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NOTE

The Molecular Characterization of Serogroup C *Neisseria meningitidis* Strains Circulating in Beijing

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The aim of this study was to characterize the molecular features of serogroup C *Neisseria meningitidis* strains circulating in Beijing, China. Twenty out of 23 strains belonged to ST 4821. The causative serosubtype for meningococcal meningitis was P1.12-1,16-8. All of the strains expressed class 3 PorB protein. Among the five pulsed-field gel electrophoresis patterns observed, pattern III predominated.

Keywords: Multilocus sequence typing, Pulsed-field gel electrophoresis, Serosubtype, Serotype Serogroup C *Neisseria meningitides*.

Meningitis due to *Neisseria meningitidis* is an important public health problem throughout the world. *N. meningitidis* is classified into 13 serogroups based on the immunological reactivity of the capsular polysaccharide (Goulding *et al.*, 2000). Serogroups A, B, and C account for over 90% of meningococcal disease (Popovic *et al.*, 1999). Among them, serogroup A is the most common cause of epidemics in Africa and Asia, followed by serogroup C (Popovic *et al.*, 1999). Three nationwide epidemic outbreaks of meningococcal meningitis caused by serogroup A *N. meningitides* occurred in China from the 1950s to the 1980s (Hu *et al.*, 1991; Hu, 2001). In recent years, the number of meningococcal meningitis cases in China attributed to serogroup C has substantially increased. Outbreaks of a new sequence type (ST 4821) of serogroup C meningitis emerged during the years 2003 to 2005 in the Anhui province of China (Shao *et al.*, 2006).

To prevent meningococcal meningitis epidemics, a mass vaccination program using a polysaccharide vaccine for serogroup A plus C was undertaken in Beijing, China in 2005. A surveillance program was implemented for isolating pathogens from the clinical specimens of meningitis patients. Bacterial strains were isolated from cerebrospinal fluid specimens of meningitis patients and from oropharyngeal swab specimens of close contacts and healthy carriers. The species were identified by oxidase and carbohydrate utilization tests according to the World Health Organization manual (Popovic *et al.*, 1999). Serogroup was determined with the agglutination test using the Remel Agglutinating Serum kit. The isolated *N. meningitidis* strains were used for further study to elucidate the molecular characterization.

112 *N. meningitidis* strains in total were obtained during the two years, including 38 serogroup A strains, 51 serogroup B strains and 23 serogroup C strains. The molecular features of serogroup A strains were described by Zhang *et al.* (2006). The serogroup B strains were all isolated from healthy carriers. In this study, the 23 serogroup C strains were designated BJ1 to BJ23, and included 6 strains from sporadic cases of meningococcal meningitis, 5 from close contacts and 12 from healthy carriers. We used serotyping, serosubtyping, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to characterize the molecular features of these strains.

The antigenic variety of meningococcal PorA and PorB outer membrane proteins formed the basis of serosubtyping and serotyping, respectively (Sacchi *et*

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al., 1998a and 1998b). The full-length DNA fragments of *porA* and *porB* genes were amplified and sequenced. The predicted amino acid sequences of PorA and PorB proteins were compared with sequences in the PorA and PorB database (Neisseria PorA and PorB typing website. http://neisseria.org/nm/typing/) to determine the serosubtypes and serotypes. PFGE was performed in accordance with the method described by Shao *et al.* (2006). Chromosomal DNA was digested with *Nhe*I and separated on 1.0% SeaKem Gold agarose in 0.5 × Tris-borate-EDTA buffer using the CHEF-DR III system (BioRad). MLST was performed as described by Morelli *et al.* (1997) and Maiden *et al.* (1998). Fragments of *abcZ, adk, aroE, fumC, gdh, pdhC,* and *pgm* were amplified by PCR, purified, and sequenced with the ABI 3100 DNA Sequencer (Applied Biosystems, USA). The obtained sequences were compared with sequences in the MLST database to determine the allele number (Neisseria multilocus sequence typing website. http://pubmlst.org/neisseria/). The sequence type of each strain was determined from the profiles of the seven alleles in the MLST database.

