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Molecular Epidemiology of *Neisseria meningitidis* Isolated in the African Meningitis Belt between 1988 and 2003 Shows Dominance of Sequence Type 5 (ST-5) and ST-11 Complexes

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At the two World Health Organization Collaborating Centers for Reference and Research on Meningococci in Marseilles, France, and Oslo, Norway, the multilocus sequence typing technique was used for the characterization of a total of 357 strains of meningococci isolated from meningitis cases in 13 African countries of the meningitis belt between 1988 and 2003. Among these strains, 278 of 357 (77.9%) belonged to the sequence type 5 (ST-5) complex; 23.2% were ST-5 and 53.5% were ST-7. ST-5 was probably introduced in Africa in 1987 and was responsible for most of the meningitis cases between 1988 and 2001. ST-7 emerged in the mid-1990s and has totally replaced ST-5 since 2002. These two STs characterized serogroup A strains and have been responsible for hundreds of thousands of cases. Fifty-two strains (14.3%) belonged to the ST-11 complex. The ST-11 complex was characterized by serogroup W135, which has been responsible for an increasing number of sporadic cases since 2000 and the first W135 epidemic ever seen in Africa (in Burkina Faso in 2002). Identification of W135 ST-11 strains in many countries is a great concern for the region. Apart from these two major clonal complexes, a few other clones, such as ST-2881, ST-181, and ST-751, were sporadically detected. Careful surveys for these clones need to be conducted, but at present they play only a minor role in the overall epidemiology of meningococcal meningitis.

Meningococcal meningitis is a great concern in the African meningitis belt, described in 1963 by Lapeyssonnie (12). Meningococcal meningitis occurs every year during the dry season, between December and May, and large epidemics are reported every 5 to 10 years. The introduction of a new strain into a susceptible population and environmental factors, such as dryness, are significant factors related to the occurrence of an epidemic (16). In 1996, the most important epidemic of meningococcal meningitis ever seen in Africa occurred, with more than 150,000 reported cases (31). In the past 40 years, most of these epidemics have been caused by serogroup A meningococci (1), but serogroup C and, more recently, serogroup W135 have been also involved.

Since the mid-1980s multilocus enzyme electrophoresis (MLEE) has been the reference method for the global epidemiology of *Neisseria meningitidis*. This method identified clusters of closely related strains, e.g., subgroup III of serogroup A, and permitted monitoring of their clonal spread throughout the world (2). However, this method relies on the indirect assignment of alleles based on the electrophoretic mobilities of enzymes (25). Maiden et al. adapted the method and used the nucleotide sequences of internal fragments of seven housekeeping genes to directly identify alleles (13). Multilocus sequence typing (MLST) permits the characterization of each strain by its sequence type (ST). Closely related STs are grouped into clonal complexes by their similarities to a central ST, along with feedback from public health laboratories and epidemiologists. MLST gives results that are unambiguous and distinguishes more alleles per locus than MLEE, allowing a high level of discrimination between isolates. The first data published by Maiden et al. showed a good congruence between MLST and MLEE (13).

At the two World Health Organization (WHO) Collaborating Centers for Reference and Research on Meningococci in Marseilles, France, and Oslo, Norway, the MLST technique was used for the characterization of 357 meningococcal isolates recovered from meningitis cases between 1988 and 2003 in 13 countries of the African meningitis belt. This work presents data on the genotypes that circulated in the region during those 15 years. In association with the epidemiological data, information on the responsibilities of STs and clonal complexes for endemic cases and outbreaks in Africa improves our understanding of the disease. Such studies may permit the development of hypotheses on the evolution of meningococcal disease in the African meningitis belt and should help to prepare responses that are the most adapted to the region.

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MATERIALS AND METHODS

Countries. Only cases from countries belonging to the African meningitis belt were included in this study. In these 17 countries, ranging from Ethiopia in the east to Senegal in the west, the typical epidemiological features of meningococcal meningitis, as described by Lapeyssonnie (12), are observed. The two collaborating centers have also received strains from other African countries, but we chose to focus on the meningitis belt to provide a homogeneous analysis.

Study period. This study was performed between 1988 and 2003. The year 1988 was chosen, as the 1987 outbreak in Mecca, Saudi Arabia, favored the introduction of serogroup A strains belonging to subgroup III (now designated the ST-5 complex) in Africa. In 1988, this epidemic strain emerged for the first time in Africa and was responsible for outbreaks in Chad, Ethiopia, and Sudan (15, 19, 24, 29).

