Characterization of *emm* types and superantigens of *Streptococcus pyogenes* isolates from children during two sampling periods

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SUMMARY

The characteristics of 359 group A streptococcal (GAS) isolates collected from Chinese paediatric patients in two periods (1993–1994, 2005–2006) were studied. Isolates were assigned to *emm* types and assayed for eight superantigen (SAg) genes (*speA*, *speC*, *speH*, *speI*, *speG*, *speJ*, *ssa*, *SMEZ*). Types *emm*1 and *emm*12 were consistently the most prevalent during the two periods, while others varied in frequency. GAS isolates carrying six or more SAg genes increased from 46·53 % (1993–1994) to 78·39 % (2005–2006); *ssa*, *speH* and *speJ* genes ($P < 0·05$) increased but *speA* declined ($P < 0·05$). SAg gene profiles were closely associated with the *emm* type, but strains of the same *emm* type sometimes carried different SAg genes in the two periods. No significant difference in *emm*-type distribution and SAg gene profile was noted between isolates from different diseases. These data may contribute towards the development of a GAS vaccine in China.

Key words: *emm* typing, epidemiology, *Streptococcus pyogenes*, superantigen.

INTRODUCTION

Group A streptococci (GAS) are one of the most common human pathogens that cause both invasive and non-invasive infections. GAS-associated diseases are more common in children than adults and clinical presentation ranges from pharyngitis and impetigo to invasive infections particularly acute rheumatic fever and acute glomerulonephritis [1]. In the late 1980s concern about GAS disease was heightened in many countries, as outbreaks of invasive clinical infection were reported [2]. Several factors have been considered to explain differences in disease frequency and severity, including changes in the virulence of the bacterium associated with expression of M protein and a group of exotoxins known as streptococcal superantigens (SAgs).

The M protein is an important virulence determinant in GAS which is determined by the *emm* gene. Currently, more than 170 *emm* types and 750 *emm* subtypes of GAS have been described [3]. Studies of GAS *emm*-typing distribution performed worldwide, have shown a differing distribution of *emm* types

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in different countries and regions [4–7]. SAgs are thought to contribute to the pathogenesis of severe GAS infections by virtue of their potent immuno-stimulatory activity and the distribution of profiles of genes encoding SAgs has been used as an additional epidemiological tool to explore the genomic hetero-geneity and possible correlation between toxin gene content and disease type [8].

Effective vaccines against GAS may be based on complex combinations of antigens, particularly type-specific M-protein components. In addition, virulence factors such as the SAgs may also be important for the design of potential vaccines. The formulations for these vaccines would require knowledge of the distri-bution of *emm* types and virulence factors among GAS isolates in a community. In some regions of China there are few studies available on *emm* types and virulence factors [9, 10] and no large-scale study has been conducted in Chinese children. We therefore set out to document the *emm* types and Sag gene profiles in GAS isolates collected from paediatric patients during two periods separated by several years.

MATERIALS AND METHODS

Bacterial isolates

This survey involved 359 GAS isolates collected from children in China during two periods. In period A (1993–1994), 137 isolates comprised those from throat swabs (110), pus (16) and ear swabs (11); in period B (2005–2006) the collection of 222 isolates were from throat swabs (203), puss (10), vulvar secretions (5), pleural effusion (2), and blood (2). The clinical diag-noses of the patients during period A included phar-yngitis (46), scarlet fever (54), impetigo (16), otitis media (11), nephritis (8), erysipelas (1), cellulitis (1). During period B, the clinical diagnoses were pharyn-gitis (44), scarlet fever (139), impetigo (10), erysipelas (9), bronchitis (6), colpitis (5), nephritis (2), urticaria (2), pleural effusion (2), sepsis (2) and psoriasis (1).

Specimens taken were inoculated onto 5% defibri-nated sheep blood tryptone soy agar plates and incubated for 24 h at 37 °C in 5% CO_2. Preliminary identification of isolates as *Streptococcus pyogenes* was based on β-haemolysis on sheep blood agar, col-ony morphology and presence of group A antigen, which was confirmed by a Streptococcal grouping kit (Oxoid Ltd, UK). Chromosomal DNA was extracted from freshly grown isolates using a Chelex-based DNA extraction kit (Beijing SBS Genetech Co. Ltd, China).

emm typing

emm typing was performed according to the protocol of the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/ncidod/biotech/strep/protocols.html). The 5′ end of the DNA sequences was compared to sequences listed in the CDC database (http://www.cdc.gov/ncidod/biotech/strep/strepblast.html).