The sequence type of 20 serogroup C *N. meningitidis* strains belonged to ST 4821, which was a new sequence type first discovered in meningitis patients in the Anhui province in 2003 (Shao *et al.*, 2006). ST 4821 is unique to China and responsible for most of the infections caused by serogroup C strains in Beijing. The ST 4821 clone was also isolated from close contacts and healthy carriers in Beijing (Table 1). The strains BJ20 to BJ23, which were isolated from throat swabs of 4 travelers from the Anhui province in 2005, also belong to the ST 4821 clone. This indicates that ST 4821 strains might be spread to

Table 1. The molecular characteristics of 23 serogroup C *N. meningitidis* strains isolated in Beijing, China

Strain	Source	Serogroup	PorA		PorB					ST
			VR 1 Loop I	VR 2 Loop IV	Class	VR 1 Loop I	VR 2 Loop V	VR 3 Loop VI	VR 4 Loop VII	
BJ1	Patient	C	12-1	16-8	3	9	15	13[c]	7	4821
BJ2	Patient	C	7-2	14	3	9	15	13	7	4821
BJ3	Patient	C	12-1	16-8	3	9	15	13	7	4821
BJ4	Patient	C	20	23-1	3	9	15	13	7	4821
BJ5[a]	Close contact	C	20	23-1	3	9	15	13	7	4821
BJ6	Patient	C	12-1	16-8	3	9	15	13	7	4821
BJ7	Patient	C	7-2	23-1	3	9	15	13	7	4821
BJ8	Close contact	C	21-2	4	3	9	15	13	7	N[d]
BJ9	Close contact	C	7-2	14	3	9	15	13	7	4821
BJ10	Close contact	C	20	23-1	3	9	15	13	7	4821
BJ11	Close contact	C	20	23-7	3	9	15	13	7	4821
BJ12	Health carrier	C	20-2	23-9	3	9	15	13	7	4821
BJ13	Health carrier	C	20	23-1	3	9	15	13	7	4821
BJ14	Health carrier	C	7-2	23-1	3	9	9	7	8	4821
BJ15	Health carrier	C	7-2	14	3	9	15	13	7	4821
BJ16	Health carrier	C	7-2	14	3	9	15	13	7	4821
BJ17	Health carrier	C	20	23-1	3	9	15	13	7	4821
BJ18	Health carrier	C	20	23-1	3	9	13	9	12	N
BJ19	Health carrier	C	20	23-1	3	9	13	9	12	N
BJ20[b]	Health carrier	C	7-2	14	3	9	15	13	7	4821
BJ21	Health carrier	C	12-6	13-4	3	9	15	13	7	4821
BJ22	Health carrier	C	20	23-7	3	9	15	13	7	4821
BJ23	Health carrier	C	20	23-7	3	9	15	13	7	4821

a BJ5 was isolated from a close contact of patient BJ4.
b BJ20 to 23 strains were isolated from throat swabs of 4 people travelling from Anhui province to Beijing, 2005.
c All of VR family 13 in VR3 were with a mutation from "DAKLALPNNNSHNSQTE" to "DAKLALPN***D***NSHNSQTE".
d Not performed.

