Epidemiological analysis of group A streptococci recovered from patients in China

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Since the mid-1980s, there has been a resurgence of severe forms of invasive group A streptococcal (GAS) disease in many countries and regions. However, there has not been any systemic epidemiologic analysis of GAS disease reported in mainland China. To analyse the molecular epidemiology of GAS disease, 86 strains from patients in different regions of mainland China were collected. The collection sites included blood, pus, wounds, the epipharynx and other sites. A total of 21 different *emm* types were identified in the isolates. In both invasive and non-invasive isolates, M1 (29.1 %) and M12 (23.3 %) were the most prevalent types, a different distribution to M type distributions reported in other countries. Furthermore, minor *emm* gene sequence alterations were noted for six types. Several important GAS virulence factors were detected by PCR using specific primers. The *speB* and *slo* genes were detected in all isolates and were species specific. Four superantigen genes, *speA*, *speC*, *smeZ* and *ssa*, were found in 52 % (45/86), 51 % (44/86), 82 % (71/86) and 23 % (27/86) of isolates, respectively. M1 isolates harboured more *speA* (84 %) and fewer *speC* genes (44 %), while M12 isolates had fewer *speA* (35 %) and more *speC* genes (100 %). There was also an association between some virulence genes and isolation sites, perhaps due to the correlation between the *emm* type distribution and virulence gene occurrence. For two important virulence genes related to necrotizing fasciitis, the *sil* gene was only carried by 11 of 86 isolates, and no *sil* gene contained the start codon ATA. The *sla* gene rarely occurred in GAS isolates, only four of 86 GAS strains being positive, including two isolates obtained from blood. In antimicrobial susceptibility tests, the overall rate of drug resistance in GAS isolates was higher than reported rates in other countries, and the resistance rates to erythromycin, tetracycline and clindamycin were 91.8, 93.4 and 80 %, respectively. This epidemiological study may help to understand the pathogenesis of GAS disease and aid in vaccine development.

Received 13 July 2005
Accepted 15 March 2006

INTRODUCTION

Group A streptococci are an important group of Gram-positive extracellular bacterial pathogens that cause a variety of diseases, including pharyngitis, scarlet fever, cellulitis, bacteraemia, impetigo, acute rheumatic fever, glomerulonephritis, necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS). Since the mid-1980s, an increase in the incidence of invasive infections caused by group A streptococci has been reported in many countries and regions (Banks *et al.*, 2002; Carapetis *et al.*, 1995; Holm *et al.*, 1992; Kaul *et al.*, 1997; Kiska *et al.*, 1997; O'Brien *et al.*, 2002; Chiou *et al.*, 2004; Ho *et al.*, 2003). This dramatic rise may

be due to changes in specific virulence factors (Vlaminckx *et al.*, 2003; Chatellier *et al.*, 2000), hence strain characterization has assumed great importance.

Among the many virulence factors produced by group A streptococci, the M protein encoded by the *emm* gene is considered to be a major factor. About 150 different M protein gene sequence types have been documented (Bisno *et al.*, 2003; Cunningham, 2000). It has been reported that strains of certain M types are epidemiologically associated with particular clinical syndromes. For example, the majority of recently reported invasive group A streptococcal (GAS) infections have been caused by strains of the M1 or M3 type, and infections with these organisms are associated with increased mortality (O'Brien *et al.*, 2002; Chatellier *et al.*, 2000; Musser *et al.*, 1995; Ikebe & Wader, 2002). Moreover, a

Abbreviations: GAS, group A streptococcal; NF, necrotizing fasciitis; STSS, streptococcal toxic shock syndrome.

particularly virulent subclone of M1 associated with invasive infections has been spreading globally for more than 20 years. However, the mechanism(s) by which any M type causes a specific clinical manifestation still remains controversial.

