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An Outbreak of Group B Meningococcal Disease: Tracing the Causative Strain of *Neisseria meningitidis* by DNA Fingerprinting

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Following an outbreak of meningococcal disease in three schoolchildren in a small community in northern Norway, DNA fingerprinting, serotyping with monoclonal antibodies, serogrouping, and sulfonamide sensitivity testing were applied for characterization and tracing of the causative agent. The three case isolates were genomically indistinguishable, sulfonamide-resistant, serogroup B, serotype 15 meningococci. Throat specimens were collected from 552 healthy contacts, including all children below age 17 and their parents. Among the 36 carrier isolates (carrier rate, 6.5%) 13 showed DNA fingerprints identical, or almost identical, to the index pattern. All of these 13 isolates were sulfonamide resistant, 12 were of serotype 15, and 8 were of polysaccharide serogroup B (5 were nongroupable). These closely related isolates were almost exclusively recovered from schoolchildren of 2 of 15 small villages, one of which included the homes of two of the patients. The remaining 23 carrier isolates were nonresistant, non-type 15 meningococci of widely differing DNA restriction patterns. Our results confirm that DNA fingerprinting has potential as an efficient tool in practical meningococcal epidemiology.

To understand the epidemiology of *Neisseria meningitidis* it is mandatory to study the interplay of neisserial strains in the population. Little is known about the dynamics and epidemiological consequences of this interplay, partly because of problems of exact differentiation between similar strains. In the early 1970s, the prevailing serogroup A and C meningococci almost disappeared from Norway as the new serogroup B, type 15, sulfonamide-resistant meningococci became predominant (4). Thus, most systemic meningococcal isolates appeared identical. However, following adaptation of the DNA fingerprinting technique to analysis of the meningococcal genome, we recently found that the fingerprint of B15 meningococci may show a wide range of different patterns (13), although the fingerprints of epidemiologically closely linked isolates may be identical (15).

The present investigation was undertaken to characterize isolates of meningococci and study their distribution in the healthy population during an outbreak of meningococcal disease in a small community in northern Norway. For this purpose DNA fingerprinting was applied in combination with serotyping by monoclonal antibodies, serogrouping, and sulfonamide susceptibility testing.

MATERIALS AND METHODS

Cases and bacterial isolates. During a 6-week period in 1983, three schoolmates, aged 11 to 12 years, in a small community in northern Norway were admitted to the University Hospital of Tromsø with fulminant meningococcal septicemia. The first child (patient 1) died 2 days after admission; the other two (patients 2 and 3) survived, possibly because of treatment with combined plasmapheresis and leukapheresis (2). Meningococci of serogroup B were isolated from all three patients. Immediately following the admission of patient 3, throat cultures were obtained from his parents (specimens were not obtained from the parents of patients 1 and 2). During a 5-day period shortly thereafter, throat specimens were collected from the remaining 177 schoolchildren, aged 7 to 16 years, including the 14 schoolmates of patients 2 and 3 (group 1), from 105 preschool children under 7 years of age (group 2), and from 270 parents 20 to 55 years of age (group 3). The population of the community was distributed over 15 widely dispersed settlements of single-family houses.

The throat specimens were immediately transported to the laboratory and plated on the same day on GC agar base with hemoglobin and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.), and meningococci were identified by conventional methods (18).

Serogrouping. Meningococci were primarily serogrouped by slide agglutination with commercial antisera against capsular polysaccharides of serogroups A, B, C, X, Y, Z, and W135 (Wellcome Reagents Ltd., Beckenham, United Kingdom) and a group 29E antiserum kindly provided by I. Lind, Statens Seruminstitut, Copenhagen, Denmark. Polyagglutinating isolates were screened for group-specific capsular polysaccharides by a gas chromatographic method (5, 6) and counterimmunoelectrophoresis of aqueous bacterial suspensions (19) with the antisera mentioned above. Weak reactions were interpreted as negative.

Serotyping. Serotyping was performed as described by Frøholm et al. (8) by a coagglutination method using monoclonal antisera kindly provided by W. D. Zollinger, Walter Reed Army Institute, Washington, D.C. (22). Antisera with specificity for one of the types 2a, 2b, 2c, or 15 or for the subtype P1.2 or P1.16 epitopes were used.

