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Clonal Diversity of *Neisseria meningitidis* from a Population of Asymptomatic Carriers

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Genetic diversity and relationships among 109 isolates of *Neisseria meningitidis* obtained from throat cultures of healthy individuals in Norway in 1984 were assessed by analyzing electrophoretically demonstrable allelic variation at 15 enzyme-encoding chromosomal genes. Seventy-eight distinctive electrophoretic types (ETs), representing multilocus genotypes, were identified. The mean genetic diversity per locus among the 78 ETs (0.538) was equivalent to that among 19 ETs represented by 66 isolates collected from patients with meningococcal disease in Norway in the first 5 months of 1984. The clonal composition of the collection of carrier strains was, however, quite different from that of strains from patients. The two groups of clones, the ET-5 complex and the ET-37 complex, that were responsible for 91% of the cases of systemic disease in Norway in 1984 were identified in only 7 and 9%, respectively, of the throat cultures from healthy individuals, and their frequencies in the human population sampled were only 0.7% for clones of the ET-5 complex and 0.9% for those of the ET-37 complex. The complex of clones that was most frequently represented by isolates from carriers (19%) has never been recovered from patients with meningococcal disease in Norway or elsewhere, which suggests that these clones have a low virulence potential. Children attending the same day care center or school seldom harbored the same clone in their throats.

Neisseria meningitidis is carried asymptomatically in the upper respiratory tract by about 15% of the human population and occasionally invades the blood stream or spinal fluid, causing septicemia and meningitis. Environmental and host factors, as well as virulence properties of certain strains, are assumed to contribute to bacterial invasion (24). Because the human nasopharynx is the only known reservoir of *N. meningitidis* and most patients with meningococcal disease have not had contact with another person with the disease, asymptomatic carriers are presumed to be the major source of transmission of pathogenic strains. For this reason, investigation of the carrier state may significantly contribute to an understanding of the epidemiology and pathogenesis of disease caused by *N. meningitidis* (4).

The major factor impeding the study of meningococcal carriage has been a lack of efficient genetic marker systems for identification and classification of strains and of methods for determining relationships among isolates. Conventional methods of typing based on immunological specificities of capsular polysaccharides (serogrouping) and outer membrane proteins (serotyping) are inadequate, because 90% of isolates from healthy carriers do not react with antisera to capsular polysaccharides and 50 to 60% do not react with antisera to outer membrane proteins (12).

A method was recently developed for classifying strains of *N. meningitidis* by the electrophoretic mobilities of their metabolic enzymes. Multilocus enzyme electrophoresis (27) provides a high-resolution system for the characterization of the chromosomal genome of all isolates and yields estimates of genetic relatedness among strains. Through the analysis of several hundred isolates from patients with meningococcal disease in various parts of the world, the extent of genetic diversity in the species has been revealed (9). The species has a clonal population structure, and only a small number of complexes of closely related clones causes most of the disease worldwide (7, 10, 11, 22, 23).

Although these studies have assessed the genetic structure of populations of strains causing disease, little is known concerning the composition of populations that colonize the throats of healthy individuals and the genetic and evolutionary relationships of these carrier organisms to those that cause disease.

An epidemic of meningococcal disease caused by sulfonamide-resistant organisms of serogroup B has occurred in Norway since 1975, resulting in about 30 fatalities per year (3). We have demonstrated that a group of closely related clones, the electrophoretic type 5 (ET-5) complex, has been largely responsible for this ongoing epidemic (6, 7). We report here an analysis, based on 15 enzyme loci, of genetic diversity and relationships among strains of *N. meningitidis* isolated from healthy carriers in Tromsø, Norway. Our study demonstrated that the group of clones responsible for almost 80% of the cases in the ongoing epidemic was represented by only 7% of the isolates recovered from healthy carriers and had a frequency of only 0.7% in the human population sampled. The complex of clones that was most frequently identified in the collection of isolates from carriers is not known to have caused disease in Norway.

MATERIALS AND METHODS

Human population sampled. Throat cultures were collected from 1,102 individuals living in Tromsø Norway (47,000 inhabitants), from May 25 to July 20 1984. The

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TABLE 1. Carriage of *N. meningitidis* in relation to population age

Age (yr) of individuals	No. of subjects	No. of carriers (%)
Up to 2	78	1 (1)
3–6	154	9 (6)
7–15	347	24 (7)
16–19	160	36 (22)
20–60	249	35 (14)
Over 60	114	4 (3)

population sampled included 78 infants (up to 2 years old) sampled mainly in the course of routine visits to health centers; 154 children (3 to 6 years old) attending day care centers; 347 school children (7 to 15 years old); 160 youths (16 to 19 years old), most of whom were attending high schools; 249 adults (20 to 60 years old) sampled during visits to physicians, in hospitals, and at workplaces; and 114 individuals older than 60 years, including 67 individuals living at a center for elderly people (B.-E. Kristiansen, K. W. Lind, K. Mevold, B. Sørensen, L. O. Frøholm, K. Bryn, T. Tjade, and K. Bøvre, submitted for publication).

