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Original Article

Increase of *emm*1 isolates among group A *Streptococcus* strains causing scarlet fever in Shanghai, China

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ABSTRACT

Objective: Scarlet fever epidemics caused by group A *Streptococcus* (GAS) have been ongoing in China since 2011. However, limited data are available on the dynamic molecular characterizations of the epidemic strains.
Method: Epidemiological data of scarlet fever in Shanghai were obtained from the National Notifiable Infectious Disease Surveillance System. Throat swabs of patients with scarlet fever and asymptomatic school-age children were cultured. Illumina sequencing was performed on 39*emm*1 isolates.
Results: The annual incidence of scarlet fever was 7.5–19.4/100,000 persons in Shanghai during 2011–2015, with an average GAS carriage rate being 7.6% in school-age children. The proportion of*emm*1 GAS strains increased from 3.8% in 2011 to 48.6% in 2014; they harbored a superantigen profile similar to *emm*12 isolates, except for the *speA* gene. Two predominant clones, SH001-*emm*12, and SH002-*emm*1, circulated in 66.9% of scarlet fever cases and 44.8% of carriers. Genomic analysis showed *emm*1 isolates throughout China constituted distinct clades, enriched by the presence of mobile genetic elements carrying the multidrug-resistant determinants *ermB* and *tetM* and virulence genes *speA*, *speC*, and *spd1*.
Conclusion: A significant increase in the proportion of*emm*1 strains occurred in the GAS population, causing scarlet fever in China. Ongoing surveillance is warranted to monitor the dynamic changes of GAS clones.

Introduction

Scarlet fever is a communicable childhood disease caused by group A *Streptococcus* (GAS), which can develop into life-threatening invasive infections, including toxic shock syndrome (Andrey and Posfay-Barbe, 2016). Scarlet fever has been public health concern worldwide since 2011 when an unprecedented large outbreak of scarlet fever occurred in China, followed by separate outbreaks in England and South Korea, with the reported annual incidence of 13.7–31.4 cases/ 100,000 persons (Chen et al., 2012; Lau et al., 2012; Yang et al., 2013; Lamagni et al., 2017; Park et al., 2017). In China, the high frequency of GAS resistance to macrolides and clindamycin has become another public concern. In clinical practice, clindamycin is an adjunctive therapy for streptococcal toxic shock syndrome, and macrolides are alternatives for treating patients allergic to β-lactam antibiotics (Chen et al., 2012).

Various molecular characterizations and specific virulence factors that are possessed by GAS strains are associated with variations in the frequency and severity of GAS diseases (Cunningham, 2000). The M protein is a major surface protein and virulence factor of GAS. Based on the variability of the N-terminal region of *emm*, the encoding gene of M protein, over 250 *emm* types have been identified (Streptococcus Laboratory of Centers for Disease Control and Prevention). The prevalence of *emm* types vary in high- and low-income countries (Steer et al., 2009). Scarlet fever is a toxin-mediated disease and the pyrogenic exotoxins, such as SpeA,

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SpeC, SSA, as well as other superantigens that bypass conventional antigen processing and activate large numbers of T cells, are essential contributors in the pathogenesis of scarlet fever and toxic shock syndrome (Commons et al., 2014). Thirteen superantigen encoding genes have been reported in GAS (Reglinski et al., 2019), including eight genes located on prophages (*speA*, *speC*, *speH*, *speI*, *speK*, *speL*, *speM*, and *ssa*) (Commons et al., 2014), which can be transferred horizontally among GAS strains (Cole et al., 2011).

The re-emergence of GAS scarlet fever has been linked to the acquisition and transmission of new mobile genetic elements carrying virulence and antimicrobial resistance determinants (Ben Zakour et al., 2015; Chalker et al., 2017; Davies et al., 2015; Tse et al., 2012; Walker et al., 2019; You et al., 2018). The 2011 epidemic of scarlet fever in China was primarily associated with *emm*12 GAS strains, which exhibited high resistance to macrolides and tetracycline (Yang et al., 2013). The two primary genetic features of the outbreak strains were the presence of integrative and conjugative elements carrying antibiotic resistance genes *tetM* and *ermB* and the presence of novel prophages ΦHKU.ssa or ΦHKU.vir carrying streptococcal superantigen genes *ssa*, *speC* and the DNase encoding gene *spd1* (Tse et al., 2012; Davies et al., 2015).

Scarlet fever in China remains at epidemic levels, and several provinces reported an incidence higher than ten cases per 100,000 population, among which the majority were distributed in the north of China; Shanghai was the only one in the south (Liu et al., 2018; You et al., 2018). However, limited data are available on the dynamic molecular characterizations of the scarlet fever epidemic strains. We aimed to determine whether any changes to the predominant *emm* types and to the level of the GAS carriage rate in vulnerable populations, occurred during the scarlet fever epidemics in Shanghai.

Materials and methods

Ethical considerations

This study was approved by the Shanghai Municipal Center for Disease Control and Prevention Ethical Review Committee (2016-4). Informed consent from all study participants (or their guardians) was obtained before the sample and data collection.

