Epidemiol. Infect. (2012), **140**, 1296–1303. © Cambridge University Press 2011
doi:10.1017/S0950268811001865

Distribution of serogroups and sequence types in disease-associated and carrier strains of *Neisseria meningitidis* isolated in China between 2003 and 2008

H. ZHOU[1], Y. GAO[1], L. XU[1], M. LI[1], Q. LI[1,2], Y. LI[3], X. LIANG[3], H. LUO[3], B. KAN[1], J. XU[1] AND Z. SHAO[1]*

[1] *National Institute for Communicable Disease Control and Prevention, and State Key Laboratory for Infectious Disease Prevention and Control, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China*
[2] *West China School of Public Health, Sichuan University, China*
[3] *National Immunization Programme, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China*

(*Accepted 24 August 2011; first published online 20 September 2011*)

SUMMARY

Given the unpredictability of *Neisseria meningitidis* outbreaks and the increased prevalence of serogroup C strains following the introduction of serogroup A-based vaccines, we conducted an analysis of serogroups and sequence types (STs) in disease-associated and carrier *N. meningitidis* isolates that have emerged in China since 2003. We used multilocus sequence-typing techniques to investigate 371 *N. meningitidis* strains isolated from patients with meningitis and healthy carriers. Two lineages were identified in serogroup A and C isolates, genotyped as the ST5 complex and ST4821 complex, respectively. Both clonal complexes were found throughout China, although ST4821 was more concentrated in the eastern region of the country. The ST5 complex has been persistent in China since the late 1980s and has since spread across the entire country. Isolates belonging to the ST4821 complex have been a dominant lineage since 2003.

Key words: Infectious disease epidemiology, meningococcal disease, molecular epidemiology, *Neisseria meningitidis*.

INTRODUCTION

Neisseria meningitidis frequently colonizes nasopharyngeal mucosal membranes and occasionally causes life-threatening diseases, such as meningitis and septicaemia [1, 2]. An estimated 100 000 cases of *N. meningitidis* infection are reported worldwide each year despite the existence of partially effective vaccines [3]. More than ten serogroups have been classified on the basis of the chemical and serological properties of the capsular polysaccharide. Most cases of invasive disease are caused by serogroups A, B, C, Y and W135 [2, 4]. A notable epidemiological feature of meningococcal disease is that the serogroup frequency of disease-associated isolates can vary over time and between different geographical regions [4, 5].

Several molecular typing methods have been developed for use in epidemiological studies of *N. meningitidis* [6–8]. Of these, multilocus sequence typing (MLST) is a very useful molecular method for tracking the national and global spread of

* Author for correspondence: Dr Z. Shao, 155 Changbai Road, Department of Respiratory Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Changping, Beijing, People's Republic of China.
(Email: shaozhujun@icdc.cn)

meningococcal clones, and for identifying lineages that have an increased propensity to cause meningococcal disease [9–15]. The population structure of *N. meningitidis* is effectively panmictic as a result of frequent horizontal genetic exchanges [16]. However, groups of *N. meningitidis* with highly related sequence types (STs) can persist for decades and spread as clonal populations. These groups are referred to as clonal complexes. Although members within a clonal complex are similar to each other, different clonal complexes are genetically dissimilar and easily distinguished by MLST [6]. *N. meningitidis* isolates derived from carriers are more genetically diverse than strains associated with invasive disease. The majority of cases of meningococcal disease are caused by a limited number of clonal complexes, now termed hyperinvasive lineages [15, 17]. Strains isolated from carriers comprise numerous STs, with only a small proportion of the strains representing invasive clones. Transmissibility during the carriage state is an essential component of the life cycle of *N. meningitidis*. Co-colonization with other bacteria may lead to genetic exchange, which may result in the emergence of new meningococcal clones.

At any particular time and geographical location, the invasive strains of one hyperinvasive lineage usually belong to the same serogroup. ST7 serogroup A has been the most important serogroup in Africa and Asia, although ST11 serogroup W135 disease has also occurred recently in Africa. In other regions of the world, such as USA, Canada and Latin America, most meningococcal disease outbreaks have been caused by serogroups B, C and Y of several hyperinvasive lineages, e.g. ST11 and ST41/44 complexes [5]. The MLST technique for characterizing *N. meningitidis* strains was adopted by the Chinese Center for Disease Control and Prevention (CDC) following the emergence of serogroup C meningococcal disease [14]. In combination with available epidemiological data, information on STs that cause sporadic cases and outbreaks in China has the potential to significantly enhance our understanding of diseases associated with *N. meningitidis*.