Bacterial isolates. *N. meningitidis* was identified in throat cultures from 109 of the 1,102 individuals sampled (Table 1), and one colony from each carrier was preserved at −70°C and analyzed. One isolate, BT186, was atypical for a meningococcus in that it did not ferment glucose and maltose.

Electrophoresis of enzymes. Methods of protein extract preparation, starch gel electrophoresis, and enzyme staining have been described by Selander et al. (27). The 15 enzymes assayed were malic enzyme, glucose 6-phosphate dehydrogenase, peptidase, isocitrate dehydrogenase, aconitase, NADP-linked glutamate dehydrogenase, NAD-linked glutamate dehydrogenase, alcohol dehydrogenase, fumarase, alkaline phosphatase, indophenol oxidases 1 and 2, adenylate kinase, phosphoglucomutase, and glutamic oxaloacetic transaminase.

Electromorphs (allozymes) of each enzyme, numbered in order of decreasing anodal mobility, were equated with alleles at the corresponding structural gene locus. Numerical allele designations are cognate with those previously recorded for *N. meningitidis* (9), and alleles not identified in earlier surveys were assigned decimal numbers. An absence of enzyme activity was attributed to a null allele, designated 0. Distinctive combinations of alleles over the 15 enzyme loci (multilocus genotypes) were designated as ETs (8).

Serogrouping and serotyping. Serogroups were determined by slide agglutination with commercial antisera (Wellcome Reagents Ltd., Beckenham, United Kingdom) specific for the A, B, C, W135 (W), X, Y, and Z capsular polysaccharides and with serum against serogroup 29E (E), provided by I. Lind, Statens Seruminstitut, Copenhagen, Denmark. For several polyagglutinating isolates, the serogroup was determined by gas chromatography (5) and counterimmunoelectrophoresis (19). Serotyping was performed by coagglutination with monoclonal antibodies for antigens 1, 2a, 2b, 2c, 5, 6, 9, 14, 15, P1.2, P1.15, and P1.16, as described by Frøholm et al. (13).

Sulfonamide susceptibility testing. The MICs of sulfadiazine were determined as described by Bøvre et al. (2). Isolates were assigned to three categories: susceptible (MIC, 5 mg/liter or lower), intermediate (MIC, 10 to 50 mg/liter), and resistant (MIC, 100 mg/liter or higher).

Statistical analysis. The genetic diversity at an enzyme locus among ETs or isolates was calculated as $h = (1 - \Sigma x_i^2)(n/n - 1)$, where x_i is the frequency of the ith allele and n is the number of ETs or isolates. Mean genetic diversity (H) is the arithmetic average of h values over all loci. Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred (mismatches), and clustering was performed from a matrix of genetic distances by the average-linkage method (28).

RESULTS

ETs and genetic diversity. All 15 enzyme loci were polymorphic for 3 (aconitase and indophenol oxidase 1) to 11 (phosphoglucomutase) alleles, and the mean number of alleles per locus was 6.0 (Table 2). The 109 isolates were assigned to 78 distinctive multilocus genotypes, ETs (Table 3), among which the mean genetic diversity per locus was 0.538 (interlocus variance, $s^2 = 0.062$). The genetic diversity among the 109 isolates ($H = 0.529$, $s^2 = 0.064$) was nearly equivalent to that among ETs (Table 2), reflecting the circumstance that the 13 ETs represented by multiple isolates (range, 2 to 8 isolates) were not closely related genetically.

Relationships among the 78 ETs are shown in Fig. 1. The smallest genetic distance in the dendrogram (0.07) corresponds to a single-locus difference between ETs, and the largest distance (0.73) corresponds to an average difference at 11 of the 15 loci assayed.

The dendrogram in Fig. 1 consists of 14 clusters of two or more closely related ETs diverging at genetic distances of less than 0.30 (designated by letters A through N) and 16 ETs that individually have no close relationship to other ETs.

Phenotypic properties of isolates in relation to genetic structure. Data on serogroup, serotype, and sulfonamide suceptibility are presented in Table 3, in which the isolates are ordered by ET, as numbered consecutively according to position in the dendrogram shown in Fig. 1. Except for ET-5

TABLE 2. Genetic diversity at 15 enzyme loci among ETs and isolates of *N. meningitidis* from healthy carriers

Enzyme locus[a]	No. of alleles	Genetic diversity	
		ETs (n = 78)	Isolates (n = 109)
MAE	7	0.650	0.667
G6P	6	0.400	0.392
PEP	7	0.745	0.749
IDH	10	0.842	0.870
ACO	3	0.585	0.577
GD1	4	0.340	0.375
GD2	5	0.630	0.552
ADH	4	0.387	0.366
FUM	4	0.370	0.438
ALP	9	0.742	0.755
IP1	3	0.076	0.054
IP2	4	0.405	0.357
ADK	5	0.193	0.141
PGM	11	0.881	0.861
GOT	8	0.827	0.787
Mean	6.0	0.538	0.529

[a] Abbreviations: MAE, malic enzyme; G6P, glucose 6-phosphate dehydrogenase; PEP, peptidase; IDH, isocitrate dehydrogenase; ACO, aconitase; GD1, NADP-linked glutamate dehydrogenase; GD2, NAD-linked glutamate dehydrogenase; ADH, alcohol dehydrogenase; FUM, fumarase; ALP, alkaline phosphatase; IP1 and IP2, two indophenol oxidases; ADK, adenylate kinase; PGM, phosphoglucomutase; GOT, glutamic oxaloacetic transaminase.