Identification of SAg genes

PCR was used to detect the presence of eight SAg genes (*speA*, *speC*, *speH*, *speI*, *speG*, *speJ*, *ssa*, *SMEZ*). The PCR conditions were: denaturing for 1 min at 94 °C, 30 cycles for 30 s at 94 °C and annealing for 30 s at the temperature determined for each primer pair. Primers were as previously de-scribed [8].

Statistical analysis

SPSS software, version 11.5 was used for all analyses (SPSS Inc., USA). In χ^2 test a P value < 0.05 was con-sidered significant.

RESULTS

Distribution of *emm* types over the two sampling periods

A total of 24 *emm* types were identified among the 359 GAS isolates (Fig. 1). In period A, 24 *emm* types were detected, with the six most prevalent being *emm*3 (26·3%), *emm*1 (17·5%), *emm*4 (13·9%), *emm*12 (10·2%), *st*1815 (7·3%) and *emm*6 (6·6%); these types accounted for 81·8% of all the isolates. Simi-larly, in period B, nine *emm* types were identified, with *emm*12 (55·9%) and *emm*1 (39·6%) accounting for 95·5% of the isolates. Of four invasive isolates, two were from pleural effusion (of types *emm*1 and *emm*12) and two from sepsis cases both of which were type *emm*1. Over the two sampling periods *emm*1 ($P < 0·01$) and *emm*12 ($P < 0·01$) significantly in-creased, while *emm*3 ($P < 0·01$) decreased in the se-cond period. Types *emm*4, *st*1815 and *emm*6 which were prevalent in the first sample were not found in the second period.
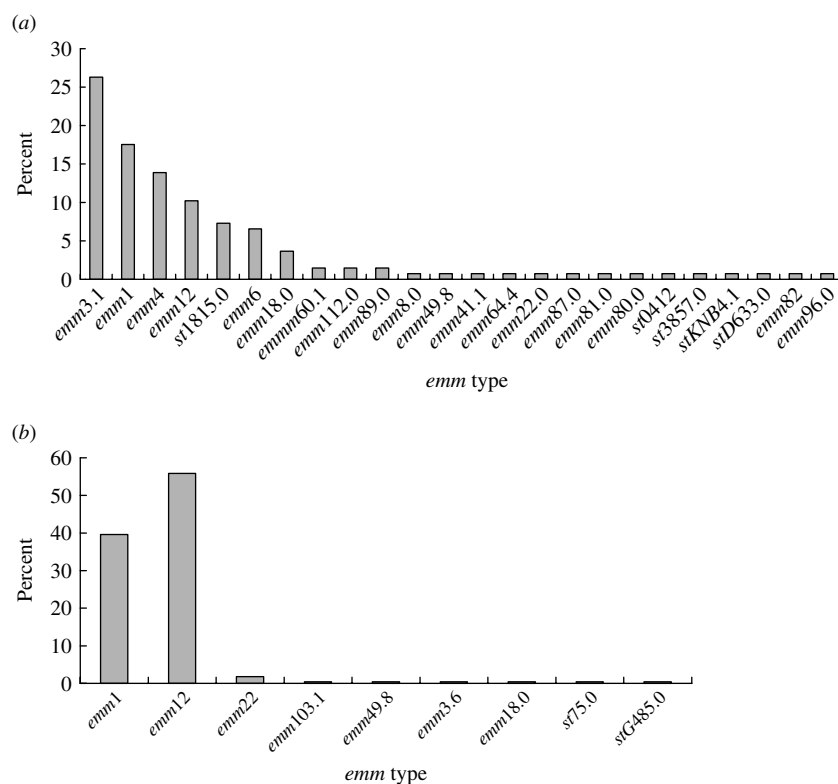
(a)

(b)

Fig. 1. Distribution of *emm* types among GAS strains isolated from patients during (*a*) period A (1993–1994), (*b*) period B (2005–2006).

Distribution of *emm* types among scarlet fever and pharyngitis isolates

In period A, 14 *emm* types were identified among the pharyngitis isolates; *emm*1, *emm*3 and *emm*12 alone constituted 21·7%, 21·8% and 13·0%, respectively. Ten types were represented among scarlet fever isolates of which *emm*1, *emm*3 and *emm*12, respectively, accounted for 14·8%, 44·4% and 7·4% of the isolates. By contrast, in period B, only four *emm* types were found among the pharyngitis isolates; *emm*1 and *emm*12 alone constituted 43·2% and 52·3% of the *emm* types, respectively. These types were also prevalent among scarlet fever isolates (*emm*1, 40·3%; *emm*12, 56·1%) where only five different types were identified. No significant difference was revealed in the *emm*-type distributions among scarlet fever and pharyngitis isolates ($P > 0.05$) over the two sampling periods.

