Original Article

Molecular Epidemiological Characteristics of *Streptococcus pyogenes* Strains Involved in an Outbreak of Scarlet Fever in China, 2011*

YOU Yuan Hai[1,†], SONG Yan Yan[2,†], YAN Xiao Mei[1], WANG Hai Bin[2], ZHANG Meng Han[5], TAO Xiao Xia[1], LI Lei Lei[4], ZHANG Yu Xin[1], JIANG Xi Hong[4], ZHANG Bing Hua[1], ZHOU Hao[3], XIAO Di[1], JIN Lian Mei[4], FENG Zi Jian[4], LUO Feng Ji[2,#], and ZHANG Jian Zhong[1,#]

1. Collabroative Innovation Center for Diagnosis Treatment of Infectious Disease, State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 2. Chaoyang District Center for Disease Control and Prevention, Beijing 100021, China; 3. Harbin Municipal Center for Disease Control and Prevention, Harbin 150056, Heilongjiang, China; 4. Office of Disease Control and Emergency Response, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 5. Suzhou City Center for Disease Control and Prevention, Suzhou 215004, Jiangsu, China

Abstract

Objective To investigate molecular characterization of *streptococcus pyogenes* isolates involved in an outbreak of scarlet fever in China in 2011.

Methods Seventy-four *Streptococcal pyogenes* involved in an outbreak of scarlet fever were isolated from pediatric patients in the areas with high incidence in China from May to August of 2011. *Emm* genotyping, pulsed-field gel electrophoresis (PFGE), superantigen (SAg) genes and antimicrobial susceptibility profiling were analyzed for these isolates.

Results A total of 4 different *emm* types were identified. *Emm*12 was the most prevalent type which contained four predominating PFGE patterns corresponding to four different virulence and superantigen profiles. *Emm12* (79.7%) and *emm1* (14.9%) accounted for approximately 94% of all the isolates. The *speA* gene was all negative in *emm*12 isolates and positive in *emm*1 isolates. All strains were resistant to erythromycin, and 89.4% of them were resistant to erythromycin, tracycline, and clindamycin simultaneously.

Conclusion Several highly diversified clones with a high macrolide resistance rate comprise a predominant proportion of circulating strains, though no new *emm* type was found in this outbreak. The data provide a baseline for further surveillance of scarlet fever, which may contribute to the explanation of the outbreak and development of a GAS vaccine in China.

Key words: Scarlet fever; *Streptococcus pyogenes*; Molecular epidemiology

Biomed Environ Sci, 2013; 26(11): 877-885 *doi: 10.3967/bes2013.016* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

*This work was supported by a fund for China Mega-Project for Infectious Disease (2011ZX10004-001).

#Correspondence should be addressed to: 1) Prof. ZHANG Jian Zhong, Tel: 86-10-58900707, Fax: 86-10-58900700, E-mail: zhangjianzhong@icdc.cn; 2) Prof. LUO Feng Ji, Tel: 86-10-87789765, Fax: 010-67773666, E-mail: luofengji@yahoo.com.cn

†These authors contributed equally to this work.

Biographical notes of the first authors: YOU Yuan Hai, male, born in 1978, master, majoring in pathogen molecular biology; SONG Yan Yan, female, born in 1975, PhD, majoring in pathogen molecular biology.

Received: July 29, 2012; Accepted: October 23, 2012

INTRODUCTION

Streptococcus pyogenes (*S. pyogenes*, group A streptococcus, GAS) is an important Gram-positive pathogen that causes a wide spectrum of diseases varying from mild pharyngitis or impetigo to life-threatening necrotizing fasciitis and streptococcal toxic shock syndrome. One of the most prevalent diseases caused by GAS is scarlet fever, which is usually attacking children under 10. About 3% to 5% of untreated scarlet fever cases will develop immune sequelae such as rheumatic heart disease and post-streptococcal glomerulonephritis, which account for a large proportion of the disease burden of *S. pyogenes* infection[1-3]. Since March 2011, there has been a dramatic increase of scarlet fever cases reported to the National Reporting System of Notifiable Infectious Diseases. Peak incidence occurred between May and June (Figure 1B), which was dramatically greater than the average incidence in the past seven years.

On 29th May and 21st June, a seven-year-old girl and a five-year-old boy were reported to have died from streptococcal infection in Hong Kong[4-5]. Meanwhile, 29 of the 31 provinces in the Mainland of China reported a significant increasing of scarlet fever cases. Figure 1A shows the incidence of scarlet fever according to provinces for the first half of year of 2011 in China. The highest incidence was reported from Beijing (15 cases per 100 000 population), followed by Shanghai (6.1 cases per 100 000), Tianjin (4.7 cases per 100 000), Liaoning Province (4.2 per 100 000) and Heilongjiang Province (4.2 cases per 100 000). Despite the molecular characterization of limited number of strains reported by some local areas[6-7], a nation-wide study was urgently needed to obtain more comprehensive information. In this study, 74 *S. pyogenes* strains were collected from scarlet fever patients in the high incidence areas and a molecular epidemiological analysis was performed to investigate the molecular epidemiological characteristic of the pathogen.

