JOURNAL OF CLINICAL MICROBIOLOGY, May 1995, p. 1174–1179 Vol. 33, No. 5
0095-1137/95/$04.00+0

PCR Amplicon Restriction Endonuclease Analysis of the Chromosomal *dhps* Gene of *Neisseria meningitidis*: a Method for Studying Spread of the Disease-Causing Strain in Contacts of Patients with Meningococcal Disease

BJØRN-ERIK KRISTIANSEN,[1,2,3]* CHRISTIAN FERMÉR,[2] ANDREW JENKINS,[1]
EIRIK ASK,[1] GÖTE SWEDBERG,[2] AND OLA SKÖLD[2]

Telemark Biomedical Center, N-3705 Skien,[1] and Department of Medical Microbiology, University of Tromsø, N-9037 Tromsø,[3] Norway, and Department of Pharmaceutical Biosciences, Division of Microbiology, Uppsala Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden[2]

Received 1 August 1994/Returned for modification 20 October 1994/Accepted 16 February 1995

We tested two sets of primers derived from the *dhps* gene of *Neisseria meningitidis* for the amplification of meningococcal DNA by PCR. Both the NM1-NM6 primers and the NM3-NM6 primers amplified *dhps* DNA from all of the meningococci included in the study, resulting, in most cases, in amplicons of 0.70 and 0.23 kb, respectively. Also, *dhps* DNAs of *N. gonorrhoeae* and some commensals were amplified but *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Escherichia coli* DNAs were not. By PCR amplicon restriction endonuclease analysis (AREA) of the larger amplicon, we could differentiate between individual strains of *N. meningitidis*. Following two cases of meningococcal disease, we used PCR AREA to identify healthy contacts carrying the disease-causing strain. We conclude that PCR AREA is a useful method for meningococcal strain differentiation and that it has potential as a method for studying the spread of a disease-causing strain in an affected population. The method is quicker and easier to perform and interpret than chromosomal DNA fingerprinting.

Prevention of the spread of meningococcal disease is based upon mass vaccination and chemoprophylaxis of close contacts of patients with meningococcal disease. In Norway, serogroup B meningococci are most prevalent. There is no efficient vaccine against this serogroup (1), and the Norwegian health authorities do not recommend chemoprophylaxis because of the risk of resistance, which did develop when sulfonamide was used for prophylaxis (2). Spread of the infection is instead counteracted by treating household members below 15 years of age with penicillin for 1 week. Penicillin does not effectively eradicate the meningococcus from the throats of healthy carriers, and thus the spread of the disease-causing strain may continue. It has also been reported that children given penicillin for 1 week have contracted meningococcal disease after the treatment was finished (5).

In the county of Telemark in southern Norway (165,000 inhabitants), there were 39 bacteriologically verified cases of meningococcal disease during the period from 1984 until 1987. Five of these were the index cases for a total of 12 verified and 4 clinically suspected secondary cases. The high number of secondary cases prompted us to initiate a project in 1987 in which we used chromosomal DNA fingerprinting to identify the disease-causing strain of *Neisseria meningitidis* in healthy contacts of patients with meningococcal disease (10), followed by selective administration of rifampin to these carriers. During the study period (1987 to 1992), there were 32 bacteriologically verified primary cases of meningococcal disease and no secondary cases.

Chromosomal DNA fingerprinting takes 3 to 4 days to perform, during which time secondary cases could arise. We therefore wanted to find a more rapid method for strain identification. We have previously reported the use of PCR to diagnose meningococcal meningitis (7). In the PCR technique, we used primers homologous to conserved sequences within the chromosomal *dhps* gene of *N. meningitidis*. This gene codes for the enzyme dihydropteroate synthase, which takes part in folic acid synthesis; mutational and recombinational changes in this gene confer sulfonamide resistance on meningococci (9, 12). Sequence analysis of the *dhps* gene of several different strains of *N. meningitidis* revealed the presence of both conserved and variable regions within the 0.7-kb sequence amplified by the primer set NM1-NM6 (12). The aim of this study was to exploit this sequence variation for strain identification and differentiation. Amplicons were cleaved with *Hha*I, and fragments were separated by gel electrophoresis (PCR amplicon restriction endonuclease analysis [AREA]). We found that PCR AREA allows strain differentiation and that it can be used to trace a disease-causing strain of *N. meningitidis* in healthy contacts of patients with meningococcal disease.

MATERIALS AND METHODS

PCR primers. We used the primers listed in Table 1; their localization within the *dhps* gene is shown in Fig. 1. The NM1 and NM0 primer sequences were identified in our reference strain MO035 (12) and were located upstream of the *dhps* gene. The NM3 and NM6 primers were found by alignment of the *dhps* sequences of seven unrelated *N. meningitidis* strains (12). Amplification of reference strain MO035 with the NM1-NM6 primer set gave an amplicon of the expected size of 0.70 kb; amplification with the NM3-NM6 primer set resulted in an amplicon of the expected size of 0.23 kb. The sizes of the PCR products were confirmed by agarose gel electrophoresis.

