

Use of Variable-Number Tandem Repeats To Examine Genetic Diversity of *Neisseria meningitidis*

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Received 7 June 2004/Returned for modification 26 July 2004/Accepted 24 November 2004

Repetitive DNA motifs with potential variable-number tandem repeats (VNTR) were identified in the genome of *Neisseria meningitidis* and used to develop a typing method. A total of 146 meningococcal isolates recovered from carriers and patients were studied. These included 82 of the 107 *N. meningitidis* isolates previously used in the development of multilocus sequence typing (MLST), 45 isolates recovered from different counties in Norway in connection with local outbreaks, and 19 serogroup W135 isolates of sequence type 11 (ST-11), which were recovered in several parts of the world. The latter group comprised isolates related to the Hajj outbreak of 2000 and isolates recovered from outbreaks in Burkina Faso in 2001 and 2002. All isolates had been characterized previously by MLST or multilocus enzyme electrophoresis (MLEE). VNTR analysis showed that meningococcal isolates with similar MLST or MLEE types recovered from epidemiologically linked cases in a defined geographical area often presented similar VNTR patterns while isolates of the same MLST or MLEE types without an obvious epidemiological link showed variable VNTR patterns. Thus, VNTR analysis may be used for fine typing of meningococcal isolates after MLST or MLEE typing. The method might be especially valuable for differentiating among ST-11 strains, as shown by the VNTR analyses of serogroup W135 ST-11 meningococcal isolates recovered since the mid-1990s.

Neisseria meningitidis is a major cause of meningitis and septicemia worldwide (8) and still remains one of the leading infectious causes of death in childhood in many industrialized countries. However, the greatest burden of the disease occurs in Africa and Asia, where large epidemics periodically affect the populations.

Epidemiological studies of *N. meningitidis* have allowed a better understanding of the nature of meningococcal dissemination and of the relation between endemic and epidemic disease. During the last 2 decades, the classical phenotyping methods for epidemiological studies of meningococci have been complemented by a number of molecular methods, such as multilocus enzyme electrophoresis (MLEE), pulsed-field gel electrophoresis, randomly amplified polymorphic DNA, repetitive element-based PCR, insertion sequence analysis, ribotyping, restriction fragment length polymorphism analysis of PCR products, sequencing of individual genes that may be related to virulence (8), PorA VR typing (28), 16S rRNA sequencing (29), and multilocus sequence typing (MLST) (21). PorA VR typing, which is used to predict the amino acid sequences of the PorA outer membrane protein, is derived from DNA sequence analysis (28). A website for comparison of *porA* sequences from different laboratories (<http://neisseria.org/nm/typing/pora>) is now in widespread use by the research community and ensures consistency in identifying and naming variants of this protein. The gene encoding 16S rRNA has been used successfully to monitor and identify epidemiologically related isolates of meningococci (29), especially those associated with the 2000 Hajj

outbreak in Saudi Arabia caused by *N. meningitidis* serogroup W135 (23, 25). 16S rRNA sequencing was the most sensitive (100%) and the most specific (98%) method in identifying the Hajj-related isolates (22).

MLEE and MLST have been considered the “gold standard” for epidemiological study of meningococci. MLEE has demonstrated significant linkage disequilibrium between alleles in populations of meningococci and the presence of a cluster of isolates that share identical or very similar multilocus allelic profiles (10). A major problem with MLEE is that the results from different laboratories are difficult to compare. MLST is an adaptation of MLEE that takes advantage of the speed and simplicity of automated DNA sequencing. MLST is based on sequence data from seven conserved housekeeping genes; unique sequences are assigned to different numeric alleles. Sequences that differ at even a single nucleotide are assigned to different alleles. The combination of alleles at the seven housekeeping genes is designated the sequence type (ST) of the isolate; STs can be compared electronically via the Internet. Isolates that differ in one to three alleles may constitute an ST complex. The accumulation of nucleotide changes in housekeeping genes is a relatively slow process, and the allelic profile of a meningococcal strain is stable over time. Therefore, MLST is a powerful resource for global epidemiology of meningococci (21). However, despite many advantages, MLST does not necessarily provide the discrimination desired for fine typing of some clonal groups of *N. meningitidis* (1).