Isolates. A total of 357 isolates received at the WHO collaborating centers in Marseilles and Oslo, were included in this study. All these isolates were from patients with meningitis; apart from one strain isolated from blood, all the strains were isolated from cerebrospinal fluid. Bacterial identification was carried out by Gram staining, the oxidase reaction, and standard biochemical tests. The strains were stored at −80°C in brain heart broth with 15% sterile glycerol or in Greaves solution.

Serogrouping, serotyping, and subtyping. *N. meningitidis* strains were serogrouped by agglutination with sera manufactured by the Institut de Médecine Tropicale du Service de Santé des Armées, Marseilles, France, or Difco (Fisher Scientific, Paris, France). Serotypes and subtypes were determined with monoclonal antibodies from the National Institute of Public Health and the Environment (Bilthoven, The Netherlands) by the whole-cell enzyme immunoassay technique or the dot blot method, as described elsewhere (8, 22, 30).

MLST. Fragments from seven housekeeping genes were used for typing: *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarase), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), and *pgm* (phosphoglucomutase), as given on the MLST website (http://pubmlst.org/neisseria/) (11). After DNA preparation and amplification by PCR, each locus sequence was analyzed on an ABI Prism 373 DNA sequencer or an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Sequence analysis was performed with Vector NTI suite software (InforMax, Bethesda, MD) and Sequence Navigator DNA and Protein Sequence comparison software (Applied Biosystems). The sequences were compared with the existing alleles on the MLST website for determination of the allele numbers, STs, and clonal complexes of the isolates.

RESULTS

Countries, year of isolation, and number of isolates. The 357 meningococcal isolates analyzed by MLST were obtained between 1988 and 2003 from the 13 following countries of the African meningitis belt: Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ethiopia, Guinea Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal, and Sudan. Many of these countries belong in only part of the meningitis belt; thus, for a few isolates it was difficult to ensure that they were really recovered from the meningitis belt. From four countries of the meningitis belt, Guinea, Ghana, The Gambia, and Togo, either no isolates were available or they had not been analyzed by MLST: between 1988 and 1994 meningococcal strains sent to the WHO collaborating center in Oslo were analyzed by MLEE; these were not included. From 1995, the number of isolates and the number of countries covered have increased (Table 1). For some countries, such as Niger, isolates have been obtained every year since 1995, but for most other countries there are gaps, as isolates are sent to the WHO collaborating centers mainly in the case of an outbreak. The number of isolates varied from 2 (Mali) to 45 (Ethiopia) and 129 (Niger).

Serogroups. Among the 357 *N. meningitidis* isolates, 279 (78.2%) were serogroup A, 62 (17.4%) were serogroup W135, 6 (1.7%) were serogroup X, 6 (1.7%) were serogroup B, 3 (0.8%) were serogroup Y, and 1 (0.3%) was serogroup C.

STs and lineages. There were 17 distinct STs that resolved into 10 lineages which occurred from 1 (0.3%) to 278 (77.9%) times in the collection of 357 isolates. Five lineages, the ST-5 complex, the ST-11 complex, the ST-32 complex, ST-181, and ST-2881, were represented by 5 or more isolates and together included 350 (98.0%) of the isolates. Others lineages were represented by only one to two strains (Table 2).

Among the strains isolated during this period, 278 of 357 belonged to the ST-5 complex. These were dominated by two closely related STs, ST-5 (23.2%) and ST-7 (53.5%), which differed only at the *pgm* locus: *pgm-3* for ST-5 and *pgm-19* for ST-7. Two strains were ST-580; one strain was ST-581, which represented single-locus variants (SLVs) from ST-5; and one was ST-2859, an SLV from ST-7 (Table 1). ST-5 strains were identified from 1988 to 2001, and ST-7 strains were identified from 1997 to 2003; all these strains were serogroup A and were found in all 13 countries (Table 1).

The ST-11 complex was the next predominant clonal complex and included 52 strains, of which 51 were ST-11 and 1 was ST-1966. All were serogroup W135. The first such strain was isolated in Chad in 1996, and the others were isolated from 2000 to 2003 in six countries (Burkina Faso, Cameroon, Central African Republic, Chad, Niger, and Senegal). ST-1966 is an SLV from ST-11 and harbors *pgm-181* instead of *pgm-6*, which is found in ST-11.

