAM F. BIBB,[1] ROBERT W. PINNER,[1] BRUCE G. GELLIN,[1]† SHEILA F. COLLIN,[2] STEPHEN H. WATERMAN,[3]‡ MICHAEL W. REEVES,[1] DON J. BRENNER,[1] AND CLAIRE V. BROOME[1]

Meningitis and Special Pathogens Branch[1] and Biostatistics and Information Management Branch,[2] Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333, and Los Angeles County Department of Health Services, Los Angeles, California 90012[3]

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We compared multilocus enzyme electrophoresis (MEE) and ribosomal DNA fingerprinting (ribotyping) for subtyping 44 strains of *Neisseria meningitidis* serogroup C that were isolated in Los Angeles County, California, between December 1985 and July 1986. The isolates were divided into six enzyme types (ETs) by MEE, but 36 of the isolates were clustered in one ET, 3. The same isolates were divided into 17 ribotypes by use of restriction endonucleases *Cla*I, *Eco*RI, and *Xho*I. Twenty of the 36 ET 3 isolates were grouped in a single ribotype, J. The rate of infection with ribotype J strains was higher in the southern part of the study area than in the northern part. Isolates from each of eight pairs (each isolate pair was cultured from the same patient from the same or different sites) were found identical by MEE, but ribotyping revealed a difference in one pair. In this study, ribotyping showed a greater discriminating capacity than MEE for subtyping *N. meningitidis* serogroup C, but the epidemiologic relevance of this increased sensitivity needs further assessment.

Neisseria meningitidis is immunologically characterized by serogrouping, which recognizes different capsular polysaccharides; by serotyping, which recognizes class 2 or 3 outer membrane proteins; and serosubtyping, which is based on variations in class 1 outer membrane proteins (5, 8, 9). These methods can categorize most *N. meningitidis* isolates into groups of related strains. Multilocus enzyme electrophoresis (MEE), a method which identifies electrophoretic variants of certain metabolic enzymes (19), has been used to define the population structure of *N. meningitidis* and to track the spread of specific clones of this organism over distance and time (5, 6, 8, 14, 15). Caugant et al. (6, 7) used MEE to recognize a highly related group of 203 strains (largely serogroup B) (electrophoretic type [ET] 5 complex) which consisted of 22 ETs and which were responsible for epidemic meningococcal disease in Norway as well as throughout the northern European continent. Olyhoek et al. (15) combined MEE with outer membrane protein analysis to study the population structure of *N. meningitidis* serogroup A. Using strains isolated over a 68-year period, they were able to define a population structure that contained four large clonal groups containing numerous clusters of closely related ETs. They found that only a few of these ET clusters were responsible for the majority of past outbreaks of disease.

Recently, methods that make use of rRNAs as probes (ribotyping) have been used to characterize bacteria for epidemiologic and taxonomic purposes. Grimont and Grimont (10) hybridized rRNA from *Escherichia coli* to restriction endonuclease-treated DNAs of 41 different bacterial species and reported that each species could be described by a specific rRNA hybridization pattern based on Southern blot analysis. Using ribotyping, others have investigated the identification of *Haemophilus influenzae* strains transmitted from mother to neonate (21), the separation of *Mycoplasma gallisepticum* veterinary vaccine strains from wild-type field strains (25), and the characterization of *Providencia stuartii* nosocomial strains (16). Both MEE and ribotyping were included in a comprehensive study of 107 strains of *H. influenzae* biogroup aegyptius by Brenner et al., who found that both techniques could identify all strains causing Brazilian purpuric fever (4).

In this study, we compared ribotyping and MEE by applying these techniques to a subset of *N. meningitidis* serogroup C strains collected as part of an active surveillance project by the Centers for Disease Control (18). Ribotyping produced more divisions within this subset of strains than MEE, but the overall results of the two methods were similar.