Sulfonamide susceptibility testing. MICs of sulfadiazine were determined in duplicate for each strain by the agar dilution method as previously described (4). The series of drug concentrations used was 1, 2, 5, 10, 20, 50, 100, 200, and 500 mg of sulfadiazine per liter. Isolates with MICs of 5 mg/liter or lower were considered sulfonamide sensitive, and

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TABLE 1. Distribution of meningococcal carrier isolates from three different age groups according to genomic similarity to the case isolates

Relationship with DNA fingerprint pattern of case isolates	No. (%) of isolates from carrier group[a]:		
	1 (n = 177)	2 (n = 105)	3 (n = 270)
Similar	12 (6.7)	0	1 (0.1)
Different	5 (2.8)	0	18 (6.7)

[a] Groups: 1, schoolchildren (7 to 16 years old); 2, preschool children (0 to 6 years old); 3, parents (20 to 55 years old).

those with values of 100 mg/liter or higher were considered resistant. Isolates with MICs between 5 and 100 mg/liter had intermediate susceptibility.

DNA fingerprinting. All strains were DNA fingerprinted as previously described (3). In short, bacteria were cultured on agar plates, harvested, and lysed by lysozyme, EDTA, and pronase; RNase was added to remove any RNA. DNA was then extracted and purified by one combined chloroform-phenol extraction followed by multiple phenol extractions, dialyzed, and then cleaved with the *Hin*dIII restriction endonuclease (Amersham Corp., Arlington Heights, Ill.). The fragments were then electrophoretically separated in a 4% polyacrylamide gel. The gel was stained with ethidium bromide, washed, and photographed in UV light, and the resulting band patterns were finally visually compared, one by one, with the DNA fingerprint shared by the three case isolates. The degree of restriction endonuclease similarity between the DNA fingerprints was calculated by the formula: degree of similarity = 100% − (Nd × 100)/Ns, where Ns is the sum of the bands of the two DNA fingerprints to be compared and Nd represents the number of bands found in only one fingerprint (14).

DNA fingerprinting and phenotypic analyses were performed in different laboratories. The code for the phenotypic traits was not broken until the isolates had been grouped by DNA restriction pattern.

RESULTS

Carrier rate. Meningococci were isolated from 17 (9.6%) schoolchildren (group 1), but not from any pupils of the same classes as the three patients. No preschool children (group 2) harbored meningococci in their throats, whereas 19 (7%) of the parents (group 3) were carriers (Table 1).

Agent characteristics. The phenotypic characteristics and genomic profile relations of the case and carrier isolates are given in Table 2. Twenty-one isolates, including the three case isolates, could be serogrouped by slide agglutination. Six carrier isolates were nongroupable (nonagglutinating) and were not further tested by gas chromatography or counterimmunoelectrophoresis. Twelve isolates were primarily polyagglutinable; of these isolates, one (K72) was found to be of serogroup B and one (K86) was of serogroup W135, the remaining isolates being nongroupable. All three case isolates (K66, K67, K68) were sulfonamide resistant and belonged to serogroup B, serotype 15. These three strains further possessed completely identical DNA restriction endonuclease patterns (Fig. 1). We identified 13 carrier isolates (K69, K71, K72, K73, K74, K75, K76, K77, K78, K95, K96, K101, K102) with a DNA fingerprint closely similar (restriction endonuclease similarity, 93 to 100%) to that of the case isolates (Table 2; Fig. 1). Of the 13 isolates, 12 reacted with the serotype 15 antibody only and one reacted with the subtype P1.16 antibody only. All 13 isolates were sulfonamide resistant. Of the 13 isolates, 8 belonged to serogroup B, whereas 5 were nongroupable, containing no or marginal amounts of capsular sialic acid detectable by gas chromatography. Of the 13 carrier isolates, 12 (92%) were from schoolchildren living in either of 2 of the 15 small villages of the community; patients 2 and 3 also lived in one of these 2 villages. Isolate 13 was collected from an adult with no known contact with the cases and who lived in another village.