Bacteria for testing of primers. The following groups of bacteria were included in the study for testing of primer sets NM1-NM6 and NM3-NM6.

(i) Meningococci. Fifty-six meningococcal isolates were from patients with meningococcal disease; 36 of these were isolated during the Norwegian MenOpp study (2), 7 isolates were from Canada, 5 were from England, 4 were from Slovenia, 2 were from Sudan, and 2 were isolated in Norway in 1969 (Table 2).

* Corresponding author. Mailing address: Telemark Biomedical Center, P.O. Box 1868 Gulset, N-3705 Skien, Norway.

TABLE 1. Primer sequences used for amplification and analysis of *N. meningitidis*

Oligonucleotide primer	Position in *dhps* sequence[a]	DNA sequence
NM0	553–579	5′-GACTGCAGGCATACCGGCAGATTCAAA-3′[b]
NM1	647–668	5′-GGGTCGACGGTTTCAGACGGCATATAA-3′[c]
NM3	1111–1131	5′-ACGGGCATTTGCCTGATGCAC-3′
NM6	1344–1324	5′-CGCCATCAATTCGGGCAAATG-3′

[a] According to reference 12.
[b] Two bases in the 5′-terminal sequence were modified to create a *Pst*I site.
[c] The first five bases (5′-GGGTC) were not derived from the *dhps* sequence but were appended to the *dhps* oligonucleotide to create a *Sal*I recognition site.

Twenty-five meningococcal isolates were throat isolates from healthy carriers of all age groups living in the city of Tromsø in northern Norway (Table 3; references 3 and 8).

(ii) Nonmeningococcal bacteria. A variety of *Neisseria* bacteria and bacteria which commonly cause meningitis were included in the study (Table 4).

Bacteria for testing of PCR AREA. For testing of the reproducibility and discriminatory ability of the PCR AREA method, we included 17 meningococcal isolates which had been typed by the isoenzyme typing (ET) method (3) by Domenique Caugant at The National Institute of Public Health, Oslo, Norway (Fig. 2A and B). Eight belonged to the ET5 complex of meningococci, and nine belonged to nine other ET complexes (2a). Finally, we included meningococcal strains from the blood of two patients with meningococcal disease and 34 throat isolates obtained from healthy contacts of these two patients (10). Five contacts of patient 1 and 29 contacts of patient 2 carried meningococci in their throats.

Serogrouping and serotyping. Meningococci were serogrouped with commercially available antisera (Wellcome Reagents Ltd., Beckenham, United Kingdom) specific for the A, B, C, W135, X, Y, and Z capsular polysaccharides as previously described (3). Meningococci were serotyped 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. (4).

Sulfonamide susceptibility testing. The MIC of sulfadiazine was determined by the agar dilution method as described by Bøvre et al. (2). Bacteria for which the MICs were 5 mg/liter or lower were sulfonamide susceptible, those for which the MICs were 100 mg/liter or higher were sulfonamide resistant, and those for which the MICs were between 5 and 100 mg/liter were intermediately susceptible to sulfadiazine.

DNA preparation. All neisseriae and *Neisseria*-like bacteria and *Haemophilus influenzae* isolates were spread on chocolate agar and grown overnight at 37°C in a 10% CO_2 atmosphere to give confluent growth. *Escherichia coli*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis* were cultured on blood agar at 37°C. Bacteria were suspended in 1.5 ml of TSE buffer (50 mM Tris-HCl [pH 8.1], 25% sucrose, 50 mM EDTA), harvested by centrifugation, and resuspended in 150 μl of TS buffer (50 mM Tris-HCl [pH 8.1], 25% sucrose). Samples were placed on ice, and 100 μl of freshly prepared lysozyme (10 mg/ml) in TN buffer

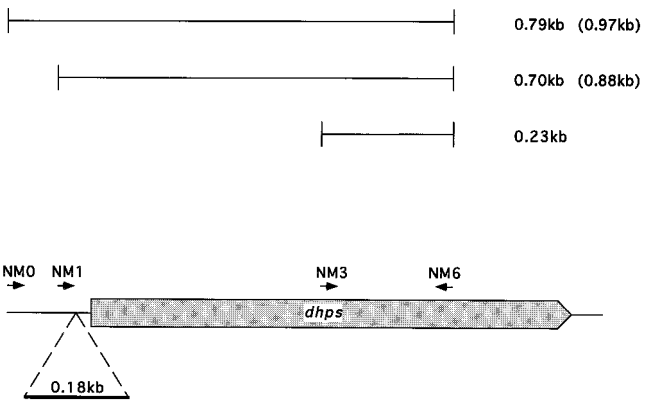

FIG. 1. Schematic representation of the *dhps* gene and the positions of the primers used in this study. With the NM3-NM6 primers, a 0.23-kb DNA sequence was amplified. Amplification with the NM1-NM6 primers gave an amplicon of 0.70 kb, and that with the NM0-NM6 primers gave an amplicon of 0.79 kb. In a few isolates (see text), there was a 180-bp insert immediately downstream of NM1, resulting in amplicons of 0.88 and 0.97 kb with the NM1-NM6 and NM0-NM6 primer sets, respectively.