MATERIALS AND METHODS

Bacterial strains. The 44 meningococcal disease isolates of *N. meningitidis* listed in Table 1 were all the strains obtained by the Centers for Disease Control in Los Angeles County, Calif., and nearby areas from December 1985 through July 1986. Thirty-five isolates (79.6%) were from Los Angeles County and represented all the strains collected during the first third of a Centers for Disease Control surveillance project period (18); 2 (4.5%) were from Orange County, and 7 (15.9%) were from Long Beach City. All isolates were serogroup C. Sixteen of the 44 isolates were paired isolates from eight patients.

MEE. Cells were harvested from plates into Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), centrifuged, and resuspended in 10 mM Tris–1 mM EDTA

* Corresponding author.
† Present address: Department of Public Health, Cornell Medical College, New York, NY 10021.
‡ Present address: Public Health Service, Community Disease Control, San Diego, CA 92101.

TABLE 1. *N. meningitidis* serogroup C isolates obtained from December 1985 to July 1986

Strain	Patient age (yr)	Patient sex[a]	Source[b]	MEE ET[c]	Banding pattern no. obtained with:			Ribotype[c]
					ClaI[c]	*XhoI*[c]	*EcoRI*[c]	
F8447	9	F	CSF	1	1	1	1	A
F7950	17	F	CSF	4	2	2	2	B
F7862	15	F	CSF	5	3	3	3	C
F8450	0.5	M	CSF	6	4	3	3	D
F8627[d]	1	M	Blood	6	4	4	3	E
F8626[d]	1	M	CSF	6	4	4	3	E
F8004[e]	3	F	Blood	2	5	5	4	F
F8045[e]	3	F	CSF	2	5	5	4	F
F8433	2	F	Blood	3	5	5	5	G
F8427	4	M	Unknown	3	5	5	6	H
F7722[f]	4	F	CSF	3	5	5	7	I
F7723[f]	4	F	Blood	3	5	5	7	I
F7928	3	F	CSF	3	5	5	7	I
F7985	2	F	CSF	3	5	5	7	I
F8398	22	F	Blood	3	5	5	7	I
F7768	2	M	CSF	3	5	5	8	J
F7845[g]	4	M	CSF	3	5	5	8	J
F7900[g]	4	M	CSF	3	5	5	8	J
F8098	12	F	Blood	3	5	5	8	J
F7929	3	M	CSF	3	5	5	8	J
F8151	2	M	CSF	3	5	5	8	J
F8178	38	M	Blood	3	5	5	8	J
F8043[h]	3	M	CSF	3	5	5	8	J
F8094[h]	3	M	Blood	3	5	5	8	J
F8093	2	F	Blood	3	5	5	8	J
F8091	6	F	Blood	3	5	5	8	J
F8248[i]	21	M	CSF	3	5	5	8	J
F8564[i]	21	M	CSF	3	5	5	8	J
F8538	9	F	CSF	3	5	5	8	J
F8448	16	F	Blood	3	5	5	8	J
F8401	4	M	CSF	3	5	5	8	J
F8610	37	M	Blood	3	5	5	8	J
F8563	3	F	CSF	3	5	5	8	J
F8649	3	M	CSF	3	5	5	8	J
F8510	1	M	CSF	3	5	5	8	J
F8283[j]	15	F	CSF	3	6	6	8	K
F8198[j]	15	F	Blood	3	5	6	8	L
F7901[k]	1	M	CSF	3	5	6	8	L
F7947[k]	1	M	CSF	3	5	6	8	L
F8520	3	F	Blood	3	5	7	8	M
F8449	3	M	Blood	3	5	8	8	N
F8217	Unknown	Unknown	Unknown	3	7	5	4	O
F8609	4	F	Blood	3	7	5	9	P
F8608	8	F	Blood	3	7	6	9	Q

[a] F, female; m, male.
[b] CSF, cerebrospinal fluid.
[c] MEE ETs, banding pattern numbers derived from probed restriction endonuclease patterns, and ribotypes are explained in Materials and Methods.
[d–k], paired letters indicate two isolates from the same patient.