Enhanced scarlet fever surveillance in Shanghai

Routine surveillance of scarlet fever in Shanghai is conducted through the National Notifiable Infectious Disease Surveillance System (NNIDSS), which has been described previously (Yang et al., 2013). To monitor the molecular characterizations of the isolates causing scarlet fever since 2011, an enhanced surveillance system was established in Shanghai. The sentinel hospital was the Children's Hospital of Fudan University, the largest medical center for pediatric notifiable infectious diseases in Shanghai, encompassing approximately 50% of scarlet fever cases in the entire city (Fig. S1). Throat swabs were collected twice a week, and 10-20 swabs were taken per week, depending on the intensity of clinical outbreaks. All these samples were sent to the laboratory of Shanghai's CDC for culture.

GAS carriage surveys in school

The asymptomatic GAS carriage survey was conducted on asymptomatic children of school age during scarlet fever peak seasons in Shanghai. In each sentinel district, 30–50 asymptomatic students from each of the three age groups (3–4 years, 5–9 years, and 10–14 years) were enrolled, and their throat swabs were collected. Each flocking swab was kept in a polypropylene screw-cap tube containing 1 mL of liquid Amies Medium (Copan ESwab, Italia) and transferred to Shanghai CDC laboratory within 4 h for culture. During 2011–2014, the carriage survey was performed in May in a suburban district (Minhang). In 2015, the survey was expanded to two time points (May and December) and encompassed three districts: an urban (Xuhui) and two suburban (Minhang and Fengxian) districts. Participants were excluded if they had a history of illness of respiratory infections within one month, a history of GAS infections within one year, a history of contact with patients with GAS infections within one month, or an antimicrobial drug treatment within one month.

GAS identification

All throat swabs were submitted to the Shanghai CDC laboratory and inoculated onto Columbia blood agar within four hours of sample collection. The GAS isolate was identified based on β-hemolysis on agar, colony morphologic characteristics, the presence of Lancefield group A antigen, which was confirmed using the Diagnostic Streptococcal Grouping Kit (Oxoid, Hampshire, United Kingdom), and by Vitek 2 system (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was evaluated by determining the minimum inhibitory concentrations (MICs) of ten antimicrobial agents, including penicillin, cefotaxime, meropenem, levofloxacin, erythromycin, clindamycin, chloramphenicol, vancomycin, linezolid, and tetracycline, using the broth microdilution method and the breakpoints according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) in 2018 (CLSI, 2018).

Screening of resistance-related genes and molecular typing for GAS

Chromosomal DNA was extracted according to the protocol of the US CDC (*Streptococcus* Laboratory of the Centers for Disease Control and Prevention). Macrolide resistance-related genes (*ermB*, *ermA*, and *mefA*) and tetracycline resistance-related genes (*tetM* and *tetO*) were screened by PCR as previously described (Chen et al., 2017). Molecular characterization of GAS isolates was determined using *emm* typing, superantigen PCR profiling, and pulsed-field gel electrophoresis (PFGE) as previously described (Beall et al., 1996; Chen et al., 2012). PFGE images were analyzed with BioNumerics software package (version 7.6.2; Applied Maths, Belgium) using the unweighted pair group method and arithmetic averages (UPGMA) clustering algorithm. According to the Tenover criteria (Tenover et al., 1995), isolates with one or more different bands were assigned to a different PFGE pattern, and isolates with the same pattern were presumed to originate from the same clone.

Genome sequencing and phylogenetic analysis

To investigate the genomic characterization of the *emm*1 isolates in Shanghai, 39 *emm*1 GAS isolates from patients with scarlet fever and carriers were selected for sequencing using the Illumina HiSeq platform with a read length of 150 base pairs. Including three historical isolates collected in 1974, the other 36 *emm*1 isolates were selected based on the PFGE pattern of the 546 *emm*1 isolates collected in this study (Table S1). Genomes were assembled and annotated using SPAdes (v 3.12.0) and Prokka (v1.13.3), respectively (Bankevich et al., 2012; Seemann, 2014).

To put the Shanghai *emm*1 GAS isolates within a global phylogenetic context, a database of 187 *emm*1 genomes from the UK, USA, Hong Kong, mainland China (not Shanghai), and single

representative genomes from other countries, was generated (Table S1). The mobile genetic elements ICE-HKU488, ICE-HLJGAS2022, and ΦHKU488.vir, which were reported to carry antibiotic resistance and superantigen genes in *emm*1 isolates (Ben Zakour et al., 2015), were determined through a read-mapping approach against a database of these *emm*1 elements (You et al., 2018). Sequence reads for all genomes mapped to the 2011 Hong Kong scarlet feverHKU488 reference genome (GenBank accession numberCP012045), and single nucleotide polymorphisms (SNPs) called using Snippy (v.3.2-dev). SNPs residing within knownHKU488 mobile genetic elements (ΦHKU488.1, ICE. HKU488, ΦHKU488.2, ΦHKU488.3, ΦHKU488.4 [or ΦHKU488. vir]) were excised from the alignment. Maximum-likelihood trees were built using RAxML (v.7.0.4) with GTR + gamma model and Bayesian temporal inferences performed in BEAST (v.2.4.8) (Drummond et al., 2012). Final BEAST phylogenetic analyses were run in triplicate for 100 million MCMC generations, with 10% burn-in using the above parameters. All reported estimates (mean rate and 95% highest posterior density [HPD]) were calculated using Tracer (v1.5).

Statistical analysis

Statistical analysis was performed using OpenEpi (v 3.01), and statistical significance was assessed at $p < 0.05$. The Pearson correlation coefficient between carriage rate and scarlet fever incidence was counted in Microsoft Excel 2010.