Ten strains belonged to ST-2881, a new ST that apparently emerged in 2002; all these strains were also serogroup W135 and were isolated in three countries, Benin, Niger, and Nigeria, in 2003. ST-2881 differs from ST-11 at six loci.

Five (1.4%) strains isolated in Niger between 1997 and 2002 belonged to ST-181 and were serogroup X. A single isolate from Burkina Faso in 2003 belonged to ST-751 and was a double-locus variant (DLV) from ST-181.

The ST-32 complex included four strains isolated in Cameroon in 2002: one serogroup C strain and two serogroup B strains belonged to ST-32, and another serogroup B strain was ST-2496. The ST-32 complex was also represented by one serogroup B ST-33 strain isolated in Ivory Coast in 1999. Two serogroup B strains isolated in 1996 and 2000 in Cameroon were ST-291 and belonged to the ST-41/44 complex. One ST-23 strain (ST-23 complex) and two ST-2880 strains (unassigned to a complex) were serogroup Y. These strains were isolated in Senegal in 2002 and in Niger in 2003. A single ST-4 (ST-4 complex) serogroup A strain was isolated in 1992 in the Central African Republic.

Diversity of housekeeping genes and STs. The total number of alleles present at each locus for the 357 isolates included six for *abcZ*; seven *for adk*, *aroE*, *fumC*, and *pdhC*; eight for *pgm*; and nine for *gdh* (Table 3). The number of polymorphic sites present at each locus was between 15 (3.2% of the sites for *adk*) and 93 (19.0% of the sites for *aroE*). These data are compared in Table 3 to those obtained for our 279 serogroup A isolates alone and to those from 107 isolates from worldwide sources (13). The number of alleles and the number of polymorphic sites were much lower in our material, but the proportions of nonsynonymous-to-synonymous substitutions were comparable except at the *aroE* locus, which showed a much higher value for our strains.

Serogroup diversity of lineages. Apart from one isolate that belonged to ST-4, 278 (77.9%) serogroup A strains belonged to

TABLE 1. Characteristics of 357 meningococci isolated in Africa between 1988 and 2003, showing the clonal expansion of ST-5 from 1988 to 2001 and of ST-7 from 1997 to 2003, and emergence of ST-11 in 2000

Yr of isolation	Country	No. of strains	Serogroup	Serotype:serosubtype	ST	Clonal complex
1988	Chad	3	A	4:P1.9	5	5
	Sudan	3	A	4:P1.9	5	5
1992	CAR[a]	3	A	4:P1.9	5	5
	CAR	1	A	4:P1.7	4	4
1993	Cameroon	3	A	4:P1.9	5	5
	Guinea Bissau	3	A	4:P1.9	5	5
1994	Cameroon	2	A	4:P1.9	5	5
	Chad	1	A	4:P1.9	5	5
1995	Burkina Faso	1	A	4:P1.9	5	5
	Burkina Faso	1	A	4:P1.9	580	5
	Cameroon	2	A	4:P1.9	5	5
	Niger	3	A	4:P1.9	5	5
1996	Burkina Faso	2	A	4:P1.9	5	5
	Cameroon	3	A	4:P1.9	5	5
	Cameroon	1	B	4:P1.9	291	41/44
	Chad	1	A	4:P1.9	5	5
	Chad	1	W135	NT:P1.2[c]	11	11
	Niger	3	A	4:P1.9	5	5
	Nigeria	3	A	4:P1.9	5	5
1997	Burkina Faso	1	A	4:P1.9	580	5
	Cameroon	2	A	4:P1.9	7	5
	Chad	1	A	4:P1.9	7	5
	Mali	2	A	4:P1.9	5	5
	Niger	6	A	4:P1.9	5	5
	Niger	2	X	NT:P1.5	181	UA[b]
	Nigeria	2	A	4:P1.9	5	5
	Nigeria	1	A	4:P1.9	7	5
1998	Cameroon	1	A	4:P1.9	7	5
	Chad	2	A	4:P1.9	7	5
	Ivory Coast	2	A	4:P1.9	5	5
	Niger	3	A	4:P1.9	5	5
	Niger	2	X	NT:P1.5	181	UA
	Nigeria	1	A	4:P1.9	7	5
	Senegal	3	A	4:P1.9	5	5
1999	Cameroon	2	A	4:P1.9	7	5
	Chad	2	A	4:P1.9	7	5
	Guinea Bissau	2	A	4:P1.9	5	5
	Ivory Coast	1	B	4:NST	33	32
	Niger	7	A	4:P1.9	7	5
	Niger	2	A	4:P1.9	5	5
	Senegal	9	A	4:P1.9	5	5
	Senegal	1	A	4:P1.9	581	5
	Sudan	11	A	4:P1.9	7	5
2000	Cameroon	10	A	4:P1.9	7	5
	Cameroon	2	W135	2a:P1.5,2	11	11
	Cameroon	1	B	4:P1.9	291	41/44
	Chad	1	A	4:P1.9	7	5
	Ethiopia	2	A	21:P1.9	7	5
	Niger	8	A	4:P1.9	5	5
	Niger	32	A	4,21:P1.9	7	5
	Senegal	2	A	4:P1.9	5	5
	Senegal	3	W135	2a:P1.5,2	11	11
2001	Burkina Faso	3	A	21:P1.9	5	5
	Burkina Faso	1	A	21:P1.9	7	5
	Burkina Faso	12	W135	2a:P1.5,2	11	11
	Cameroon	3	A	4:P1.9	7	5
	Cameroon	1	W135	2a:P1.5,2	11	11

