

Molecular Epidemiology of Meningococci Isolated in Niger in 2003 Shows Serogroup A Sequence Type (ST)-7 and Serogroup W135 ST-11 or ST-2881 Strains

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In 2003, in the Zinder and Maradi regions (Niger), epidemics were due to serogroup A:4:P1.9 meningococci belonging to sequence type 7 (ST-7). In Niamey, only sporadic cases were reported: 55% of the meningococcus strains were in serogroup A, and 38% were in serogroup W135 and could be placed in ST-11, identical to the 2002 Burkina Faso epidemic clone, and in ST-2881, a new ST.

In the countries belonging to the African meningitis belt, severe meningococcal meningitis epidemics have a regular cyclic pattern and break out every 5 to 8 years. Until recently, most of these epidemics were due to serogroup A meningococci. However, in 2002, the first serogroup W135 meningococcus outbreak ever seen in the region was reported in Burkina Faso (12).

In this threatening context, the World Health Organization encouraged and supported reinforced surveillance in the sahelian countries of the meningitis belt, where Niger is one of the countries with the highest disease prevalence. This surveillance was performed at the Centre de Recherches Medicales et Sanitaires with identification and serogrouping by either PCR or culture of strains isolated in Niamey and by PCR of the three main causative agents of acute bacterial meningitis in remote areas (2, 3, 8, 10). A second PCR was performed on specimens positive for *Neisseria meningitidis* to identify the serogroup (10).

Of the 73 meningococcus strains isolated in Niger in 2003, 42 were sent to the World Health Organization Collaborating Centre in Marseille, France, for typing, subtyping, and genotyping by the multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) techniques. MLST is based on the sequencing of DNA fragments belonging to seven housekeeping genes characterizing each strain by its sequence type (ST) (4). Closely related STs are gathered in ST complexes. PFGE permits the separation of DNA macrorestriction fragments obtained with the endonuclease SpeI. It can show differences in DNA fingerprint patterns and can compare strains that belong to the same ST, giving better resolution.

From 1 January to 30 June 2003, a total of 8,113 suspected cases were reported in Niger, and the laboratory results for 1,840 patients were interpretable by culture and/or PCR methods: 759 (41.3%) were confirmed as meningococcal meningitis (659 by PCR only, 31 by culture only, and 69 by both culture and PCR), of which 681 (89.7%) were serogroup A and 63

(8.3%) were serogroup W135 (8). Epidemics occurred only in the Zinder and Maradi regions (8). They were due to serogroup A:4:P1.9 meningococci belonging to ST-7 and the ST-5 complex. In the capital, Niamey, 69 sporadic meningococcal cases were confirmed by culture and/or PCR, of which serogroups A, W135, and Y represented, respectively, 55, 37.7, and 4.3%. The strains selected for genotyping were all of the 11 W135 strains, the 3 available serogroup Y strains, and 1 of the 18 serogroup A strains. The latter belonged to ST-7 (ST-5 complex). Of the three serogroup Y strains, two belonged to ST-2879 and one belonged to ST-2880. The 11 serogroup W135 strains could be classified into two STs. Five strains (W135:2a:P1.5,2 or W135:2a:P1.2) belonged to ST-11, the ST that was responsible for the outbreak in Saudi Arabia in the year 2000, six strains (W135:NT:P1.5,2) belonged to ST-2881, a new ST that differed from ST-11 by six out of seven loci analyzed. By the PFGE technique, the fingerprint patterns of the ST-11 strains were all identical and showed a one-band difference from the year 2000 epidemic strains. Of the ST-2881 strains, five showed identical fingerprint patterns and one differed by one band. Their PFGE patterns were totally different from the ST-11 epidemic strain patterns.

Serogroup A:4:P1.9 meningococci belonging to ST-7 and the ST-5 complex have been isolated in Niger since 1999 and were responsible for epidemics that occurred in the Zinder and Maradi regions in 2003. These strains are identical to the strains responsible for recent outbreaks in Chad (in 1998) and Sudan (in 1999) (6). ST-7 strains are replacing ST-5 strains, which have been responsible for very important outbreaks in Niger in 1995 and 1996 and severe outbreaks in Africa since 1988 (6). ST-5 and ST-7 are closely related, differing only in the *pgm* locus (*pgm-3* is characteristic of ST-5, and *pgm-19* is characteristic of ST-7). ST-7 will probably be responsible for the next epidemic wave of serogroup A in Africa. Recently, we showed that a third pandemic due to serogroup A strains began in China in 1993, causing large epidemics in Mongolia in 1994 and in Moscow in 1996, reaching Africa in 1995 (13). The strain associated with this pandemic can be readily recognized by its genotype, ST-7.

Serogroup W135:2a:P1.5,2 meningococcus strains that belonged to ST-11 and the ST-11 complex were responsible for a

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global outbreak of meningococcal meningitis in the year 2000 that began during the pilgrimage in Saudi Arabia (5, 7, 9). Many cases were reported among returning pilgrims and their close contacts in Europe, the United States, Asia, and Africa. Its characteristic fingerprint pattern determined by PFGE with the endonuclease SpeI permitted the tracing of this epidemic clone all over the world (5). In 2001, serogroups W135:2a:P1.5,2 and A:4:P1.9 were found equally in Niger and Burkina Faso (11). The former was responsible for the 2002 outbreak in Burkina Faso. In Niger, meningococcal meningitis has so far been due mainly to serogroup A and rarely to serogroup X (1). During the 1980s, few cases due to serogroup W135 were reported in Niamey and none were reported between 1995 and 2001. Thus, the recent reemergence of serogroup W135 in this capital, although only in sporadic cases, is of great concern. In 2003, two STs were found circulating simultaneously in Niamey, the ST-11 epidemic strains closely related to the year 2000 hajj and year 2002 Burkina Faso strains and ST-2881, differing at six of the seven loci. This is the first evidence of an ST-2881 strain involved in sporadic meningitis cases. In September 2004, the MLST database (<http://pubmlst.org/neisseria/>) showed that most of the strains with genotypes closely related to ST-2881 that were isolated between 1971 and 2000 were carried strains. The possibility that this new W135 ST-2881 strain will be responsible for epidemics in the next seasons or, in contrast, will confer a specific herd immunity, thus preventing a W135 outbreak, cannot be ruled out. The W135 genotypes that occurred in the past and outside of Niamey are poorly known, since the strains available for analysis are scarce. In 2004, the national epidemiological and laboratory surveillance was enhanced in order to help in making decisions on immunization policies by documenting the meningococcal serogroups involved in meningitis cases. Together, the genetic follow-up of the *N. meningitidis* serogroup W135 isolates from patients and carriers, with respect to their immune status, will help us to understand the pathogenic role of the ST-2881 clone compared to that of the ST-11 complex.

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