(pH 6.8) containing 0.04% NADP (19), and constitutive cytoplasmic enzymes were extracted by the method of Selander et al. (19). Horizontal starch gels (11% concentration) were electrophoresed until the tracking dye (23) ran 8 cm from the origin to the anode. The 14 enzymes assayed were adenylate kinase, alcohol dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase (NAD dependent), glutamate dehydrogenase (NADP dependent), glutamic-oxaloacetic transaminase, glyceraldehyde-3-phosphate dehydrogenase (NAD dependent), indophenol oxidase, isocitrate dehydrogenase, malic enzyme, phenylalanyl-leucine peptidase, phosphoglucomutase, and 6-phosphogluconate dehydrogenase (19). The starch gel buffers, electrode buffers, and voltage used for *N. meningitidis* enzymes were described by Selander et al. (19). Relative enzyme mobilities (alleles) were numbered in order of increasing anodal migration, and each unique set of alleles was defined as an ET. Similarities among the six ETs were indicated by dendrogram analysis (19, 22). The ET numbers assigned are not related to the ET numbers used in other studies of *N. meningitidis*.

Ribotyping. DNA was extracted by a modification of the method of Maniatis et al. (13), except that before phenol extraction, sodium chloride was added to make the lysate 0.72 M NaCl (final concentration). Hexadecyltrimethylammonium bromide (1% final concentration) in 0.7 M NaCl was added, and the lysate was heated at 65°C for 10 min. The extracted material was precipitated with isopropanol, washed once in 70% ethanol, and resuspended in 10 mM Tris (pH 8.0) containing 1 mM EDTA.

N. meningitidis DNA was digested with *Eco*RI, *Cla*I, or *Xho*I in accordance with the protocol of the supplier (New England BioLabs, Beverly, Mass.). Restricted DNA was electrophoresed in Tris-acetate buffer overnight on 1% agarose gels at 1 V/cm and capillary transferred by Southern blotting (13) onto nylon filters (Micron Separations, Inc., Westboro, Mass.).

16S + 23S rRNA from *E. coli* (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was 5′ end labeled with [γ-^{32}P]ATP as described by Altwegg et al. (1). A 1-kb DNA ladder (Bethesda Research Laboratories, Gaithersburg, Md.) was nick translated with [α-^{32}P]dCTP (New England Nuclear Research Products, Wilmington, Del.) and a nick translation kit (catalog no. 8160SB; Bethesda Research Laboratories) as described by the manufacturers.

Hybridization of Southern blots to labeled rRNA was done as described by Altwegg et al. (1). Posthybridization treatment was modified at the wash step by washing the filters twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 0.1% sodium dodecyl sulfate (SDS) and then twice in 0.5× SSC with 0.1% SDS.

Restriction fragment sizes were estimated by the method of Southern (20) with a 1-kb ladder as a reference for *Eco*RI and *Xho*I digests and *Serratia fonticola* 3965 *Hin*dIII fragments as a reference for *Cla*I digests (10). Ribosomal banding patterns were confirmed by repeated runs, and strains with identical patterns for each restriction endonuclease were given the same banding pattern number. When banding pattern numbers for all three restriction endonucleases were combined, each unique group was designated a separate ribotype and given an arbitrary alphabetic character (Table 1).

A dendrogram of the ribotypes was made by determining the total number of unique bands produced by each restriction endonuclease and then assembling these as a set of bands of decreasing size. The banding patterns of each strain were compared with this total set of bands (master set), and for each band in a strain profile that matched a band in the master set, the number 1 was placed in that position. When a band was missing, the position was marked with a 0. The pattern of sequences of 1's and 0's for each strain were compared with the patterns of sequences for the other strains, and a relatedness index was calculated as the proportion of mismatches of bands. A dendrogram showing the relatedness of the ribotypes was produced by the average-linkage method of clustering from a matrix of coefficients of relatedness (22).

ET and ribotype distribution. The geographic distribution of ETs and ribotypes for 24 of the 36 patients (16 of the 44 isolates were paired isolates from eight patients) was analyzed with Fisher's exact test (2). Patient addresses were located on a map of the Los Angeles area, and the Santa Monica and Santa Ana freeways were used as a north-south dividing line.