MATERIALS AND METHODS

Data of Scarlet Fever Incidence

Scarlet fever incidence during 2003 and 2011 were obtained from the National Reporting System of Notifiable Infectious Diseases. All data were imported into MICROSOFT EXCEL and a line graph was generated to show the fluctuation of scarlet fever incidence in the past nine years.

GAS Isolates

Isolates of *S. pyogenes* that caused scarlet fever and tonsillopharyngitis from April to July were collected from infectious disease hospitals of three areas with high incidence in China in 2011. Scarlet fever was clinically diagnosed based on symptoms including fever, sore throat, rash and bright red tongue with a 'strawberry' appearance. Tonsillopharyngitis was clinically diagnosed according to the manifestations as sore throat, fever and tonsillopharyngeal erythema and exudates. Seventy four *S. pyogenes* isolates were obtained from Beijing (*n*=48), Heilongjiang (*n*=18), and Tianjin

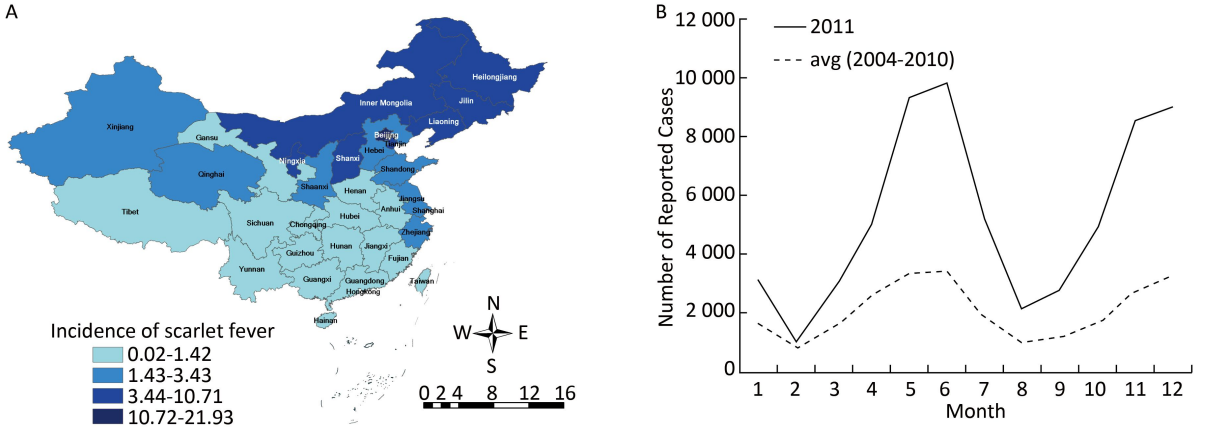

Figure 1. A. Incidence of scarlet fever according to provinces for the first half of year of 2011 in China. The incidence shown is per 100 000 population; B. Monthly reported cases of scarlet fever between 2004 and 2011 in China.

(*n*=8), where the incidence of scarlet fever are generally the highest in China. Among them, 65 isolates were from scarlet fever patients (87.8%) and 9 from the tonsillopharyngitis patients (12.2%). All isolates showed beta-hemolysis on Trypticase soy agar containing 5% sheep blood, negative in the catalase test, susceptible to 0.04 U of bacitracin, and Lancefield grouped as GAS using the streptococcal grouping kit (BioMérieux, France). All isolates were stored at -70 °C in brain heart infusion broth containing 15% glycerol before laboratory operation.

Emm Gene Typing

All isolates were subjected to *emm* typing, the sequence of the first 240 bases obtained was submitted to the National Centers for Disease Control Biotechnology Core Facility Computing Laboratory, and *emm* type and subtype were determined by the parameters for assigning types and subtypes described in the Centers for Disease Control and Prevention (CDC) sequence database (http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm). *emm* type data of previous reports in China were reviewed for their distributions[8-16].

PFGE

All isolates were characterized by PFGE after DNA digestion with *Sma*I (New England Biolabs) using CHEFDR II (Bio-Rad) for 19 h (initial forward time, 4 s; final forward time, 40 s). Gels were stained with GelRed and analyzed using Bionumerics software (Applied Maths, Kortrijk, Belgium). The unweighted pair group method with arithmetic averages and the DICE coefficient (1.0% optimization, 1.0% position tolerance) were used to construct a dendrogram.

