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Laboratory Confirmation of A Suspicious Meningococcal Meningitis Death Case

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A suspicious meningococcal meningitis death case was reported to the Beijing CDC. The blood specimen was analyzed via multi-PCR and MLST. 6 isolates from close contacts were analyzed via PFGE and MLST. According to the results of the above analyses, the cause of this case was identified as a serogroup A *Neisseria meningitidis*, which, in terms of sequence typing, belonged the ST7 group.

Keywords: *Neisseria meningitidis*, pulsed-field gel electrophoresis, multilocus sequence typing, serogroup

Meningococcal meningitis disease occurs worldwide, and is characterized by very high mortality. Thirteen *Neisseria meningitidis* serogroups have been recognized on the basis of capsular polysaccharide antigens, five of which (Serogroups A, B, C, W135, and Y) have been identified as the most common causes of meningococcal meningitis (Jonathan *et al.*, 2000). In Asia, serogroups A and C have been implicated as the leading causative agents of epidemics. Three nationwide epidemic outbreaks of meningococcal meningitis occurred in China from the 1950s to the 1980s. All of these cases were caused by serogroup A *Neisseria meningitidis* (Hu *et al.*, 1991). In an attempt to prevent future meningococcal meningitis epidemics, serogroup A vaccines have come into wide use within China. The annual incidence of meningococcal meningitis has rapidly declined as a result of this measure (Hu, 2001). Although vaccines against *Neisseria meningitidis* performed pivotal roles in the control of meningococcal meningitis, the antiserum generated from serogroup A vaccines is only able to neutralize serogroup A strains. In recent years, the number of meningococcal meningitis attributed to serogroups B and C has increased substantially in China. The efficiency of current vaccines relies on laboratory identification of the disease-causing pathogen, in addition to good epidemiological information. A great deal of attention has been focused on laboratory diagnoses of meningococcal meningitis, in order to characterize the changes occurring within the serogroups of *Neisseria meningitidis*. Currently, a host of techniques have been developed for the laboratory diagnoses of meningococcal meningitis, including rapid polymerase chain reaction (PCR), multilocus sequence typing (MLST), multiplex-PCR, real-time PCR, and pulsed-field gel electrophoresis (PFGE) (Bygraves and Maiden, 1992; Martin *et al.*, 1998; Muhamed, 2000; David *et al.*, 2003; Elizabeth *et al.*, 2004). In this study, a suspicious case of death associated with meningococcal meningitis disease was reported to the Beijing CDC, and we succeeded in the laboratory confirmation of this case, utilizing multiplex-PCR, MLST, and PFGE.

The onset of this case was characterized by a high fever and headache, which began on November 11th, 2005. A petechiate rash initially emerged on the patient's face, and then expanded to the body, accompanied by a stiff neck, as well as Kernig's and Brudzinski's signs. The disease progressed rapidly. The patient entered a deep coma, and then died on November 13th, 2005. In accordance with the observed clinical symptoms, this case was diagnosed as suspicious meningococcal meningitis. The clinical blood specimen was taken from the suspicious meningococcal meningitis patient at the Beijing Ditan Hospital, then transferred to the Beijing CDC laboratory for detection via multiplex-PCR and MLST. Blood incubation was negative, and no cerebrospinal fluid was collected, due to rejection occurring in the patient's

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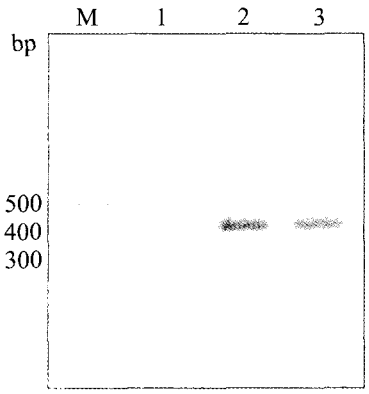

Fig. 1. Multiplex-PCR amplification of the specific DNA fragments of 5 different *Neisseria meningitidis* serogroups from the clinical blood specimen. Electrophoresis was conducted on 1% agarose gel. Lane 1, negative control. Lane 2, positive control using the DNA of serogroup A strains as a template. Lane 3, the amplification results of the clinical blood specimen. Lane M, 100 bp DNA ladder. Some bands are indicated in base pairs on the left.

family. The results of blood specimen multiplex-PCR detection were shown in Fig. 1. A gene fragment of 400 bp in size was amplified from both the blood specimen and the positive control. Serogroup prediction via multiplex-PCR was a match for serogroup A. In accordance with this result, we deduced that this suspicious meningococcal meningitis death case might have been caused by serogroup A *Neisseria meningitidis*.