RESULTS

Of the 14 enzymes that were evaluated by MEE, 10 showed differences in electrophoretic mobility, and these were used to subtype the isolates. The 44 *N. meningitidis* isolates were grouped into six ETs. ET 3 was the largest, containing 36 (82%) of the isolates. ETs 2 and 3 differed from each other only in the allele for glucose-6-phosphate dehydrogenase, while ETs 5 and 6 were different from each other only in the allele for phenylalanyl-leucine peptidase (Table 2). The other ETs differed from each other in five to eight enzyme alleles. ETs 5 and 6 were separated from ETs 1 through 4 by a relatedness index of 0.37 (Fig. 1A). ET 4 was unique even when the two enzyme alleles for which it showed no activity (labeled 0 in Table 2) were eliminated from the data.

TABLE 2. *N. meningitidis* serogroup C electrophoretic results from MEE

ET[a]	No. of strains/ ET[b]	Variable allele designation for[c]:									
		PLP	G6P	ME	6PG	IDH	ADH	GPD	PGM	GOT	AP
1	1	1	3	3	3	2	3	4	5	6	5
2	2	1	1	1	2	2	4	4	5	5	5
3	36	1	2	1	2	2	4	4	5	5	5
4	1	1	4	1	3	2	3	4	0	0	8
5	1	1	4	2	1	1	4	5	5	2	5
6	3	2	4	2	1	1	4	5	5	2	5

[a] Derived as explained in Materials and Methods.

[b] Strains in each ET were as follows: ET 1, F8447; ET 2, F8004 and F8045; ET 3, F7722, F7723, F7768, F7845, F7900, F7901, F7928, F7929, F7947, F7985, F8043, F8091, F8093, F8094, F8098, F8151, F8178, F8198, F8217, F8248, F8283, F8398, F8401, F8427, F8433, F8448, F8449, F8510, F8520, F8538, F8563, F8564, F8608, F8609, F8610, and F8649; ET 4, F7950; ET 5, F7862; and ET 6, F8450, F8626, and F8627.

[c] Variable enzymes: PLP, phenylalanyl-leucine peptidase; G6P, glucose-6-phosphate dehydrogenase; ME, malic enzyme; 6PG, 6-phosphogluconate dehydrogenase; IDH, isocitrate dehydrogenase; ADH, alcohol dehydrogenase; GPD, glyceraldehyde-3-phosphate dehydrogenase (NAD dependent); PGM, phosphoglucomutase; GOT, glutamic oxaloacetic transaminase; AP, alkaline phosphatase. Nonvariable enzymes glutamate dehydrogenase (NAD and NADP dependent), indophenol oxidase, and adenylate kinase are not shown.

When the 44 isolates were analyzed by ribotyping, seven different ribosomal DNA restriction profiles were obtained with *Cla*I (Fig. 2), eight were obtained with *Xho*I, and nine were obtained with *Eco*RI (Table 1). When the ribosomal DNA restriction profiles for all three restriction endonucleases were considered together, 17 different ribotypes were obtained (Table 1). Ribotype J contained 20 (46%) of the isolates. The 44 isolates tested in this study contained eight pairs from eight patients (either from the same site or different sites). When tested by MEE, each pair fell in the same ET; however, when these eight pairs were analyzed by ribotyping, one pair of isolates differed in *Cla*I restriction profiles (Table 1 and Fig. 2).

Ribotyping allowed further discrimination of the isolates in two of the six ETs (ETs 3 and 6). The 36 isolates that made up ET 3 were grouped into 11 ribotypes, G through Q. However, 20 of the 36 ET 3 isolates were indistinguishable from each other by ribotyping and were assigned to ribotype J. The three isolates in ET 6 were divided into two ribotypes, D and E (Table 1).

Analysis of the dendrograms generated from the MEE and ribotyping data revealed a general agreement between the two methods with respect to the relatedness of isolates (Fig. 1). Isolates in ETs 2 and 3 that were found closely related by MEE retained this similarity when analyzed by ribotyping, and this was also true for isolates in ETs 5 and 6. ETs 1 and 4, which were found separate from other ETs by MEE, were also found distinct by ribotyping.