Accession numbers

Illumina short reads of the 39 genomes sequenced in this study were submitted to the European Nucleotide Archive (ENA) - study ID of PRJEB35406.

Results

Epidemiological characterizations of scarlet fever during 2011-2015 and 2005-2010

During 2011–2015, a total of 2,257 scarlet fever cases were collected, and 1,451 (64.3%) were culture-positive for GAS. All the collected patients were aged <15 years, with the mean age of 6.0 ± 1.9 years, and 60.2% (1,358/2,257) were male.

According to data from the NNIDSS, the annual average incidence of scarlet fever in Shanghai was 7.5–19.4/100,000 people during 2011–2015, which was much higher than the incidence (2.1–5.1/100,000 population) during 2005–2010 (Figure 1A and S1). The highest incidence of scarlet fever was observed in 2015 (Fig. S1), with 4,708 reported cases. No fatal case and severe complications associated with scarlet fever were reported during 200–2015.

Seasonal epidemiological features of scarlet fever in 2011–2015 were similar to those in 2005–2010. There were two pronounced peak seasons (Figure 1A): one peak from April to June (late spring and midsummer), another peak from November to the next

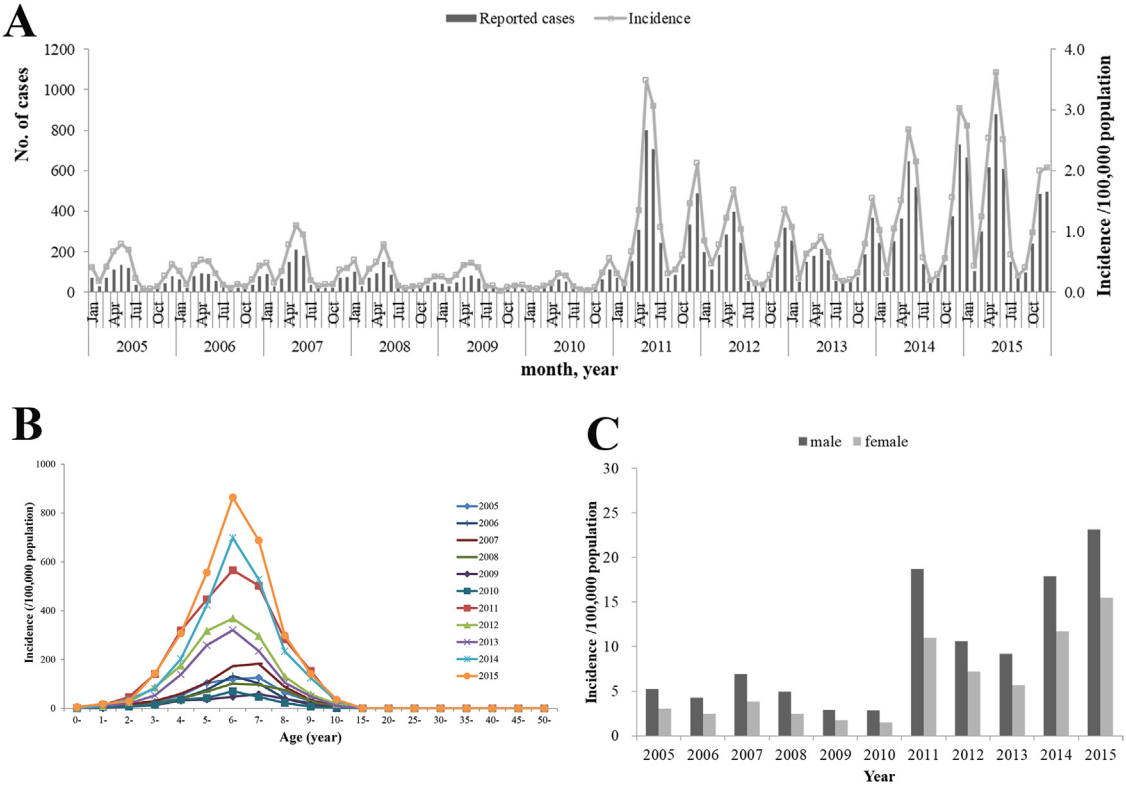

Figure 1. Scarlet fever incidence, Shanghai, China, 2005–2015, as reported in the National Notifiable Infectious Disease Surveillance System. A) Number of cases and average incidence rate of the overall population by month. Scarlet fever displayed two pronounced peak seasons. A sudden increase in the incidence of scarlet fever was observed in 2011, and the incidence sustained a high level during 2011–2015.
B) Incidence rate by age. The incidence of particular age groups was calculated with cases in each age group per 100,000 overall population. The incidence was highest in children aged 6-7 years.
C) Incidence rate by sex. The incidence of a particular sex was calculated with cases in this sex group per 100,000 overall population. Incidence was always higher in males than in females.

January (winter). Scarlet fever mainly affected children aged 3-9 years, accounting for 93.0% (14,552/15,650) of cases during 2011–2015 and 85.8% (3,310/3,860) of cases during 2005–2010. When incidence was analyzed by age, the incidence was highest in children aged 6–7 years (Figure 1B), reaching as high as 864.2 /100,000 in children six years old during 2015. Incidence was higher in males than in females by 50–100% (Figure 1C).