From the 1950s to the 1980s, more than 95% of meningococcal cases in China were caused by *N. meningitidis* serogroup A, with an annual disease rate of up to 500 cases/100 000 inhabitants. Serogroups B and C were less common and caused only sporadic cases of meningococcal meningitis [18]. A national immunization programme, using a serogroup A polysaccharide-based vaccine was initiated in the early 1980s, which involved the administration of four injections to each child: the first at age 6 months, the second 3 months later, the third at age 3 years, and the final one at age 6 years. During the following two decades only sporadic cases were reported. However, between 2003 and 2004 a new hyperinvasive lineage, the ST4821 complex, was identified in Anhui province. This lineage spread to 11 other Chinese provinces between 2004 and 2005, causing several outbreaks of serogroup C meningococcal disease and an increase in the number of serogroup C cases [14]. Following the initial serogroup C disease outbreak in 2003, a vaccination programme using a serogroup C polysaccharide-based vaccine, was initiated in conjunction with administration of serogroup A vaccine. A conjugate A+C vaccine was also used in some provinces. At present, more than 95% of meningococcal disease seen in China is caused by serogroups A or C *N. meningitidis*.

Given the unpredictability of *N. meningitidis* outbreaks and the increased prevalence of serogroup C strains after the introduction of serogroup A-based vaccines, we conducted an analysis of serogroups and STs among disease-associated and carrier *N. meningitidis* isolates that have emerged in China since 2003. The study investigated the distribution of serogroups and STs in a collection of 371 strains of meningococci isolated from individuals with meningococcal disease and asymptomatic carriers from 2003 to 2008. Studies such as ours have the potential to increase our knowledge of the molecular epidemiology of meningococcal disease, thereby increasing our ability to respond appropriately and effectively to disease outbreaks.

METHODS

Bacterial isolates and culture conditions

The study was conducted over a period of five academic years (from October to September of the following year). During this period, a total of 371 *N. meningitidis* strains were collected for analysis. The strains were isolated from 27 provinces in China between 2003 and 2008 (Table 1). All 27 provinces have laboratories that routinely collect pathogenic *N. meningitidis* isolates and periodically carry out surveys of *N. meningitidis* carriers for outbreak investigation, surveillance and research. Our laboratory requested that strains isolated by provincial CDCs were sent to our laboratory. Bacteria were identified

Table 1. *Numbers of* Neisseria meningitidis *isolates according to province and academic year*

	No. of isolates										
	2003–2004		2004–2005		2005–2006		2006–2007		2007–2008		
	Patient	Carrier	Patient	Carrier	Patient	Carrier	Patient	Carrier	Patient	Carrier	Total
Anhui	1	28	7	12	15	6	8	1			78
Beijing	1		3	24	3	6	3				40
Fujian			1		2		1				4
Gansu			1	3					1	1	6
Guangdong			8	2	2	1	1	2			16
Guangxi									1	1	2
Guizhou			1	1							2
Hainan				1							1
Hebei		3	2	3						13	21
Henan			1								1
Hubei				1		2		2	2	2	9
Jilin					2						2
Jiangsu			15	3	3					1	22
Jiangxi				29	2	19			5	1	56
Liaoning						3		2			5
Neimenggu								3		2	5
Ningxia				2			1	2			5
Qinghai			2		1	1					4
Shandong				8	3	6					17
Shanxi			1	3							4
Shanghai			3						12	4	19
Sichuan			1	8	1	9		1			20
Tianjin			2			4					6
Xinjiang							1				1
Yunnan				2		7					9
Zhejiang			4				4	6			14
Chongqing			1				1				2
Total	2	31	53	102	34	64	20	19	21	25	371

by Gram stain, oxidase reaction, and standard biochemical tests using API® NH test kits (bioMérieux, France). *N. meningitidis* strains were serogrouped by slide agglutination using polyclonal antisera to different serogroups (Difco, France). Strains were stored at −80 °C in brain heart broth with 15% sterile glycerol.