Short DNA tandem repeats in coding or promoter areas of genes have been recognized as an important mechanism for controlling the expression of meningococcal surface antigens (15, 22). The number of tandem repeats can be modified during replication through slipped-strand mispairing, thus influ-

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TABLE 1. Properties of isolates recovered in connection with local outbreaks in different counties in Norway

Group	County	No. of isolates	Yr of isolation	Case or carrier	Phenotype	Clone complex	Source or reference
I	Møre and Romsdal	2	2002	Case	B:4, 7:P1.22,22a	ST-32	This study
II	Rogaland	2	1996	Case	B:15:P1.12,13a	ET-5	This study
	Hordaland	1			B:15:P1.12,13a		
	Akershus	1			B:15:P1.12,13a		
III	South Trøndelag	12	1995–1997	Case	B:15:P1.7,16	ET-5	This study
		4	1996	Carrier	B:15:P1.7,16 or NG:15:P1.7,16 ^a		
IV	Hordaland	8	1995–1996	Case	C:15:P1.7,16	ET-5	33
		12	1996	Carrier	C:15:P1.7,16 or NG:15:P1.7,16 ^b		
V	Møre and Romsdal	3	1995	Case	C:2a:P1.5,2	ET-15/37	This study

^a Three isolates belonged to serogroup B, and one isolate could not be assigned to a group (NG).

^b Four isolates belonged to serogroup C, and eight isolates could not be assigned to a group (NG).

encing translation or transcription (13). Complete-genome analyses of *N. meningitidis* MC58 and Z2491 have shown the largest repertoire of putative phase-variable genes ever described in any species (30, 34).

Hypervariable minisatellite regions have been utilized to detect DNA fingerprints in the human genome (24) and in other eukaryotes such as *Candida albicans* (20), *Candida krusei* (31), *Leishmania infantum* (6), and *Sphaeropsis sapinea* (7). In the last 5 years, multilocus variable-number tandem repeats (VNTR) have also been used for molecular typing of several bacterial species, including *Bacillus anthracis* (12), *Yersinia pestis* (16), *Mycobacterium tuberculosis* (32), *Haemophilus influenzae* (36), *Francisella tularensis* (14), *Xylella fastidiosa* (11), *Legionella pneumophila* (26), *Staphylococcus aureus* (29), *Salmonella enterica* (17, 19), and *Escherichia coli* O157 (18).

The availability of the whole-genome sequences of *N. meningitidis* MC58 and Z2491 has opened the way for the evaluation of repetitive DNA motifs and their application to epidemiological investigations. In this study, VNTR loci were identified by a bioinformatics approach. These were applied to typing of a panel of *N. meningitidis* strains which had previously been characterized by MLST or MLEE.

MATERIALS AND METHODS

Bacterial strains. A total of 146 *N. meningitidis* isolates were included in this study. They comprised (i) 82 of the 107 isolates previously studied by Maiden et al. (21) for the establishment of MLST (75 of these isolates represented the seven major clone complexes causing disease worldwide [the ST-1, ST-4, ST-5, ST-8, ST-11, ST-32, and ST-41/44 complexes], while 7 were selected from the 32 isolates not assigned to a clone complex), (ii) 45 isolates recovered from various counties in Norway in connection with local outbreaks (Table 1), and (iii) 19 ST-11 serogroup W135 isolates recovered in several parts of the world between 1994 and 2002, including isolates related to the Hajj outbreak of 2000 and isolates from outbreaks in Burkina Faso in 2001 and 2002. Some of these W135 isolates (M 7034, S 292/97, S 29/99, and MK 89/94) have been studied previously by using various molecular techniques (23).

Phenotyping characterization. Serogrouping, serotyping, and serosubtyping of the isolates were performed using monoclonal antibodies by a dot blot method as described previously (37).