Superantigen and Virulence Genes Detection

All isolates were analyzed for amplification of *ssa*, *spe*A, *spe*B, *spe*C, *spe*F, *spe*G, *spe*H, *spe*I, *spe*J, *spe*L, *spe*M, *sl*o, and *sme*Z genes using the primers and reaction conditions reported previously[13,17-19] (Table1). A heatmap of genes detected among the 74 *S. pyogenes* strains analyzed by PCR was made using Mev_4_0 (Multiple Experiment Viewer, TIGR). For the convenience of analysis, *emm* types and PFGE patterns were also added on the right side of the clustered results.

Antimicrobial Susceptibility Testing and Detection of Erythromycin Resistance Genes

Susceptibility tests were performed using Etest (AB Biodisk, Solna, Sweden) in Mueller-Hinton agar supplemented with 5% sheep blood, in accordance with the guidelines drafted by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents tested were ampicillin, ceftriaxone, vancomycin, erythromycin, tetracycline, levofloxacin, chloramphenicol, and clindamycin. Streptococcus pneumoniae strain ATCC 49619 was used as a quality control strain. All isolates were analyzed for amplification of mefA, ermTR, and ermB genes using the primers and reaction conditions reported previously[20-22]. DNA in the reaction mixture

Table 1. Primers Used for PCR of Virulence and Superantigen Genes

Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Length (bp)	Annealing (°C)
*spe*A	CCAAGCCAACTTCACAGATC	CTTTAT (T /C) CTTAG(G /A) TATGAAC	523	50
*spe*B	GTCAACATGCAGCTACAGGA	AATACCAACATCAGCCATCA	257	55
*spe*C	TCTAGTCCCTTCATTTGGTG	GTAAATTTTTCAACGACACA	459	55
*spe*F	CGAAATTAGAAAAGAGGAC	GGCTGAGCAAAAGTGTGTG	1193	50
*spe*G	CTGGATCCGATGAAAATTTAAAAGATTTAA	AAGAATTCGGGGGGAGAATAG	652	55
*spe*H	GTGAATGTCCAGGGAAAAGG	GCATGCTATTAAAGTCTCCATTG	317	55
*spe*I	AATGAAGGTCCGCCATTTTC	TCTCTCTGTCACCATGTCCTG	516	55
*spe*J	GATAGTGAAAATATTAAAGACG	GCTCCTATCTTATTTAGTCC	639	55
*spe*L	CAGCACCTTCCTCTTTCTCG	GGAAAAAGAGGGACGCAAG	459	55
*spe*M	GGATGAGTGAATAAATCGGTAAAC	AGTCTGGGACGATGATAA	425	55
ssa	TGATCAAATATTGCTCCAGGTG	TCCACAGGTCAGCTTTTACAG	502	55
*sme*Z	TAGAAGTAGATAATAATTCC	TTAGGAGTCAATTTCTATAT	629	48

(20 μL) was denatured at 94 °C for 4 min, and the amplification cycles consisted of elongation at 72 °C for 60 s, denaturation at 94 °C for 60 s, and annealing at 57 °C for 60 s. After 30 amplification cycles, the last elongation step was performed at 72 °C for 5 min.

RESULTS

Data of Scarlet Fever Incidence

From 2003, when the National Reporting System of Notifiable Infectious Diseases was well established in China, the number of scarlet fever occurrences increased mildly in the next seven years. In contrast to the low level scarlet fever incidences in the past eight years, the occurrences remarkably increased in 2011, with an incidence of 4.75 cases per 100 000.

Emm Typing

A total of 4 *emm* types were found in the 74 GAS isolates (Table 2). Of the distinct *emm* types identified, the 2 most frequently occurring were *emm*12 (79.7%) and *emm*1 (14.9%), which accounted for approximately 94% of all the isolates. Other *emm* types included *emm* 22 and *emm*75. Four *emm*12 subtypes including *emm*12.0, *emm*12.12, *emm*12.19, and *emm*12.40 were detected. The predominant subtype in all of the four populations was *emm*12.0.

The dendrogram was constructed with BioNumerics software, with a 1.5% position tolerance and 1% optimization by using the unweighted paired group with arithmetic averaging algorithm and Dice similarity coefficients. Five predominant patterns were obtained with similarity coefficients greater than 85%, containing isolates of *emm*12 and *emm*1.

PFGE Analysis

Since among the 74 isolates, BJCYGAS17 and BJCYGAS36 strains were not sensitive to *Sma*I, a total of other 72 strains were finally analyzed with BioNumerics software (Figure 2). Of *emm*12, 48 isolates shared four predominant patterns (SPYS16C0006, SPYS16C00011, SPYS16C00012, SPYS16C00014). SPYS16C0006 pattern strains were more prevalent in Heilongjiang and Tianjin than in Beijing. SPYS16C00011 was the predominant pattern in Beijing. Seven other PFGE patterns (SPYS16C0007, SPYS16C0008, SPYS16C0009, SPYS16C00010, SPYS16C00013, SPYS16C00015, and SPYS16C00016) were detected in *emm*12 isolates, suggesting a high diversity of genetic characterization among *emm*12 strains. *emm* subtype 12. 19 strains were shown with an identical PFGE pattern, except for only one strain, BJCYGAS34, which had one band difference from other *emm* 12. 19 strains. Three PFGE patterns (SPYS16C00020, SPYS16C00020, and SPYS16C00022) were identified in 11 isolates of *emm*1, with one or two band differences between them. SPYS16C00022 was the predominant pattern shared by 7 isolates.