Among the virulence factors that regulate GAS pathogenicity, the known extracellular products, including streptolysin O (SLO), streptococcal pyrogenic exotoxin B (SpeB) and superantigen toxins, are principal contributors. The virulence-related superantigens are the pyrogenic exotoxins SpeA, SpeC, SSA and SMEZ. They are mitogenic for certain T-cell subsets and do not require processing by antigen-presenting cells, and thus can activate a much larger number of T cells than conventional antigens and release excessive amounts of inflammatory cytokines (Bisno *et al.*, 2003; Cunningham, 2000; Kotb, 1995). They are encoded on various phage genomes and are associated with gene variation. SLA is a prophage-encoded secreted extracellular phospholipase A₂ in GAS strains and is associated with host-pathogen interaction (Banks *et al.*, 2003; Beres *et al.*, 2002; Nagiec *et al.*, 2004). The *sil* gene is the streptococcal invasion locus, which controls GAS spread into deeper tissues and may be involved in DNA transfer (Hidalgo-Grass *et al.*, 2002, 2004).

Studies of GAS distribution have been performed worldwide, but there are few data from mainland China, where the epidemiology of GAS may be different. In Asia, studies from Malaysia, Thailand and Hong Kong have revealed a different GAS type distribution (Chiou *et al.*, 2004; Ho *et al.*, 2003). In mainland China, a similar GAS infection increase has also been observed in recent years. Severe infections, such as NF and STSS, caused by group A streptococci were earlier almost unknown. To obtain a better understanding of the epidemiology of GAS infections in mainland China, we performed a molecular epidemiology analysis on GAS isolates obtained from patients in China, including GAS *emm* typing, detection of some virulence factors, and antibiotic-susceptibility tests.

METHODS

Bacterial strains. A total of 86 strains of group A streptococci were recovered from patients in different regions of mainland China from 2000 to 2004. The sources of the specimens included hospitalized patients, as well as outpatients from emergency rooms and specific outpatient clinics. All GAS strains were grown on blood agar plates in 5% CO₂ and at 37 °C overnight. All isolates were β -haemolytic and their identity was confirmed using API strips. Invasive isolates were exclusively from blood ($n=10$). Non-invasive isolates were obtained from the epipharynx ($n=47$), pus ($n=7$), wounds ($n=7$) and other sources ($n=15$).

DNA preparation. The DNA preparation of GAS stains was performed as described by the USA Centers for Disease Control and Prevention (CDC) (<http://www.cdc.gov/ncidod/biotech/strep/protocols.htm>). Briefly, one loopful of bacteria was suspended in 300 μ l 0.85% NaCl and heated for 15 min at 70 °C. The samples were centrifuged and resuspended in 50 μ l TE buffer (10 mM Tris, 1 mM EDTA, pH 8) supplemented with 10 μ l mutanolysin (3000 U ml⁻¹)

and 2 μ l hyaluronidase (30 mg ml⁻¹). The mixture was then incubated for 30 min at 37 °C, heated at 100 °C for 10 min and briefly clarified by centrifugation. The supernatant including genomic DNA was used as the template for PCR.

***emm* genotyping.** The M genotypes of strains were identified as described at the CDC website (<http://www.cdc.gov/ncidod/biotech/strep/strepindex.html>). Briefly, the *emm* gene encoding the M protein was amplified with the genomic DNA described above as template and P1 [5'-tatt(c/g)gcttagaaaattaa-3'] and P2 (5'-gcaagttcttcagctgttt-3') as primers. The cycling parameters were 94 °C for 15 s, 46 °C for 30 s, and 72 °C for 1 min 15 s for the first 10 cycles, and then 94 °C for 15 s, 46 °C for 30 s, 72 °C for 1 min 15 s (with a 10 s increment for each of the subsequent 19 cycles) for the subsequent 20 cycles. The PCR product was sequenced with primer *emmseq2* (5'-tattcgcttagaaaattaaaaacagg-3') and the first 160 bp of the 5' *emm* sequence of every strain was compared with the sequences in the CDC *emm* database (<http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>) to determine *emm* types. This sequence includes 70 bases encoding the signal peptide and 90 bases encoding the first 30 amino acids of the mature protein. The subtypes were assigned to exact 150 bp sequences encoding the N-terminal 50 residues of the mature M protein.

