Clonal Spread of Serogroup W135 Meningococcal Disease in Turkey

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Six cases of Neisseria meningitidis serogroup W135 meningococcal infection have been reported in Turkey since 2003. Seven isolates recovered from four meningococcal meningitis patients and two asymptomatic carriers produced three distinct pulsed-field gel electrophoresis (PFGE) patterns. Multilocus sequence typing and antigen gene sequencing showed that five isolates were indistinguishable from ST-11 (ET-37) serogroup W135 meningococci, which were first isolated in Saudi Arabia and were responsible for the worldwide outbreak among Hajj pilgrims and their contacts in 2000. The remaining two isolates, which had related PFGE patterns, differed from each other at only one of the genetic loci characterized but were not related to the ST-11 clonal complex. None of the six individuals recalled contact with a pilgrim or had traveled on the Hajj. These six individuals exhibited no time or place relationships to each other, except for the two asymptomatic carriers, who were soldiers and served in the same military unit. These data demonstrate that serogroup W135 meningococci with different genotypes, including the Hajj epidemic strain, are endemic in Turkey.

Neisseria meningitidis is a common commensal bacterium of the human upper respiratory tract and can cause invasive infections such as septicemia or meningitis. Such colonization is known to be an epidemic threat in the countries of the meningitis belt (6). N. meningitidis strains have been classified into 13 serogroups (6, 13). Serogroup W135 meningococci were rarely associated with epidemiologically distinct outbreaks of meningococcal disease (2) until an international outbreak was reported following the annual Hajj seasons in Saudi Arabia in 2000 and 2001 (11, 12). The epidemic strain has been identified by multilocus enzyme electrophoresis (10). The Hajj (ST-11), which corresponds to electrophoretic type 37 (ET-37) by multilocus sequence typing (MLST) as sequence type 11 since 2003. Seven isolates recovered from four meningococcal meningitis patients and two asymptomatic carriers produced three distinct pulsed-field gel electrophoresis (PFGE) patterns. Multilocus sequence typing and antigen gene sequencing showed that five isolates were indistinguishable from ST-11 (ET-37) serogroup W135 meningococci, which were first isolated in Saudi Arabia and were responsible for the worldwide outbreak among Hajj pilgrims and their contacts in 2000. The remaining two isolates, which had related PFGE patterns, differed from each other at only one of the genetic loci characterized but were not related to the ST-11 clonal complex. None of the six individuals recalled contact with a pilgrim or had traveled on the Hajj. These six individuals exhibited no time or place relationships to each other, except for the two asymptomatic carriers, who were soldiers and served in the same military unit. These data demonstrate that serogroup W135 meningococci with different genotypes, including the Hajj epidemic strain, are endemic in Turkey.

In Turkey, limited epidemiologic studies of N. meningitidis have been reported (7, 9). In 1990 a carriage study in the Aegean region reported an N. meningitidis carriage rate of 28%, and the serogroups were identified as A (12%), B (9%), C (63%), D (0.4%), Y (0.6%), and X-Y (0.4%) (7). A recent study reported that the N. meningitidis carriage rate among primary school children was 6.2%, with A, B, and C as the dominant serogroups (9). In 2001 a serogroup W135 meningococcus was isolated for the first time in Turkey from a healthy preschool child (3), and the first case of invasive disease caused by a serogroup W135 organism was reported in 2003 (8). The isolate recovered in the first case was epidemiologically unrelated to the ST-11 epidemic strain that was originally isolated in Saudi Arabia and was responsible for the worldwide outbreak among Hajj pilgrims and their contacts in 2000 (8, 11).

In this study we describe the genetic and antigenic characterization of the first Turkish serogroup W135 isolate in 2003 (8) and of six serogroup W135 isolates recovered in 2004 from five individuals in Ankara, the capital city of Turkey, and we correlate these data with those of clinical and epidemiologic studies.