Table 2. Distributions of *emm* Types in Different Patient Groups for the 74 Isolates

emm	Scarlet Fever (%)	Tonsillopharyngitis (%)	Number (%)
*emm*12	52 (80.0)	7 (77.8)	59 (79.7)
*emm*1	10 (15.4)	1 (11.1)	11 (14.9)
*emm*22	2 (3.1)	0	2 (2.7)
*emm*75	1 (1.5)	1 (11.1)	2 (2.7)
total	65	9	74

Virulence and Superantigen Genes Detection

PCR amplification for *ssa*, *spe*A, *spe*B, *spe*C, *spe*F, *spe*G, *spe*H, *spe*I, *spe*J, *spe*L, *spe*M, *slo*, *and sme*Z genes was conducted for all the isolates (Figure 3). The *spe*B, *spe*C, *ssa*, *slo,* and *sme*Z genes were detected in all of them. But the occurrences of *spe*A and *spe*C were different, *spe*A were all negative in *emm*12 isolates and positive in *emm*1 isolates, except one *emm*1 isolate from a case of tonsphargngitis, which was PCR negative for the *spe*A gene.

Antimicrobial Susceptibility

Of the 74 GAS isolates 100%, 97.0%, and 89.4% were resistant to erythromycin, clindamycin and tetracycline, respectively. All isolates were susceptible to ampicillin, ceftriaxone, vancomycin, and levofloxacin (Table3). The *erm*B gene was detected in all isolates. Four strains were found to have both *mef*A and *erm*B genes. *erm*TR was detected in neither of the isolates (Table 4).

DISCUSSION

Scarlet fever has been included in the list of notifiable infectious diseases and must be reported through the National Reporting System of Notifiable Infectious Diseases in China. According to the reporting system, areas with high scarlet fever incidence are mainly located in the north of China, especially in the northeast part of China. However, the