TABLE 2. Isolates[a] of *N. meningitidis* from patients with meningococcal disease which were used to test the primers

Isolate no.	Origin[b]	Phenotype[c]
MO001	NOR	B:NT:−:S
MO003	NOR	B:15:P1.16:R
MO007	NOR	B:15:P1.16:R
MO012	NOR	B:15:P1.16:R
MO015	NOR	B:−:P1.16:R
MO035	NOR	B:15:P1.2:R
MO036	NOR	B:15:P1.2:R
MO039	NOR	B:NT:−:S
MO044	NOR	B:15:−:R
MO052	NOR	B:15:P1.16:R
MO053	NOR	B:15:P1.16:R
MO055	NOR	B:−:P1.2:S
MO056	NOR	C:NT:−:S
MO061	NOR	B:−:P1.16:R
MO063	NOR	B:15:P1.15:R
MO064	NOR	B:NT:−:R
MO067	NOR	B:15:P1.16:R
MO068	NOR	B:15:P1.16:R
MO069	NOR	B:15:P1.16:R
MO072	NOR	B:15:P1.16:R
MO073	NOR	C:2a:P1.2:R
MO074	NOR	B:15:P1.16:R
MO075	NOR	B:−:P1.16:R
MO076	NOR	B:15:P1.16:R
MO077	NOR	B:15:P1.16:R
MO078	NOR	B:15:−:R
MO079	NOR	B:15:−:R
MO089	NOR	B:15:P1.16:R
MO093	NOR	B:15:P1.2:R
MO094	NOR	B:NT:−:S
MO097	NOR	B:2b:−:S
MO100	NOR	C:NT:−:S
MO101	NOR	B:NT:P1.16:S
MO114	NOR	B:2a:−:S
MO117	NOR	B:15:P1.15:I
MO124	NOR	C:15:P1.16:R
418	SUD	A:4,21:P1.X,9:R
1014	SUD	A:4,21:P1.7:S
952/68	NOR	B:NT:P1.2:S
3976/69	NOR	B:NT:P1.2:R
89-488	CAN	C:2a:P1.2:R
92-014	CAN	C:2a:P1.2:R
92-026	CAN	C:2a:P1.2:R
91-133	CAN	C:2a:P1.2:S
92-019	CAN	C:2a:P1.2:R
92-041	CAN	B:15:P1.16:R
91-053	CAN	C:2a:P1.2:R
G2221	ENGL	B:15:P1.16:R
G2501	ENGL	B:15:P1.16:R
H800	ENGL	B:15:P1.16:R
G2376	ENGL	B:15:P1.16:R
G2379	ENGL	B:15:P1.16:R
1M	SLO	B:4:−:S
3M	SLO	B:NT:−:R
5M	SLO	B:NT:P1.5:R
9M	SLO	Y:8:P1.5:R

[a] All isolates were amplified by both the NM1-NM6 and NM3-NM6 primer sets, which resulted in amplicons of 0.70 and 0.23 kb, respectively. With one isolate (9M), an amplicon of 0.88 kb was obtained (see text).
[b] NOR, isolates collected from all over Norway during the MenOpp study (2) and in Bergen in 1969. SUD, isolates from Sudan kindly provided by Per Olcen, Ørebro Hospital, Ørebro, Sweden. CAN, isolates from Canada kindly provided by Fraser Ashton, National Laboratory for Bacteriology, Ottawa, Ontario, Canada. ENGL, isolates from the Plymouth and Gloucester areas in England kindly provided by D. M. Jones, Manchester Public Health Laboratories, Manchester, England. SLO, isolates from Slovenia kindly provided by Metka Paragi, National Institute of Public Health, Ljubljana, Slovenia.
[c] Phenotype: serogroup:serotype:subtype:sulfonamide susceptibility. R, resistant; S, susceptible; I, intermediately susceptible; NT, nontypeable.

TABLE 3. Isolates[a] of *N. meningitidis* from healthy carriers of all age groups[b]

Isolate no.	Phenotype[c]
BT017	NG:15:−:S
BT041	NG:15:−:R
BT048	NG:15:−:S
BT053	NG:15:P1.16:S
BT054	C:15:−:S
BT074	B:NT:−:S
BT096	B:NT:−:R
BT119	B:NT:P1.16:S
BT122	B:15:−:S
BT135	NG:NT:−:I
BT137	W135:NT:−:S
BT146	NG:15:P1.16:R
BT147	B:15:P1.16:R
BT168	29E:NT:−:I
BT173	C:2a:P1.2:R
BT201	W135:NT:−:S
BT227	C:2a:P1.2:R
BT294	B:15:P1.16:R
BT322	NG:NT:−:S
BT325	NG:15:P1.16:R
BT341	NG:NT:P1.16:S
BT368	NG:NT:−:I
BT416	B:15:P1.16:S
BT490	B:15:−:S
BT544	A:NT:−:S

[a] All isolates were amplified by both the NM1-NM6 and NM3-NM6 primer sets, which resulted in amplicons of 0.70 and 0.23 kb, respectively. With two isolates (BT074 and BT096), an amplicon of 0.88 kb was obtained (see text).
[b] See references 3 and 8.
[c] Phenotype: serogroup:serotype:subtype:sulfonamide susceptibility. R, resistant; S, susceptible; I, intermediately susceptible; NG, nongroupable; NT, nontypeable.