Preparation of crude bacterial DNA. Meningococcal isolates, stored at -70°C , were plated onto chocolate agar and incubated overnight at 37°C under a 5% CO_2 atmosphere. A loopful (10 μl) of bacterial growth was removed from the plate, suspended in 100 μl of 1 M Tris-EDTA buffer (pH 8.0) in an Eppendorf

tube, and boiled for 10 min. After centrifugation at $3,700 \times g$ for 10 min, the supernatant was transferred to a new tube and used for further analyses.

MLST. MLST was performed as described previously (21). Primers, determination of allele sequences, and designation of STs and ST complexes were as described at <http://pubmlst.org/neisseria>.

MLEE. MLEE was performed for 43 isolates (Table 1) as previously described (9). Distinctive multilocus genotypes were assigned electrophoretic type (ET) designations.

Identification of VNTR loci. The genomes of *N. meningitidis* strains MC58 (NC_003112) and Z2491 (NC_003116) were screened for repetitive DNA with the Tandem Repeats Finder, version 3.21 (4), which is available for free at tandem.bu.edu/tools.html. The program generates an output file giving the repeat location, the repeat segment size, the nucleotide composition, and the copy number of the array. Fifteen VNTR loci were selected on the basis of a repeat length between 4 and 30 bp and a copy number ranging from 3 to 36. These VNTR loci are presented in Table 2.

VNTR-PCR and data analysis. PCR primers were designed from the sequences flanking the repeats of the 15 loci. Primers were designed by using the free program available at http://biotools.umassmed.edu/bioapps/primer3_www.cgi. Amplification was conducted in a 50- μl reaction mixture on a Gene Amp 9700 PCR system (Applied Biosystems) with AmpliTaq polymerase (Applied Biosystems) and $1 \times$ PCR buffer containing 1.5 mM MgCl_2 . After an initial denaturation at 94°C for 5 min, the reaction was performed for 32 cycles at 94°C for 1 min, at different annealing temperatures depending on the primers for 1 min, and at 72°C for 1 min, 30 s, followed by an extension step at 72°C for 5 min. Primers and annealing temperatures for the four VNTR loci retained (VNTR01, -02, -06, and -08) are shown in Table 3. Five microliters of each of the PCR products was electrophoresed in 2% SeaKem ME agarose (Cambrex Bio Science, Rockland, Maine). Then portions (5 μl) of the PCR products resulting from the amplification of the four VNTR for each isolate were mixed together in a single tube. The PCR product pools were added to 5 μl of loading buffer prior to being run on a 2% agarose gel at 40 V for 18 h. A 1-kb DNA ladder (Invitrogen) was used as a standard. The gel was stained with ethidium bromide for 10 min, washed in water for 20 min, and photographed under UV illumination.

The gel photograph was analyzed by using BioNumerics software (Applied Maths BVBA, Sint-Martens-Latem, Belgium), and a dendrogram was constructed by using the Dice coefficient of similarity and cluster analysis with the unweighted-pair group method with arithmetic averages (UPGMA). Both the position tolerance and the optimization were set up at 1%, as recommended in the BioNumerics software manual.

Nucleotide sequence determination. Nucleotide sequences of the amplified regions were determined for nine arbitrarily chosen isolates. Among these, eight isolates were from the collection used for establishment of MLST (21) and one W135 isolate was from the epidemic in Burkina Faso. The primers for nucleotide sequence determination were the same as the primers for PCR amplification of the four VNTR loci. DNA sequencing was performed using the ABI Prism Big