The remaining 23 carrier isolates had DNA patterns easily

TABLE 2. Phenotypic and genomic characteristics of meningococcal case and carrier isolates

Source and isolate[a]	Serogroup	Serotype (subtype)	RE similarity (%)[b]	Sulfonamide susceptibility[c]
Cases				
K66	B	15:−[d]	100	R
K67	B	15:−	100	R
K68	B	15:−	100	R
Group 1 carriers				
K69	B	15:−	99	R
K70	NG[e]	NT[f]:−	58	S
K71	NG	15:−	98	R
K72	B	15:−	93	R
K73	B	15:−	95	R
K74	NG	15:−	96	R
K75	B	15:−	99	R
K76	NG	15:−	97	R
K77	NG	15:−	100	R
K78	B	15:−	100	R
K80	B	NT:P1.16	64	S
K81	NG	NT:−	72	I
K82	B	NT:−	66	I
K83	W135	NT:−	74	S
K96	B	15:−	100	R
K101	B	15:−	98	R
K102	NG	15:−	99	R
Group 3 carriers				
K85	NG	NT:P1.2	72	S
K86	W135	NT:P1.16	76	S
K87	NG	NT:−	74	S
K88	NG	NT:−	74	S
K89	Y	NT:−	75	S
K90	B	2b:P1.2	74	S
K91	C	NT:−	77	I
K92	NG	NT:−	70	S
K93	NG	NT:−	79	I
K94	NG	NT:−	65	S
K95	B	NT:P1.16	93	R
K97	NG	NT:−	73	S
K98	W135	NT:−	69	S
K99	29E	NT:−	73	S
K100	W135	NT:−	64	S
K103	B	NT:−	72	S
K104	NG	NT:−	72	S
K105	NG	NT:−	73	S
K106	29E	NT:−	71	S

[a] Group 1 carriers were schoolchildren (7 to 16 years old); group 3 carriers were parents (20 to 55 years old).

[b] Restriction endonuclease (RE) similarity was determined by comparing the DNA fingerprint of each isolate with that of a case isolate (K66) (see the text).

[c] R, Resistant; S, susceptible; I, intermediate susceptibility.

[d] −, Unknown subtype.

[e] NG, Nongroupable.

[f] NT, Nontypable.

distinguishable from the other 13 carrier isolates (Fig. 1). Their degree of similarity with the DNA fingerprint of the case isolates varied between 58 and 79% (Table 2). These 23 carrier isolates were all susceptible or of intermediate susceptibility to sulfonamide and belonged to different serogroups and serotypes. Eleven (48%) were nongroupable.

DISCUSSION

The present epidemiological setting provided a unique opportunity to study the distribution of a highly virulent meningococcal strain, or closely related meningococci, in a typical rural community. However, compared with previous epidemiological studies from this part of the world (10, 12, 20), the percentage of carriage was surprisingly low.

Restriction patterns identical or almost identical to that of the proven invasive organism were found in carrier isolates from 13 of 36 individuals. With only one exception, these carriers were schoolchildren living in either of 2 of the 15 villages of the community. At the time of investigation, neither classmates of the three patients nor the family contacts of patient 3 harbored meningococci in their throats. However, the accumulation of carriers of meningococci with an "invasive" restriction pattern in these two villages suggests that such carriage was acquired more readily out of school than at school. Interestingly, B15 isolates with clearly different restriction patterns were not found in the population of this community. In a previous study on the epidemiology of B15 meningococci (13), we showed that the DNA fingerprints of the systemic isolates were closely related, whereas most of the carrier isolates differed considerably both from this invasive pattern and from each other. It should be emphasized, however, that these carrier isolates represented different areas of northern Norway and a period of several years.

The remaining 23 carrier isolates that, by DNA fingerprinting, could be easily differentiated from the case isolates belonged to various serogroups or frequently were nongroupable. None of them reacted with the type 15 antibody, and none of them were resistant to sulfonamides. In fact, sulfonamide resistance and also the serotype 15 character were associated with the invasive restriction pattern. Similar results have previously been reported from Norway (4, 9, 11). An association between sulfonamide resistance and severity of meningococcal disease was previously proposed by Andersen (1), who, in studies of clinical material from Oslo, Norway, found that sulfonamide-resistant variants of meningococci were more frequently isolated from patients with unfavorable prognostic scores and that six of seven deaths due to meningococcal disease were caused by such isolates. Later, Bøvre et al. (4), in studies of systemic isolates from the whole country, found that sulfonamide resistance was probably associated with severity of the disease (septicemia and death).