To evaluate whether the division of ET 3 by ribotyping defined groups that may have had significance, we analyzed 24 ET 3 strains by geographic location. Strains isolated from patients in the southern part of the study area represented 9 of 12 strains of ribotype J and 3 of 12 strains not of ribotype J ($P < 0.04$ by a two-tailed Fisher's exact test).

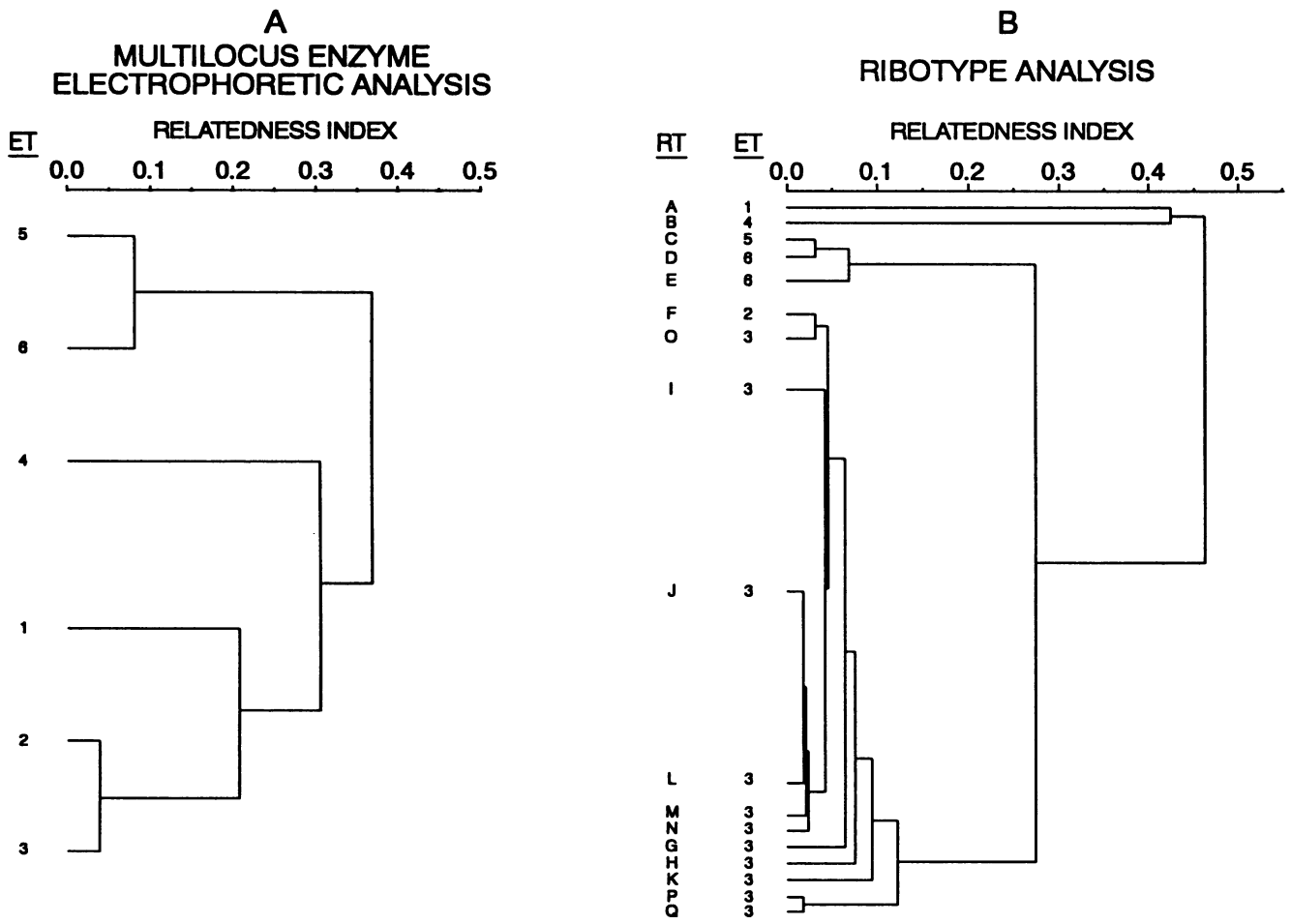

FIG. 1. Dendrograms of MEE analysis (A) and ribotype analysis (B) of *N. meningitidis* strains. Strains in each ribotype (RT) were as follows: ribotype A, F8447; ribotype B, F7950; ribotype C, F7862; ribotype D, F8450; ribotype E, F8626 and F8627; ribotype F, F8004 and F8045; ribotype G, F8433; ribotype H, F8427; ribotype I, F7722, F7723, F7928, F7985, and F8398; ribotype J, F7768, F7845, F7900, F8098, F7929, F8151, F8178, F8043, F8094, F8093, F8091, F8248, F8564, F8538, F8448, F8401, F8610, F8563, F8649, and F8510; ribotype K, F8283; ribotype L, F8198, F7901, and F7947; ribotype M, F8250; ribotype N, F8449; ribotype O, F8217; ribotype P, F8609; and ribotype Q, F8608.

DISCUSSION

The 44 serogroup C strains tested in this study were isolated from Los Angeles County and surrounding areas over an 8-month period. The MEE data on these strains indicated the presence of a large group, ET 3 (Table 2). Strains of ET 3 were also found in a 1986 six-state surveillance study of *N. meningitidis* reported by Pinner et al. (18). That study, which included a much larger number of strains, identified this ET as ET 22. It was found in 48% of all serogroup C strains; 74% of the ET 22 strains were from Los Angeles County, Calif.; 16% were from Tennessee; and 9% were from the other four states (18). Within the Los Angeles County area in recent years, this ET has been reported to be responsible for an increase in serogroup C meningococcal disease (18). Thus, the use of MEE has increased epidemiologic understanding of the prevalence of certain clones and clonal groups in human populations.

Although ribotyping and MEE are being increasingly applied in molecular epidemiology studies of bacterial diseases (3, 4, 17), very few comparative evaluations of the two methods have been made. Arthur et al. (3) compared MEE and ribotyping for assessing the genetic diversity of uropathogenic *E. coli* isolates. In their study, MEE was more sensitive to divergence than was ribotyping. However, they used only one restriction endonuclease (*Eco*RI) for ribotyping. For the isolates of *N. meningitidis* serogroup C used in this study, ribotyping with three restriction enconucleases was more sensitive than was MEE, particularly for the analysis of the isolates grouped in ET 3. The importance of using more than one restriction endonuclease to establish genetic homogeneity or heterogeneity was emphasized by Yogev et al. (24) in their work with *Mycoplasma pneumoniae*.

Ribotype J isolates were clustered in the southern part of the study area. However, the epidemiologic significance of this clustering is unclear. This point needs to be addressed in future ribotyping studies with a set of epidemiologically well-characterized isolates.

One disturbing finding was that a pair of isolates (F8283 and F8198) that were from a single patient and that were expected to be found identical were found different by ribotyping (ribotypes K and L; Table 1 and Fig. 2). Assuming that proper processing and labeling were used for these isolates in all the laboratories that handled them, it is

