African Journal of Microbiology Research Vol. 5(16), pp. 2385-2387, 18 August, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR09.389
ISSN 1996-0808 ©2011 Academic Journals

Short Communication

Laboratory analysis of a fatal meningococcal case due to serogroup B *Neisseria meningitidis* belonging to ST-4821 complex

Zengguo Wang*, Tiejun Hou, Zhijun Chen, Jinsong Li, Shouzhi Wu, Xiaoguang Wei, Yahui Sun and Quanli Du

Xi'an Center for Disease Control and Prevention, 599 Xi Ying Road, Xi'an, 710054, PR China.

Accepted 12 November, 2009

We describle a suspicious Meningococcal death case, confirmating by the laboratory PCR methods. The infection developed rapidly within only 24 h until the patient died. Analyzing the close contact strain and the DNA template extracted from the serum of this patient, we characterized a serogroup B *Neisseria meningitidis* as the pathogen of this case, which, in terms of sequence typing, belonged to ST-4821 complex which first characterized in serogroup C *Neisseria meningitidis* as a new unique hypervirulent meningococcal lineages.

Key words: *Neisseria meningitidis*, multilocus sequence typing, serogroup.

INTRODUCTION

As a major bacterial meningititis pathogen, *Neisseria meningitidis* could cause a rapidly progressive and often fatal illness mainly in children and young adults all over the world. 13 serogroups were known of this organism based on the chemical and serological properties of the capsular polysaccharide. Serogroup A meningococci were the main cause of meningitis and responsibled for several nationwide epidemics during the last century in China (Hu, 1991). After the mass vaccination campaign with mainly the MenA vaccine initiated in 1982, the incidence of meningococcal disease rapidly declined and only some sporadic case reported. However, several outbreaks caused by serogroup C, ST -4821 meningococci occurred in Anhui province in 2003 and then spread to other provinces (Shao et al., 2006). To control this disease, a mass vaccination using polysaccharide vaccine for serogroup A plus C were undertaken in several provinces and the A plus C vaccine was used more prevalent than before in China.

An etiologic diagnosis of *N. meningitidis* is confirmed by the isolation of strain from cerebrospinal fluid, blood or other body fluids. However, this diagnosis was hindered by the failure to isolate bacteria because of the early treatment of the patient (Cartwright et al., 1992). So the PCR-based methods have been extensively used for the diagnosis and also the serotying based on several genes special for Neisseria *meningitidis* or the serotype. In this study, we confirmed a suspicious death case of meningococci infection with laboratory methods such as PCR (multiplex-PCR) (Jordens et al., 2002), MLST (Maiden et al., 1998). We also found the etiologic serogroup B meningococci belonging to the ST-4821 complex which was a newly identified lineage that most prevalent in serogroup C meningococci in China (Zhang et al., 2007).

CASE REPORT

A 16 years old female student was characterized by a high fever and headache without other symptoms like cough, diarrhea, vomit, which began on March 11, 2009. The body temperature was 39.4°C. The symptom was exacerbated when the patient was checked in LanTian Hosiptal next day at 7:30 am. Several petechiate rashes emerged on the whole body with the blood pressure was 118/68 mmHg and pulse rate of 132 per minute. The numbers of white cell in the blood were 1.6 × 1010/L. The patient receiving azithromycin, and within drug administration, the patient had nausea several times. After the

*Corresponding author. E-mail: upwilly@gmail.com. Tel: 86-10-8551-2367.

drug administration, the body temperature was normal but also with the headache. Then the patient was transferred to Tang Du Hospital at 10:50 am, the body temperature was 36°C and the blood pressure was 84/65 mm Hg with pulse rate of 116 per minute. Neck stiffness and purpura fulminans were noted during clinical examination. The blood and CSF samples were obtained and submitted for laboratory examination. Laboratory investigations revealed thrombocytopenia, the TP, ALB and GLO were all decreased, the blood culture was negative for any bacterial. The patient was initially stabilized with volume resuscitation, dopamine infusion, until 13:30, the patient was shortness of breath and dysphoria again, and died of acidemia at 14:35. In accordance with the observed clinical symptoms, this case was diagnosed as suspicious meningococcal meningitis. The serum of the patient was transferred to Xi'an CDC laboratory for detection via PCR and MLST.