TABLE 3. Characteristics of 109 isolates of 78 ETs of *N. meningitidis* from carriers

ET	Isolate no.	Allele at the following enzyme locus[a]															Serogroup: serotype	Sulfonamide susceptibility[b]
		MAE	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALP	IP1	IP2	ADK	PGM	GOT		
1	BT786	1	3	2	5	0	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
2	BT517	1	3	2	11	0	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
3	BT17	1	3	2	11	4	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
	BT48	1	3	2	11	4	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
	BT123	1	3	2	11	4	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
	BT206	1	3	2	11	4	2	3	1	1	5	2	5	2	7	5	NG:15:−	S
4	BT747	1	3	2	8	0	2	3	1	1	3	2	3	2	11	10	X:NT:−	S
5	BT41	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	NG:15:−	R
	BT146	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	NG:15:P1.16	R
	BT147	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	B:15:P1.16	R
	BT294	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	B:15:P1.16	R
	BT325	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	NG:15:P1.16	R
	BT798	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	B:15:P1.16	R
	BT895	1	1	7	8	4	2	3	2	1	1	2	3	2	2	10	B:15:−	R
6	BT930	1	1	7	8	0	2	3	2	1	1	2	3	0	0	0	NG:15:P1.16	R
7	BT1042	1	5	2	8	2	2	1	2	1	2	2	3	2	11	4	C:NT:−	S
8	BT801	1	1	2	9	0	1	3	2	1	8	2	3	2	6	6	B:NT:P1.2	S
9	BT776	1	3	2	2	0	1	3	2	1	8	1	3	2	10	2	E:NT:P1.16	S
10	BT74	1	2	2	7	2	1	3	2	1	3	2	3	2	0	0	B:NT:−	S
11	BT921	1	2	2	7	0	1	3	2	1	3	2	3	2	10	6	B:NT:−	S
12	BT622	3	3	2	7	0	1	3	2	1	3	2	3	2	10	9	E:NT:−	S
13	BT53	1	4	1	8	2	1	3	2	1	3	2	3	2	9	2	NG:15′:P1.16	S
	BT204	1	4	1	8	2	1	3	2	1	3	2	3	2	9	2	NG:15′:P1.16	S
14	BT487	1	3	2	9	0	1	3	3	3	8	2	3	2	4	4	NG:NT:−	S
15	BT245	1	3	5	2	4	1	3	2	3	8	2	3	2	8	4	NG:NT:P1.2	S
16	BT985	1	3	5	0.5	4	1	3	2	3	8	2	3	2	8	4	E:NT:−	S
17	BT131	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT140	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT162	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT394	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT585	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT624	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	E:NT:−	S
	BT643	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT877	1	3	5	1	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
18	BT422	1	3	5	2	0	1	3	2	3	8	2	3	2	8	4	NG:NT:P1.2	S
	BT549																E:NT:−	S
19	BT914	1	3	5	12	0	1	3	2	3	8	2	3	2	8	4	NG:NT:P1.16	S
20	BT429	1	3	5	1	0	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT748	1	3	5	1	0	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT998	1	3	5	1	0	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S
	BT1022	1	3	5	1	0	1	3	2	3	8	2	3	2	8	4	NG:NT:−	S