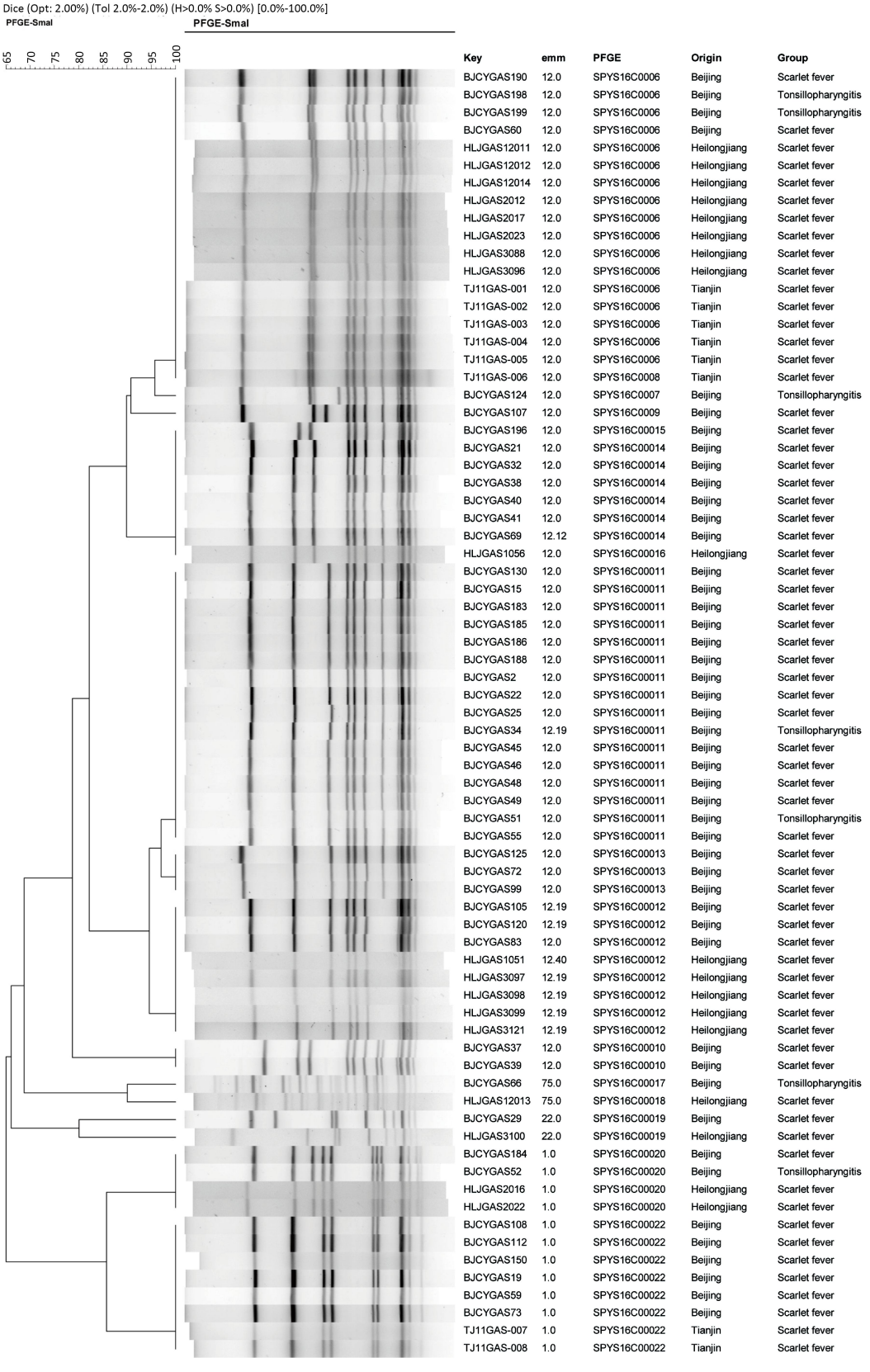

Figure 2. PFGE profiles and background of 72 GAS isolates from the three patient groups.

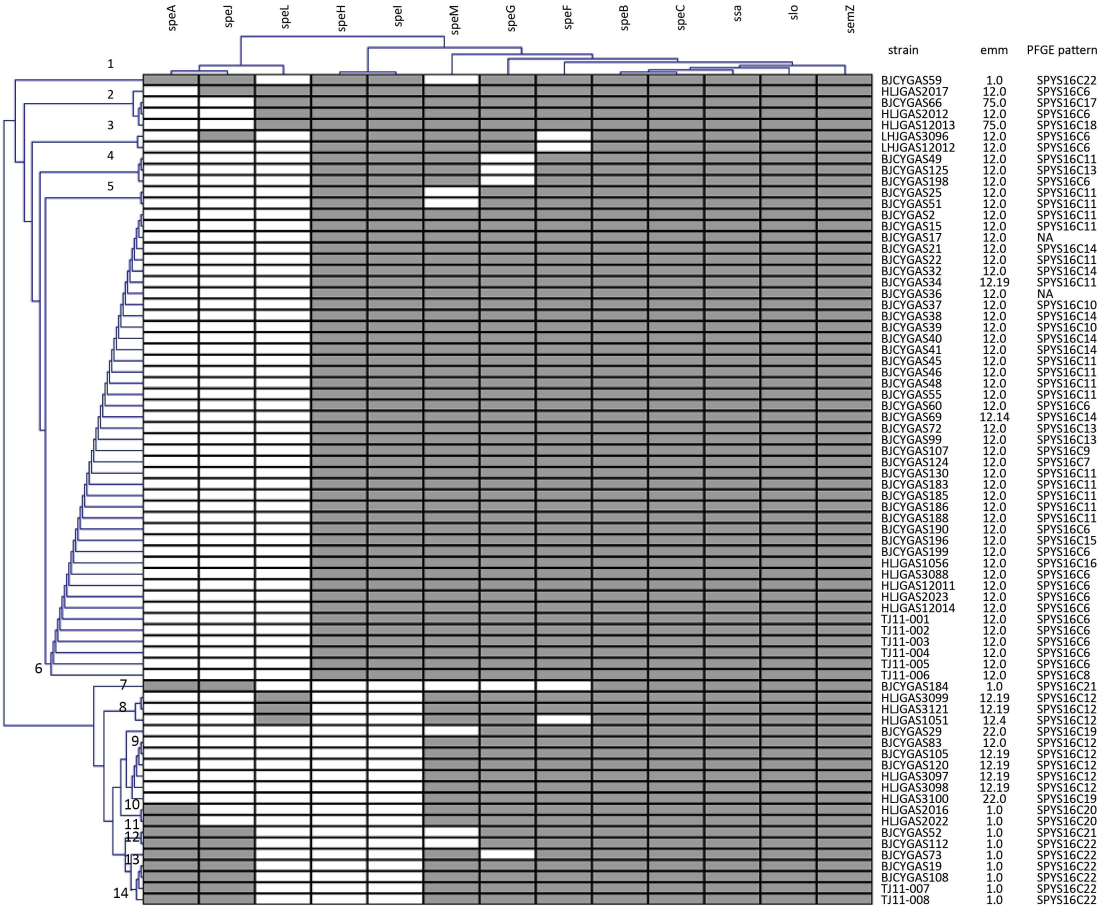

Figure 3. A heatmap of virulence genes detected among the 74 *S. pyogenes* strains analyzed by PCR. White and grey denote the absence and presence of genes detected, respectively. Distinctions of the strains are clustered using a dendrogram on the left side of the figure, which was constructed using a hierarchical analysis of the presence or absence of genes.