Distribution of SAg genes

All eight SAg genes, *speA* (68·4%), *spec* (92·6%), *speH* (32·6%), *speI* (78·1%), *speG* (91·9%), *speJ* (28·4%), *ssa* (51·1%) *and SMEZ* (97%) were

identified in isolates from the first sampling period. In period B, their respective frequencies were *speA* (46·8%), *speC* (98·6%), *speH* (63·5%), *speI* (76·8%), *speG* (96%), *speJ* (41·8%), *ssa* (92·5%) and *SMEZ* (100%). The incidence of *ssa*, *speH* and *speJ* genes ($P < 0.05$) increased significantly over the two periods while *speA* decreased ($P < 0.05$). Overall isolates from the first period carried six or more SAg genes (46·5%) compared with 78·4% in period B ($P < 0.05$).

emm type and SAg gene profile

Table 1 shows the distribution of the erythrogenic toxin genes (*speA* and *speC*) in two common *emm* types. In period A, all *emm*1 isolates harboured *speA* but two-thirds carried *speC* genes (66·7%). This was reversed for *emm*12 isolates where *speA* was 35·7% and all were positive for s*peC* genes. However, in the second sampling *emm*1 isolates harboured 94·3% and 97·7% of *speA* and *speC*, respectively, while *emm*12 isolates carried 8·87% and 100% of *speA* and *speC*. We also analysed the distribution of prevalent SAg gene profiles (Table 2), among the most common *emm* types (*emm*1, *emm*3, *emm*12). In period A, the profiles

Table 1. *Distribution of erythrogenic toxin genes* (speA *and* speC) *in GAS isolates between 1993–1994* (period A) *and 2005–2006* (period B) *in China*

| | No. (%) of isolates | | | |
| | *emm*1 | | *emm*12 | |
SAg genes	1993–1994 ($n=24$)	2005–2006 ($n=88$)	1993–1994 ($n=14$)	2005–2006 ($n=124$)
speA	24 (100)	83 (94·31)	5 (35·71)**	11 (8·87)**
speC	16 (66·67)*	86 (97·73)*	14 (100)	124 (100)
speA + *speC*	16 (66·67)*	81 (92·05)*	5 (35·71)**	11 (8·87)**

* $P<0.05$ by comparison of *emm*1 isolates between periods A and B.
** $P<0.05$ by comparison of *emm*12 isolates between periods A and B.

Table 2. *The nine predominant superantigen gene profiles and their relationship with the prevalent M types among 359 GAS isolates from Chinese paediatric patients*

| | Superantigen profile | | | | | | | | 1993–1994 | | | 2005–2006 | | |
Profile	*speA*	*ssa*	*speC*	*speH*	*speI*	*SMEZ*	*speG*	*speJ*	*emm*1 ($n=24$)	*emm*3 ($n=36$)	*emm*12 ($n=14$)	*emm*1 ($n=88$)	*emm*3 ($n=1$)	*emm*12 ($n=124$)
A	+	+	+	+	+	+	+	+	0	2	0	14	0	2
B	+	+	+	−	+	+	+	+	0	0	0	21	0	1
C	+	+	+	−	−	+	+	+	0	0	0	30	0	2
D	−	+	+	+	+	+	+	−	0	0	1	2	0	64
E	−	+	+	+	−	+	+	−	0	0	0	0	0	21
F	−	+	+	−	−	+	+	−	0	1	0	1	0	3
G	+	−	+	−	+	+	+	+	10	0	0	0	0	0
H	−	−	+	−	+	+	+	−	0	0	6	0	0	0
I	+	+	+	+	+	+	+	−	0	18	1	4	0	1
Others									14	15	6	16	1	30

A and E combined accounted for only 7·8% of isolates while 41·6% of *emm*1 isolates were of profile G. Other common associations were *emm*12 and profile H (42·8%) and, *emm*3 and profile I (50%). This contrasted markedly with the second-sample isolates where profiles A, B and C combined accounted for 73·9% of *emm*1 isolates and profiles D and E combined represented 68·5% of *emm*12 isolates.

SAg genes among scarlet fever and pharyngitis isolates

In period A, *speA*, *speC*, *speH*, *speI*, *speG*, *speJ*, *ssa* and *SMEZ* among the pharyngitis isolates alone constituted 67·4%, 89·1%, 34·8%, 76·1%, 86·9%, 26·1%, 47·8% and 89·1%, respectively, compared with the period B where they were 47·7%, 100%, 56·8%, 56·8%, 86·4%, 45·5%, 97·7% and 100%,

respectively. For scarlet fever isolates the positive rates of *speA*, *speC*, *speH*, *speI*, *speG*, *speJ*, *ssa* and *SMEZ* genes were 75·9%, 88·9%, 48·1%, 79·6%, 92·6%, 31·5%, 62·9% and 87·0%, respectively, in period A, but 44·6%, 98·6%, 66·9%, 69·1%, 89·3%, 38·8%, 95·7% and 100%, respectively, in period B. No significant difference was revealed in the distribution of SAg genes among scarlet fever and pharyngitis isolates ($P>0.05$) for the same period.