Continued on following page

TABLE 1—*Continued*

Yr of isolation	Country	No. of strains	Serogroup	Serotype:serosubtype	ST	Clonal complex
	CAR	2	W135	2a:P1.5,2	11	11
	Chad	14	A	4,21:P1.9	7	5
	Chad	1	W135	2a:P1.5,2	11	11
	Ethiopia	1	A	21:P1.9	7	5
	Niger	1	A	4:P1.9	5	5
	Niger	1	A	4:P1.9	7	5
	Senegal	2	A	4:P1.9	5	5
	Senegal	1	W135	2a:P1.5,2	11	11
2002	Benin	1	A	4:P1.9	7	5
	Burkina Faso	9	W135	2a:P1.5,2	11	11
	Burkina Faso	1	W135	2a:P1.5,2	1966	11
	Cameroon	6	A	4:P1.9	7	5
	Cameroon	5	W135	2a:P1.5,2	11	11
	Cameroon	1	C	4:P1.16	32	32
	Cameroon	1	B	4:P1.16	32	32
	Cameroon	1	B	4:P1.7,16	32	32
	Cameroon	1	B	4:P1.16	2496	32
	Ethiopia	24	A	21:P1.9	7	5
	Niger	12	A	4:P1.9	7	5
	Niger	5	W135	2a:P1.5,2	11	11
	Niger	1	W135	2a:P1.2	11	11
	Niger	1	X	NT:P1.5	181	UA
	Senegal	1	W135	2a:P1.5,2	11	11
	Senegal	1	Y	14:NST	23	23
2003	Benin	2	A	21:P1.9	7	5
	Benin	1	W135	NT:P1.5,2	2881	UA
	Burkina Faso	9	A	4,21:P1.9	7	5
	Burkina Faso	1	A	21:P1.9	2859	5
	Burkina Faso	2	W135	2a:P1.5,2	11	11
	Burkina Faso	1	X	NT:P1.5,2	751	UA
	Ethiopia	17	A	21:P1.9	7	5
	Niger	22	A	4:P1.9	7	5
	Niger	2	W135	2a:P1.5,2	11	11
	Niger	2	W135	2a:P1.2	11	11
	Niger	1	W135	NT:P1.2	11	11
	Niger	7	W135	NT:P1.5,2	2881	UA
	Niger	2	Y	14:P1.5	2880	UA
	Nigeria	3	A	21:P1.9	7	5
	Nigeria	2	W135	NT:P1.5,2	2881	UA

[a] CAR, Central African Republic.
[b] UA, unassigned to any clonal complex.
[c] NT, nontypeable.

the ST-5 complex. Among the serogroup W135 strains, 52 belonged to ST-11 and 10 belonged to ST-2881. Among the serogroup X strains, five were ST-181 and one was ST-751. Serogroup B strains were in either the ST-41/44 complex or the ST-32 complex, one serogroup Y strain was in the ST-23 complex, and two other strains had STs that could not be assigned to a clonal complex.