Table 3. MIC Distributions for 74 *Streptococcus Pyogenes* Isolates

Antimicrobial	MIC in mg/mL, No. of Isolates															
	<0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Erythromycin	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	72
Levofloxacin	-	-	-	15	35	15	3	6	-	-	-	-	-	-	-	-
Ampicillin	38	33	-	2	1	-	-	-	-	-	-	-	-	-	-	-
Ceftriaxone	-	26	18	30	-	-	-	-	-	-	-	-	-	-	-	-
Vancomycin	-	-	-	4	54	13	3	-	-	-	-	-	-	-	-	-
Chloramphenicol	-	-	-	-	-	-	-	18	53	3	-	-	-	-	-	-
Clindamycin	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	72
Tetracycline	-	-	1	3	2	-	1	-	4	17	26	17	3		-	-

Table 4. *emm* Typing and Resistance Genes Distribution of 74 *Streptococcus Pyogenes* Isolates

emm	*erm*B (%)	*erm*TR (%)	*mef*A (%)	*erm*B+*mef*A (%)
*emm*12	59 (79.7)	0	3 (0.05)	3 (0.05)
*emm*1	11 (14.9)	0	0 (0)	0 (0)
*emm*22	2 (2.7)	0	0 (0)	0 (0)
*emm*75	2 (2.7)	0	1 (50)	1 (50)
total	74	0	4	4

molecular pathogenesis of the disease is only superficially understood and little molecular biological information on scarlet fever streptococci is made available. Moreover, its uncommon sharp rising incidence in China in 2011 has highlighted the burden of this disease. Therefore, a nation-wide investigation on scarlet fever associated streptococcus isolates is recommended, which has led us to initiate the present study.

To tentatively investigate whether new *emm* types emerged in this nation-wide epidemic, we firstly reviewed all previous reports that described *emm* type distribution of the *S. pyogenes* strains in China. Although very limited *emm* data could be used for analysis, we tried to collect and combine all available reports of strain *emm* typing. Owing to lack of a continuous pathogen surveillance for *emm* types of *S. pyogenes* in China, it was hard to perform a strict comparison among scarlet fever strains of previous years. Nonetheless, by comparing with these data, we found a sharp increasing proportion of *emm* 12 clones, and *emm*1 was dramatically declined compared to previous data[8,13]. It was reported that the fluctuation of scarlet fever cases was probably associated with the shuffling of the prevalent *emm* clones, and the incidence of *S. pyogenes* disease could vary over time and in different geographic regions, which was possibly due to the population's susceptibility to particular strains and variation in the predominant *emm* types[23-26]. The differential pathogenic potentials of *emm* types may result in the fluctuations of scarlet fever incidence. Therefore, the *emm* clone information provided by our study could be a useful reference and serve as a baseline for the scarlet fever pathogenic surveillance in the future years.

The major *emm* types found in this study were further discriminated into several PFGE patterns. The *emm*12 strains belonged to four major clones, and *emm*1 strains belonged to one major clone (Figure 2). Thus, five *emm* clones caused most (74.3%) of the scarlet fever cases in the areas of the mainland of China in that specific year. It seemed that SPYS16C0006 PFGE pattern strains were more prevalent in Heilongjiang and Tianjin than in Beijing, where SPYS16C00011 PFGE pattern strains were more predominant. This suggests that there is a geographic variation of prevalent clones in the Chinese Mainland.