GAS carriage survey in schools

A total of 28 kindergartens (age range, 3–5 years old) and schools (including primary schools, age range, 6–10 years old; and junior middle schools, age range, 11–14 years old) were enrolled for the carriage survey between 2011 and 2015, covering three age groups of 3–4 years (n = 309), 5–9 years (n = 588), and 10–14 years (n = 360). The overall carriage rate was 7.6% (96/1,257) during 2011–2015, with the highest rate (29.6%) found in the age group 5–9 years in 2014.

During 2011–2014, the survey was only conducted in Minhang District (District A) during May. The annual carriage rates of GAS decreased from 16.2% (16/99) in 2011 to 3.3% (4/120) in 2013 in the kindergarten and school students, which were positively correlated with the decreased incidence of scarlet fever (Table 1). The average carriage rate of different age groups during the four years was highest in the age group 5–9 years (14.3%, 39/273).

In 2015, the survey was expanded to three districts with sampling occurring in both May and December. The average carriage rates were 10.2% (26/256) in Minhang District (District A), 5% (12/240) in Xuhui District (District B), and 4% (12/300) in Fengxian District (District C), respectively (Figure 2). In May, the average carriage rate was 5.9% (24/409) and highest in the age group 5-9 years (7.0%, 13/186); in December, the average overall carriage rate was 6.7% (26/390) and highest in the age group 10-14 years (16.9%, 22/130) (Table S2).

Antimicrobial agent susceptibility

Almost all the 1,547 GAS isolates from patients (n = 1,451) and carriers (n = 96) were resistant to erythromycin (97.5%, 1,508/1,547; resistant MIC range, 4 - >128 μg/ml), clindamycin (97.3%, 1,506/1,547; 8 - >128 μg/ml), and tetracycline (95.7%, 1,481/1,547; 8 - >64 μg/ml). The resistance to macrolides and tetracycline was associated with *ermB* (100%, 1,508/1,508) and *tetM* (99.4%, 1,472/1,481), respectively. All GAS isolates were susceptible to penicillin, cefotaxim, meropenem, vancomycin, and linezolid, and almost all susceptible to chloramphenicol (99.8%, 1,544/1,547) and levofloxacin (98.4%, 1,522/1,547).

Increase of emm*1 between 2011–2015*

Among the 1,451 GAS isolates recovered from scarlet fever patients between 2011-2015, 12 different *emm* types were identified (Figure 3), yet *emm*12 (61.8%, 897/1,451) and *emm*1 (35.9%%, 521/1,451) constituted the vast majority (>97%) of cases. This data was mirrored in the GAS carriage isolates conducted over the same time frame, where four *emm* types were identified, yet *emm*12 (70.8%, 68/96) and *emm*1 (26.0%, 25/96) constituted >96% of GAS carriage isolates.

The proportions of *emm*1 GAS strains recovered from patients increased from 3.8% in 2011 to 48.6% in 2014, while the proportion *of emm*12 decreased from 94.9% in 2011 to 49.6% in 2014 (Figure 3).

Superantigen profiles

Almost all the isolates from patients and carriers possessed *speC* (99.7%, 1,543/1,547), *speG* (99.7%, 1,542/1,547), *ssa* (99.4%, 1,538/1,547) and *smeZ* (99.1%, 1,533/1,547); more than half harbored *speH* (51.6%, 799/1,547) and *speI* (73.2%, 1,118/1,547). SpeA was carried by 38.0% (588/1,547) of isolates, and less than 10% of isolates harbored *speJ*, *speK*, *speL* and *speM* (Table 2). The gene *speA* was found to be carried at a significantly higher frequency by *emm*1 isolates than by *emm*12 isolates (98.1% vs 4.2% in patient isolates and 80.0% vs 10.3% in carrier isolates, both $p < 0.001$; Table 3).

PFGE patterns and clonal dissemination

A preliminary genomic comparison of the Shanghai GAS population was determined by PFGE. The 1,451 patient isolates displayed 78 PFGE patterns, while the 96 carrier isolates displayed 17 patterns. Among the *emm*12 isolates, 70.7% (634/897) of the scarlet fever isolates, and 52.9% (36/68) of asymptomatic isolates showed the same clonal pattern, SPYS16.SH0001, now termed SH001-*emm*12. Similarly, of the *emm*1 isolates, 64.1% (334/521) of the scarlet fever isolates, and 28% (7/25) of asymptomatic isolates were of a single PFGE genotype, SPYS16.SH0010 now termed SH002-*emm*1. These two clones were circulating in 66.9% (970/1,451) of scarlet fever cases and 44.8% (43/96) of carriers. Both of them appeared stable in time and were geographically dispersed, as evidenced by being isolated in 405 kindergartens and 208 schools in this study, respectively (Figure 2). No clear clonal separation could be observed between the carriage and clinical scarlet fever isolates, indicating that disease-associated clones can be carried asymptomatically.

Genome analysis of emm*1 isolates*

To better understand the evolution of the *emm*1 genotype over time, a total of 39 temporally and regionally disparate *emm*1 GAS isolates from scarlet fever patients (n = 35, comprising 32 from the 1,451 patient isolates and three from historical cases in 1974) and carriers (n = 4) in Shanghai were selected for genome sequencing. Representing 26 prevalent PFGE patterns (Supplementary Fig. 2), these 39 genomes were compared to 148 global representative *emm*1 genomes. Temporal regression analysis of these *emm*1 isolates indicated support for the molecular evolutionary clock (Supplementary Fig. 3). The three isolates from 1974 formed a

Table 1
Surveys of GAS carriage in District A, a suburban district of Shanghai, China during 2011-2014[a].