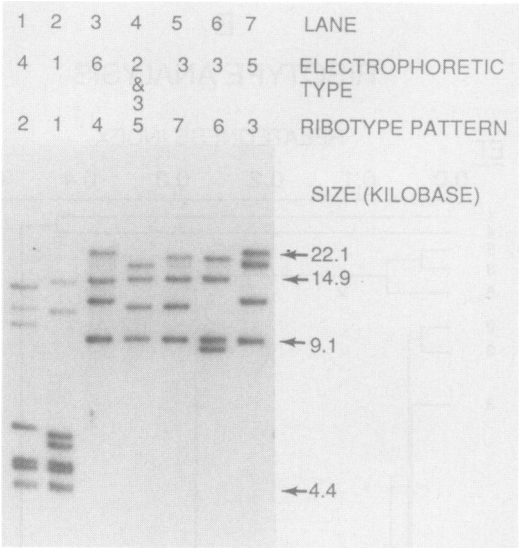

FIG. 2. *Cla*I restriction fragments of *N. meningitidis* serogroup C hybridized with ^{32}P-labeled 16S + 23S rRNA from *E. coli*. One representative strain for each pattern is shown: lane 1, F7950; lane 2, F8447; lane 3, F8450; lane 4, F7722 (F8198 is in this pattern); lane 5, F8217; lane 6, F8283; and lane 7, F7862. ETs within each ribotype pattern are indicated.

possible that this patient was simultaneously infected by two different isolates. Another possibility is that ribotyping may have discriminated on the basis of differences among cells of a single infecting isolate. A third possibility is that the difference observed by ribotyping may have been caused by a chromosomal inversion(s). Such inversions have been demonstrated to occur in the intervening sequences between *rrnD* and *rrnE* operons in *E. coli* (11). The inversion phenomenon may account for the observation of two ribotypes within the isolates making up the clone of *H. influenzae* biogroup aegyptius identified as the causative agent of Brazilian purpuric fever in Brazil (4, 12).

The finding that categorizations of isolates by both MEE and ribotyping were quantitatively similar may be of use in validating both methods as subtyping tools. Our results are in agreement with those of other investigators. Arthur et al. (3) found a good correlation between the *Eco*RI restriction endonuclease polymorphism associated with the *rrn* operons and the allelic variations in ETs detected in uropathogenic *E. coli* isolates. Picard-Pasquier et al. (17) also observed a strong correlation between ribotyping and MEE in the subtyping of *Yersinia* spp.

In conclusion, both ribotyping and MEE were useful for the subtyping of *N. meningitidis* serogroup C isolates. The additional degree of discrimination obtained by ribotyping with multiple restriction enzymes may be useful, but its epidemiologic relevance needs to be established.