Detection of virulence genes. Virulence genes, including *sepA*, *sepB*, *sepC*, *slo*, *ssa*, *smeZ*, *sla* and *sil*, were detected by PCR with primers as shown in Table 1. Amplification of all genes was performed with an initial 5 min denaturation step at 94 °C followed by 30 cycles of denaturation at 94 °C for 30 s, 30 s of annealing at the appropriate temperature for each gene, as specified in Table 1, and 60 s of extension at 72 °C with a final extension step at 72 °C for 7 min.

Susceptibility tests. Susceptibility testing to erythromycin, clindamycin, tetracycline, ceftriaxone, penicillin G, vancomycin, ciprofloxacin and chloramphenicol was performed on some isolates. MICs were determined by the broth microdilution method. All results were interpreted according to National Committee for Clinical Laboratory Standards criteria with a final inoculum of 10⁵ c.f.u. ml⁻¹. *Streptococcus pneumoniae* ATCC 49619 was used as a control strain. The data were analysed with WHONET 5 software.

Statistical analysis. The chi square test was used for statistical analysis with a *P* value <0.05 considered significant. The SPSS 10.0 statistical package was used for all analyses.

RESULTS

Distribution of *emm* types

A total of 21 M types were identified among the 86 clinical isolates. In descending order of frequency, the prevalent M types (with a frequency >3% in the population) in mainland China were M1 (29.1%), M12 (23.3%), M8 (7.0%), M18 (5.8%), M80 (4.7%) and M28 (4.7%). The other M types (M4, M66, M77, M94, M101, M3, M6, M23, M44, M63, M64, M75, M85, M86 and M88) were detected in only one or two isolates. M1 and M12 together accounted for 60.0 and 51.3% of the invasive and non-invasive isolates, respectively.

A total of six different M types, including M1, M12, M18, M80, M6 and M86, were found in the 10 invasive isolates. A total of 19 different M types were identified in the 76 non-invasive isolates. Besides the most prevalent M1 and M12

Table 1. Primers used for PCR of virulence genes

Gene	Primer direction	Primer sequence	Annealing temperature	Amplicon size
<i>speA</i>	Forward	5'-CGGAATCCATGTCAACAAGACCCAAGCCCAGC-3'	55 °C	660 bp
	Reverse	5'-CGGGATCCTTACTTGGTTGTTAGGTAGAC-3'		
<i>speB</i>	Forward	5'-GTGGAGTCTCTGACGGCTTC-3'	50 °C	170 bp
	Reverse	5'-GTGTTTTTCGGCACAAAAGGT-3'		
<i>speC</i>	Forward	5'-GCGAATTCATGGACTCTAAGAAAGACATTTTCG-3'	50 °C	627 bp
	Reverse	5'-GCGGATCCTTATTTTTCAAGATAAATATCG-3'		
<i>smeZ</i>	Forward	5'-CGGGATCCTTAGAAGTAGATAATA-3'	52 °C	630 bp
	Reverse	5'-AAGAATTCCTTAGGAGTCAATTTTC-3'		
<i>slo</i>	Forward	5'-GCCAATGTTTCAACAGCTATTG-3'	50 °C	439 bp
	Reverse	5'-CGGAGCTGCACTAAAGGCCGC-3'		
<i>ssa</i>	Forward	5'-CGGAATTCATGAATAAAAGAATAAGAATACTCG-3'	55 °C	785 bp
	Reverse	5'-CGGGATCCTTACTTGGTTGTTAGGTAGAC-3'		
<i>sil</i>	Forward	5'-GGAGTTGGTTTATCAAATGTCAG-3'	55 °C	637 bp
	Reverse	5'-ATCTGCCACAAAGACTGATCAAG-3'		
<i>sla</i>	Forward	5'-ACCATGGAAGGGATAAATGATAAAATGG-3'	45 °C	496 bp
	Reverse	5'-CGAATTCTTAACATCCTATAGAACCTAC-3'		

types, other common types in non-invasive isolates (with a frequency >3 %) were M8 (7.9 %), M18 (5.3 %), M28 (5.3 %), M80 (3.9 %) and M101 (3.9 %). M8 and M28 were absent in the 10 invasive isolates and more frequent in the non-invasive isolates ($P < 0.05$).