MLST

Fragments from seven housekeeping genes were used for typing: *abc*Z (putative ABC transporter), *adk* (adenylate kinase), *aro*E (shikimate dehydrogenase), *fum*C (fumarase), *gdh* (glucose-6-phosphate dehydrogenase), *pdh*C (pyruvate dehydrogenase subunit), and *pgm* (phosphoglucomutase), as given on the MLST website (http://pubmlst.org/neisseria/) [19]. Following DNA preparation and amplification by PCR, each locus sequence was analysed on an ABI Prism 3130 or 3730 DNA sequencer (Applied Biosystems, USA).

Data analysis

The sequence data were assembled using SeqMan II of the DNASTAR program (DNASTAR, USA). The sequences were compared with existing alleles on the MLST website in order to determine allele numbers, STs, and to assign the isolates to the correct clonal complexes. BioNumerics software (version 5.10; Applied Maths, USA) was used to create a minimum spanning tree [20]. In the minimum spanning tree, the founder ST was defined as the ST with the greatest number of single-locus variants.

https://doi.org/10.1017/S0950268811001865 Published online by Cambridge University Press

RESULTS

Source and serogroup typing of *N. meningitidis* isolates

The 371 *N. meningitidis* isolates analysed in our study were isolated from 130 (35·0%) samples of cerebrospinal fluid or blood from patients, and 241 (65·0%) throat swab samples from healthy carriers. However, the carrier strains observed in this study are not truly representative of normal carriage, as some were associated with cases of invasive meningococcal disease. Of the 130 disease isolates, the two most common serogroups were C (76 isolates, 58·5%) and A (45, 34·6%), whereas serogroups B (5, 3·9%), W135 (3, 2·3%) and X (1, 0·8%) were less frequently observed. Of the 241 carrier isolates, the main serogroups were C (150, 62·2%), B (47, 19·5%) and A (23, 9·5%), followed by W135 (3, 1·2%), Z (1, 0·4%), X (1, 0·4%) and 29E (1, 0·4%). Serogrouping was not possible in 15 isolates, all of which were from the carrier group (Supplementary Table 1, available online).

Genotypic characterization of *N. meningitidis* isolates

There were 19 STs among the 130 disease isolates. Fifteen of the STs, representing 96·9% (126/130) of the isolates, could be assigned to five clonal complexes. Four STs, representing 3·1% (4/130) of the isolates, could not be assigned to any known clonal complexes (Supplementary Table 1). Of the 241 carrier isolates, 62 STs were identified. Twenty-nine of these STs could be assigned to eight clonal complexes (representing 81·3% of the isolates). Thirty-three of the STs could not be assigned to any known clonal complexes. Of the total number of 74 STs, only seven were shared between disease and carrier isolates.

ST4821 and ST5 complexes were the commonest clonal complexes in both disease and carrier isolates. Just over half (55·4%, 72/130) of the disease isolates and 62·2% (150/241) of the carrier isolates could be assigned to the ST4821 complex. The ST5 complex was present in 36·9% (48/130) and 11·2% (27/241) of the disease and carrier isolates, respectively. The remaining disease isolates were assigned to the ST11 complex (four isolates), ST103 complex (one isolate), and the ST32 complex (one isolate). Four isolates could not be assigned to a clonal complex.

There were more STs and clonal complexes in the carrier isolates. In addition to the ST4821 and ST5 complexes, the ST198 complex (seven isolates), ST41/44 complex (six isolates), ST1 complex (two isolates), ST32 complex (two isolates), ST11 complex (one isolate), and ST174 complex (one isolate) were also observed in the carrier isolates. Thirty-three STs (45 total isolates, with between 1 and 3 isolates in each ST) could not be assigned to any known complex.

Serogroup diversity of lineages

The serogroups varied in their genotypic diversity. Serogroup A and C isolates were conserved irrespective of source. Other serogroups and non-serogroupable isolates exhibited higher genotypic diversity in both disease and carrier isolates (Fig. 1). Most (94·1%) serogroup A isolates were associated with the ST5 complex and most (87·6%) serogroup C isolates with the ST4821 complex. There were no dominating STs in the other serogroups.

Some clonal complexes were strongly associated with particular serogroups, although these associations were not absolute (Supplementary Table 1, Fig. 1). Most of the ST4821 complex isolates belonged to serogroup C (198/222, 89·2%), for example, but a minority were identified as belonging to other serogroups, including serogroups B (14/222, 6·3%), A (1/222, 0·5%) or W135 (1/222, 0·5%). Eight (3·6%) members of this clonal complex could not be serogrouped. The ST5 complex was associated with isolates that belonged predominantly to serogroup A (64/75, 85·3%), with a minority belonging to serogroups C (8/75, 10·7%) and B (2/75, 2·7%). One isolate of the ST5 complex could not be serogrouped. Four of five ST11 complex isolates were W135. All isolates associated with ST41/44 and ST32 were serogroup B.