By integrating the *emm*, PFGE, and superantigen profiling results, we could find some correlations. Most strains of predominant superantigen cluster 6 links to PFGE pattern SPYS16C00011, SPYS16C0006 and SPYS16C00014, which all belong to *emm* 12.0. For the stains of superantigen cluster 6 that have been known as predominant strains by *emm* and PFGE analysis, they are all in lack *spe*A, *spe*J and *spe*L, but all have other eight virulence genes. Four of the seven *emm* subtype 12.19 strains fall into superantigen cluster 10 and PFGE pattern SPYS16C00012. Nine of the eleven *emm*1 strains show a similar superantigen profile (falling into cluster 11, 12, 13, and 14). Most of them belong to SPYS16C00022. Other two *emm*1 strains, BJCYGAS59 and BJCYGAS184, belong to cluster 1 and cluster 7 respectively. It is intriguing that BJCYGAS59 harbors eleven of the thirteen virulence genes, only *spe*L and *spe*M are absent. BJCYGAS184 only carried *spe*A and *spe*J along with the other five genes that were present in all isolates. We also observed a strain HLJGAS2017, which was isolated from a scarlet fever patient in Heilongjiang province, carrying all virulence and superantigen genes except for *spe*A. It is well known that many streptococcal superantigen genes are encoded by prophage, and prophage-related open-reading frames (ORFs) are composed of the major variable components of GAS genome. It is reported that the variation in prophage content and prophage-associated virulence factor profile may exist among strains of the same M type. This is confirmed by the findings of our study that superantigen cluster 2, 3, 4, 5, 6, 8, 10 are all corresponding to *emm* 12 strains. Different *emm* type strains can have the same superantigen profile, such as BJCYGAS66 (*emm*75.0), HLJGAS2012 (*emm*12.0), and HLJGAS12013 (*emm*75.0) which belong to the same superantigen cluster 2. By comparing the above described characteristics of virulence and superantigen genes distributions with the previously reported data in China, no apparent changing profile was found, suggesting that strain prevalence, rather than a changing virulence potential, might serve as the major factor responsible for the increase of scarlet fever cases.

More sampling and investigations in different areas for these molecular characteristics are needed.

The high susceptibility to ampicillin, ceftriaxone, vancomycin, and levofloxacin in all tested isolates strongly suggest the practice of using ampicillin as first-line therapy for *S. pyogenes* infection. It is reported that the resistance rate to erythromycin in 1994 was 43.3%, from 2004 to 2006 this rate was over 90%[27-28], and for the isolates tested in this study the rate was as high as 100%. A more recent research performed by Liang et al.[12] stated that among the 466 isolates recovered from Chinese children in 2005-2008, >97% were resistant to erythromycin and clindamycin. Similar results were reported more recently in Shanghai and Hong Kong for the isolates recovered in 2011[4,21], which was supposed to be related to the higher level of macrolide exposure in China[29]. In this study the high susceptibility to ampicillin, ceftriaxone, vancomycin, and levofloxacin in all tested isolates strongly suggests that ampicillin be used as first-line therapy for *S. pyogenes* infection. Resistance to macrolide can be due to the *mef*A gene encoding a macrolide efflux pump or ribosomal modification by methylation caused by the *erm*B or *erm*TR gene[30]; our isolates mainly harboured the *erm*B gene, which suggested a potential mechanism of macrolide resistance related to the ermB mediated methylation of ribosomal modification.

In summary, the study indicates a high *emm12* proportion and high macrolide resistance rate of isolates recovered from the outbreak although no new *emm* type appears, and provides a baseline for further monitor of scarlet fever, which may contribute to the explanation of the outbreak and development of a GAS vaccine in China. *erm*B mediated methylation of ribosomal modification may be the potential mechanism contributing to the high resistance. In regard to the GAS strain characteristics, environmental factors, and population susceptibility are likely important for the spike of scarlet fever, and more comprehensive studies involving both pathogens and hosts are needed to make the event clear.

ACKNOWLEDGEMENT

We appreciate our colleagues HE Li Hua, GU Yi Xin, ZHANG Hui Fang, and ZHENG Ming Huan for their excellent technical assistance.