Only few *emm* sequence variations were found in our study. In nine of 86 isolates, types *emm*1.22, *emm*12.13, *emm*3.1, *emm*6.10, *emm*18.12 and *emm*86.1 were found to contain one to six base substitutions relative to M type reference strains, while no deletions and insertions were found in other isolates. Most of these subtype variants occurred in one or two isolates (Table 2). The remaining 77 isolates all had DNA sequences identical to the corresponding reference *emm* sequences.

Table 2. Allelic variation(s) in different *emm* subtypes

<i>emm</i> subtype	Number of isolates	Allelic variation(s) in <i>emm</i> *
<i>emm</i> 1.22	2	55 C to T
<i>emm</i> 12.13	1	119 G to A
<i>emm</i> 3.1	2	92 A to C, 122 G to A
<i>emm</i> 18.12	2	6 T to C, 25 G to A, 89 T to A, 114 T to A
<i>emm</i> 86.1	1	51 C to A, 71 C to G, 113 T to G, 118 G to A, 127 A to G, 133 G to A
<i>emm</i> 6.10	1	38 A to G

*Numbered from the base encoding the N terminus of the mature 50 amino acid protein.

Identification of virulence genes

The virulence genes *speA*, *speB*, *speC*, *slo*, *ssa*, *smeZ*, *sla* and *sil* were amplified by PCR. As shown in Table 3, *speB* and *slo* were detected in all GAS isolates, proving to be species specific. Four superantigen genes, *sepA*, *spec*, *smeZ* and *ssa*, were found in 52 % (45/86), 51 % (44/86), 82 % (71/86) and 23 % (27/86) of GAS isolates, respectively. The genes *sla* and *sil* rarely occurred in our isolates and were identified in 4.7 % (4/86) and 12.8 % (11/86) of isolates, respectively.

The relationship between the distribution of *emm* types and virulence genes is also shown in Table 3. For instance, M1 isolates harboured more *speA* (84 %) and fewer *speC* genes (44 %), while M12 isolates carried fewer *speA* (35 %) and more *speC* genes (100 %). The *speA* gene was prevalent in the M80 (100 %), M1 (84 %) and M18 (60 %) isolates, while the *speC* gene was prevalent in the M12 (100 %), M118 (60 %) and M1 (44 %) isolates, respectively. Analysis of all strains revealed that 24 % of the isolates carried both *speA* and *speC*. Interestingly, three important superantigen genes, *speA*, *speC* and *ssa*, were absent in all six non-invasive M8 isolates. The *emm* types of four isolates containing the *sla* gene were M18 (2/5), M101 (1/3) and M86 (1/1). The *emm* types of 11 isolates containing the *sil* gene were M1 (1/24), M18 (2/5), M4 (2/2), M94 (2/2), M101 (3/3) and M86 (1/1), but no *sil* gene contained the start codon ATA. Interestingly, two M4 strains contained one nucleotide substitution (C→T) at -20, while the *sil* sequences of other strains were same.

The association of virulence genes and source is shown in Table 4. The *speA* gene was found in 70 % of isolates from blood and 51 % of isolates from the epipharynx, while *speC* was found in 40 % of isolates from blood and 62 % of