Year (Number of samples)	2011 (n = 99)	2012 (n = 122)	2013 (n = 120)	2014 (n = 120)	In total
Scarlet fever incidence (/100,000 district population)	44.2	34.2	26.8	50.0	44.7
Age group (years)					
3–4	NA[b]	0% (0/32)	0% (0/30)	0% (0/26)	0% (0/88)
5–9	16.2% (16/99)	8.3% (5/60)	3.3% (2/60)	29.6% (16/54)[c]	14.3% (39/273)
10–14	NA	13.3% (4/30)	6.7% (2/30)	2.5% (1/40)	7% (7/100)
Average	16.2% (16/99)	7.4% (9/122)	3.3% (4/120)	14.2% (17/120)	10.0% (46/461)

[a] Correlation coefficient between incidences and carriage rates was 0.933.
[b] NA, not applicable.
[c] A class composed of students aged 5–6 years showed a carriage rate of up to 40% (8/20), with five isolates assigned to clone SH001-*emm*12.

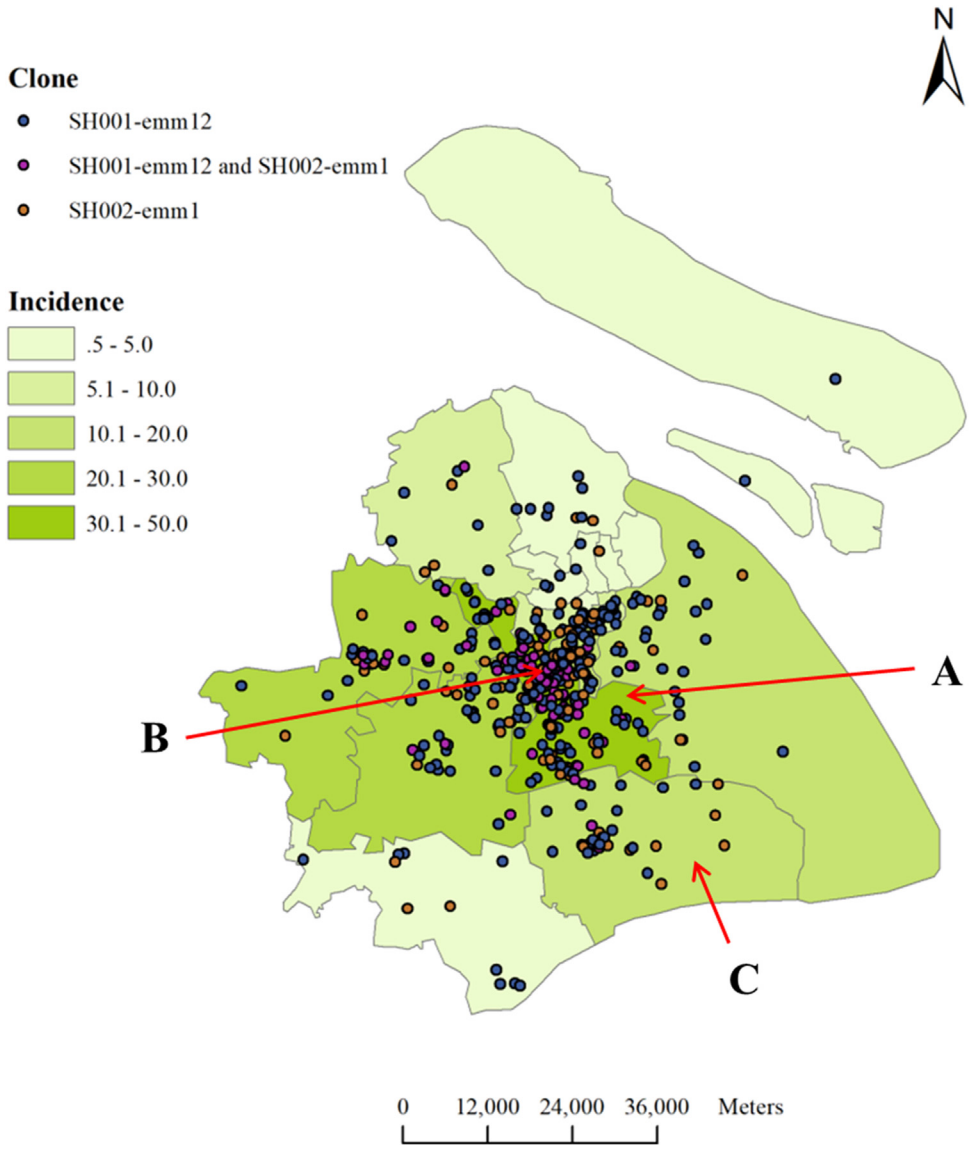

Figure 2. An administrative map of Shanghai labeled with incidences of scarlet fever. Kindergartens and schools with cases caused by two predominant group A *Streptococcus* clones, SH001-*emm*12 and SH002-*emm*1. Districts were separated by lines, and the three districts selected to conduct carriage surveys were indicated as A, B, and C. The average incidence in the overall population in a each district was indicated by color. A circle stands for a kindergarten or a school where either or both of the two GAS clones were circulating. The two predominant clones, SH001-*emm*12 and SH002-*emm*1, were circulating in almost all the districts of Shanghai.

related cluster towards the evolutionary root of the modern population, while 33/39 (84.6%) clustered with isolates originally from Hong Kong and mainland China (non-Shanghai) ("Clades 1-3"). The remaining three Shanghai isolates clustered with "Clade 5" that is frequently associated with isolates from the USA and UK (Figure 4). PFGE profiles were not commensurate with clades observed in the phylogenetic tree, indicating that PFGE lacks the resolution required to define these clonal populations (Fig S2 and 4).