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TABLE 3—*Continued*

ET	Isolate no.	Allele at the following enzyme locus[a]															Serogroup: serotype	Sulfonamide susceptibility[b]
		MAE	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALP	IP1	IP2	ADK	PGM	GOT		
21	BT126	6	3	4	0.5	4	1	3	2	3	8	2	3	2	8	4	NG:NT:-	R
	BT135	6	3	4	0.5	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	I
	BT168	6	3	4	0.5	4	1	3	2	3	8	2	3	2	8	4	E:NT:−	I
	BT368	6	3	4	0.5	4	1	3	2	3	8	2	3	2	8	4	NG:NT:−	I
22	BT718	1	5	5	5	4	1	2	2	3	8	2	3	2	2	2	B:NT:−	S
23	BT722	1	5	5	5	0	1	2	2	3	8	2	3	2	2	2	B:NT:−	S
24	BT709	3	3	5	11	4	1	3	1	1	8	2	3	2	2	2	B:15′:−	S
25	BT222	1	3	4	9	4	1	2	2	1	2	2	3	2	8	9	E:NT:−	S
26	BT728	1	3	4	9	4	1	2	2	1	7	2	3	2	10	9	NG:NT:−	S
27	BT230	1	3	7	9	4	1	2	2	1	7	2	3	2	10	9	E:NT:−	S
28	BT649	3	1	7	7	4	1	1	2	1	2	2	3	2	2	9	C:NT:−	S
29	BT888	3	1	7	7	4	1	1	2	1	8	2	3	2	0	9	B:NT:−	S
30	BT1032	3	1	7	7	0	1	4	2	2	2	2	3	2	0	9	B:15:−	S
31	BT594	5	1	7	7	0	1	6	2	1	2	2	3	2	0	9	C:NT:−	S
32	BT403	2	3	5	1	0	1	2	2	1	3	2	3	2	9	5	Y:NT:−	—
33	BT186	3	3	5	9	0	1	2	2	0	3	2	3	2	9	5	NG:NT:−	S
34	BT735	3	3	5	8	0	1	3	2	2	3	2	3	2	9	2	NG:NT:P1.2	S
35	BT145	3	3	5	7	4	2	3	2	1	2	2	3	2	5	10	NG:15′:−	I
36	BT544	3	3	5	7	0	2	3	2	1	2	2	0	2	5	5	A:NT:−	S
37	BT416	3	3	5	7	0	2	3	2	1	2	2	3	2	0	5	B:15:P1.16	S
	BT661	3	3	5	7	0	2	3	2	1	2	2	3	2	0	5	B:NT:P1.16	S
38	BT538	3	3	5	5	0	2	3	2	1	0	2	3	2	0	5	B:NT:P1.16	—
39	BT556	3	3	5	5	0	2	3	2	1	1	2	3	2	0	5	B:NT:P1.16	—
40	BT341	3	3	5	7	4	2	3	2	1	2	2	5	2	0	5	NG:NT:P1.16	S
41	BT119	3	3	5	5	4	2	3	2	1	3	2	5	2	0	5	B:NT:P1.16	S
42	BT926	4	3	5	5	2	1	3	2	1	3	2	3	2	0	10	B:NT:−	R
43	BT582	2	3	4	12	0	1	4	2	1	3	2	0	2	2	9	NG:15:−	S
44	BT1061	2	3	4	12	0	1	4	2	1	3	2	5	2	2	9	NG:15:−	S
45	BT981	2	3	4	12	4	1	0.5	2	1	3	2	5	2	2	9	B:15:−	S
46	BT122	3	3	4	5	4	1	3	2	1	2	2	3	3	0	2	B:15′:−	S
47	BT490	3	3	4	12	4	1	2	0.5	1	3	2	0	2	10	2	B:15′:−	S
48	BT236	3	3	4	12	4	1	3	2	1	3	2	3	2	2	4	B:NT:−	S
49	BT738	3	3	4	12	4	1	3	2	1	3	2	3	2	2	2	NG:NT:−	S
	BT792	3	3	4	12	4	1	3	2	1	3	2	3	2	2	2	B:NT:−	S