Lineages and outbreaks. Between 1988 and 1997, most of the outbreaks in the African meningitis belt were caused by serogroup A strains belonging to ST-5: Chad (1988), Central African Republic (1992), Cameroon (1993), Niger (1995, 1996), Burkina Faso (1996), Mali (1997), Senegal (1998, 1999), and Guinea Bissau (1999). ST-7 was responsible for outbreaks in Chad (1998), Sudan (1999), and Ethiopia (2000 to 2003). ST-5 and ST-7 strains were isolated together in Niger and Burkina Faso in 2000 and 2001. Then, ST-5 disappeared from those countries and only ST-7 persisted. Among the other STs, only serogroup W135 ST-11 strains were responsible for important outbreaks in Burkina Faso in 2001 and 2002.

DISCUSSION

Using the MLST technique, the two WHO collaborating centers in Marseilles and Oslo have performed a study with a collection of 357 disease-associated *N. meningitidis* strains isolated during a 15-year period (1988 to 2003) in 13 countries of the African meningitis belt.

The number of alleles distinguished in our study with 357 isolates is rather small compared to that observed in other MLST studies involving fewer isolates (Table 3) (10, 13). This difference is due to the fact that these strains were recovered mainly from outbreaks of meningitis in Sahelian countries and that most of them were serogroup A, which is genetically less diverse (3).

TABLE 2. Distribution of lineages found among 357 *N. meningitidis* strains isolated in the African meningitis belt between 1988 and 2003

Clonal complex	ST	No. (%) of isolates	Serogroup
ST-5	5	83 (23.2)	A
	7	191 (53.5)	A
	580	2 (0.6)	A
	581	1 (0.3)	A
	2859	1 (0.3)	A
ST-11	11	51 (14.3)	W135
	1966	1 (0.3)	W135
Unassigned	2881	10 (2.8)	W135
Unassigned	181	5 (1.4)	X
ST-32	32	3 (0.8)	B, C
	33	1 (0.3)	B
	2496	1 (0.3)	B
ST-41/44	291	2 (0.6)	B
Unassigned	2880	2 (0.6)	Y
ST-4	4	1 (0.3)	A
ST-23	23	1 (0.3)	Y
Unassigned	751	1 (0.3)	X

Seventeen distinct STs resolved into 10 lineages were identified, and 92.4% of the strains were classified in two ST complexes. Thus, in the 15 years covered in the 13 countries studied, the molecular epidemiology of meningococcal meningitis cases can be summarized by serogroup A isolates belonging to the ST-5 complex and serogroup W135 isolates belonging to the ST-11 complex.

Serogroup A. Our study confirms that serogroup A, with 78.2% of the strains, was the most important serogroup involved in the African meningitis belt in the 15 years of this study. Only six different STs were found among serogroup A strains, and all but one isolate belonged to the ST-5 complex. The ST-5 complex was represented by five closely related STs: ST-5 (29.9%) and ST-7 (68.7%), which differ only at the *pgm* locus; ST-580 and ST-581, which are two SLVs of ST-5; and ST-2859, which is an SLV of ST-7. It is interesting that in the MLST database (accessed in January 2005), the ST-5 complex comprised 243 isolates that belong to 10 STs. Of these, 107 were ST-5, 115 were ST-7, 13 were ST-2859, and only 1 isolate was of each of the other seven STs. Thus, except for ST-2859, the genotype frequencies in the ST-5 complex in the database are similar to those in our study, which indicates a stable population with two major STs and rare closely related variants. However, the frequency of occurrence of ST-5 and ST-7 both in our study and in the database might not reflect the relative importance of these two STs as causes of meningitis. ST-5 was responsible for the severe epidemics in 1996, but ST-7 has not caused epidemics of such a scale.

Microevolution through mutation and recombination can explain genetic changes that result in the emergence of a few variants (1, 7, 17). Most of the variants are lost, but sometimes, the homogenizing effect of a sequential bottleneck might have randomly selected among a limited number of different genotypes a variant, such as ST-7, that becomes uniform in a population. Another explanation for the replacement of ST-5 by ST-7 might be the existence of differences in antigenic structures between ST-5 and ST-7, such as TbpB (33). These differences might provide a selective advantage for ST-7 in a human population that has not been exposed to these antigenic variants and, thus, that has not developed a protective immunological response.