Nine STs contained isolates from different serogroups (Supplementary Table 1, Fig. 1). Most serogroups had isolates that belonged to ST2146 (ST198 complex). One isolate from serogroup B, two from serogroup C, one from serogroup X, one from serogroup 29E and two isolates that could not be serogrouped, all belonged to ST2146.

Temporal and geographical distribution of clonal complexes

The ST4821 and ST5 complexes were observed in each year of study, and were always the two most frequent complexes. Isolates of other clonal complexes and STs appeared more sporadically (Supplementary Table 2,

https://doi.org/10.1017/S0950268811001865 Published online by Cambridge University Press

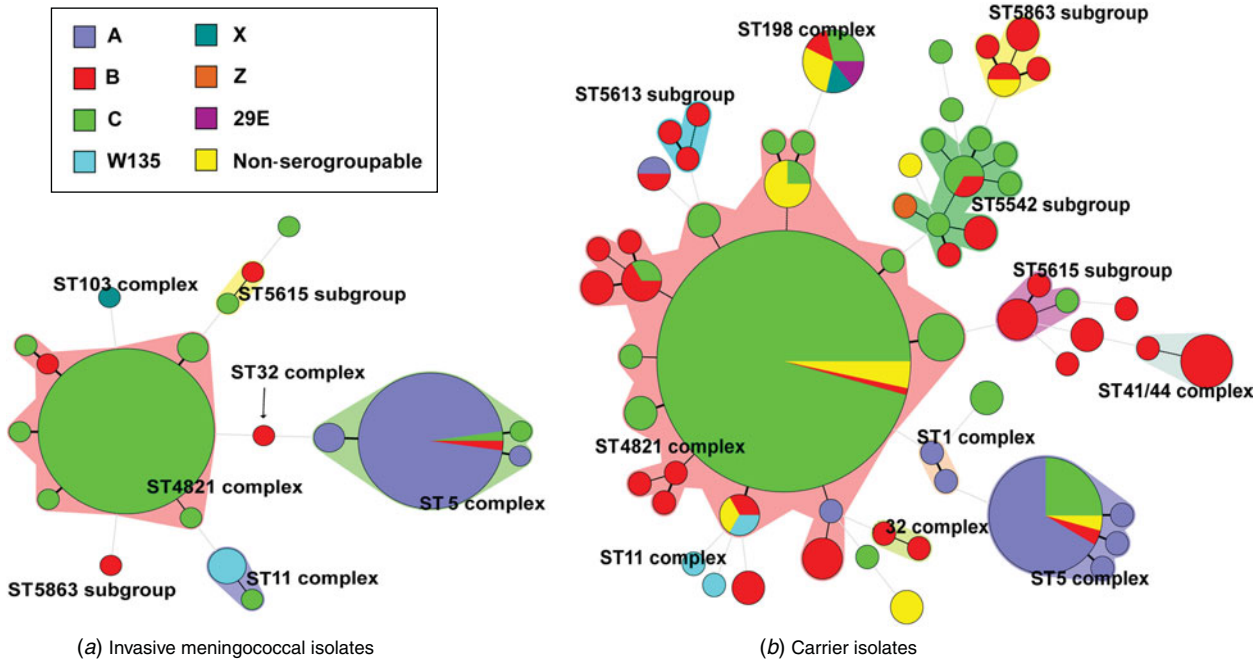

Fig. 1. Minimum spanning tree analysis of *Neisseria meningitidis* strains according to sequence type (ST), 2003–2008. (*a*) Relationships of 130 invasive meningococcal isolates; (*b*) relationships of 241 carrier isolates. (This is not representative of any kind of normal carriage, because parts are associated with cases of invasive meningococcal disease.) In the minimum spanning tree, the STs are displayed as circles. The size of each circle indicates the number of isolates within this particular type. Serogroups are represented by different colours. Heavy solid lines represent single-locus variants; light solid lines represent double-locus variants; heavy dotted lines represent triple-locus variants; light dotted lines represent quadruple-locus variants; and grey circles represent STs that are not part of any clonal complex. The colours of the halo surrounding the STs denote types that belong to the same clonal complex. The clonal complexes not identified by the MLST website were named 'subgroups'. Two lineages were identified in serogroup A and C isolates, which were genotyped as ST5 complex and ST4821 complex, respectively. The strains of other serogroups exhibited higher genotypic diversity.

available online). Between 2003 and 2008, frequencies of 5·5, 4·0, 4·3, 3·9 and 3·3 strains/ST were observed, with no upward or downward trend over the timeline of the study.