TABLE 2. Selected VNTR loci on the genome of *N. meningitidis*

Locus	Repeated length (bp)	No. of repeat units	Consensus pattern	Location ^a	Function	GenBank accession no.
VNTR01	7	36	CAAACAA	657231–657481	Putative galactosyltransferase-related protein	NC_003112
VNTR02	13	29	GGGCTGTAGAGAT	1131155–1131527	Unknown	NC_003112
VNTR03	15	15	GCTGAAGCTCCTGCT	1572374–1572592	Unknown	NC_003112
VNTR04	18	15	GGTATTCCTGACGATTCA	1952031–1952308	Unknown	NC_003112
VNTR05	15	13	TTTTCTGTTTCGCTG	2158562–2158750	Major outer membrane protein	NC_003112
VNTR06	9	9	GGCAACTTT	2158511–2158594	Putative rotamase	NC_003116
VNTR07	6	5	TCITCA	863508–863539	ATP-dependent Clp protease	NC_003112
VNTR08	9	7	GCCAAAGCT	285906–285971	Unknown	NC_003112
VNTR09	24	4	GCAAAACAACAGGCGCGGCATCA	1902204–1902308	Putative PilQ protein	NC_003112
VNTR10	5	16	TTGGG	1273590–1273680	Unknown	NC_003112
VNTR11	10	3	TAATCCACTA	1303221–1303253	Noncoding region	NC_003112
VNTR12	28	5	GGGAATGACGGAATGTTGCGGGAATCAT	197212–197358	Noncoding region	NC_003112
VNTR13	24	4	TTCGTGGGAATGACGGGATGTAGG	52222–52319	Noncoding region	NC_003112
VNTR14	19	4	GCGGGAATGACGAAAGGTT	2095123–2095210	Unknown	NC_003112
VNTR15	5	17	AAGAG	940260–940343	Putative opacity protein	NC_003112

^a The locations of VNTR loci were based on *N. meningitidis* MC58, except for VNTR06, which was based on Z2491.

Dye Terminator cycle sequencing ready reaction kit and an ABI Prism 377 sequencer (both from Applied Biosystems).

RESULTS

We selected 15 different sequence areas containing tandem repeats in the genome of *N. meningitidis* MC58 and confirmed their presence in strain Z2491. By running PCR for a set of at least 17 genetically diverse meningococcal isolates, loci 3, 7, 9, 10, and 11 gave a single PCR band, but there was little polymorphism among the isolates. Loci 4, 5, 13, 14, and 15 were polymorphic among the tested isolates but resulted in multiple PCR products (one to four bands), while locus 12 was monomorphic but gave multiple PCR products. Thus, only loci 1, 2, 6, and 8 gave a single amplicon polymorphic among various meningococcal isolates. These were selected for further analysis of the 146 isolates. The specificity of the primers for these loci was confirmed by determining the nucleotide sequences of the VNTR for nine arbitrarily chosen isolates. The in vitro stability of the VNTR patterns was determined by daily passaging *N. meningitidis* strain MC58 for 32 days. DNA was isolated every 4th day, and PCR was run with VNTR primers

for loci 1, 2, 6, and 8. The VNTR patterns did not change with laboratory passage.

The VNTR patterns and subsequent dendrograms generated by the BioNumerics software were analyzed for the seven major hypervirulent clone complexes. When the PCR products of the four VNTR were mixed, only three bands were detected in some isolates (see Fig. 1 and 2), as a result of equal or very similar PCR product sizes in these samples. The intensity of the PCR product was used to identify the double bands. All seven hypervirulent clone complexes included strains with highly diverse VNTR patterns. Meningococcal isolates within the ST-1, ST-4, ST-5, ST-32, and ST-41/44 complexes displayed 35 to 100% similarity. Similarities between 40 and 85% were observed among isolates within ST complexes 8 and 11, and among isolates with STs not assigned to a complex. The variation in the VNTR pattern within each ST complex is summarized in Table 4. The construction of a dendrogram, based on VNTR analysis, with these isolates displayed cluster formation also among genetically unrelated meningococcal isolates (data are available on request).

VNTR patterns of meningococcal isolates from associated cases and local outbreaks in various counties of Norway were analyzed (Table 1). Figure 1 shows the VNTR variation in meningococcal isolates belonging to the ET-5/ST-32 complex. The VNTR patterns were similar for isolates from the same geographical area but different for isolates from different geographical areas in Norway.