(10 mM Tris, 10 mM NaCl [pH 8.0]). For lysis of *S. epidermidis*, lysozyme was replaced with lysostaphin (2 mg/ml) prepared in 50 mM Tris (pH 8). Samples were incubated until cell lysis was evident from the change in sample viscosity, usually for about 20 min, when 10 μl of RNase A (10 mg/ml) was added, and the samples were incubated for 5 min. Subsequently, 50 μl of proteinase E (25 mg/ml) was added and the samples were incubated for 15 min. TN buffer (150 μl) and lysis mix (300 μl containing 10 mM EDTA, 2% Triton X-100, and 50 mM HCl [pH 8.0]) were added, and the lysate was extracted three times with an equal volume of 1:1 phenol-chloroform (vigorous shaking is necessary at this stage, as the lysate is extremely viscous). Finally, the DNA solution was dialyzed for 3 h against TN buffer. This method usually yields DNA with a concentration of approximately 2 mg/ml.

Chromosomal DNA fingerprinting. Chromosomal DNA fingerprinting was performed as previously described, by cleavage of extracted DNA with *Hin*dIII followed by electrophoretic separation of the resulting DNA fragments on 4% polyacrylamide gels (10).

PCR technique. The PCR test was performed as previously described (7). For restriction endonuclease cleavage of the PCR amplicon, we chose *Hha*I, which gave band patterns consisting of five to eight easily distinguishable bands. Twenty microliters of the PCR product was digested with 30 U of *Hha*I in the buffer supplied by the manufacturer (Promega, Madison, Wis.) at 37°C for 1 h. Ten microliters of the mixture was run on a 6% acrylamide (24:1 acrylamide-bisacrylamide ratio) gel containing 30% glycerol in 60 mM Tris-borate–1.5 mM EDTA buffer for 5.5 h at 20 mA and 580 V. The gel was stained in 2 μg of ethidium bromide per ml for 30 min and then photographed in UV light at 254 nm.

RESULTS

Primer amplification. All 81 meningococcal isolates that were included for primer analysis were amplified by both the NM1-NM6 and NM3-NM6 sets of primers. The NM1-NM6 primers, which were also used to amplify meningococcal DNA for PCR AREA, amplified 17 meningococcal laboratory strains with known ET types (Fig. 2A and B) and 2 additional meningococcal case strains and 34 isolates from close contacts of these two cases (Fig. 3 and 4). NM1-NM6 amplification gave, in most instances, an amplicon of 0.70 kb. With one systemic isolate (9M) and two carrier isolates (BT074 and BT096), however, a 0.88-kb amplicon was obtained. We amplified the segment between NM0 and NM6 in these isolates and sequenced it. Sequence analysis revealed a 184-bp insert immediately downstream of the NM1 primer and that this insertion was homologous to part of the insertion sequence IS*1106*, which has been found downstream of the gene for the major surface antigen (*porA*) in B15 *N. meningitidis* (6). The 184-bp insert corresponds to nucleotides 1752 to 1936 of Fig. 3 in reference 6 and represents a repetitive region of IS*1106*. When the NM3-NM6 primers were used for amplification, one single amplicon of 0.23 kb was obtained with all of the meningococcal isolates included in the study.

TABLE 4. Amplification of nonmeningococcal bacteria by the primer sets NM1-NM6 and NM3-NM6

Bacterium	Origin	PCR result with:	
		NM1-NM6[a]	NM3-NM6[b]
Neisseria lactamica ATCC[c] 23970[T][d]		+	+
Neisseria mucosa ATCC 19696[T]		+	+
Neisseria sicca ATCC 9913		+	+
Neisseria sicca 37826/92	TBC[e]	+	+
Neisseria subflava ATCC 14221		−	+
Neisseria flavescens NCTC[f] 8263[T]		−	−
Neisseria subflava ATCC 19243		−	−
Neisseria elongata ATCC 25295[T]		−	−
Neisseria cuniculi ATCC 14688[T]		−	−
Neisseria cinerea 159/62	RH[g]	+	+
Neisseria caviae NCTC 10293		−	−
Neisseria canis ATCC 14687[T]		−	−
Branhamella catarrhalis 37839/92	TBC	−	−
Branhamella catarrhalis NE 11	TBC	−	−
Neisseria gonorrhoeae 23419/92	TBC	+	+
Neisseria gonorrhoeae 44262/92	TBC	+	+
Moraxella osloensis 5873	RH	−	−
Kingella denitrificans NCTC 10995[T]		−	−
Eikenella corrodens ATCC 23834[T]		−	−
Haemophilus influenzae 13051/92	TBC	−	−
Staphylococcus epidermidis 27439/92	TBC	−	−
Escherichia coli 28845/92	TBC	−	−
Escherichia coli 35141/92	TBC	−	−
Streptococcus pneumoniae 33483/92	TBC	−	−
Streptococcus pneumoniae 97899/92	TBC	−	−