The close contact in this case was defined as 5 roomates with the patient. Within the 5 roommates' oropharyngeal swabs, the ctrA gene was positive in two samples and 1 strain was isolated. The ctrA gene was also positive in the patient's serum. To check the serotype we use the molecular serogroup methods to predict the possible serotype described elsewhere (Taha, 2000), (Bennett et al., 2004). A 450 bp fragment predicted serogroup B Neisseria *meningitidis* was positive in the two ctrA gene positive oropharyngeal swabs sample and also the patient's serum. MLST analyses were verified here with the close contact strain. The sequence type of this close contact strain was ST -7623 which was a new ST type found in our study belonging to ST-4821 complex. MLST was also conducted using the DNA template extracted from the serum of this patient. Only three fragments (adk, aroE and gdh) were obtained and sequenced. To search the type in the PubMLST net, the allelic profile was identical to 12 sequence types that all belonging to ST-4821 complexs. Also, the sequence of these three fragments identical to the close contact strain. According to the above-listed, we conclude that the pathogen of this fatal case was serogroup B *N. meningitidis* which belonging to ST-4821 complexs.

DISCCUSSION

Currently, rapid laboratory diagnosis of meningococcal disease has been widely used as routine methods (Bryant et al., 2004; Hoang et al., 2005). Especially, when the bacterial could not been isolated, PCR based methods will be very important in confirming the meningococci infection. With the only sample like serum of patient, MLST could be used in epidemiology research (Zhang et al., 2006). In this case, PCR and MLST method was used in laboratory confirmation and epidemiology research as only the serum sample was obtained. To our knowledge, this is the first death case causing by serogroup B meningococci belonging to ST- 4821 complexs in China.

Serogroup C meningococci have been associated with the outbreaks in 2003. Then a unique ST-4821 clone has been characterized (Shao et al., 2006). ST-4821 complex isolates were observed as early as 1980 in China and existed in several serogroups like C, B, H, I and K (Yang et al., 2007; 2008; Zhang et al., 2007). In addition to ST-4821 complex has been seen in serogrop B meningococcal isolated from healthy carriers on the Chinese mainland, it also have been seen from invasive cases in Taiwan (Chiou et al., 2006). Here we also found the serogroup B meningococcal belonging to ST- 4821 complex in a fatal case. Because the pathogen strain of the patient was not able to isolate and we can only get 3 gene fragments from the patient's serum. Moreover we isolated two *N. meningitidis* strains from the oropharyngeal swabs of patient's other 10 classmates. This two strains also belongs to B serogroup and the sequence type was ST-4821 and ST-5586 each. The 3 gene fragments from the patient's serum also indentical to the ST-4821 strain. We also got ctrA gene positive result from 2 of 5 close contacts, one close contact strain with the exact sequence type is unsatisfactory. So we presumed there was not sufficient evidence to confirm the exact sequence type of this etiological strain. After all it seems belonging to ST-4821 complex.

Multivalence vaccines A plus C have used widely in China after the serogroup C meningococci outbreaks. Thus, the B serogroup has been observed in healthy carrier is relative higher than before and even maybe the capsule switch between B and C (Beddek et al., 2009). The case presented here indicate the need for better surveillance of the epidemiology of meningococcal meningitis in China, especially the new unique hypervirulent meningococcal lineage ST- 4821 complexs.

ACKNOWLEDGEMENTS

We thank all the researchers in hospitals and Wang Qian in the Baqiao district CDC. We also thank Dr Julia Bennett at the University of Oxford for the registration of the close contact strain as new ST in the MLST database.

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