In our study, ST-5 was isolated in Africa between 1988 and 2001 and ST-7 was isolated in Africa between 1997 and 2003. The ST-5 clone was introduced in Africa in 1987 by pilgrims returning from Saudi Arabia (15, 19). It was responsible for important epidemics in Chad and Sudan in 1988. It was also the cause of the large epidemic in Ethiopia that same year (Norheim et al., unpublished data). Between 1988 and 1999, it reached all the countries of the region and was responsible for numerous outbreaks (Table 1) (18, 19). ST-7 was identified for the first time in sub-Saharan Africa in 1997 (19, 33) and was responsible for outbreaks in Chad (1998), Sudan (1999), Ethiopia (2000 to 2003), and Niger (2003). In Niger, while the 1995 and 1996 outbreaks were due to ST-5, they were followed by outbreaks caused by a mixed population of ST-5 and ST-7 in 1999, 2000, and 2001; but in 2002 only ST-7 strains were isolated. The same shift probably occurred in Burkina Faso. Ten years separated the ST-5 from the ST-7 outbreaks in Chad, and 11 years separated the ST-5 from the ST-7 outbreaks in Sudan. As it took 10 to 11 years for ST-5 to reach Senegal and Guinea

TABLE 3. Genetic variation at MLST loci in all 357 isolates and the 279 serogroup A isolates from the African meningitis belt compared to that in 107 strains isolated worldwide[a]

Locus	Size (bp)	357 African isolates			279 serogroup A isolates			107 worldwide strains		
		No. of alleles	No. (%) of polymorphic sites	*dn*/*ds*[b]	No. of alleles	No. (%) of polymorphic sites	*dn*/*ds*	No. of alleles	No. (%) of polymorphic sites	*dn*/*ds*
abcZ	432	6	59 (13.6)	0.054	2	26 (6.0)	0.031	15	75 (17.4)	0.050
adk	465	7	15 (3.2)	0.014	3	2 (0.4)	0	10	17 (3.7)	0.020
aroE	489	7	93 (19.0)	1.835	2	5 (1.0)	0	18	166 (34.0)	0.293
fumC	465	7	23 (4.9)	0.025	1	0		19	38 (8.2)	0.024
gdh	501	9	21 (4.2)	0.044	2	11 (2.2)	0.059	16	28 (5.6)	0.050
pdhC	480	7	44 (9.1)	0.061	1	0		24	80 (16.7)	0.070
pgm	450	8	59 (13.1)	0.123	2	47 (10.4)	0.127	21	77 (17.0)	0.121

[a] Adapted from reference 10.
[b] *dn*/*ds*, the proportion of nonsynonymous-to-synonymous nucleotide substitutions.

Bissau after its introduction in East Africa, it might be speculated that ST-7 might continue to spread until 2008 to 2009. In addition, monitoring for ST-2859, which appeared in 2003 in Niger and Burkina Faso, needs to be conducted to determine whether it will emerge as a new problem in the future. We have not observed the emergence of a particular clone in a single country or a region of the Sahel: first ST-5 and then ST-7 were involved in all the countries.

The MLST technique uses housekeeping genes that are known to accumulate variations very slowly, which permits the easy monitoring of the spread of clones throughout the world. However, by using three outer membrane protein genes, one secreted protein gene, one housekeeping gene, and a defective insertion sequence, it was shown that strains of ST-5 and ST-7 had accumulated substantial microheterogeneity; and it might be speculated that ST-7 isolates have also become more fit (33). The last study designated a frequent genotype plus its epidemiologically associated descendants as a genocloud: after 1987, in the meningitis belt, ST-5 isolates could be classified into genocloud 5, 6, 7, or 9, while ST-7 strains could be classified into genocloud 8; this last genocloud is responsible for the third pandemic of subgroup III meningococci (33).

Serogroup W135. Sixty-two strains (17.4%) were serogroup W135, of which 52 belonged to the ST-11 complex and 10 belonged to ST-2881. In the MLST database (accessed in January 2005), among the 138 serogroup W135 strains belonging to the ST-11 complex, 132 were ST-11 and 6 single strains belonged to six different STs, respectively.

Since 1993, W135 ST-11 isolates have been recovered in the African continent: Algeria, Mali, Ghana, and The Gambia (14). In 2000 a global outbreak that began in Saudi Arabia was caused by a W135:2a:P1.5,2 strain of *N. meningitidis* belonging to ST-11 (23, 27). Even though such strains have already been isolated in Africa, it is probable that the annual Hajj amplified the spread of this clone, resulting in an increase in the number of cases caused by serogroup W135 strains belonging to the ST-11 complex in Africa. Between 2000 and 2003, our two collaborating centers obtained such strains from Burkina Faso, Cameroon, the Central African Republic, Chad, Niger, and Senegal. In particular, the severe epidemic in Burkina Faso in 2002 (>12,000 cases) was associated with this clone (32).