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REFERENCES

1. **Altwegg, M., R. Altwegg-Bissig, A. Demarta, R. Peduzzi, M. W. Reeves, and B. Swaminathan.** 1988. Comparison of four typing methods for *Aeromonas* species. J. Diarrhoeal Dis. Res. **6**:88–94.
2. **Armitage, P., and G. Berry.** 1987. Statistical methods in medical research, p. 129–132. Blackwell Scientific Publications, London.
3. **Arthur, M., R. D. Arbeit, C. Kim, P. Beltran, H. Crowe, S. Steinbach, C. Campanelli, R. A. Wilson, R. K. Selander, and R. Goldstein.** 1990. Restriction fragment length polymorphisms among uropathogenic *Escherichia coli* isolates: *pap*-related sequences compared with *rrn* operons. Infect. Immun. **58**:471–479.
4. **Brenner, D. J., L. W. Mayer, G. M. Carlone, L. H. Harrison, W. F. Bibb, M. C. deCunto Brandileone, F. O. Sottnek, K. Irino, M. W. Reeves, J. M. Swenson, K. A. Birkness, R. S. Weyant, S. F. Berkeley, T. C. Woods, A. G. Steigerwalt, P. A. D. Grimont, R. M. McKinney, D. W. Flemming, L. L. Gheesling, R. C. Cooksey, R. J. Arko, C. V. Broome, and the Brazilian Purpuric Fever Study Group.** 1988. Biochemical, genetic, and epidemiologic characterization of *Haemophilus influenzae* biogroup aegyptius (*Haemophilus aegyptius*) strains associated with Brazilian purpuric fever. J. Clin. Microbiol. **26**:1524–1534.
5. **Caugant, D. A., K. Bøvre, P. Gaustad, K. Bryn, E. Holten, E. A. Hoiby, and L. O. Froholm.** 1986. Multilocus genotypes determined by enzyme electrophoresis of *Neisseria meningitidis* isolated from patients with systemic disease and from healthy carriers. J. Gen. Microbiol. **132**:641.
6. **Caugant, D. A., L. O. Froholm, K. Bøvre, E. Holten, C. E. Frasch, L. F. Mocca, W. D. Zollinger, and R. K. Selander.** 1986. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. Proc. Natl. Acad. Sci. USA **83**:4927–4931.
7. **Caugant, D. A., L. O. Froholm, K. Bøvre, E. Holten, C. E. Frasch, L. F. Mocca, W. D. Zollinger, and R. K. Selander.** 1987. Intercontinental spread of *Neisseria meningitidis* clones of the ET-5 complex. Antonie Leeuwenhoek **53**:389–394.
8. **Caugant, D. A., L. F. Mocca, E. E. Frasch, L. O. Froholm, W. D. Zollinger, and R. K. Selander.** 1987. Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern. J. Bacteriol. **169**:2781–2792.
9. **Frasch, C. E., W. D. Zollinger, and J. T. Poolman.** 1985. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev. Infect. Dis. **7**:504–510.
10. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur/Microbiol. **137B**:165–175.
11. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7069–7072.
12. **Irino, K., F. Grimont, I. Casin, P. A. D. Grimont, and the Brazilian Purpuric Fever Study Group.** 1988. rRNA gene restriction patterns of *Haemophilus influenzae* biogroup aegyptius strains associated with Brazilian purpuric fever. J. Clin. Microbiol. **26**:1535–1538.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Moore, P. S., M. W. Reeves, B. Schwartz, B. G. Gellin, and C. V. Broome.** 1989. Intercontinental spread of an epidemic group A *Neisseria meningitidis* strain. Lancet **ii**:260–262.
15. **Olyhoek, T., B. A. Crowe, and M. Achtman.** 1987. Clonal population structure of *Neisseria meningitidis* serogroup A isolated from epidemics and pandemics between 1915 and 1983. Rev. Infect. Dis. **9**:665–692.
16. **Owen, R. J., A. Beck, P. A. Dayal, and C. Dawson.** 1988. Detection of genomic variation in *Providencia stuartii* clinical isolates by analysis of DNA restriction fragment length polymorphisms containing rRNA cistrons. J. Clin. Microbiol. **26**: 2161–2166.
17. **Picard-Pasquier, N., B. Picard, S. Heeralal, R. Krishnamoorthy, and P. Goullet.** 1990. Correlation between ribosomal DNA polymorphism and electrophoretic enzyme polymorphism in *Yersinia*. J. Gen. Microbiol. **136**:1655–1666.
18. **Pinner, R. W., B. G. Gellin, W. F. Bibb, C. N. Baker, S. H.**

Waterman, L. Moca, C. E. Frasch, and C. V. Broome. Meningococcal disease in the United States—1986. J. Infect. Dis., in press.
19. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51**:873–884.
20. **Southern, E. M.** 1979. Measurement of DNA length by gel electrophoresis. Anal. Biochem. **100**:319–323.
21. **Stull, T. L., J. J. LiPuma, and T. D. Edlind.** 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. **157**:280–286.
22. **Wishart, D.** 1978. CLUSTAN user manual 1, version 1c, release 2, 3rd ed. Program Library Unit, Edinburgh University, Edinburgh.
23. **Woods, T. C., R. M. McKinney, B. D. Plikaytis, A. G. Steigerwalt, W. F. Bibb, and D. J. Brenner.** 1988. Multilocus enzyme analysis of *Legionella dumoffii*. J. Clin. Microbiol. **26**:799–803.
24. **Yogev, D., D. Halachmi, G. E. Kenny, and S. Razin.** 1988. Distinction of species and strains of mycoplasmas (Mollicutes) by genomic DNA fingerprints with an rRNA gene probe. J. Clin. Microbiol. **26**:1198–1201.
25. **Yogev, D., S. Levisohn, S. H. Kleven, D. Halachimi, and S. Razin.** 1988. Ribosomal RNA gene probes to detect intraspecies heterogeneity in *Mycoplasma gallisepticum* and *M. synoviae*. Avian Dis. **32**:220–231.