[a] Amplicon of 0.70 kb.
[b] Amplicon of 0.23 kb.
[c] ATCC, American Type Culture Collection.
[d] T = type strain.
[e] TBC, clinical bacteria isolated at Telemark Biomedical Center, Skien, Norway.
[f] NCTC, National Collection of Type Cultures, London.
[g] RH, obtained from Kjell Bøvre, Rikshospitalet, Oslo, Norway.

The following nonmeningococcal *Neisseria* strains were amplified by both sets of primers: *N. gonorrhoeae*, *N. sicca*, *N. lactamica*, *N. mucosa*, and *N. cinerea* (Table 4). The amplicons were, in all cases, 0.70 kb long with the NM1-NM6 primers and 0.23 kb long with the NM3-NM6 primers. *N. subflava* was amplified only with the NM3-NM6 primers. None of the remaining nonmeningococcal bacteria were amplified by either of the two primer sets (Table 4).

PCR AREA. To verify the band patterns generated by PCR AREA, we generated *Hha*I fragment size catalogs from the known *dhps* sequences of seven *N. meningitidis* strains by using the GCG MAPSORT program (Genetic Computer Group, University of Wisconsin, Madison) and compared them with

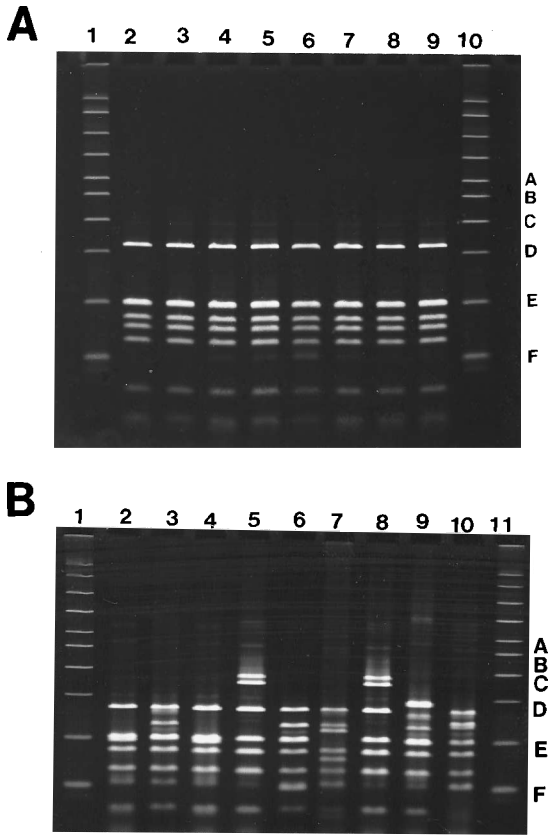

FIG. 2. (A) PCR AREA of eight strains of *N. meningitidis* of the ET5 complex. Lanes: 2, MO121 (B:15:P1.16:R); 3, MO064 (B:NT:R); 4, MO063 (B:15:P1.16:R); 5, MO061 (B:−:P1.16:R); 6, MO044 (B:15:−:R); 7, MO035 (B:15:P1.2:R); 8, MO015 B:−:P1.16:R); 9, MO003 (B:15:P1.16:R). Lanes 1 and 10 contained size markers (Bio-Rad 50 to 2,000-bp ladder). Sizes: A, 500 bp; B, 400 bp; C, 300 bp; D, 200 bp; E, 100 bp; F, 50 bp. Bacterial phenotype designations: serogroup:serotype:subtype:sulfonamide susceptibility. R, resistant; S, susceptible; I, intermediately susceptible; NT, nontypeable. (B) PCR AREA of nine isolates of nine ET complexes other than ET5. Lanes: 2, MO117 (B:15:P1.15:I); 3, MO114 (B:2a:S); 4, MO101 (B:NT:P1.16:S); 5, MO100 (C:NT:−:S); 6, MO097 (B:2b:−:S); 7, MO073 (C:2a:P1.2:R); 8, MO056 (C:NT:−:S); 9, MO039 (B:NT:−:S); 10, MO001 (B:NT:−:S). Lanes 1 and 11 contained the same size markers as in panel A.

the fragment sizes observed on gel electrophoresis. In all seven cases, the observed sizes were in agreement with those predicted from the DNA sequence.

