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Neisseria meningitidis Serogroup W135, China

To the Editor: *Neisseria meningitidis* is a gram-negative bacterium found only in humans and is a major cause of serious invasive diseases. Before 2006, in the People's Republic of China, all meningococcal diseases were caused by serogroups A, B, and C. However, there are ≥ 13 serogroups of this organism. Three cases of infection with *N. meningitidis* serogroup W135 were reported in China during 2006–2008. We describe these 3 meningitis patients and the *N. meningitidis* serogroup W135 strains isolated from these patients by genotyping methods.

Patient 1, a 36-year-old man, was seen at a local hospital in Fujian Province in January 2006. He became ill while on a business trip and was given a diagnosis by culture of an *N. meningitidis* infection. Patient 2, a 25-year-old man, was seen in Guangdong Province in May 2007. He had not traveled outside this area in the 10 days before becoming ill. Patient 3, a 14-year-old girl, was seen in Guangxi Province in February 2008. She was a middle school student and had toured the suburbs of this province with her classmates 2 days before becoming

ill. Close contacts of all 3 patients were investigated; no additional *N. meningitidis* infections were detected. However, *N. meningitidis* was isolated from a throat swab specimen obtained from the younger cousin of patient 3.

N. meningitidis infection was confirmed for all 3 patients on the basis of clinical symptoms and laboratory results. All patients reported neck stiffness. Physical examinations showed Kernig signs, Brudzinski signs, and high temperatures ($>38^{\circ}\text{C}$). Cerebrospinal fluid (CSF) samples were turbid with increased protein levels and pressure; leukocyte counts were increased ($>5,000$ cells/ μL). CSF culture on chocolate agar grew *N. meningitidis* after 24 h. Isolates were identified as serogroup W135 by using specific antiserum (Remel, Lenexa, KS, USA) at provincial Centers for Disease Control and Prevention (CDC) in China and confirmed at the Chinese CDC.

Patients were treated with antimicrobial drugs and recovered fully. An isolate from the cousin of patient 3 was also identified as W135. Etest strips and broth microdilution were used for antimicrobial drug susceptibility testing for the 4 W135 isolates. All isolates were susceptible to 12 antimicrobial drugs tested, which included therapeutic and prophylaxis agents used frequently in China.

Pulsed-field gel electrophoresis (PFGE), multilocus sequence typing, and outer membrane protein (*porA*) gene variant region subtyping were used to characterize the 4 case-related W135 *N. meningitidis* isolates and other isolates from asymptomatic carriers. Strain R29057 (from France) was used as a reference strain. The 4 case-related isolates showed similar PFGE patterns. These patterns were distinct from those of other W135 isolates obtained from asymptomatic carriers. Three invasive disease isolates and 1 from the close contact of patient 3 had the same multilocus sequence type (ST) and *PorA* subtype; all were

ST11: P1.5, 2. This subtype was not detected among other tested isolates of W135 obtained from asymptomatic carriers (online Technical Appendix, www.cdc.gov/EID/content/16/2/348-Techapp.pdf).

ST11: P1.5, 2 *N. meningitidis* serogroup W135 was responsible for the epidemic of W135 meningococcal disease in 2000, which was associated with the Hajj pilgrimage in Saudi Arabia (1,2). The strain related to the Hajj pilgrimage was derived from clonal expansion within the ST11 complex/ET-37 complex (3). However, no epidemiologic data showed that the 3 cases in our study were linked to the Hajj pilgrimage. Since 2000, invasive diseases caused by W135 meningococci of ST11 have been reported in Africa, Asia, and the Middle East (4). ST11 W135 infections have been reported to cause invasive disease in Taiwan during 1996–2002 and were apparently introduced into Taiwan before the Hajj pilgrimage-associated outbreak because they were genotypically distinct from the Hajj-related W135 clone (5,6).

The 3 cases we report were observed in southeastern China near Taiwan (online Technical Appendix), but no direct epidemiologic links are known. Because of the lack of W135 strains from Hajj pilgrimages and Taiwan in this study, we could not provide a detailed and integrated genotypic relationship between the strains in China and those of Hajj pilgrimages and Taiwan. However, we can confirm that these 3 cases were caused by strains from the same hypervirulent clone characterized as ST11: P1.5, 2.

W135 strains have been isolated after vaccination with a bivalent meningococcal vaccine in Cameroon (7). In China, the bivalent meningococcal vaccine has been successfully introduced into the national expanded immunization program in response to an outbreak of *N. meningitidis* serogroup C during 2003–2004 (8).

The 3 patients infected with W135 in our study did not receive bivalent meningococcal vaccines. W135 meningococcal disease appears to be an emerging problem that should be investigated epidemiologically. These patients highlight the need for further epidemiologic surveillance to monitor changes in the incidence of meningococcal disease caused by W135 and for improved public health disease control strategies in the future.