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TABLE 3—*Continued*

ET	Isolate no.	Allele at the following enzyme locus[a]															Serogroup: serotype	Sulfonamide susceptibility[b]
		MAE	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALP	IP1	IP2	ADK	PGM	GOT		
50	BT406	3	3	4	12	4	1	3	2	1	3	2	3	2	0	2	B:NT:−	S
51	BT573	3	3	4	12	4	1	3	2	1	3	2	0	2	6	2	NG:15′:−	S
52	BT755	3	3	4	12	4	1	3	2	1	3	2	5	2	6	2	NG:15′:−	S
53	BT262	3	3	4	12	4	1	3	3	1	3	2	5	2	2	4	B:15′:−	S
54	BT540	3	3	4	12	4	1	3	2	0	3	2	2	5	2	2	NG:NT:−	S
55	BT955	3	3	4	12	4	1	3	2	1	3	2	2	5	2	2	NG:NT:−	S
56	BT421	3	3	4	7	0	1	1	2	1	3	2	3	2	5	4	NG:NT:−	S
57	BT156	3	3	4	7	4	1	1	2	1	3	2	3	2	7	10	NG:NT:−	S
58	BT200	3	3	4	7	4	1	1	2	1	9	2	3	2	7	10	NG:NT:−	I
59	BT627	3	3	4	7	4	1	1	2	1	3	2	3	1	7	10	W:NT:−	S
60	BT137	3	3	2	7	4	1	1	2	1	3	2	3	2	6	10	W:NT:−	S
61	BT796	3	3	4	7	0	1	1	2	1	3	2	3	2	7	10	NG:NT:−	S
62	BT423	3	3	2	7	0	1	1	2	1	3	2	3	2	7	10	E:NT:−	S
	BT770	3	3	2	7	0	1	1	2	1	3	2	3	2	7	10	Y:NT:−	S
63	BT1101	3	3	2	7	0	1	3	2	1	3	2	3	2	7	10	Y:NT:−	S
64	BT201	3	3	4	9	4	1	1	2	1	3	2	3	2	0	10	W:NT:−	S
65	BT1030	3	3	5	5	4	1	2	2	1	3	2	5	2	2	4	B:15′:−	S
	BT1031	3	3	5	5	4	1	2	2	1	3	2	5	2	2	4	B:15′:−	S
66	BT878	3	2	5	7	0	1	3	2	1	3	2	5	2	2	6	B:NT:−	S
67	BT54	3	6	9	9	4	1	4	2	1	8	2	3	2	9	4	C:15:−	S
68	BT625	3	6	9	5	0	1	1	2	1	6	2	3	2	9	4	C:NT:−	S
69	BT391	4	3	4	5	2	1	4	1	1	1	2	3	2	5	4	C:2a:P1.2	R
70	BT756	4	3	4	5	0	1	4	1	1	1	2	3	2	5	4	C:2a:P1.2	R
	BT884																C:2a:P1.2	R
71	BT173	4	3	4	5	2	1	4	1	1	8	2	3	2	5	4	C:2a:P1.2	R
	BT227	4	3	4	5	2	1	4	1	1	8	2	3	2	5	4	C:2a:P1.2	R
	BT396	4	3	4	5	2	1	4	1	1	8	2	3	2	5	4	NG:2a:P1.2	R
72	BT401	4	3	4	5	2	1	2	1	1	8	2	3	2	5	4	C:NT:P1.2	R
73	BT595	4	3	4	5	0	1	4	1	1	8	2	3	2	5	4	C:2a:P1.2	R
74	BT676	4	3	4	5	0	1	3	1	1	8	2	3	2	5	4	NG:2a:P1.2	R
75	BT900	4	3	4	5	0	1	4	1	0	8	2	3	2	5	4	C:2a:−	R
76	BT96	3	5	2	12	4	1	3	3	1	2	2	3	3	9	6	B:NT:−	R
77	BT988	3	3	9	11	0	1.5	4	0.5	3	8	0	5	3	12	2	NG:NT:−	S
78	BT322	0.5	4	8	0.2	0	3	3	0.5	0	0	0	0	3	8	1	NG:NT:−	S

[a] Enzyme abbreviations are defined in Table 2, footnote *a*.
[b] S, Susceptible; R, resistant; I, intermediate; —, not tested.

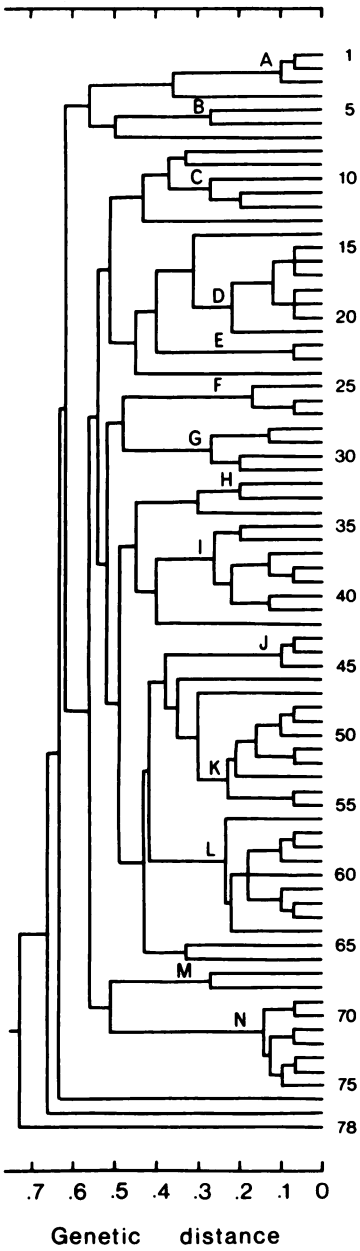

FIG. 1. Genetic relationships among 78 ETs of *N. meningitidis* isolates from asymptomatic carriers. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distance based on 21 enzyme loci. ETs are numbered sequentially from top to bottom in the order of their listing in Table 3. Letters A through N indicate clusters of ETs that diverge at genetic distances of less than 0.30.

(6, 7), the ET numbers are not cognate with those previously assigned to *N. meningitidis* (9).

Of the 109 isolates, 50 (46%) were nonserogroupable, and 59 (54%) were assigned to one of the following eight serogroups: B (29 isolates), C (13 isolates), E (9 isolates), W (3 isolates), Y (3 isolates), A (1 isolate), and X (1 isolate). ETs represented by multiple isolates generally included either isolates of the same serogroup or isolates of the same serogroup together with nonserogroupable ones (Table 3). The only exception was provided by two isolates of ET-62; one, BT423, was serogroup E, and the other, BT770, was serogroup Y. Within clusters of closely related ETs, several serogroups were represented in cluster C (two serogroup B isolates and one serogroup E isolate), cluster G (two serogroup B and two serogroup C isolates), cluster I (five serogroup B isolates, one serogroup A isolate, and one nonserogroupable isolate), and cluster L (three serogroup W, two serogroup Y, one serogroup E, and four nonserogroupable isolates).