Within the *emm*1 Hong Kong and mainland China clades, the combination of *ssa*, *speC*, *spd*, *tetM*, and *ermB* was found in 90.9% (30/33) of isolates. These genes were associated with the mobile elements ΦHKU488.vir (n = 30; carrying *ssa, speC*, and *spd*) and ICE-HKU488 (n = 22; carrying *tetM* and *ermB*) or ICE-HLJGAS2022 (n = 8; carrying *tetM* and *ermB*) that were previously characterized in the progenitor *emm*1 population of mainland China and Hong Kong (Ben Zakour et al., 2015). These data indicate that the retention of these genes and associated elements within this dominant clade may have contributed to the selective expansion of this scarlet fever population within East Asia. Conversely, these genes and their corresponding mobile elements were largely absent from the UK/USA sub-clade indicating geographical differentiation of these clones (Figure 4).

Discussion

This study represents a longitudinal epidemiological and genomic assessment of GAS from the carriage and scarlet fever cases within school-age children in Shanghai during the scarlet fever epidemic. The annual incidence of scarlet fever peaked at 19.4/100,000 people, which is much higher than the national average (4.1/100,000), during 2011–2015 (You et al., 2018). Our findings confirm previous observations that the scarlet fever landscape in East Asia is dominated by multidrug-resistant *emm*12 and *emm*1, yet extend this to show that the relative frequency of these populations is dynamic over time. While *emm*12 was the dominant GAS *emm* type when the scarlet fever outbreak was first documented in Hong Kong in 2011 (76.4%–95.6%) (Tse et al., 2012;

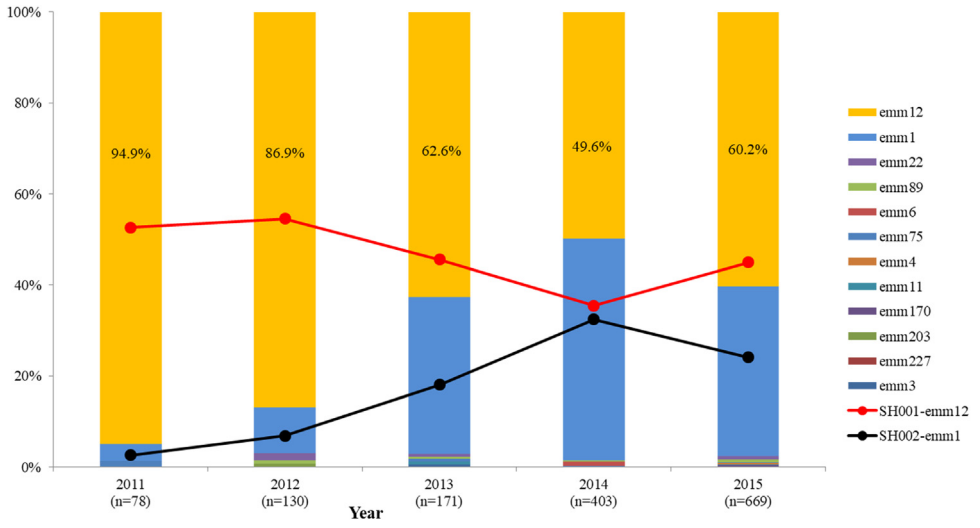

Figure 3. Increase of proportion of *emm*1 isolates among isolates from patients with scarlet fever in Shanghai, China, during 2011-2015. The proportion of *emm*1 GAS strains increased from 3.8% in 2011 to 48.6% in 2014.

Table 2
Distribution of *emm* types and superantigens in disease and carriage GAS in Shanghai, China, during 2011–2015.