Isolates in group I (N 7/02 and N 11/02), recovered from a mother and her baby a few days apart, had identical VNTR patterns (Fig. 1). Group II comprised four isolates with phenotype B:15:P1.12,13a. Two of these isolates (N 100/96 and N 66/96) were from cousins and shared the same VNTR pattern, whereas the other two isolates (N 44/96 and N 109/96), which were from patients in different counties, had only 75% similarity to the first two. The ET-5 isolates with phenotype B:15:P1.7,16, recovered from 12 patients in South Trøndelag county (group III) during a localized but long-lasting outbreak in 1995 to 1997, had very similar VNTR patterns. Furthermore, four isolates (MK 105/96, MK 64/96, MK 155/96, and MK 124/96) from healthy carriers in the same area, which had the same

TABLE 3. PCR primers used for VNTR analysis of meningococcal strains in this study

Primer designation	Primer sequence (5'→3')	Annealing temp (°C)	Product size ^a (bp)
VNTR01 F	GACGGGTCAAAGACGGAAG	59	485
VNTR01 R	GGCATAATCCTTCAAAACTTCG		
VNTR02 F	CTCCCGATAGGCCCGAAATACC	57	606
VNTR02 R	AAAGCGGCGGGAATGACGAAGAGT		
VNTR06 F	CCGGCGGCGGTGATGACTT	63	466
VNTR06 R	GCAGAAACCCGACAGCAGGATGG		
VNTR08 F	GACCCTGACCGTCGGAACCC	57	312
VNTR08 R	ATACCGCCTGCTGTTGTGC		

^a PCR product sizes for VNTR01, -02, and -08 are from *N. meningitidis* MC58 (GenBank accession no. NC_003112), and that for VNTR06 is from *N. meningitidis* Z2491 (GenBank accession no. NC_003116).