Table 3. Distribution of virulence genes among M types

M type	Number of strains	Number of isolates carrying virulence gene:							
		<i>speA</i>	<i>speB</i>	<i>speC</i>	<i>smeZ</i>	<i>ssa</i>	<i>slo</i>	<i>sil</i>	<i>sla</i>
M1	25	21	25	11	19	10	25	1	0
M12	20	7	20	20	17	8	20	0	0
M8	6	0	6	0	6	0	6	0	0
M18	5	3	5	3	5	1	5	2	2
M80	4	4	4	1	4	0	4	0	0
M28	4	1	4	0	3	1	4	0	0
M101	3	1	3	0	3	0	3	3	1
M4	2	0	2	2	2	2	2	2	0
M66	2	0	2	0	2	0	2	0	0
M77	2	1	2	2	2	0	2	0	0
M94	2	1	2	1	2	1	2	2	0
M3	2	2	2	2	0	2	2	0	0
M6	1	1	1	1	0	1	1	0	0
M23	1	0	1	0	1	1	1	0	0
M44	1	1	1	0	0	0	1	0	0
M63	1	1	1	0	1	0	1	0	0
M64	1	0	1	0	1	0	1	0	0
M75	1	1	1	0	1	0	1	0	0
M85	1	0	1	1	0	0	1	0	0
M86	1	0	1	0	1	0	1	1	1
M88	1	0	1	0	1	0	1	0	0
Total	86	45	86	44	71	27	86	11	4

isolates from the epipharynx. The *speC* gene was significantly over-represented among isolates from the epipharynx. The occurrence rates of *speA*, *speC* and *ssa* in wound isolates were much lower than in isolates from other sources. This could be explained by the reduced need for GAS virulence factors to establish infection after the destruction of the skin barrier. Because two out of four strains containing the *sla* gene were obtained from blood, the *sla* gene was more frequent in invasive isolates than non-invasive isolates overall (2/10 versus 2/64; $P < 0.01$). Consistent with our expectation, none of six M8 isolates lacking *speA*, *speC* and *ssa* caused invasive infection in blood.

Susceptibility testing

We performed antimicrobial susceptibility testing with eight common antibiotics. MICs are presented in Table 5 for 61 GAS isolates. We found resistance to erythromycin, tetracycline and clindamycin among all GAS isolates. Overall, 80, 91.8 and 93.4 % of isolates were resistant to clindamycin, erythromycin and tetracycline, respectively. All 61 strains tested were susceptible to ceftriaxone, vancomycin and chloramphenicol, and 85.2 and 98.4 % of these isolates were susceptible to ciprofloxacin and penicillin G, respectively.

Table 4. Association of virulence genes and strain source

Source	Number of cases	Number (%) of isolates with gene:							
		<i>speA</i>	<i>speB</i>	<i>speC</i>	<i>slo</i>	<i>ssa</i>	<i>sla</i>	<i>sil</i>	<i>smeZ</i>
Blood	10	7 (70 %)	10 (100 %)	4 (40 %)	10 (100 %)	3 (30 %)	2 (20 %)	1 (10 %)	9 (90 %)
Wound	7	1 (14 %)	7 (100 %)	2 (29 %)	7 (100 %)	1 (14 %)	0	0	6 (86 %)
Epipharynx*	47	24 (51 %)	47 (100 %)	29 (62 %)	47 (100 %)	19 (40 %)	1 (2.1 %)	7 (15 %)	36 (77 %)
Pus	7	4 (57 %)	7 (100 %)	2 (29 %)	7 (100 %)	1 (14 %)	1 (14 %)	2 (29 %)	7 (100 %)
Other†	15	9 (60 %)	15 (100 %)	7 (41 %)	15 (100 %)	3 (20 %)	0	1 (6.7 %)	13 (87 %)

*Epipharynx includes throat, nose and sputum.

†Other includes vaginal secretions, urine and unknown.