Isolate collection and identification. N. meningitidis serogroup W135 isolates were recovered from the Gulhane Military Hospital in Ankara and the surrounding area in 2004. Clinical specimens, including throat swabs, blood, and cerebrospinal fluid (CSF), were immediately plated on BBL-modified Thayer-Martin medium (MTM II; Becton Dickinson Microbiology Systems). Plates were incubated for 2 days at 35°C under 5% CO2. Plates were examined for the presence of colonies showing the typical morphology of N. meningitidis. Suspect colonies were screened for oxidase reactivity, and positive samples were Gram stained. If gram-negative diplococci were present, a biochemical profile by the API NH system (bioMérieux, France) was used for confirmation. The isolates were stored at −70°C in brain heart infusion broth containing 15% glycerol until further analysis. An N. meningitidis strain isolated at Vanderbilt University Medical Center was used as a reference strain.

Serogrouping. Serogrouping of the meningococcal isolates was performed by a slide agglutination technique as recommended by Difco Laboratories (Detroit, Mich.). The serogroup of these meningococcal isolates was further confirmed...
by the Tennessee Department of Health Laboratory Services by using monoclonal and polyclonal sera for groups A, B, C, D, X, Y, and W135, provided by both Difco Laboratories and Murex (Remel, Lenexa, Kans.) (14).

**MLST and antigen gene sequencing.** Characterization of isolates by MLST and nucleotide sequencing of the antigen-encoding genes *porA* and *porB* were performed as described previously (5, 18), with allele, ST, and antigen type assignment carried out by interrogation of the appropriate Web-accessible databases (http://www.neisseria.org/nm/typing).

**PFGE typing.** The relationship between strains was examined by genomic DNA pulsed-field gel electrophoresis (PFGE) as described previously with modifications (13). Briefly, genomic DNA was extracted from logarithmic-phase cultures grown on 5% blood agar, prepared in low-melting-point agarose plugs, and digested with the *NheI* enzyme (New England Biolabs, Beverly, MA) for 24 h, as previously described (16). The DNA size standard used was a bacteriophage lambda ladder consisting of concatemers starting at 48.5 kbp and increasing to approximately 1,000 kbp (Bio-Rad Laboratories, Hercules, CA). Electrophoresis was performed with a GenePath system (Bio-Rad) using a dedicated program two for 20 h. The gels were stained with ethidium bromide, rinsed, and photographed under UV light using the Gel Doc 2000 computerized documentation system (Bio-Rad). Each strain was classi
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**TABLE 1. Clinical and epidemiologic characteristics of seven *Neisseria meningitidis* W135 isolates in Turkey**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Patient age (yr)</th>
<th>Site of isolation</th>
<th>Date isolated</th>
<th>Place isolated</th>
<th>Relation of patient to other patient(s)</th>
<th>Travel history</th>
<th>Clinical disease</th>
<th>PFGE type</th>
<th>Sequence type</th>
<th>porA</th>
<th>porB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nm118</td>
<td>20</td>
<td>CSF</td>
<td>03/26/2003</td>
<td>Downtown Ankara</td>
<td>No</td>
<td>No</td>
<td>Meningitis</td>
<td>Turkey</td>
<td>ST-2754</td>
<td>P1.5,2</td>
<td>porB2-86</td>
</tr>
<tr>
<td>Nm120</td>
<td>6</td>
<td>CSF</td>
<td>03/18/2004</td>
<td>Sihhiye, Ankara</td>
<td>No</td>
<td>No</td>
<td>Meningitis</td>
<td>ET-37</td>
<td>ST-11</td>
<td>P1.5,2</td>
<td>porB2-2</td>
</tr>
<tr>
<td>Nm119</td>
<td>21</td>
<td>CSF</td>
<td>03/24/2004</td>
<td>Downtown Ankara</td>
<td>No</td>
<td>No</td>
<td>Meningitis</td>
<td>ET-37</td>
<td>ST-11</td>
<td>P1.5,2</td>
<td>porB2-2</td>
</tr>
<tr>
<td>Nm121</td>
<td>21</td>
<td>Blood</td>
<td>03/24/2004</td>
<td>Downtown Ankara</td>
<td>No</td>
<td>No</td>
<td>Meningitis</td>
<td>ET-37</td>
<td>ST-11</td>
<td>P1.5,2</td>
<td>porB2-2</td>
</tr>
<tr>
<td>Nm124</td>
<td>7</td>
<td>CSF</td>
<td>05/16/2004</td>
<td>Sihhiye, Ankara</td>
<td>No</td>
<td>No</td>
<td>Meningitis</td>
<td>Turkey</td>
<td>ST-185</td>
<td>P1.5,2</td>
<td>porB2-86</td>
</tr>
<tr>
<td>Nm123</td>
<td>20</td>
<td>Throat</td>
<td>08/12/2004</td>
<td>Balgat, Ankara</td>
<td>Yes*</td>
<td>No</td>
<td>Asymptomatic</td>
<td>ET-37</td>
<td>ST-11</td>
<td>P1.5,2</td>
<td>porB2-2</td>
</tr>
<tr>
<td>Nm122</td>
<td>21</td>
<td>Throat</td>
<td>08/12/2004</td>
<td>Balgat, Ankara</td>
<td>Yes*</td>
<td>No</td>
<td>Asymptomatic</td>
<td>ET-37</td>
<td>ST-11</td>
<td>P1.5,2</td>
<td>porB2-2</td>
</tr>
</tbody>
</table>