The close contact in this case was defined as the man who lived in the same room as the patient. 6 bacterial strains were isolated from the oropharyngeal swab specimens of 18 close contacts on 5% sheep blood agar plates. The other 5 serogroup A *Neisseria meningitidis* strains were isolated from the cerebrospinal fluid samples of meningococcal meningitis disease patients in Beijing in 2005. Those strains were all identified in accordance with the guidelines provided in the "Laboratory Manual for the Diagnosis of Meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae*" manual, compiled by the World Health Organization. Those strains were identified via oxidase test, agglutination with serogrouping antisera, and carbohydrate utilization assays. According to the results (shown in Table 1), we can observed that all of them were serogroup A strains of *Neisseria meningitidis*. The relationships between the six close contact strains were analyzed via PFGE after DNA digestion with *Nhe*I and *Spe*I, respectively. As is shown in Fig. 2A, the six strains shared the same PFGE pattern and originate from one clone. This result was further verified via MLST analysis. The sequence types of the six strains showed that they were all members of ST7. MLST was also conducted

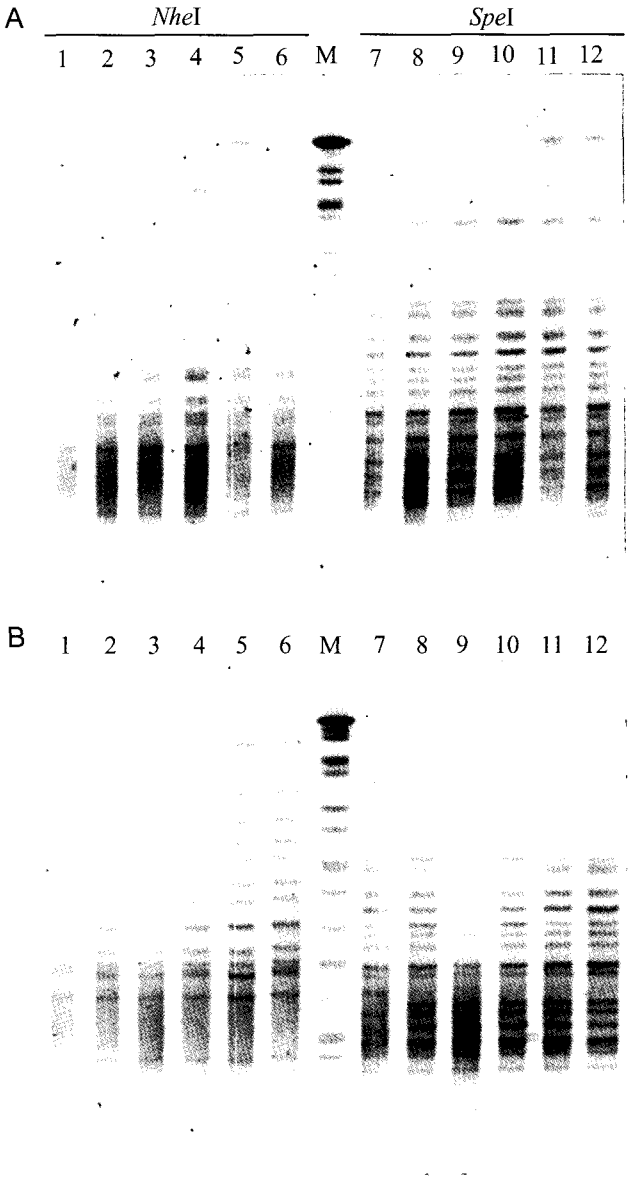

Fig. 2 Pulsed-field gel electrophoresis patterns of serogroup A *Neisseria meningitidis,* including the six close contact strains (A) and 5 strains isolated from the other meningococcal meningitis patient (B). A, lanes 1 to 6 and 7 to 12, the pattern of close contact strains after DNA digestion with *Nhe*I and *Spe*I, respectively. B, lanes 1 and 7, the patterns of one close contact strain. Lanes 2 to 6 and 8 to 12, the patterns of other serogroup A *Neisseria meningitidis* isolated from meningococcal meningitis patients after DNA digestion with *Nhe*I and *Spe*I, respectively. M, the DNA of *Salmonella* (H9812) was restricted with *Xba*I.