We amplified eight isolates belonging to the ET5 complex and nine isolates with other ET types and cleaved the resulting amplicons with *Hha*I. All of the eight ET5 isolates had identical PCR AREA band patterns (Fig. 2A). The nine isolates with other ET types had band patterns different from the ET5 band patterns (Fig. 2B). The following pairs of isolates had identical PCR AREA band patterns (Fig. 2B): MO101 and MO117, MO097 and MO114, and MO056 and MO100. The PCR AREA band patterns of the remaining three isolates (MO001, MO039, and MO073) were unique. The apparent discrepancy between the ET method and the PCR AREA method was investigated by chromosomal DNA fingerprinting. By using chromosomal DNA fingerprinting, we found MO097 and MO114 to be indistinguishable, supporting the PCR AREA result. However, by chromosomal DNA fingerprinting MO101 was found to be different from MO117 and MO056 was found to be different from MO100, supporting the ET results.

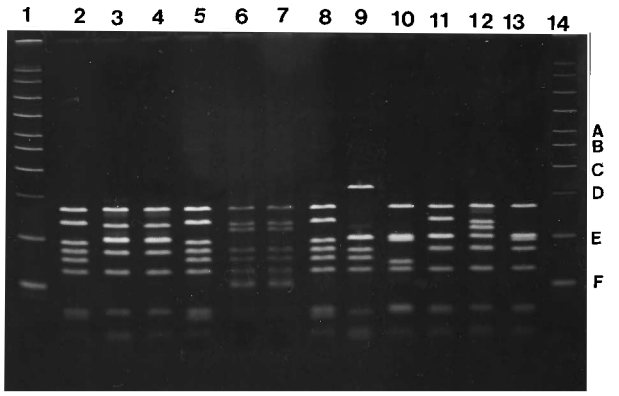

FIG. 3. PCR AREA of a meningococcal isolate from patient 1 (lane 6) and isolates from the throats of 10 of his close contacts. The throat isolate of one contact (lane 7) was identical to that of the patient. Lanes 1 and 14 contained the same size markers as in Fig. 2.

We also observed a discrepancy between the measured size of the amplicon and its actual size in some of the tracks of Fig. 2B. In tracks 2 (MO117) and 4 (MO101), which had identical band patterns, it was necessary to assume that the particularly intense and broad 100-bp band is, in fact, a triplet. In tracks 5 (MO100) and 8 (MO056), with identical band patterns, the sum of the fragment sizes was approximately 900 bp. Comparison with other digests revealed that the largest fragment (290 bp) may be a partial digest product which, in complete digests, resolved to the next largest band (270 bp) plus a very small fragment. The partial digestion pattern is reproducible, a fact that may be attributed to the presence of a restriction-refractory *Hha*I site.

PCR AREA used to identify a disease-causing strain of *N. meningitidis* in healthy contacts. (i) Patient 1. An 18-year-old male in high school with meningococcal septicemia was admitted to a hospital in October 1991. Two days later, throat samples were collected from his family, friends, and schoolmates, a total of 98 persons. Serogroup C, sulfonamide-resistant meningococci were found in a blood culture from the patient. Meningococci were isolated from 29 contacts. By testing the isolates with PCR AREA, we showed that the disease-causing strain was present in the throat of one close friend but in no others (Fig. 3). This result was confirmed by chromosomal DNA fingerprinting (10). Among the other carrier strains, many different PCR AREA patterns were seen. However, some of them shared a PCR AREA pattern, indicating that

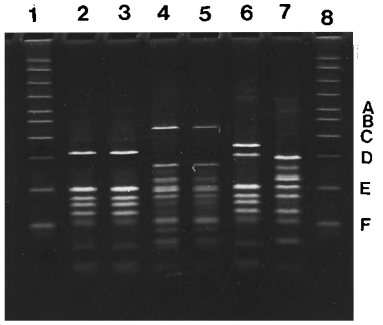

FIG. 4. PCR AREA of a meningococcal isolate from the blood of patient 2 (lane 3) and those from the throats of her close contacts. Her grandmother carried meningococci in her throat identical to those in the patient (lane 2). Lanes 1 and 8 contained the same size markers as in Fig. 2.

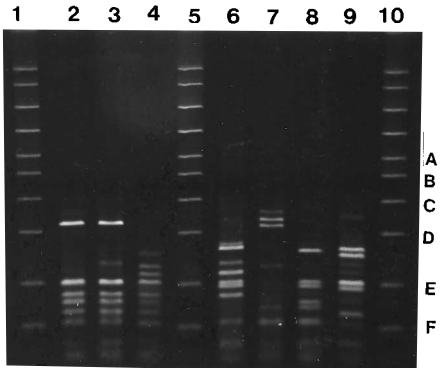

FIG. 5. PCR AREA of nonmeningococcal neisseriae that were amplified with the NM1-NM6 primers. Lanes 1, 5, and 10 contained the same size markers as in Fig. 2. Lanes: 2 (MO035, serogroup B, serotype 15:P1) and 3 (BT147, serogroup B, serotype 15:P1.16), two different but similar strains of meningococci; 4, *N. cinerea* 159/62; 6, *N. sicca* ATCC 9919; 7, *N. mucosa* ATCC 19696; 8, *N. lactamica* ATCC 23970; 9, *N. gonorrhoeae* 23419/92.

strains other than the patient strain were also distributed among the close contacts.