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Avian Influenza (H5N1) Outbreak among Wild Birds, Russia, 2009

To the Editor: Highly pathogenic avian influenza (HPAI) virus (H5N1) has been endemic in poultry in Southeast Asia since 2003 (1). In April 2005, an outbreak of influenza virus (H5N1) infection was detected in wild birds on Qinghai Lake in western China (2). Subsequently, the Qinghai-like (clade 2.2) HPAI virus (H5N1) lineage was detected in wild birds and poultry in many countries (1,3,4). The source of these introduc-

tions, although still debated, is likely through bird migration (5).

In June 2006, an influenza (H5N1) outbreak was detected in wild birds on Uvs-Nuur Lake in western Siberia, Russia. We showed that A/duck/Tuva/01/2006, isolated during the outbreak, was highly pathogenic for chickens and mice and belonged to the Qinghai-like group (2.2 clade) (6).

The first case of Fujian subclade 2.3.2 influenza virus (H5N1) lineage in the Russian Far East was recorded in April 2008 (7). Before this case, no HPAI (H5N1) outbreaks of the Fujian lineage had been reported in Russia.

In June 2009, an outbreak of HPAI in wild birds was recorded in Mongolia (4) and on Uvs-Nuur Lake in Russia. RNA extracted from organs (liver, spleen, intestine) of 10 dead birds belonging to 4 species (great crested grebe [*Podiceps cristatus*], little grebe [*Tachybaptus ruficollis*], black-headed gull [*Larus ridibundus*], and spoon-bill [*Platalea leucorodia*]) was positive for type A influenza RNA and for the H5 subtype by real-time reverse transcription-PCR (8). We isolated 2 viruses from embryonated specific antibody-negative fowl eggs. Hemagglutination (HA) and neuraminidase (NA) inhibition assays with monospecific antiserum confirmed the H5N1 subtype. Viruses were designated as A/black-headed gull/Tyva/115/2009 and A/great crested grebe/Tyva/120/2009, and sequences of their HA and NA segments were defined. No HPAI virus (H5N1) was found in cloacal swabs obtained from 36 live birds (of the 4 species listed above) from Uvs-Nuur Lake.

Phylogenetic analysis (9) of the HA gene (Figure) showed that viruses belong to clade 2.3.2. These viruses are clearly distinguishable from the HPAI viruses previously isolated in this Russian region in 2006, A/duck/Tuva/01/2006 (clade 2.2) but are more related to A/whooper swan/Mongolia/8/2009 and A/whooper swan/Mongolia/2/2009. For the NA

Neisseria meningitidis Serogroup W135, China

Technical Appendix

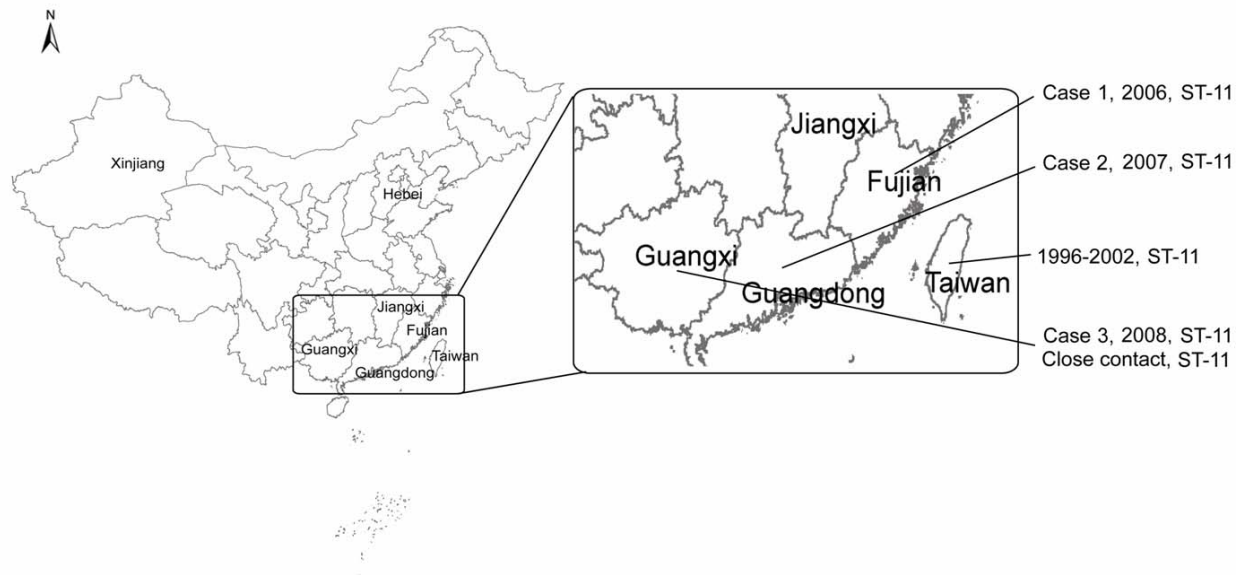


Figure 1. Distribution and molecular characterization of *Neisseria meningitidis* W135 isolates from 3 patients, People's Republic of China, and from Taiwan. ST, sequence type.