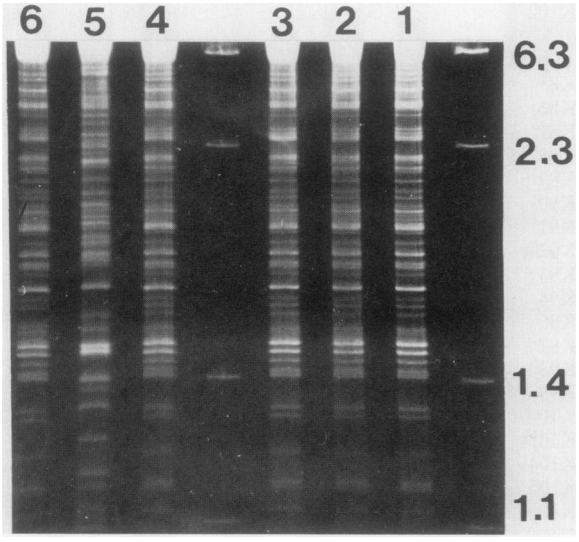

FIG. 1. DNA restriction endonuclease patterns of the three B:15 case isolates (lanes: 1, K66; 2, K67; 3, K68), one B:15 carrier isolate (lane 4, K69), and one NG:15 isolate (lane 6, K71), both of the latter with DNA fingerprints similar to that of the case strain, and of one NG:NT:− isolate (lane 5, K70) with a DNA fingerprint different from that of the case strain. All except the NG:NT:− isolate were sulfonamide resistant (Table 2). P4 DNA digested with *Hin*dIII was used as a size marker (kilobases).

In the present study there was no clear association between the invasive restriction pattern and serogroup. Thus, among the 13 genomically similar carrier isolates, 5 were nongroupable and 8 were of serogroup B. Possibly, these isolates are genetically closely related variants differing only in expression of capsular polysaccharide synthesis. The determinant DNA segment(s) may be very small and unlikely to contain recognition sites for the endonuclease. Alternatively, the expression of capsular antigen may be coded for by DNA segments that are not present in the relevant fingerprint. Changes from a capsule-positive to a capsule-negative phase in isogenic meningococci in culture (17), as well as quantitative differences in the expression of meningococcal capsular antigen, have previously been observed (5, 7).

The potential of the DNA fingerprint method in studies on bacterial epidemiology depends on the resolution ability of the gel. We used 4% polyacrylamide gels, which separate DNA fragments below 2 kilobases very well. However, bands larger than 2 kilobases are better separated on agarose gels, which, however, easily crack during washing and staining procedures. Hence, the combination of agarose and polyacrylamide gel electrophoresis methods would yield the most extensive information about DNA organization. Another methodological problem is reading the DNA fingerprints. In our study this was done by eye against an illuminated background. However, the speed of migration may vary slightly from gel to gel, hampering the comparison of different DNA patterns. Studies by us (21) show that the use of optical densitometry followed by digitization of the resulting data may be a solution to this problem.

In previous studies (3, 13, 15) we showed that the DNA fingerprinting technique permits the differentiation between meningococcal isolates that are serologically indistinguishable. The present study illustrates the potential of the DNA fingerprinting method to disclose similarities in the genetic organization of meningococcal isolates that, by conventional serological techniques, would have been considered unrelated or of uncertain relationship. However, the study also illustrates the dilemma that one faces when carrier isolates linked to the same local outbreak are almost, but not completely, identical. If it is acceptable that isolates of the same strain show minor variations in their restriction patterns, what then are the criteria for possible or probable identity versus nonidentity? This problem is well known from epidemiological and epizoological studies on herpesviruses, in which variations in the sizes of homologous restriction fragments may impair strain differentiation (16). The variation observed among the closely related 13

carrier isolates of the present material never affected more than 4 of a total of approximately 50 different bands. We are presently inclined to consider this degree of variation possible within the concept of a clone spreading in the population. We hope that further studies applying DNA fingerprinting with the use of more than one restriction endonuclease in combination with sophisticated techniques for phenotypic differentiation will clarify this issue.

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