(ii) Patient 2. Shortly after a 1-year-old girl was admitted to Telemark Central Hospital on 27 March 1993 for serogroup B meningococcal septicemia, throat samples were collected from 18 close contacts, including family members. Meningococci were detected in 5 of these 18 samples. By PCR AREA, we found that only the patient's grandmother carried the disease-causing strain in her throat. Two other carriers harbored meningococci with PCR AREA band patterns identical to each other but different from that of the disease-causing strain; the remaining two throat isolates had unique band patterns (Fig. 4). These results were confirmed by chromosomal DNA fingerprinting.

PCR AREA of commensals. The band patterns obtained by cleaving the amplicons of the NM1-NM6-amplifiable commensals are shown in Fig. 5. The band patterns varied from species to species and were easily differentiated from meningococcal band patterns.

DISCUSSION

We have previously reported the development of a PCR method with primers from the chromosomal *dhps* gene for detection of serogroup B *N. meningitidis* in the cerebrospinal fluid of a patient with meningococcal disease (7). Further analysis of these primers, however, revealed that some serogroup C meningococci were not amplified. We have therefore, on the basis of sequence analysis of the *dhps* gene of seven meningococcal strains, identified conserved sequences of the same gene that seem promising for use in a PCR test for detection of meningococcal DNA. In the present study, two sets of primers consisting of three different sequences, NM1, NM3, and NM6, were evaluated for the ability to amplify meningococci. Both sets of primers amplified all of the meningococci included in the study. These strains had been isolated from different parts of the world, they represented a wide variety of phenotypes, and they were from both patients and healthy carriers. With NM1-NM6, the expected amplicon size of 0.70 kb was the result in most instances, but with 3 of the 134 meningococcal isolates included in this study, an amplicon size of 0.88 kb was produced. The reason for this was shown to be due to an insertion of a 0.18-kb DNA sequence homologous to the insertion sequence IS*1106*, which is present in all meningococci (6, 11). The primers were not specific for meningococci, however; *N. gonorrhoeae*, *N. lactamica*, *N. mucosa*, *N. sicca*, and *N. cinerea* were amplified by both sets of primers. In contrast, *S. pneumoniae*, *H. influenzae*, and *E. coli*, which are common causes of meningitis, were not amplified. The predictive value of a positive PCR test of cerebrospinal fluid with these primers is good because the nonmeningococcal *Neisseria* bacteria that were amplified do not cause meningitis. However, if the PCR test is to be applied directly to throat specimens in which nonmeningococcal *Neisseria* bacteria are commonly present, false-positive results may occur. Initial comparisons of band patterns indicated that by further analysis of the amplicon by PCR AREA, nonmeningococcal *Neisseria* bacteria can be differentiated from meningococci.

By sequencing the *dhps* gene of several different strains of *N. meningitidis*, the amplicons obtained by NM1-NM6 amplification have been shown to contain variable regions (12). By cleavage of the NM1-NM6 amplicons with *Hha*I, these variations were shown to allow strain differentiation. The PCR AREA band pattern of strains with identical ET types were identical. Among the nine isolates with nine different ET types, three pairs of isolates shared PCR AREA band patterns; the remaining three isolates had unique PCR AREA band patterns. Chromosomal DNA fingerprinting confirmed the apparent identity of one pair; for the two other pairs, the ET results were supported by chromosomal DNA fingerprinting. This apparent discrepancy between ET results, chromosomal DNA fingerprinting, and PCR AREA typing needs further investigation. Studies along this line are in progress.

We found that *Hha*I digestion gave restriction patterns that discriminated meningococcal strains well and were usually easy to interpret. However, we observed that partial digestion and, in some cases, overdigestion (star activity) may occur with *Hha*I and its isoschizomers. Although in practice this rarely causes strain comparison problems, there is a potential for two similar strains to be scored as dissimilar because of partial digestion. To avoid this problem, we are currently investigating the applicability of other restriction endonucleases for PCR AREA.

The restriction sites occupy up to nearly 6% of the bases in the present amplicon, and the amplicon contains variable regions. This means that PCR AREA patterns are subject to slow mutational decay, and this may limit the utility of the method for comparison of isolates separated by longer periods of time.

By using the PCR AREA method on meningococcal isolates cultured from 2 patients and on isolates from the throats of their close contacts, the disease-causing strain was traced and the contacts carrying the disease-causing strain were given successful chemoprophylaxis. These results were confirmed by chromosomal DNA fingerprinting.