TABLE 4. Mean genetic diversity over 15 loci in samples of ETs of *N. meningitidis* from carriers of various age

Age (yr) of carriers	No. of isolates	No. of ETs	Mean genetic diversity	Isolate/ET ratio
Up to 6	10	10	0.566	1.00
7–15	24	22	0.592	1.09
16–19	36	27	0.501	1.33
Over 19	39	33	0.498	1.18

Forty isolates (37%) were serotypable for the class 2 and 3 outer membrane protein. Only two serotypes, 2a and 15, were represented. All nine serotype 2a isolates were genetically closely similar and belonged to ETs in cluster N. Two categories were distinguished among the serotype 15 isolates. Twenty isolates reacted with the two monoclonal antibodies for serotype 15 tested (2-1-P15 and 3-1-P15), whereas 11 isolates reacted only with monoclonal antibody 2-1-P15 and were designated as 15′ (Table 3). Both serotypes 15 and 15′ were represented by isolates of distantly related genotypes. Serotype 15 was represented by all isolates of clusters A, B, and J and by three isolates of ETs belonging to clusters G, I, and M, and the 11 serotype 15′ isolates were distributed in seven different lineages (ET-13, ET-24, ET-35, ET-46, ET-47, ETs 51 to 53 of cluster K, and ET-65).

Twenty-nine strains (27%) were typable for the class 1 protein. Thirteen isolates were P1.2 and 16 were P1.16. P1.2 was represented by ET-8, ET-15, one of two isolates of ET-18 in cluster D, ET-34 in cluster H, and 9 of the 10 isolates (ETs 69 to 74) of cluster N. P1.16 was found in association with six of the eight isolates (ETs 5 and 6) of cluster B, the single isolates of ET-9 and ET-19 (cluster D), both isolates of ET-13, and six of the eight isolates (ETs 37 to 41) in cluster I.

Most of the isolates (75%) were sulfonamide susceptible. Sulfonamide resistance was restricted to the isolates of ETs in clusters B and N, the single isolate of ET-76, and one isolate (BT126) of ET-21 in cluster D; three other isolates of ET-21 were intermediate in suceptibility. The single isolates of ET-35 (cluster I) and ET-58 (cluster L) were also intermediate in suceptibility.

Genetic diversity in relation to carrier age. There was less genetic diversity among ETs of isolates recovered from carriers older than 15 years than among those from younger children (Table 4), and the proportion of ETs represented by multiple isolates was highest among individuals in the age range of 16 to 19 years. There was, however, no close association of specific ETs with different age groups of the population, as demonstrated by the recovery of isolates of the same genotype in both children and adults. Of the 13 ETs represented by multiple isolates, only 1, ET-49, was confined to carriers of one age group. The highest proportion of genotypes shared between age groups was 11% for the 7- to 15- and 16- to 19-year-old carriers. No genotypes of isolates from children under the age of 6 were identified among isolates from 7- to 15-year-old carriers.

Sharing of ETs among associated hosts. Throat cultures were obtained from children in nine day care centers, where

TABLE 5. Mean genetic diversity over 15 enzyme loci in samples of ETs of *N. meningitidis* from asymptomatic carriers and patients with meningococcal disease

Source	No. of isolates	No. of ETs	Mean genetic diversity	Isolate/ET ratio
Carriers	109	78	0.538	1.40
Patients	66	19	0.499	3.47

10 to 32 (mean, 17.0) children were sampled. No carriers or only a single carrier was identified in four of the day care centers, and in none of the five day care centers where more than one carrier was identified (four centers with two carriers and one with three) did the children harbor isolates of the same ET. However, in the day care center where three carriers were identified, two of the children had strains (BT718 and BT722) that differed in genotype solely by the occurrence of a null allele at one enzyme locus (ET-22 and ET-23; Table 3).

Children in the 7- to 15-year age group were sampled at six different schools (18 to 104 children per school; mean, 55.5), and in five schools two to seven carriers were identified. One pair of children harboring the same clone was identified in each of two schools: BT17 and BT48, representing ET-3, and BT294 and BT325, representing ET-5.

In three high schools, where 26 to 67 throat samples were collected (mean, 46.7), 4, 11, and 15 carriers were identified, respectively. In the high school with 15 carriers, three ETs were identified in more than one individual: ET-21, represented by isolates BT126, BT135, and BT168; ET-17, represented by BT131, BT140, and BT162; and ET-5, represented by BT146 and BT147. In the school with 11 carriers, all isolates had distinctive genotypes, but one pair of isolates (BT394 and BT429) differed only in the occurrence of a null allele at one enzyme locus. Each of the four youths from the remaining high school yielded isolates of a distinctive ET.