ST-2881 emerged in 2002; and in our study, serogroup W135:NT:P1.5,2 ST-2881 strains were isolated in 2003 in Nigeria, Benin, and Niger. We recently showed that this is the first evidence of ST-2881 strains involved in cases of sporadic meningitis (20). Until 2000, most of the strains genetically related to ST-2881 belonged to several serogroups and were isolated from carriers (20). The emergence of this genotype is possibly related to the important W135 strain carriage that we observed in another study (21), which favors capsule switching and exchange within the *sia* locus with meningococcus strains from carriers (26).

This recent emergence of serogroup W135 strains in several countries is of great concern. Surveys for the circulation of this new W135 clone in Africa must be conducted in the coming meningitis seasons to determine whether it will replace ST-11 as the cause of the disease or, in contrast, will confer a specific herd immunity, thus preventing further W135 outbreaks (20).

Serogroup X. The six serogroup X strains of this study belonged to two STs: ST-181 or ST-751. The latter is a DLV of ST-181. In the MLST database, 37 serogroup X isolates are assigned to four closely related STs: 24 belong to ST-181, 5 belong to ST-182, 2 belong to ST-751, and 1 belongs to ST-3507. Most of these strains were isolated from carriers in Africa. It is interesting that these genotypes were found in Mali from 1970 to 1990 and in Chad in 1995 (9), showing that the genotypes of serogroup X strains are also stable. In Niger, many serogroup X *N. meningitidis* strains were isolated between 1995 and 2000; and a small outbreak occurred in 1997, with 83 notified cases (4). This outbreak occurred just between the two important serogroup A outbreaks in 1995 and 2000. Etienne et al. (6) reported a similar outbreak in Niamey, Niger, in 1990. Even if outbreaks caused by serogroup X strains are very small in comparison with epidemics caused by serogroup A and W135 strains, the high prevalence of serogroup X carriage among children in northern Ghana (9) and in Niger (5) has been reported, which indicates the need for careful surveillance for this serogroup.

Serogroups B, C, and Y. Only a few strains belonging to serogroup B or C were isolated. These strains belonged to the ST-32 complex and the ST-41/44 complex, which are known to be responsible for hyperendemic waves of disease all over the world (2). The presence of these clonal complexes in sub-Saharan Africa is important to keep in mind in view of the lack of a vaccine effective against all cases of serogroup B disease. Only a few serogroup Y strains belonging to the ST-23 complex and ST-2880 were identified during the period; they were responsible for sporadic cases.

In summary, our data show that in the meningitis belt of Africa, three STs belonging to two clonal complexes were responsible for most of the outbreaks between 1988 and 2003. Our results confirm that these two complexes are hypervirulent lineages that display the capability to expand clonally. During periods of endemicity, the same STs were also likely responsible for most sporadic cases.

Genotyping of meningococci is important to obtain an understanding of what is happening in Africa. It also permits surveillance for the eventuality of polysaccharide switches, especially when mass vaccination campaigns are involved, although such events have not yet been clearly documented. Serogroup A strains belonging to the ST-5 complex have become well established in the meningitis belt after the 1988 outbreaks, and there is no sign yet of the emergence of a new clonal complex. For the serogroup W135 strains, the recovery of ST-2881 from patients, in addition to the ST-11 complex in several countries of the region, might be important; and the eventuality of the replacement of a clone should be monitored. Since 2000, the epidemiology of meningococcal meningitis has changed in the meningitis belt, with both the ST-5 and the ST-11 complexes associated with outbreaks simultaneously in one country (28). This underlines the need for reinforcing the bacteriological diagnosis capacities of laboratories in Africa, with the aim of identifying and rapidly characterizing the causative agent of acute bacterial meningitis. Strains must also be sent to the WHO reference laboratories to identify their genotypes and permit a better understanding of the bacterial population dynamics in relation to outbreaks and epidemics to be obtained. This strategy should help to prepare and adapt the vaccine response, as the epidemiological features of meningococcal meningitis are very similar throughout the countries of the meningitis belt.

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