	No. (%) patients, by age			No. (%) patients, by sex			No. (%) isolates, by source			
	<5 y, n = 508	>5 y, n = 943	p value	Male, n = 929	Female, n = 522	p value	Scarlet fever, n = 1,451	Carriage, n = 96	p Value[b]	Total, n = 1,547
emm type										
12	351 (69.1)	546 (57.9)	**0.010**	569 (61.3)	328 (62.8)	0.710	897 (61.8)	68 (70.8)	0.038	965 (62.4)
1	142 (28.0)	379 (40.2)	<**0.001**	336 (36.2)	185 (35.4)	0.828	521 (35.9)	25 (26.0)	0.024	546 (35.3)
Other[a]	15 (3.0)	18 (1.9)	0.219	24 (2.6)	9 (1.7)	0.305	33 (2.3)	3 (3.1)	0.285	36 (2.3)
superantigen										
speA	156 (30.7)	404 (42.8)	<**0.001**	363 (39.1)	197 (37.7)	0.697	560 (38.6)	28 (29.2)	0.032	588 (38.0)
speC	506 (99.6)	941 (99.8)	0.974	928 (99.9)	519 (99.4)	0.934	1,447 (99.7)	96 (100)	0.374	1,543 (99.7)
speG	505 (99.4)	941 (99.8)	0.945	927 (99.8)	519 (99.4)	0.950	1,446 (99.7)	96 (100)	0.351	1,542 (99.7)
speH	291 (57.3)	452 (47.9)	**0.018**	479 (51.6)	264 (50.6)	0.804	743 (51.2)	56 (58.3)	0.089	799 (51.6)
speI	393 (77.4)	656 (69.6)	0.096	686 (73.8)	363 (69.5)	0.355	1,049 (72.3)	69 (71.9)	0.459	1,118 (72.3)
speJ	34 (6.7)	69 (7.3)	0.678	58 (6.2)	45 (8.6)	0.107	103 (7.1)	6 (6.3)	0.400	109 (7.0)
speK	0	1 (0.1)	0.650	0	1 (0.2)	0.360	1 (0.07)	0	0.114	1 (0.06)
speL	4 (0.8)	2 (0.2)	0.140	4 (0.4)	2 (0.4)	0.928	6 (0.4)	2 (2.1)	0.047	8 (0.5)
speM	3 (0.6)	2 (0.2)	0.289	4 (0.4)	1 (0.2)	0.517	5 (0.3)	2 (2.1)	0.036	7 (0.5)
ssa	506 (99.6)	937 (99.4)	0.965	924 (99.5)	519 (99.4)	0.997	1,443 (99.5)	95 (99.0)	0.271	1,538 (99.4)
smeZ	503 (99.0)	935 (99.2)	0.980	921 (99.1)	517 (99.0)	0.988	1,438 (99.1)	95 (99.0)	0.404	1,533 (99.1)

[a] Other *emm* types included 3 (1), 4 (3), 6 (4), 11 (2), 22 (8), 75 (4), 89 (9), 170 (3), 203 (1), 227 (1).
[b] Due to the disproportionate sample sizes of the isolates from scarlet fever and carriage, the p values of this column might have a bias; consequently, some that appear to be significant are not highlighted in bold font.

Chen et al., 2012; Luk et al., 2012; Yang et al., 2013), we have shown a striking rise in *emm*1 GAS (from 3.8% in 2011 to nearly 50% in 2014). It is unclear what competition may be driving this shift, yet it is tempting to speculate based on genome analysis that the increase of *emm*1 isolates might be associated with the acquisition of the mobile elements similar to those of *emm*12 outbreak strains.

Note that a similar increasing trend of *emm*1 isolates associated with scarlet fever was also found in UK, Hong Kong, and Beijing (Ben Zakour et al., 2015; Lynskey et al., 2019; You et al., 2020). The *emm*1 UK isolates represent a different sub-clone to the *emm*1 clone driving scarlet fever in East Asia, and they generally have a different superantigen profile. It is unclear how these different profiles contribute to disease severity and/or longitudinal population dynamics, yet a combination of gene content, gene regulation, genetic host, immune status, and ecological competition likely contribute to the success of such clones. In Hong Kong, the increase of *emm*1 was linked to the presence of mobile genetic elements associated with the expansion of *emm*12 scarlet fever clones (Ben Zakour et al., 2015), but the mechanism of the increase of *emm*1 in Beijing remains unknown. It seems a mechanism similar to that of Hong Kong exists behind the increase of *emm*1 isolates in Shanghai. In the genomic analysis, the majority of Shanghai *emm*1 isolates possessed two mobile genetic elements, ICE-HKU488 and ΦHKU 488.vir, which shared high sequence identity with that from *emm*12 clones (ICE-HKU16, also named ICE-*emm*12, and ΦHKU.vir) (Ben Zakour et al., 2015; Davies et al., 2015). Due to the presence of common mobile genetic elements between *emm*1 in Shanghai and *emm*12 in Hong Kong, the dominant *emm*1 clone in this study showed a superantigen and antimicrobial resistance profile closer to the *emm*12 outbreak strains than *emm*1 clones circulating in other countries (Figure 4), including the superantigen encoding genes *speC* and *ssa*, which have been associated with scarlet fever (Silva-Costa et al., 2014), and the macrolide and tetracycline resistance encoding genes *ermB* and *tetM*. Previous studies have indicated that the acquisition of mobile elements conferring a new virulence gene profile (superantigens *ssa, speC,* and deoxyribonuclease *spd1*) and multidrug resistance (*erm*B and *tet*M) may have changed the virulence propensity of GAS isolates leading to a selective advantage (Davies et al., 2015; Tse et al., 2012; Ben Zakour et al., 2015; You et al., 2018). Our data support these observations

Table 3
Comparison of antimicrobial resistance, superantigens, and PFGE patterns between *emm*12 and *emm*1 group A *Streptococcus* in the scarlet fever epidemic period in Shanghai, China, during 2011–2015[a].