The occurrence of ST4821 and ST5 complexes was geographically diverse (Fig. 2). Isolates of the ST5 complex were found in 21 provinces spread across China, whereas isolates from the ST4821 complex were mainly concentrated in the eastern provinces. In most of the central and western provinces, only isolates from the ST5 clonal complex were observed. In the two central provinces of Sichuan and Gansu, where ST5 and ST4821 complexes co-exist, the ST5 complex was found more frequently. However, in the eastern regions where both complexes also co-exist, the ST4821 complex was found more frequently than the ST5 complex. Of the less frequently observed clonal complexes, ST11 was distributed in the south-eastern region, and most (5/6) isolates in the ST41/44 complex were observed in Sichuan province.

DISCUSSION

In this study, MLST was used to investigate the molecular characteristics of *N. meningitidis* strains isolated in China between 2003 and 2008. The *N. meningitidis* strains tested in this study were isolated from 27 provinces in China between 2003 and 2008, which presumably represented the most abundant diversity of *N. meningitidis* strains in this period. The 130 disease-associated strains examined represent almost 1% of the cases seen in China between 2003 and 2008, and those from carriers were obtained from routine surveys of *N. meningitidis*. However, these carriers were not 'normal' in the strict sense, as there had been some association with invasive meningococcal disease. Notwithstanding, this study, which combines data from both disease-associated and normal carrier strains, provides the most representative data to date of the entire Chinese meningococcal population.

https://doi.org/10.1017/S0950268811001865 Published online by Cambridge University Press