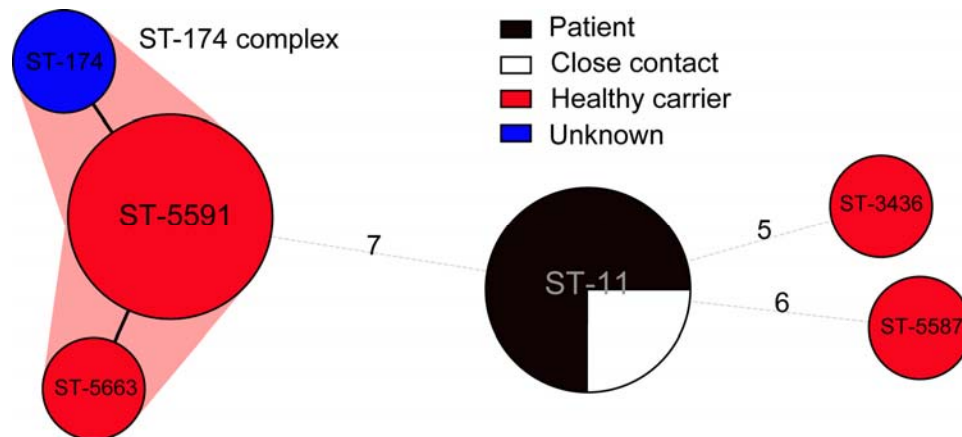


Figure 2. Distribution and molecular characterization of *Neisseria meningitidis* W135 isolates from case-patients, People's Republic of China. Minimum spanning tree of 12 W135 isolates typed by multilocus

sequence typing (MLST). Clustering of MLST profiles was performed by using a categorical coefficient. MLST types are indicated by circles. The size of each circle is proportional to the number of isolates with this particular type. Numbers (5, 6, and 7) indicate number of different loci between 2 MLST types. Thick solid lines connect types that differ at 1 locus and thin dotted lines connect types that differ at 2 loci. The color of each circle indicates types that belong to the same complex. MLST complexes were assigned if 2 neighboring types did not differ at >1 locus and if ≥ 2 types fulfilled this criterion. ST, sequence type.

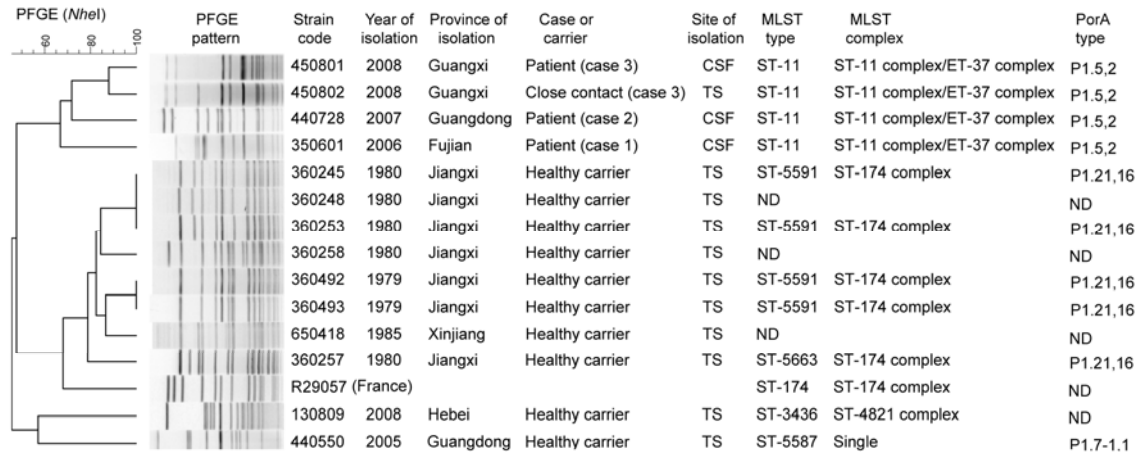


Figure 3. Distribution and molecular characterization of *Neisseria meningitidis* W135 isolates from case-patients, People's Republic of China. Cluster analysis of 15 strains base on pulsed-field gel electrophoresis (PFGE) patterns. Clustering was performed by using the Dice coefficient and a 1.2% optimization setting. The dendrogram was generated by using the unweighted pair group method using averages. MLST, multilocus sequence type; PorA, outer membrane protein; CSF, cerebrospinal fluid; ST, sequence type; TS, throat swab; ND, not determined.