The three isolates from the carriers in the center for elderly persons were of three distinctive multilocus genotypes.

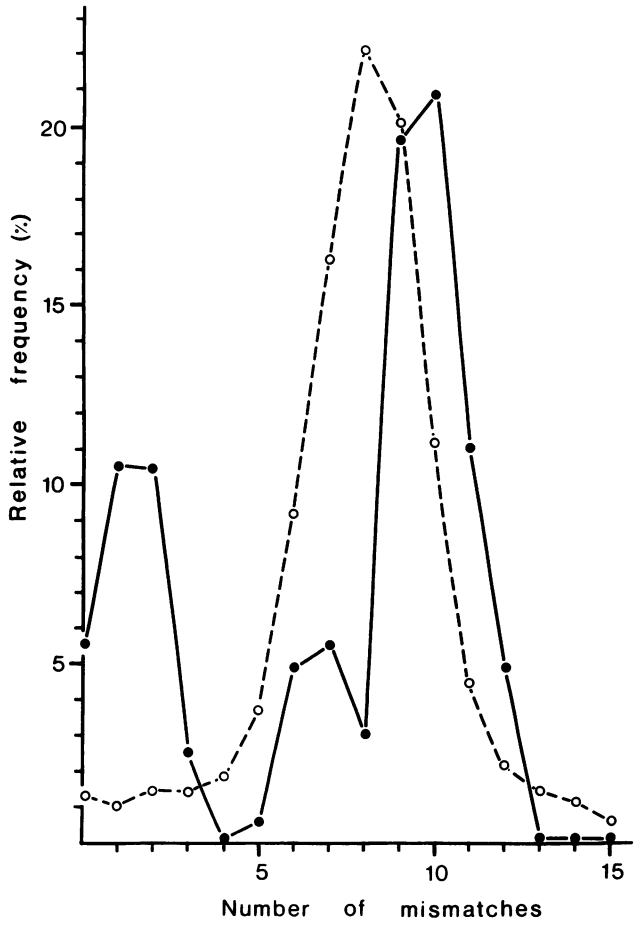

FIG. 2. Frequency distribution of numbers of enzyme loci at which unlike alleles (mismatches) occurred in pairwise comparisons of 78 ETs of *N. meningitidis* isolates from carriers and 18 ETs of *N. meningitidis* isolates from patients. Symbols: ○, carriers; ●, patients.

DISCUSSION

Genetic relatedness among isolates of *N. meningitidis* from asymptomatic carriers. The analysis of electrophoretically demonstrable allelic variation among meningococcal isolates recovered from asymptomatic carriers in a single town in Norway revealed extensive heterogeneity in chromosomal genotype in this local segment of the species. The mean genetic diversity among the 78 ETs of the Tromsø isolates was as great as that recorded for 272 ETs of 601 isolates from patients with meningococcal disease in 20 countries (9).

In an analysis of DNA restriction fragment length polymorphism (1, 18) in the same collection of isolates examined in the present study, Kristiansen (personal communication) was able to recognize the eight isolates of the ET-5 complex, but application of this technique did not yield estimates of the frequency of other clones in the population.

Some of the isolates examined in our study were so distantly related to other strains of *N. meningitidis* that their specific identities may be questioned. This is especially true for BT322, representing ET-78, which diverged from the other ETs at a genetic distance of 0.73 (Fig. 1). This isolate showed no activity for 5 of the 15 enzymes assayed and had alleles not previously identified in *N. meningitidis* at two other enzyme loci (malic enzyme and isocitrate dehydrogenase). The multilocus genotype of BT322 was identical to that of an isolate recovered from another child attending the same school; that isolate was identified as *N. lactamica* on the basis of its ability to ferment lactose. Although BT322 did not produce acid from lactose, we nonetheless suspect that it is a strain of *N. lactamica*. When BT322 was omitted from the collection of carrier isolates, the mean genetic diversity among ETs was reduced to 0.528.

Genetic diversity among isolates from patients with meningococcal disease versus isolates from healthy carriers. Estimates of mean genetic diversity per locus among ETs of carrier isolates from Tromsø were compared with similar estimates for a sample of strains obtained from patients with meningococcal disease in the whole of Norway from January to May 1984 (6). The mean genetic diversity among the 19 ETs of the isolates from patients was only slightly less than that among the 78 ETs in the sample of throat isolates (Table 5). There was, however, a larger proportion of isolates with identical genotypes in the sample from patients than in the sample from carriers, as reflected by a 2.5-fold increase in the ratio of isolates per ET.

Although mean genetic diversity in the sample from patients was not markedly different from that in the sample from carriers, the distributions of pairwise genetic distances,

estimated by the proportion of mismatches between ETs, were very different in the two sets of strains (Fig. 2). For ETs of carrier isolates the distribution was unimodal, whereas for ETs of isolates from patients there were three modes, reflecting the occurrence of several distinctive groups of ETs: two complexes of five and eight ETs, respectively, each including genotypes differing at no more than three loci but distinguishable from one another at an average of 11 of the 15 loci assayed, and six ETs that were not closely related to one another or to ETs of the two complexes.