By using PCR AREA, it would be possible to obtain a DNA band pattern or fingerprint of a disease-causing strain directly from the cerebrospinal fluid of a patient. Subsequently, by direct PCR AREA of throat specimens from close contacts of a patient, the disease-causing strain could be traced and eradicated from the environment within 24 h after the patient has been admitted to the hospital. In addition to allowing simpler sample preparation, PCR AREA gives a less complex band pattern, which is easier to interpret than chromosomal DNA fingerprinting. The technique could also be used to classify meningococci, but this requires that the band patterns be subjected to computerized analysis. Work along this line is in progress.

ACKNOWLEDGMENTS

We thank Mette Olsen and Anne Gry Andersen for excellent technical assistance and the following persons for supplying bacteria: Kjell Bøvre and Tone Tønjum, Department of Medical Microbiology, Rikshospitalet, Oslo, Norway; Alfred Halstensen, Department of Medicine, Haukeland Hospital, Bergen, Norway; Per Olcen, Central Hospital of Ørebro, Sweden; Fraser Ashton, National Laboratory for Bacteriology, Ottawa, Ontario, Canada; D. M. Jones, Manchester Public Health Laboratory, Withington Hospital, Manchester, England; Domenique Caugant, National Institute of Public Health, Oslo, Norway; and Metka Paragi, National Institute of Public Health, Ljubljana, Slovenia.

REFERENCES

1. **Bjune, G., E. A. Høiby, J. K. Grønnesby, Ø. Arnesen, J. Holst-Fredriksen, A. Halstensen, E. Holten, A. K. Lindbak, H. Nøkleby, E. Rosenqvist, L. K. Solberg, O. Closs, J. Eng, L. O. Frøholm, A. Lystad, L. S. Bakketeig, and B. Hareide.** 1991. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet **338:**1093–1096.
2. **Bøvre, K., L. O. Frøholm, P. Gaustad, E. Holten, and E. A. Høiby.** 1983. Some agent characteristics and their coexistence related to occurrence and severity of systemic meningococcal disease in Norway, Winter 1981–1982. NIPH Ann. **6:**75–84.

2a. **Caugant, D. A.** Personal communication.

3. **Caugant, D. A., B. E. Kristiansen, L. O. Frøholm, K. Bøvre, and R. K. Selander.** 1988. Clonal diversity of *Neisseria meningitidis* from a population of asymptomatic carriers. Infect. Immun. **56:**2060–2068.
4. **Frøholm, L. O., K. Bøvre, E. Holten, and W. D. Zollinger.** 1983. Serotyping of meningococci by coagglutination with monoclonal antibodies. NIPH Ann. **6:**125–131.
5. **Høiby, E. A., P. J. Moe, A. Lystad, and L. O. Frøholm.** 1986. Phenoxymethylpenicillin treatment of household contacts of meningococcal disease patients. Antonie van Leeuwenhoek **52:**255–257.
6. **Knight, A. I., H. Ni, K. A. V. Cartwright, and J. McFadden.** 1992. Identification and characterization of a novel insertion sequence, IS*1106*, downstream of the *porA* gene in B15 *Neisseria meningitidis*. Mol. Microbiol. **6:**1565–1573.
7. **Kristiansen, B. E., E. Ask, A. Jenkins, C. Fermer, P. Rådstrøm, and O. Sköld.** 1991. Rapid diagnosis of meningococcal meningitis by polymerase chain reaction. Lancet **337:**1568–1569.
8. **Kristiansen, B. E., K. W. Lind, K. Mevold, B. Sørensen, L. O. Frøholm, K. Bryn, T. Tjade, and K. Bøvre.** 1988. Meningococcal phenotypic and genotypic characteristics and human antibody level. J. Clin. Microbiol. **26:**1988–1992.
9. **Kristiansen, B. E., P. Rådstrøm, A. Jenkins, E. Ask, B. Facinelli, and O. Sköld.** 1990. Cloning and characterization of a DNA fragment that confers sulfonamide resistance in a serogroup B, serotype 15 strain of *Neisseria meningitidis*. Antimicrob. Agents Chemother. **34:**2277–2279.
10. **Kristiansen, B. E., Y. Tveten, E. Ask, T. Reiten, A. B. Knapskog, J. Steen-Johnsen, and G. Hopen.** 1992. Preventing secondary cases of meningococcal disease by identifying and eradicating disease-causing strains in close contacts of patients. Scand. J. Infect. Dis. **24:**165–173.
11. **Ni, H., A. I. Knight, K. Cartwright, W. H. Palmer, and J. McFadden.** 1992. Polymerase chain reaction for diagnosis of meningococcal meningitis. Lancet **340:**1432–1434.
12. **Rådstrøm, P., C. Fermèr, B. E. Kristiansen, A. Jenkins, O. Sköld, and G. Swedberg.** 1992. Transformational exchanges in the dihydropteroate synthase gene of *Neisseria meningitidis*: a novel mechanism for acquisition of sulfonamide resistance. J. Bacteriol. **174:**6386–6393.