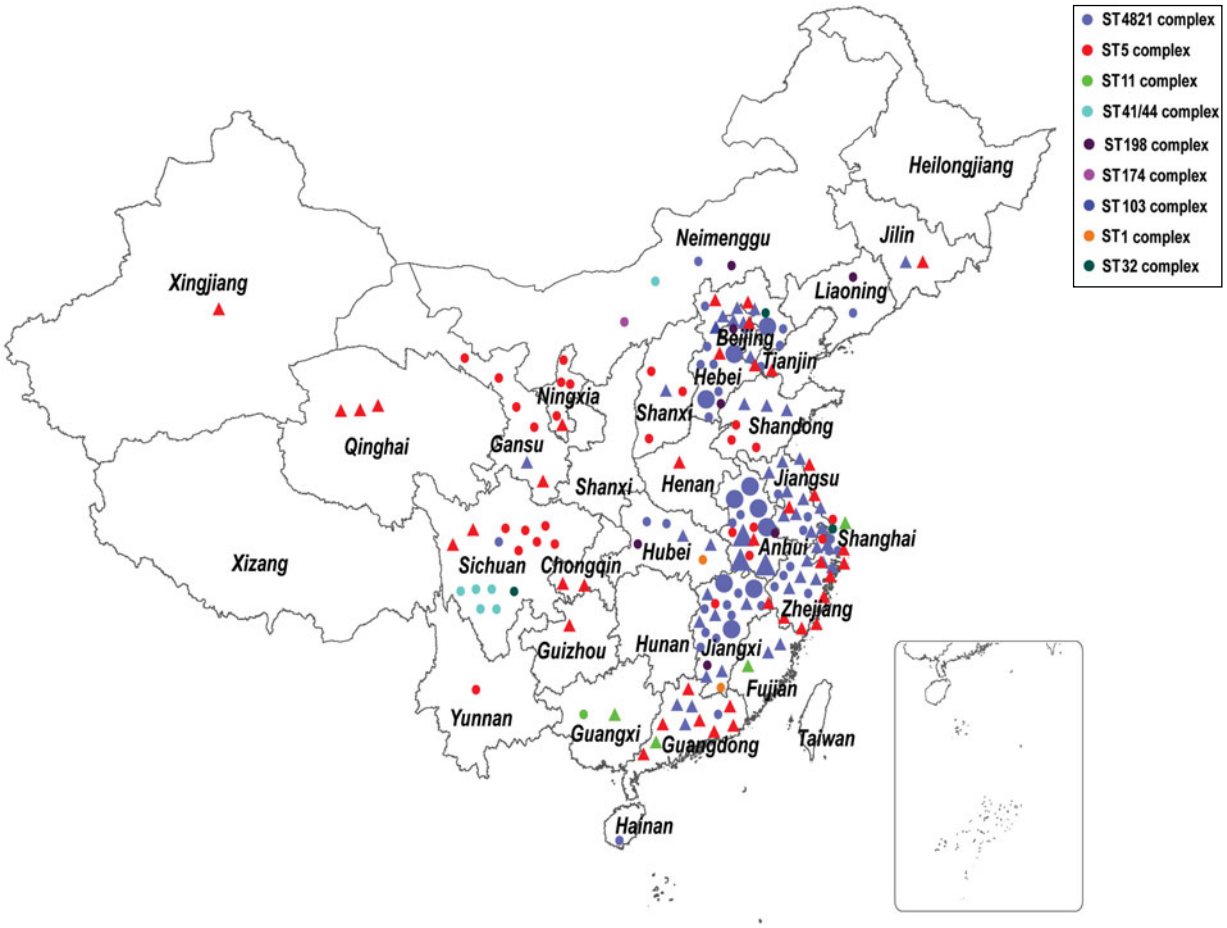

Fig. 2. Distribution of *Neisseria meningitidis* clonal complexes in China between 2003 and 2008. The triangles represent strains isolated from patients and the circles represent those from healthy carriers. The small triangles and circles represent a single isolate and the large triangles and circles represent 10 isolates.

Of the isolates collected from patients, the main serogroups were C and A, and those collected from carriers, C, B and A. Two clones predominated, ST7 and ST4821, associated with serogroup A and serogroup C isolates, respectively. Isolates from other serogroups, and also isolates which could not be serogrouped, were highly diverse, with no single ST dominating.

Collections of meningococci recovered from healthy carriers exhibit greater genetic diversity than that observed in patients with invasive disease [15, 21, 22]. The majority of cases of meningococcal disease reported to date are caused by a limited number of clonal complexes [17]. Our data are consistent with these observations [23]. Strains belonging to the ST1 (ST3) and ST5 complexes (ST5 and ST7) were responsible for almost all the meningococcal disease in China prior to 2003. Since 2003, most meningococcal disease has been caused by ST7 and ST4821 isolates. Isolates of other clonal complexes were only responsible for sporadic cases [24].

The ST7 clone emerged in China in the late 1980s, replacing the ST5 clone as the dominant cause of disease [23]. This clone subsequently appeared in Mongolia (1994), Russia (1994–1997, 2003), Egypt (2000), and African countries (1995–2008) [25, 26]. The ST7 clone, which emerged in Africa in 1995 and 1997, appears to be responsible for a new wave of epidemics [13, 25, 27]. In this study, most of the serogroup A isolates were classified as ST7. No ST5 isolate was detected in China between 2005 and 2008. Previous data demonstrate that the ST7 serogroup A clone has been persistent in China since the late 1980s. Recent outbreaks of serogroup A disease have occurred in India [28] and the Philippines in 2005 [29]. Using the MLST database, the disease-causing strain responsible for the outbreak in the Philippines was identified as ST7 [26]. Based on limited studies of

https://doi.org/10.1017/S0950268811001865 Published online by Cambridge University Press

typing *N. meningitidis* isolates, no ST7 serogroup A isolate was found in any other Asian country between 2003 and 2008 [30, 31].

In 2003, in Anhui province of China, ST4821 serogroup C isolates were responsible for increased incidence of meningococcal infections. To date, this lineage has only been found in China and has become more common than the ST7 serogroup A strain in the eastern region of the country. In addition, this new hyperinvasive lineage is now spreading across China, with meningococcal epidemics occurring throughout the entire country over the past 50 years. Fortunately, the ST4821 complex is not the most dominant lineage found in these provinces so far.