REFERENCES

1. Carapetis JR, Steer AC, Mulholland EK, et al. The global burden of group A *streptococcal* diseases. Lancet Infect Dis, 2005; 5, 685-94.
2. Cunningham MW. Pathogenesis of group A *streptococcal* infections. Clin Microbiol Rev, 2000; 13, 470-511.
3. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and reemerging infectious diseases. Nature, 2004; 430, 242-9.
4. Hsieh YC, Huang YC. Scarlet fever outbreak in Hong Kong, 2011. J Microbiol Immunol Infect, 2011; 44, 409-11.
5. Lau MCK. Increase in scarlet fever cases in 2011. Communicable Diseases Watch, 2011; 8, 48-9.
6. Chen M, Yao W, Wang X, et al. Outbreak of Scarlet Fever Associated with *emm12* Type Group A *Streptococcus* in 2011 in Shanghai, China. Pediatr Infect Dis J, 2012; Apr 23. [Epub ahead of print]
7. Tse H, Bao JY, Davies MR, et al. Molecular Characterization of the 2011 Hong Kong Scarlet Fever Outbreak. J Infect Dis, 2012; Jun 11. [Epub ahead of print]
8. Chang H, Shen X, Huang G, et al. Molecular analysis of *Streptococcus pyogenes* strains isolated from Chinese children with pharyngitis. Diagn Microbiol Infect Dis, 2011; 69, 117-22.
9. Chen ZH, Gong SF, Dong TM, et al. Typing of 104 *Streptococci pyogenes* isolates from China based on emm gene sequence. Chin J Microbiol Immunol, 2004; 24, 489-91. (In Chinese)
10. Liang YM, Chang HS, Shen XZ, et al. Relationship between emm Typing and Superan tigen Genes speA and speC of *Streptococcus Pyogenes* Isolated from Children in Beijing. J App l Clin Pediatr, 2010; 25, 1700-2. (In Chinese)
11. Liang YM, Chang HS, Shen XZ, et al. emm typing of *Streptococcus pyogenes* isolated from children with adenopharyngitis. J Clin Pediatr, 2011; 29, 382-5. (In Chinese)
12. Liang YM, Liu XR, Chang HS. Epidemiological and molecular characteristics of clinical isolates of *Streptococcus pyogenes* from Chinese children between 2005 and 2008. Published online ahead of print. J Med Microbiol, 2012; jmm.0.042309-0
13. Liang YM，Shen XZ，Huang GY, et al. Characteristics of *Streptococcus pyogenes* strains isolated from Chinese children with scarlet fever. Acta Paediatrica, 2008; 97, 1681-5.
14. Ma YL, Yang YH, Huang GM. Characterization of emm types and superantigens of *Streptococcus pyogenes* isolates from children during two sampling periods. Epidemiol Infect, 2009; 137, 1414-9.
15. Ma YL, Yang YH, Yu SJ, et al. Emm types and superantigen analysis of *streptococcus pyogenes* isolated from Chinese children. Basic& Clinica Medicine, 2009; 29, 1166-9. (In Chinese)
16. Jing HB, Ning BA, Hao HJ, et al. Epidemiological analysis of group A *streptococci* recovered from patients in China. J Med Microbiol, 2006; 55, 1101-7.
17. Norrby TA, Newton D, Kotb M, et al. Superantigenic properties of the group A *streptococcal* exotoxin SpeF (MF). Infect Immun, 1994; 62, 5227-33.
18. Proft T, Moffatt SL, Berkahn CJ, et al. Identification and characterization of novel superantigens from *Streptococcus pyogenes*. J Exp Med, 1999; 189, 89-2.
19. Tyler SD, Johnson WM, Huang JC, et al. *Streptococcal* erythrogenic toxin genes: detection by polymerase chain reaction and association with disease in strains isolated in Canada from 1940 to 1991. J Clin Microbiol, 1992; 30, 3127-31.
20. Brenciani A, Bacciaglia A, Vecchi M, et al. Genetic elements carrying erm (B) in *Streptococcus pyogenes* and association with tet (M) tetracycline resistance gene. Antimicrob Agents Chemother, 2007; 51, 1209-16.
21. Feng L, Lin H, Ma Y. Macrolide-resistant *Streptococcus*

pyogenes from Chinese pediatric patients in association with Tn916 transposons family over a 16-year period. Diagn Microbiol Infect Dis, 2010; 67, 369-75.
22. Liu XR, Shen XZ, Chang HS. High macrolide resistance in *Streptococcus pyogenes* strains isolated from children with pharyngitis in China. Pediatr Pulmonol, 2009; 44, 436-41.
23. Lamagni T, Efstratiou A, Vuopio-Varkila J, et al. The epidemiology of severe *Streptococcus pyogenes* associated disease in Europe. Euro Surveill, 2005; 10, 179-84.
24. Rogers S, Commons R, Danchin M H. Strain prevalence, rather than innate virulence potential, is the major factor responsible for an increase in serious group A *streptococcus* infections. J Infect Dis, 2007; 195, 1625-33.
25. Zachariadou L, Papaparaskevas J, Paraskakis I, et al. Predominance of two M-types among erythromycin-resistant group A *streptococci* from Greek children. Clin Microbiol Infect, 2003; 9, 310-4.
26. O'Loughlin RE, Roberson A, Cieslak PR, et al. The epidemiology of invasive group A *streptococcal* infection and potential vaccine implications: United States, 2000-2004. T Clin Infect Dis, 2007; 45, 853-62.
27. Dong TM, Su J, Huang ZD. An antibiotic resistance epidemiologic study of group A *streptococci* isolated from elementa ry school children in 4 provinces of China. Chin J Pediatr, 1999; 37, 35-7.
28. Ma YL, Yang YH, Liang YM, et al. The drug resistance analysis of Group A beta-hemolytic *streptococci* and the detection of macrolide resistant gene. Chin J Infect Chemother, 2008; 8, 338-42. (In Chinese)
29. Zhang WS, Shen XZ, Wang Y. Outpatient antibiotic use and assessment of antibiotic guidelines in Chinese children's hospitals. Eur J Clin Pharm, 2008; 64, 821-8.
30. Brenciani A, Bacciaglia A, Vecchi M, et al. Genetic elements carrying erm (B) in *Streptococcus pyogenes* and association with tet (M) tetracycline resistance gene. Antimicrob Agents Chemother, 2007; 51, 1209-16.