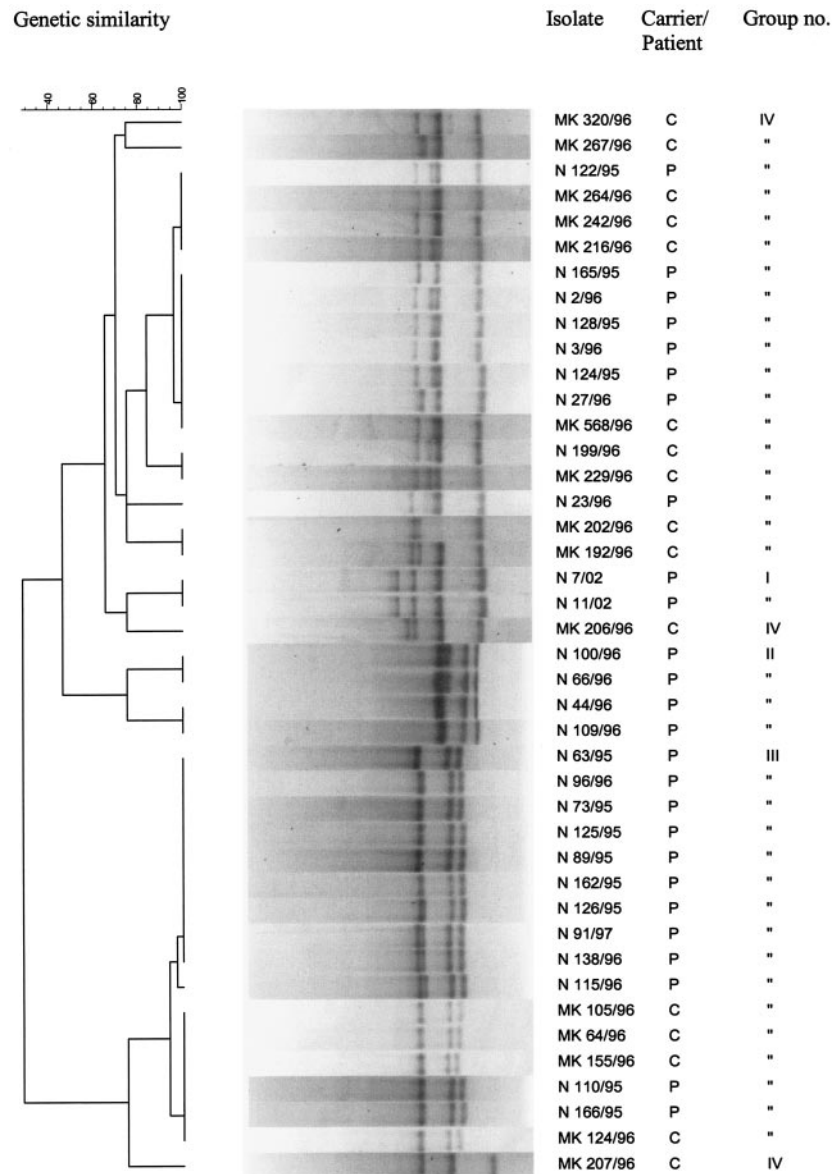


FIG. 1. VNTR-based genetic relationships among selected meningococcal isolates (ET-5/ST-32 complex) recovered from related cases and local outbreaks in different counties of Norway, 1995 to 2002. The left side of the gel photo is the top of the gel.

genotype and the same phenotype as the outbreak strain (except for MK 64/96, which could not be assigned to a group), had identical VNTR patterns and showed high degrees of similarity with the disease-associated isolates. Meningococcal isolates in group IV were isolated from 8 patients and 12 carriers in Hordaland in 1995 and 1996 (33). All disease-associated isolates belonged to ET-5 and had the same phenotype, C:15:P1.7,16. The isolates from carriers belonged to ET-5 and had the same serotype and serosubtype as the patient strains, but only four belonged to serogroup C, while the remaining carrier isolates could not be assigned to a group. All but 2 of these 20 isolates showed closely related VNTR patterns, distinct from those presented by strains in groups I, II, and III (Fig. 1). Six of the eight disease-associated isolates (N 165/95, N 2/96, N 128/95, N 3/96, N 124/95, and N 27/96) were grouped

TABLE 4. Variation in VNTR patterns among meningococcal isolates of the different ST complexes, used for the establishment of MLST

ST complex	No. of isolates	No. of STs	No. of VNTR types	Source ^a	Yr
ST-1	14	3	9	AU, NA, AS, EU, AF	1963–1980
ST-4	11	1	7	NA, AS, AF	1937–1990
ST-5	12	3	11	SA, AS, EU, AF	1967–1996
ST-8	8	4	8	EU, AU, AF	1967–1992
ST-11	10	1	10	EU, AF, NA, AS, SA	1969–1993
ST-32	10	3	8	EU, SA	1976–1982
ST 41/44	10	5	7	EU, SA, AU	1963–1996
Others	7	7	7	EU	1988–1994

^a AF, Africa; AS, Asia, including India; AU, Australia, including New Zealand; EU, Europe, including Russia and Iceland; NA, North America; SA, South America.