Capsular switching in a meningococcal isolate is said to occur when a ST in a serogroup not generally associated with that clonal complex is observed [32, 33]. This phenomenon is not infrequent and has been observed worldwide [12, 34–38]. In this study, we also observed several STs that belonged to different serogroups, suggesting a high degree of capsular switching. For example, there were some serogroup B isolates associated with ST4821. This was also observed in China in the 1980s [39], suggesting that capsular switching between serogroup B and C isolates could have occurred before or after the ST4821 serogroup C disease outbreak in 2003. In the current study, several serogroup C isolates belonged to ST7. Thus, since most ST7 isolates belong to serogroup A, capsular switching could explain the appearance of the ST7 serogroup C strains. By sequencing and comparing the whole capsular gene clusters and surrounding genes, Wang *et al*. confirmed that recombination is the genetic basis of capsular switching between ST7 serogroup A and C strains [40]. As details surrounding capsular switching in *N. meningitidis* remain unclear, continued surveillance is needed.

This study is the first to investigate the diversity of *N. meningitidis* STs and their geographical distribution in China since the emergence of serogroup C strains post-introduction of serogroup A-based vaccines. Using the MLST technique, we typed 371 different *N. meningitidis* strains isolated over a 5-year period. The ST4821 and ST5 complexes were the two most commonly occurring clonal complexes in both disease and carrier (albeit not truly representative of a normal carrier) isolates in China. The ST5 complex has been persistent in China since the late 1980s and has spread throughout the entire country. Isolates belonging to the ST4821 complex have appeared as a dominant lineage since 2003, particularly in eastern China.

NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

ACKNOWLEDGEMENTS

This study was supported by grants from the Ministry of Health and the Ministry of Science and Technology (2011CB504900 and 2008ZX10004-008), People's Republic of China.

DECLARATION OF INTEREST

None.

REFERENCES

1. **DeVoe IW.** The meningococcus and mechanisms of pathogenicity. *Microbiological Reviews* 1982; **46**: 162–190.
2. **Rosenstein NE, *et al.*** Meningococcal disease. *New England Journal of Medicine* 2001; **344**: 1378–1388.
3. **World Health Organization.** WHO report on global surveillance of epidemic-prone infectious diseases. Geneva: World Health Organization, 2000.
4. **Peltola H.** Meningococcal disease: still with us. *Reviews of Infectious Diseases* 1983; **5**: 71–91.
5. **Harrison LH, *et al.*** Global epidemiology of meningococcal disease. *Vaccine* 2009; **24**: 27.
6. **Maiden MC, *et al.*** Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences USA* 1998; **95**: 3140–3145.
7. **Popovic T, *et al.*** Evaluation of pulsed-field gel electrophoresis in epidemiological investigations of meningococcal disease outbreaks caused by *Neisseria meningitidis* serogroup C. *Journal of Clinical Microbiology* 2001; **39**: 75–85.
8. **Yakubu DE, Abadi FJ, Pennington TH.** Molecular typing methods for *Neisseria meningitidis*. *Journal of Medical Microbiology* 1999; **48**: 1055–1064.
9. **Doyle TJ, *et al.*** Cluster of serogroup W135 meningococci, southeastern Florida, 2008–2009. *Emerging Infectious Diseases* 2010; **16**: 113–115.
10. **Enright MC, Spratt BG.** Multilocus sequence typing. *Trends in Microbiology* 1999; **7**: 482–487.
11. **Kilic A, *et al.*** Clonal spread of serogroup W135 meningococcal disease in Turkey. *Journal of Clinical Microbiology* 2006; **44**: 222–224.