These two groups of clones, previously designated, respectively, as the ET-5 complex and the ET-37 complex (6, 7), together were responsible for 91% of the cases of meningococcal disease in Norway in 1984. Of 66 cases, 51 (77%) were caused by clones of the ET-5 complex, and clones of the ET-37 complex were responsible for 9 cases (14%). In Norway, a large proportion of isolates of these two complexes of clones is phenotypically characterized by serogroup B, serotype 15:P1.16 (ET-5 complex), and serogroup C, serotype 2a:P1.2 (ET-37 complex), and all are sulfonamide resistant (6).

The two complexes were represented in the collection of isolates from asymptomatic carriers in Tromsø by the ETs of clusters B and N. The frequency of occurrence of these clones in throat cultures of carriers were 7.4 and 9.2%, respectively. Their frequency in the population of healthy individuals sampled was 7.2 per 1,000 individuals for clones of the ET-5 complex and 9.1 per 1,000 individuals for the ET-37 complex. Thus, a low frequency of carriage apparently is sufficient to assure transmission of the pathogenic clones in populations.

The frequency of clones of the ET-37 complex (cluster N) in the Tromsø population was higher than that of clones of the ET-5 complex. The difference actually was not significant, but it is noteworthy that the relative frequency of disease caused by clones of the ET-37 complex has increased in Norway in recent years, from no known cases in 1981 to 1982 to 14% in 1984, and in 1986 25% of the cases of disease were caused by that group of clones (Caugant, unpublished data). The remaining six isolates recovered from patients in 1984 were of six distinctive multilocus genotypes, only one of which (ET-13) was represented among the isolates from carriers in Tromsø.

Genotypes of strains causing systemic disease clearly are not a random sample of those that colonize the throats of healthy individuals. Several of the genotypes of carrier isolates, notably those of cluster D, have not been found to be associated with disease in Norway. Clones of cluster D were also not represented in a large collection of strains from patients in other parts of the world (9). Although the possibility cannot be excluded that these clones can cause disease in immunodeficient individuals (16), it is likely that their virulence potential is low. Variation in the virulence potential of carrier isolates has been experimentally demonstrated in the mouse model (14, 21).

Clonal structure of meningococcal populations in asymptomatic carriers. Several genotypes identified in the collection of carrier isolates from Tromsø were similar to those found in a survey of 34 strains obtained from carriers in a military training camp near Oslo, Norway, in 1981 to 1982 (6). Whereas ET-5 was the most frequent genotype among isolates from military recruits, the second most common genotype (14%) was a clone of cluster D. This suggests that, even among strains of low virulence, the rate of chromosomal gene recombination is insufficient to disrupt the association of characters over a period of a few years.

Variation in serogroup and serotype in relation to population structure. We have previously demonstrated that variation in both serogroup and serotype has little relationship to the genetic structure of a meningococcal population as revealed by multilocus enzyme electrophoresis (9). In the present study, we found that many of the ETs represented by multiple isolates included both serogroupable and nonserogroupable strains (Table 3). This finding suggests that the amount of capsular polysaccharide synthesized by a strain can vary during carriage. Especially noteworthy is a case of two children sampled at the same school at the same time. One harbored a strain (BT147) of ET-5 that was in serogroup B, and the other carried a nonserogroupable isolate (BT146) of ET-5. This type of variation apparently occurs not only in serogroup B organisms but also in strains of other serogroups, such as C, W, and E. For example, the two isolates of ET-13 were nonserogroupable, whereas the isolate of the same ET recovered from a patient was in serogroup W, as were several other isolates of that ET responsible for cases of disease in the United States.

Bacterial capsules have been implicated in virulence, and in the mouse model, comparisons of virulence of isogenic meningococcal strains that differ only in production of polysaccharide have demonstrated the importance of the capsule for invasiveness (20). Spontaneous revertants from nonencapsulated variants can be obtained at significant frequencies both in vitro and in vivo (20, 26). However, the mechanism(s) that regulates the synthesis of capsular polysaccharides in *N. meningitidis* is unknown.

Similarly, isolates of the same ET differed in serotype, either for the class 2 and 3 protein or for the class 1 protein (see, for example, isolates of the ET-5 complex). Inasmuch as this type of variation also occurs among isolates of clones that cause disease, there is no reason to believe that it has any special adaptative or other relationship to the carrier state.

Epidemiology of carriage. There was little sharing of clones among individuals attending the same day care centers or schools. In most cases in which several schoolmates harbored the same clone, it was also identified in several other individuals with no obvious connection. Hence, this study confirms an earlier report of a low rate of transmission of meningococcal strains between individuals attending the same school (17). Transmission of clones responsible for outbreaks has, on several occasions, been shown to be related to school attendance (15, 25), but it is probable that these cases are exceptional.

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