using the DNA template extracted from the blood sample of this patient. Six DNA fragments, all except for the pdhC gene fragment, were obtained from the blood via MLST and sequenced. The sequences of the six DNA fragments were identical to those of the close contact strains. According to the above-listed, we conclude that the clone strains were likely the

Table 1. Identification of 11 *Neisseria meningitidis* strains including 6 isolated from close contacts (C1 to C6) and 5 from the cerebrospinal fluid specimens of other patients with meningococcal meningitis disease (P1 to P5). +, Positive. –, Negative

Strains	Oxidase Test	Serogroup	Carbohydrate Utilization				Sequence typing
			Glucose	Maltose	Lactose	Sucrose	
C1	+	A	+	+	–	–	ST7
C2	+	A	+	+	–	–	ST7
C3	+	A	+	+	–	–	ST7
C4	+	A	+	+	–	–	ST7
C5	+	A	+	+	–	–	ST7
C6	+	A	+	+	–	–	ST7
P1	+	A	+	+	–	–	ST7
P2	+	A	+	+	–	–	ST7
P3	+	A	+	+	–	–	ST7
P4	+	A	+	+	–	–	ST7
P5	+	A	+	+	–	–	ST7

pathogen for this case.

In Fig. 2B, lanes 1 and 7 evidenced the representative PFGE patterns of close contact strains after DNA digestion with NheI and SpeI. Lanes 2 to 6 and 8 to 12 evidenced patterns of other serogroup A strains isolated from the cerebrospinal fluid specimens of meningococcal meningitis disease patients in Beijing in 2005. The patterns of lanes 2 to 6 and 8 to 12 were indistinguishable from those of lanes 1 and 7, respectively. They were also sequence-typed as ST7 by MLST analysis (Table 1). These results showed that those serogroup A *Neisseria meningitidis* strains had been derived from one clone, which appeared to be responsible for serogroup A *Neisseria meningitidis*-associated meningococcal meningitis disease in Beijing.

Over the past few years, serogroup A was the most frequently detected cause of meningococcal meningitis in China. Thus, a substantial number of serogroup A vaccines were employed in order to prevent new cases of meningococcal meningitis. As a result, the carrier rate of serogroup A *Neisseria meningitidis* was lower than that of serogroups B and C. In this study, the serogroup A *Neisseria meningitidis* carrier rate was found to be approximately 33.3%. No serogroups B and C were isolated from the 18 close contacts. Although no bacterial strains were isolated from this patient, we can estimate that six serogroup A *Neisseria meningitidis* may have originated from only one clone, and that this clone strain resulted in the death of this patient. This conclusion was consistent with our analysis of the epidemiologic data. We also confirmed these results by laboratory diagnoses using molecular technology. MLST, which was employed to differentiate *Neisseria meningitidis* strains within case clusters (Feavers *et al.*, 1999), was directly conducted using the DNA from the blood specimen as a PCR template in this study. We can clarify, in part, the relationship between the close contact strains and the blood specimen strains via comparisons of the sequences of the DNA fragments. This provides a feasible method for the laboratory confirmation of meningococcal meningitis, especially for cases from which no bacteria were isolated.

Laboratory pathogen surveillance is extremely relevant to the establishment of immune policy. In China, monovalent serogroup A vaccines were extensively employed, whereas multivalence vaccines, such as A plus C, were used only rarely. The specific serogroup antisera evidenced a lack of ability to cross-react with other serogroups. Immune policy, then, should be adjusted in accordance with observed changes in epidemic serogroups. In addition, the pathogens isolated in this study may be additionally employed in antibiotic-resistance tests and bactericidal activity experiments. The results of those studies may prove useful in the direction of clinical treatment and evaluations of vaccine efficiency.

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Neisseria meningitidis strains and clinical blood specimen.

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