Table 5. Susceptibility of 61 GAS isolates to various antibiotics

Antibiotic	Antibiotic MIC (mg l ⁻¹)			Susceptibility (%)		
	Range	MIC ₅₀	MIC ₉₀	Resistant	Intermediate	Susceptible
Ceftriaxone	0.015–0.25	0.06	0.064	0	0	100
Clindamycin	0.06–4	4	4	80	1.7	18.3
Ciprofloxacin	0.06–2	0.5	2	0	14.8	85.2
Erythromycin	0.03–4	4	4	91.8	1.6	6.6
Penicillin G	0.015–0.25	0.032	0.064	0	1.6	98.4
Tetracycline	0.12–32	32	3	93.4	0	6.6
Vancomycin	0.25–0.5	0.25	0.25	0	0	100
Chloramphenicol	2–4	4	4	0	0	100

DISCUSSION

This is the first molecular epidemiological study of GAS isolates causing invasive and non-invasive infection in different regions of mainland China. Our study revealed unique M type profiles in mainland China which were different to M type distributions reported in Hong Kong. The prevalent M types in mainland China were M1 (29.1%), M12 (23.3%), M8 (7.0%), M18 (5.8%), M80 (4.7%) and M28 (4.7%), compared to the prevalent M types in Hong Kong, M12 (20.6%), M1 (15.0%), M58 (10.3%), M4 (9.3%), M53 (4.7%) and M49 (4.7%) (Ho *et al.*, 2003). M1 was the most prevalent type among isolates from invasive disease as well as in non-invasive isolates. M1 was equally prevalent in both invasive and non-invasive isolates in previous epidemiological studies performed in North Carolina, USA, and Hong Kong (Kiska *et al.*, 1997; Ho *et al.*, 2003). The M3 type has been reported to be prevalent in invasive infections such as NF and STSS in the USA, Canada and other countries (Banks *et al.*, 2002; Carapetis *et al.*, 1995; Kaul *et al.*, 1997; O'Brien *et al.*, 2002; Ikebe & Wada, 2002; Banks *et al.*, 2003), but only two M3 type GAS strains were found in the present study, and they were both non-invasive. Hidalgo-Grass *et al.* (2002, 2004) have reported that the M14 type is associated with invasive infections in Israel, but this M type was not observed at all in our study, nor in Hong Kong or Taiwan (Chiou *et al.*, 2004; Ho *et al.*, 2003). This suggests that the GAS M type distribution in China differs from that in other countries. The lack of M3 and M14 strains may account for the fact there were fewer clinically severe infections such as NF and STSS caused by group A streptococci in mainland China.

Only minor *emm* gene sequence alterations were noted for six *emm* types in our study. Allelic variation in the *emm* genes of our isolates, compared to reference sequences, was predominantly due to single-base substitutions. This study also showed that the GAS *emm* type distribution in mainland China differs from those in Thailand and Hong Kong (Chiou *et al.*, 2004; Ho *et al.*, 2003).

In order to study the factors contributing to the aggressive clinical picture seen with some GAS infections, the isolates were tested for the presence of different toxin genes. The

streptococcal pyrogenic exotoxin B (SepB) is a highly conserved extracellular cysteine protease that is associated with pyrogenicity, T-lymphocyte mitogenicity, and the ability to increase susceptibility to endotoxic shock in individuals infected with group A streptococci (Collin & Olsen, 2001; Watanabe *et al.*, 2002). Louie & Simor (1988) reported that *speB* was positive in all GAS isolates by PCR assay, but the assay was negative in all patients without culture or serological evidence of streptococcal infection. Our results were the same, indicating that PCR detection of the *speB* gene in GAS strains may be a useful method to confirm GAS infection.

The isolates were tested for the presence of the phage-encoded superantigens. It has been reported that *speA* is located in phages T12, 270 and 49 (Yu & Ferretti, 1991) and *speC* in phage CS112 (Goshorn & Schlievert, 1989). We showed that these toxin genes were exclusively associated with particular M types. Some of these observed associations between M types and toxin genes have been reported by others. For instance, Schmitz *et al.* (2003) have found that all but one of 239 M1 and M3 strains carry a *speA* allele and lack a *speC* gene, suggesting that the presence of *speA* affects the acquisition of a *speC* gene in M1 and M3 types. Vlamincx *et al.* (2003) speculated that the exclusive combination of *speA* and *smeZ* in M1, which encode proteins known to be immunoactive, might contribute to intrinsic virulence. This specific set of virulence genes might make them more efficient as pathogens, findings in accordance with our study.