DISCUSSION

The distribution of *emm* types in GAS isolates varies with geographical region and between and within individual countries. A survey in Italy spanning 11 years showed that the majority of isolates were of *emm* types 1, 4 and 12, but types 1 and 12 declined towards the end of the sampling period while types 3, 22 and

77 appeared [11]. Similar findings were reported from other countries [12–16]. The present study is the first to review a large number of GAS isolates in different parts of China over an extended period. We found the majority of isolates fell in *emm* types 1, 12 and 3, followed by types 4, 6 and *st*1815, with *emm*3, *emm*4, *st*1815 and *emm*6 declining over time. Meanwhile, *emm*1 and *emm*12 isolates appeared more recently and accounted for the most of the isolates at the end of the 12-year sampling period. During 2005–2006, GAS isolates became less diverse compared to 1993–1994 with 24 *emm* types decreasing to just nine *emm* types, indicating that a substantial change had occurred.

Seroepidemiological studies have shown that different *emm* types of *S. pyogenes* are associated with different diseases. Historically, *emm* types such as 1, 2, 3, 4, 12, 15, 49, 55, 56, 59, 60 and 61 have been associated with post-streptococcal glomerulonephritis, while types 5, 6, 18, 19 and 24 have been linked to rheumatic fever [17–19]. We did not find a significant relationship between *emm* type and clinical origin of GAS, in accord with recent studies [8, 9, 20], but it should be noted that there were relatively few representatives from invasive infections in the studied collection.

There are two reports in the literature on GAS *emm*-type distribution from regions of China. In Hong Kong, Ho *et al*. [9] found types 1, 4 and 12 to be among the most common and type 3 was totally absent from their survey of 1997–1998. Jing *et al*. [10] reported types 1 and 12 as the most common in China during 2003–2004 and also identified a broader range of types than found here in 1993–1994 which was the period of greatest diversity of types. However, this survey confirms the predominance of *emm* types 1 and 12 in the Chinese paediatric population resident in four large industrial cities in China. Moreover, it is clear that the pattern of *emm*-type distribution of GAS strains changes over time, and may be influenced by the sample population and/or different age groups. The results obtained here suggest that the currently available 26-valent M-protein based vaccine might provide >95% coverage for Chinese paediatric patients.

We found that the majority of GAS isolates collected from both periods carried around six or more SAg genes and there was a significant increase in the presence of *ssa*, *speH* and *speJ* genes over the 12-year period. The distribution of erythrogenic toxin genes *speA* and *speC* has been shown to be highly variable in different GAS populations. In a Polish study the *speA* gene was present, and *speC* absent, in *emm*1 isolates while all *emm*12 isolates lacked *speA* [5]. Similar results have been reported elsewhere [14, 21–23]. A survey from mainland China reported frequencies of 84% for *speA* and 44% for *speC* in *emm*1 isolates while 35% of *emm*12 isolates carried *speA* and all carried *speC* genes [10]. Associations between *speA*, *speC* and *emm* type were likewise apparent in our findings, especially for *speA*, whose frequency was similar to that found in previous local and international studies; however, the *speC* gene was more frequent in our *emm*12 strains than in other studies. This may be a particular feature of Chinese paediatric isolates.

The relationship of toxin gene profiles to *emm* types has been noted by several groups [5, 14, 20, 22, 23]. Here, nine predominant exotoxin gene profiles were identified, with some profiles associated with individual *emm* types, and these changed over the sampling period. Isolates with the same *emm* type commonly shared the same toxin gene profile and some strains of the same *emm* type had different toxin gene profiles in the two sampling periods. This suggests that the toxin gene profiles of GAS prevalent in Chinese children differ from those obtained by other studies [8, 14, 15, 22]. Moreover, the distribution of SAg genes provides an additional epidemiological tool to explore genomic heterogeneity and the possible correlation between toxin gene content and disease type [8, 23]. Previous studies from Poland, The Netherlands, Denmark and Belgium [5, 20, 22, 23] support the strong association between invasive GAS isolates with *emm*1 type and the presence of the *speA* gene. However, no significant difference in toxin gene profile was evident between the invasive and non-invasive isolates in other studies [8, 16]. It would have been of interest to explore associations of SAg genes with disease presentation but the lack of invasive isolates in this collection prevented this.

DECLARATION OF INTEREST

None.

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