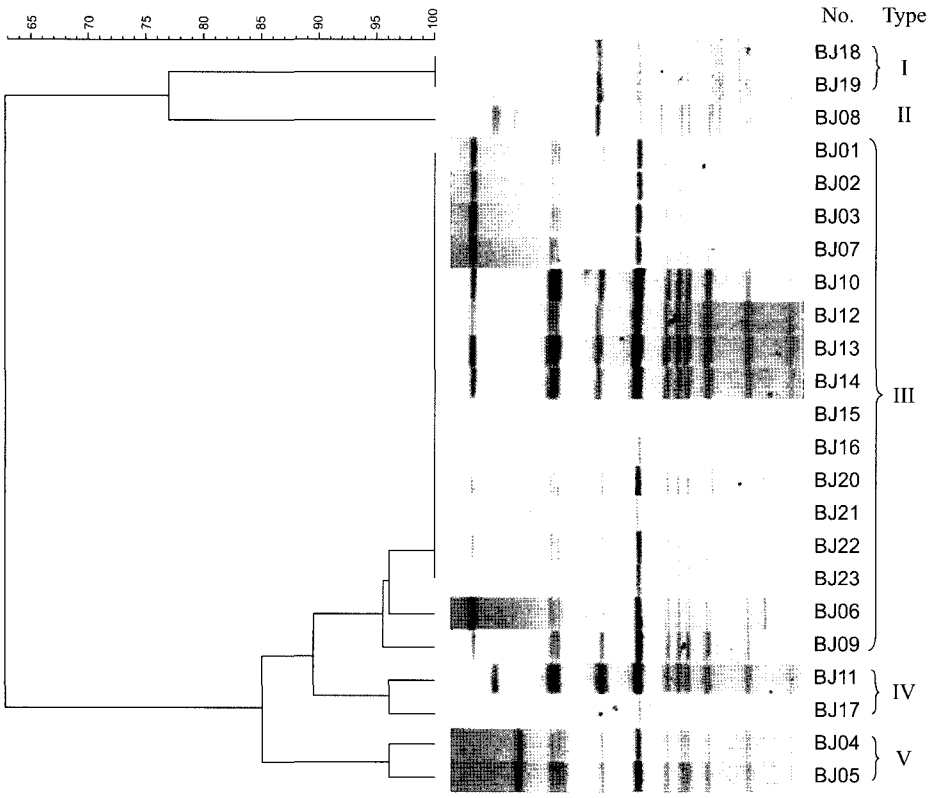

Fig. 1. PFGE types of genomic DNA of 23 serogroup C *N. meningitides* strains digested with NheI. The strain numbers and PFGE types are shown on the right. The identity between different types was less than 95%.

other parts of China by the movement of healthy carriers. Eight PorA variable-region (VR) types were found among these serogroup C strains based on the predicted amino acid sequences of loops I and IV, including P1.7-2,14, P1.7-2,23-1, P1.12-1,16-8, P1.12-6, 13-4, P1.20,23-1, P1.20,23-7, P1.20-2,23-9 and P1.21-2,4. P1.20,23-1 and P1.7-2,14 were dominant serosubtypes. P1.7-2,14 was the only dominant serosubtype in the Anhui province (Shao *et al.*, 2006). P1.12-1,16-8 and P1.20,23-1 were the causative serosubtypes in Beijing. The serosubtypes of BJ20 to BJ23 belonged to P1.7-2,14, P1.12-6,13-4, P1.20,23-7 and P1.20,23-7, respectively. All of these strains expressed class 3 PorB protein. The *PorB* gene of serogroup C strains was more conserved than the *PorA* gene. Twenty out of the 23 serogroup C strains (all except BJ14, BJ18 and BJ19) had identical serotypes, with sequences of VR family 9 in VR1, VR family 15 in VR2, VR family 13 in VR3 and VR family 7 in VR4. Notably, VR family 13 in VR3 was mutated from "DAKL ALPN*N*NSHNSQTE" to "DAKLALPN*D*NSHNSQTE". The molecular features of those serogroup C strains were further analysed by PFGE. PFGE of NheI-restricted genomic DNA showed that the 23 serogroup C strains clustered into 5 types with type III being predominant (Fig. 1). The PFGE types of patient strains belonged to types III and V. BJ20 to BJ23 from the Anhui province were also type III.

Table 2. MIC50s and MIC90s for 12 antibiotic agents

Antibiotic	Function	MIC50 (μg/ml)	MIC90 (μg/ml)
Ampicillin	Therapeutic agent	0.06	0.12
Cefotaxime		0.03	0.03
Ceftriaxone		0.015	0.015
Chloromycetin		2	2
Meropenem		0.03	0.06
Penicillin G		0.06	0.12
Ciprofloxacin	Chemoprophylactic agent	0.24	0.24
Levofloxacin		0.24	0.24
Rifampicin		0.03	0.06
Azithromycin		0.5	1
Tetracycline		4	>8
Trimethoprim/ sulfamethoxazole		2/38	4/76

An antibiotic susceptibility test was performed by

the broth microdilution method according to the protocol of the Clinical and Laboratory Standards Institutes (CLSI, 2005). The MIC50s and MIC90s of 12 different antibiotic agents were shown in Table 2. All of the strains were susceptible to ampicillin, cefotaxime, ceftriaxone, chloromycetin, meropenem, and penicillin G, which are frequently used as therapeutic agents. Twenty out of 23 strains were resistant to ciprofloxacin and 21 were resistant to levofloxacin. All of the strains were susceptible to azithromycin and rifampicin and resistant to trimethoprim/sulfamethoxazole. Ten out of 23 strains were resistant to tetracycline. We conclude that six different therapeutic agents can be used for the treatment of meningitis patients, namely ampicillin, cefotaxime, ceftriaxone, chloromycetin, meropenem, and penicillin G. Azithromycin and rifampicin could be administered to close contacts for chemoprophylaxis, whereas ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole should be avoided for chemoprophylaxis.

This study has verified the spread of ST 4821 serogroup C strains in Beijing. This spread of the ST 4821 clone confirms the importance of the use of the polysaccharide vaccine for serogroup A plus C in the prevention and control of meningitis in Beijing. In addition, the features of the ST 4821 serogroup C strains were further defined by molecular typing technology. It would be helpful to monitor the spread of this new virulent meningococci sequence type clone in China and worldwide.

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