The presence of *speA*, as opposed to *speC*, was positively correlated with invasive infection in blood. This finding was consistent with the notion that *speA* is an important virulence factor required to cause invasive infection. The *speC* gene occurred more frequently in strains obtained from the epipharynx than in those obtained from other sources, a finding supportive of the conclusion that *speC* and bacteriophage induction are pharyngeal-cell dependent. Broudy *et al.* (2001) have reported that *speC* is induced along with phage CS112 when group A streptococci are co-cultured with human pharyngeal cells.

It has been reported that the prophage-encoding *sla* is not present in M3 GAS strains recovered before 1987, and that

strains containing an *sla* gene are more likely to come from a severe infection such as NF. When the resurgence of severe invasive disease occurred in North America, Europe and Japan (Banks *et al.*, 2003; O'Brien *et al.*, 2002), Nagiec *et al.* (2004) found that the *sla* gene was more widely distributed in GAS than just serotype M3 strains. The *sla* gene was present in 10.8% of 1189 GAS strains tested, including *emm1*, *emm2*, *emm3*, *emm4*, *emm22*, *emm28* and *emm7* (Nagiec *et al.*, 2004). In our studies, we found the *sla* gene occurred at a lower rate (4.65%) than those in other countries, and only four strains were *sla* positive, including *emm18*, *emm101* and *emm86*, suggesting a horizontal transmission of the prophage-encoded gene in mainland China. Moreover, two of the four *sla* gene-positive strains could cause severe infection and death in mice (data not shown). Therefore, the *sla* gene is also an important virulence gene and plays a major role in invasive infection in M types other than M3.

The *sil* gene is the streptococcal invasion locus. Within this locus, a gene, *silCR*, is predicted to encode a 41 amino acid bacterial pheromone peptide that abrogates chemokine proteolysis. Hidalgo-Grass *et al.* (2004) showed that the M14 GAS invasive strains have a missense mutation (ATG→ATA) in the start codon of *silCR*, suggesting that the peptide might not be produced. We found that 11 strains carried the *sil* gene (Table 4), of which only one was from invasive infection. Sequence analysis of *sil* showed that these strains did not contain a missense mutation in the start codon of *silCR*, suggesting that the pheromone peptide might be produced in these strains and decrease host damage.

The overall rate of erythromycin resistance in our GAS isolates was high (91.8%) compared to reported rates of 2.6% in the USA, 2.1–4.6% in Canada, 8.6% in Finland and 32% in Hong Kong (Ho *et al.*, 2003). We also found a high rate of resistance to tetracycline (93.4%) compared to 4% in the USA and 53% in Hong Kong. The high rates of resistance that we found in mainland China are probably a reflection of the high level of antibiotic usage in China. Fortunately, the GAS strains in this study were still highly susceptible to penicillin, ceftriaxone, vancomycin, ciprofloxacin and chloramphenicol. Resistance to penicillin and ceftriaxone has not emerged in GAS, despite extensive use of β -lactam drugs in China. In general, the observations of GAS resistance to erythromycin, tetracycline and clindamycin are consistent with previous reports, despite a much higher drug-resistance rate (Ho *et al.*, 2003; Bemer-Melchior & Juvin, 2000; Deazavedo *et al.*, 1999; Palavecino *et al.*, 2001).

This epidemiological study has demonstrated different *emm* types and virulence factors in mainland China, and such data may help understand both pathogenesis and vaccine development. Fewer M3 and M14 types, lower carriage of the *sla* and *sil* genes, lack of the missense mutation in the start codon of *silCR*, and the continued susceptibility of GAS to antibiotics, may explain fewer severe clinical infections, such as NF and STSS, in mainland China.

ACKNOWLEDGEMENTS

We thank Dr Li Cheng for critical reading of and helpful suggestions for this manuscript. This work was supported by key programmes of the National Science and Technology Foundation of China (no. 2003BA712A04-06).

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