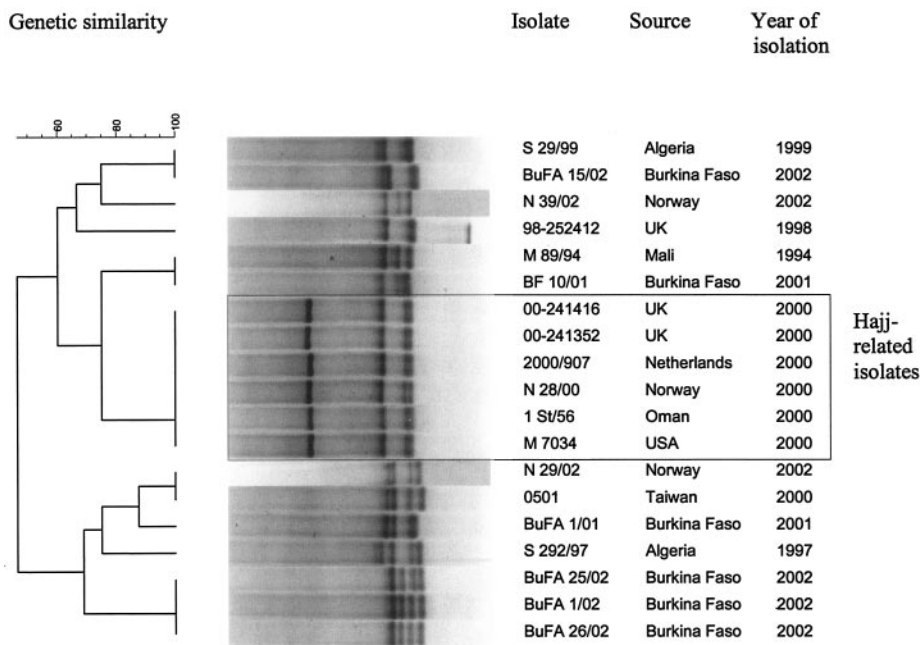


FIG. 2. VNTR-based genetic relationships among serogroup W135 meningococcal isolates of the ST-11 complex. The left side of the gel photo is the top of the gel.

at 100% similarity in the dendrogram, although slight variations in the VNTR patterns were visible. The seventh disease-associated isolate (N 122/95) showed a high degree of similarity in the VNTR pattern with the first six, and the last disease-associated isolate (N 23/96) showed similarity with the other seven isolates at two loci but differed at two others, VNTR01 and VNTR02. Four of the 12 isolates (Mk 264/96, MK 242/96, MK 262/96, and MK 568/96) carried by healthy children showed a high degree of similarity to the major group of the disease-associated isolates. One carrier isolate (MK 207/96) harbored a very different VNTR pattern. While all of these 12 isolates belonged to the ET-5 clonal complex, the restriction fragment length polymorphism of chromosomal DNA with the restriction enzyme HaeIII demonstrated five different fingerprints (unpublished data), while the VNTR study differentiated seven patterns among these isolates.

Isolates in group V (N 9/95, N 10/95, and N 16/95) had phenotype C:2a:P1.5,2 and belonged to ET-15 (3) of the ET-37 complex (ST-11). These isolates were recovered from patients in the same town within a period of 6 weeks. They all had exactly the same VNTR pattern (data not shown).

The VNTR patterns of W135 isolates belonging to ST-11 are shown in Fig. 2. The results indicated that the serogroup W135 isolates recovered from cases of meningococcal disease related to the Hajj 2000 outbreak in the United Kingdom, The Netherlands, Norway, the United States, and Oman were similar to each other but distinct from those recovered from outbreaks in Burkina Faso in 2001 and 2002, and from other W135 ST-11 isolates recovered from various parts of the world. Six Hajj-related isolates from the United Kingdom, Norway, The Netherlands, the United States, and Oman showed exactly the same VNTR pattern (Fig. 2) characterized by a large PCR product (approximately 1,350 bp) obtained with the VNTR01 primers.

DNA sequence analyses of the PCR product and a BLAST search (2) of the DNA sequence in GenBank showed that the large PCR product contained 36 tandem repeats (CAAACAA) and an additional DNA sequence, which proved to be an insertion (IS) element (IS1016). The six W135 isolates (BuFA 15/02, BuFA 10/01, BuFA 1/01, BuFA 25/02, BuFA 1/02, and BuFA 26/02) recovered from the outbreaks in Burkina Faso in 2001 and 2002 presented four different VNTR patterns. Two ST-11 W135 isolates (N 39/02 and N 29/02) recovered from non-ethnic Norwegians in 2002 also showed different VNTR patterns.

DISCUSSION

Meningococcal isolates with known phenotypic and MLST or MLEE data recovered from carriers and patients in Norway and various parts of the world were analyzed for the presence of variable tandem repeats. The majority of the tandem repeat loci in meningococci are in or near genes that encode proteins with phase-variable properties (30, 34). The analysis of tandem repeat areas showed that 5 of 15 loci studied in this work had low polymorphism in a genetically diverse set of isolates. The tandem regions with longer repeats, e.g., VNTR09, -12, -13, and -14, were not suitable for VNTR analysis. In vitro experiments showed that tandem repeats of loci 1, 2, 6, and 8 in *N. meningitidis* MC58 were stable during laboratory passage. We have also studied VNTR stability during long-term carriage of meningococcal clones. The results of our preliminary analyses indicate that change in tandem repeats is not common (unpublished data).