*All six individuals were male, and Nm118 was the first index case. Isolates Nm119 and Nm121 were recovered from the same patient.

b Given as month/day/year.

c These two cases served in the same military unit.
East (2). Two million pilgrims from approximately 140 countries congregate annually in Mecca and Medina in Saudi Arabia for the Hajj and/or Umrah pilgrimages during Hajj seasons (4). Overcrowding is the most important factor for person-to-person transmission of meningococci and the spread of invasive disease (2). Most of the Turkish population is Muslim, and approximately 150,000 Turkish pilgrims make the annual Islamic pilgrimage to Mecca and Medina in Saudi Arabia (8). The first reported pandemic that was associated with the Hajj season began in 1987 in Saudi Arabia and was caused by hyperinvasive serogroup A meningococci. Following this outbreak, the Saudi government mandated vaccination with a bivalent serogroup A and serogroup C vaccine for all pilgrims entering the country for the Hajj season (4). Subsequently, the vaccination policy was extended further to include all Umrah visitors (1).

During April of 2000, approximately 400 cases of serogroup W135 meningococcal disease were reported in 10 countries among pilgrims returning from Hajj and their close contacts (2, 11). In 2001, the second international outbreak of serogroup W135 meningococcal disease was reported. This outbreak was smaller than the first, with 200 cases of meningococcal infection, associated with international travel or contact with travelers who had been to Saudi Arabia, identified in 10 different countries (20). It has also been reported that 55 deaths from meningococcal infection occurred during the Hajj pilgrimage (4). Following the 2001 outbreak, the Saudi Arabian government made it a requirement that all pilgrims entering the country should be vaccinated with a quadrivalent vaccine containing serogroups A, C, W135, and Y (2).

Although serogroup W135 ST-11 meningococci have been isolated worldwide since 1970, they have been reported rarely in European countries (15). In this study of W135-associated meningococcal disease in Turkey, W135 ST-11 meningococci have been isolated in disease cases and from healthy carriers, none of whom were linked epidemiologically to the Hajj pilgrimages of 2000 and 2001. These data suggest that carriage and transmission of the Hajj epidemic strain have led to the emergence of serogroup W135 ST-11 organisms as a cause of endemic meningococcal disease in Turkey. Moreover, these data highlight the need for continued epidemiological surveillance to identify any changes in the incidence of serogroup W135 meningococcal disease and to inform future public health strategies.

We thank the Tennessee Department of Health Laboratory Services for confirming the serogroup of the isolates; Levant Doganci, Mehmet Baysallar, Gulsen Hascelik, and Alaaddin Pahsa for excellent assistance; and Tanja Popovic and Leonard Mayer of the Centers for Disease Control and Prevention for helpful discussions. This publication made use of the Neisseria Multi Locus Sequence Typing website (http://pubmlst.org/neisseria/).

REFERENCES


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