12. **Mayer LW, *et al.*** Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electophoretic type-37 complex. *Journal of Infectious Diseases* 2002; **185**: 1596–1605.
13. **Nicolas P, *et al.*** Molecular epidemiology of *Neisseria meningitidis* isolated in the African Meningitis Belt between 1988 and 2003 shows dominance of sequence type 5 (ST-5) and ST-11 complexes. *Journal of Clinical Microbiology* 2005; **43**: 5129–5135.
14. **Shao Z, *et al.*** Identification of a new *Neisseria meningitidis* serogroup C clone from Anhui province, China. *Lancet* 2006; **367**: 419–423.
15. **Yazdankhah SP, *et al.*** Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *Journal of Clinical Microbiology* 2004; **42**: 5146–5153.
16. **Smith JM, *et al.*** How clonal are bacteria? *Proceedings of the National Academy of Sciences USA* 1993; **90**: 4384–4388.
17. **Caugant DA, *et al.*** Clonal diversity of *Neisseria meningitidis* from a population of asymptomatic carriers. *Infection and Immunity* 1988; **56**: 2060–2068.
18. **Hu X.** Study on periodically prevalent feature for epidemic cerebrospinal meningitis in China. *Zhonghua Liu Xing Bing Xue Za Zhi* 1991; **12**: 136–139.
19. **Jolley KA, Chan MS, Maiden MC.** mlstdbNet – distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics* 2004; **5**: 86.
20. **Feil EJ, *et al.*** eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology* 2004; **186**: 1518–1530.
21. **Climent Y, *et al.*** Clonal distribution of disease-associated and healthy carrier isolates of *Neisseria meningitidis* between 1983 and 2005 in Cuba. *Journal of Clinical Microbiology* 2010; **48**: 802–810.
22. **Jolley KA, *et al.*** Carried meningococci in the Czech Republic: a diverse recombining population. *Journal of Clinical Microbiology* 2000; **38**: 4492–4498.
23. **Zhang X, *et al.*** Genetic characteristics of serogroup A meningococci circulating in China, 1956–2005. *Clinical Microbiology and Infection* 2008; **14**: 555–561.
24. **Shao Z, *et al.*** *Neisseria meningitidis* Serogroup W135, China. *Emerging Infectious Diseases* 2010; **16**: 348–349.
25. **Nicolas P, *et al.*** Clonal expansion of sequence type (ST-) 5 and emergence of ST-7 in serogroup A meningococci, Africa. *Emerging Infectious Diseases* 2001; **7**: 849–854.
26. **Neisseria sequence typing database.** (http://pubmlst.org/neisseria/).
27. **Nicolas P, *et al.*** Molecular epidemiology of meningococci isolated in Niger in 2003 shows serogroup A sequence type (ST)-7 and serogroup W135 ST-11 or ST-2881 strains. *Journal of Clinical Microbiology* 2005; **43**: 1437–1438.
28. **Nair D, *et al.*** Outbreak of meningococcal disease in and around New Delhi, India, 2005–2006: a report from a tertiary care hospital. *Epidemiology and Infection* 2009; **137**: 570–576.
29. **World Health Organization.** Meningococcal disease in the Philippines – update, 19 January 2005.
30. **Bae SM, Kang YH.** Serological and genetic characterization of meningococcal isolates in Korea. *Japanese Journal of Infectious Diseases* 2008; **61**: 434–437.
31. **Takahashi H, *et al.*** Characterization of *Neisseria meningitidis* isolates collected from 1974 to 2003 in Japan by multilocus sequence typing. *Journal of Medical Microbiology* 2004; **53**: 657–662.
32. **Swartley JS, *et al.*** Capsule switching of *Neisseria meningitidis*. *Proceedings of the National Academy of Sciences USA* 1997; **94**: 271–276.
33. **Vogel U, Claus H, Frosch M.** Rapid serogroup switching in *Neisseria meningitidis*. *New England Journal of Medicine* 2000; **342**: 219–220.
34. **Beddek AJ, *et al.*** Evidence for capsule switching between carried and disease-causing *Neisseria meningitidis* strains. *Infection and Immunity* 2009; **77**: 2989–2994.
35. **Harrison LH, *et al.*** Antigenic shift and increased incidence of meningococcal disease. *Journal of Infectious Diseases* 2006; **193**: 1266–1274.
36. **Harrison LH, *et al.*** Population structure and capsular switching of invasive *Neisseria meningitidis* isolates in the pre-meningococcal conjugate vaccine era – United States, 2000–2005. *Journal of Infectious Diseases* 2010; **201**: 1208–1224.
37. **Linz B, *et al.*** Frequent interspecific genetic exchange between commensal *Neisseriae* and *Neisseria meningitidis*. *Molecular Microbiology* 2000; **36**: 1049–1058.
38. **Tsang RS, *et al.*** Potential capsule switching from serogroup Y to B: the characterization of three such *Neisseria meningitidis* isolates causing invasive meningococcal disease in Canada. *Canadian Journal of Infectious Diseases & Medical Microbiology* 2005; **16**: 171–174.
39. **Yang L, *et al.*** Genotypic characterization of *Neisseria meningitidis* serogroup B strains circulating in China. *Journal of Infection* 2008; **56**: 211–218.
40. **Wang Q, *et al.*** Genetic study of capsular switching between *Neisseria meningitidis* sequence type 7 serogroup A and C strains. *Infection and Immunity* 2010; **78**: 3883–3888.