Our data did, however, demonstrate the rapid evolution of VNTR patterns in comparison to phenotype and genotype. VNTR analysis of isolates used for the establishment of the

MLST scheme (21) showed that this VNTR assay is not appropriate for typing meningococci isolated over a long time span and from different geographical areas. However, the method has potential for providing further information to enable differentiation of meningococcal isolates that are identical by MLST.

The analysis of isolates from local outbreaks caused by the ET-5 (ST-32) complex showed that the different outbreaks can be delineated by the VNTR patterns of the isolates. This can be exemplified by the outbreak in South Trøndelag county in 1995 to 1997, where all isolates belonged to the ST-32/ET-5 complex and showed high similarities in VNTR patterns, ranging between 90 and 100%. A high degree of variability was found overall in this clonal complex. MLST has shown limited ability to distinguish between isolates formerly assigned to the ET-37 complex by MLEE. Nearly all isolates of the ET-37 complex belong to ST-11, which makes MLST inadequate for distinguishing between different outbreaks due to this hyper-virulent lineage. In contrast, the VNTR analyses of 10 ST-11 isolates used for the establishment of MLST revealed 10 different patterns, and the 19 serogroup W135 ST-11 isolates were found to be very heterogeneous as well. The ST-11 complex includes isolates of a variety of serogroups, and capsule switching between these serogroups occurs readily, due to horizontal gene transfer of the capsule biosynthesis operon between strains. Meningococcal isolates with serogroup C and W135 belonging to the ST-11 complex were differentiated by our VNTR analysis (data not shown), indicating that various genetic events have occurred, in addition to the change in the genes involved in capsule synthesis.

The six Hajj-related isolates recovered from five countries in spring 2000 had identical VNTR patterns, confirming that the 2000 Hajj outbreak, which was the first large worldwide outbreak caused by W135, was due to a single clone (23). An intact *IS1016* in the VNTR01 locus was found in the Hajj-related isolates in this study. In *N. meningitidis*, the *pglE* gene, which encodes the glycosyltransferase (PglE), is essential for pilin glycosylation (27). The heptanucleotide repeats (CAACAAA) in the *pglE* gene are responsible for the phase variation between trisaccharide and disaccharide structures in pilin. The insertion of *IS1016* may have inactivated pilin glycosylation in Hajj 2000-related isolates. An intact *IS1016* was reported, for the first time, in *N. meningitidis* serogroup X (35), which was found within the intergenic region separating *ctrA* and *xcbA* in the capsule genetic loci. None of the W135 ST-11 isolates recovered before the Hajj outbreak harbored *IS1016* in the VNTR01 locus, and the element did not occur in the VNTR01 loci of strains from cases in Burkina Faso in the following years. However, the possibility that *IS1016* is present in other areas of the genomes of meningococcal isolates other than Hajj-related isolates should not be discounted. The instability of the IS element may be the reason for the absence of *IS1016* in the VNTR01 locus in the W135 ST-11 isolates recovered after the 2000 Hajj outbreak. Our analysis also demonstrated the microheterogeneity of the W135 strains causing the epidemics of meningococcal disease in Burkina Faso in 2001 and 2002, with four different VNTR patterns identified among the six strains studied.

In conclusion, we showed that VNTR typing might be a useful differentiating method for short-term epidemiology of

meningococcal isolates in connection with outbreaks. VNTR analysis may be used for fine typing of meningococcal isolates belonging to the same clonal complex, as characterized by MLST or MLEE. The method might be especially valuable for distinguishing among ST-11 strains, as shown in particular by the VNTR analyses of serogroup W135 ST-11 isolates recovered since the mid-1990s. Based on PCR primers tagged with different fluorescent dyes, the method can be automated and become a rapid genotyping assay.

ACKNOWLEDGMENTS

This work was supported by the EU-MenNet project of the European Commission, contract QLK2-CZ-2001-01436.

The excellent technical assistance of Torill Alvestad is greatly appreciated.

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