	No. (%) patients, by *emm*			No. (%) carriers, by *emm*		
	*emm*12, n = 897	*emm*1, n = 521	p value	*emm*12, n = 68	*emm*1, n = 25	p value
Antimicrobial resistance						
erythromycin	887 (98.9)	500 (96.0)	0.594	68 (100.0)	24 (96.0)	0.202
clindamycin	887 (98.9)	498 (95.6)	0.546	68 (100.0)	24 (96.0)	0.202
tetracycline	867 (96.7)	494 (94.8)	0.735	62 (91.2)	24 (96.0)	0.247
levofloxacin	9 (1.0)	0	NA	0	0	NA
Superantigen						
speA	38 (4.2)	511 (98.1)	<**0.001**	7 (10.3)	20 (80.0)	<**0.001**
speC	896 (99.9)	519 (99.6)	0.963	68 (100.0)	25 (100.0)	NA
speG	895 (99.8)	521 (100.0)	0.966	68 (100.0)	25 (100.0)	NA
speH	719 (80.2)	19 (3.6)	<**0.001**	55 (80.9)	0	<**0.001**
speI	782 (87.2)	254 (48.8)	<**0.001**	58 (85.3)	9 (36.0)	**0.004**
speJ	29 (3.2)	73 (14.0)	<**0.001**	2 (2.9)	4 (16.0)	**0.010**
speK	0	0	NA	0	0	NA
speL	0	1 (0.2)	0.367	0	0	NA
speM	0	0	NA	0	0	NA
ssa	896 (99.9)	521 (100.0)	0.982	68 (100.0)	25 (100.0)	NA
smeZ	887 (98.9)	519 (99.6)	0.892	68 (100.0)	25 (100.0)	NA
Number of PFGE patterns	46	21	0.364	7	7	**0.025**

The p values appear to be significant are highlighted in bold font.
[a] PFGE, pulsed-field gel electrophoresis.

by showing that the most dominant scarlet fever *emm*1 clades in mainland China and East Asia retain this virulence gene profile, which may, in turn, enhance their fitness relative to other GAS clones.

Ongoing surveillance of GAS *emm* types is needed to guide the local development of future GAS vaccine formulations and public health interventions. The epidemiological data from China are different from those reported in England and South Korea, where multiple *emm* types were associated with nationwide outbreaks of scarlet fever that included *emm*3, *emm*4, and *emm*28 in addition to *emm*1 and *emm*12 (Lamagni et al., 2017; Park et al., 2017). GAS vaccines based on several type-specific M proteins, conserved M protein epitopes, or other GAS antigens, such as fusions of streptococcal pyrogenic exotoxins, are in various stages of preclinical or clinical evaluation that can differ in their global coverage (Dale et al., 2011; Dale et al., 2013; Morefield et al., 2014; Davies et al., 2019). It is clear from the re-emergence of scarlet fever that a vaccine needs to be effective in preventing GAS infections in children across multiple *emm* types. The identification of dominant *emm*12 and *emm*1 scarlet fever clones circulating among children in kindergartens and schools suggests that asymptomatic individuals are likely to be an important reservoir in the transmission of scarlet fever. Consequently, any therapeutic or public health intervention, such as a vaccine, will need to target the wider school-age population to reduce the transmission chain of scarlet fever clones. Without preventative measures, the most effective management of this ongoing scarlet fever outbreak is early recognition and timely antibiotic treatment, such as third-generation cephalosporins.

The overall carriage rates among students aged 3-14 years in this study were 7.6%, positively correlated with the scarlet fever incidences, and reached 35–40% in some classes (Tables 1 and S2). In this study, scarlet fever mainly affected children aged 3–9 years (93.0%), which supports observations from a recent nationwide study (Liu et al., 2018). The identification of the same genetic clones from the carriage and scarlet fever patients, in addition to being from the same kind of institution, implies that these settings likely play an important role in the transmission of scarlet fever clones; further studies are required to confirm this observation. This is supported by the fact that the two main seasonal peaks in scarlet fever incidence across China overlap with school semesters and that scarlet fever decreases substantially during school holidays (Liu et al., 2018). These peaks do not seem to be influenced by season, where different climates exist in southern China (warmer and more humid) and northern China (colder and dry) (Shu et al., 2010). Increased longitudinal surveillance from other provinces of China is required, yet we found no evidence in the literature of GAS carriage surveys being undertaken in China since the scarlet fever outbreak began in 2011.

We noticed that the prevalence of macrolide- and tetracycline-resistance among GAS isolates was higher in mainland China (80–97%) than in Hong Kong (60%) and South Korea (2.8–7.0%) (Hsieh and Huang, 2011; Yang et al., 2013; Park et al., 2017), which has narrowed the therapeutic options for GAS infections. The high prevalence of multidrug resistance among GAS strains is attributed to the high consumption of antibiotics in mainland China (Van Boeckel et al., 2014). The resistance of the GAS isolates to macrolides was mediated by *ermB*, one of erythromycin ribosome methylase (*erm*) encoding genes, which usually confers constitutive resistance to macrolides, clindamycin, and streptogramin B ($cMLS_B$) by preventing antibiotics binding with the ribosome target (Leclercq, 2002). As a result, Shanghai GAS isolates displayed high MIC values to erythromycin (4 - >128 μg/ml) and clindamycin (8 - >128 μg/ml). In the genomic analysis, the existence of the two mobile genetic elements in the Shanghai *emm*1 population was identified, highlighting the dynamic evolution of GAS *emm* types and their impact on clinical management.

In support of previous observations (Liu et al., 2018; You et al., 2018), we did not observe a change in the seasonal epidemiological features during the scarlet fever epidemics in 2011–2015 compared to pre-epidemic data in 2005–2010. Two peak seasons appeared from late spring to middle summer and in winter months, and the vulnerable subjects were children aged 3–9 years and boys, which are consistent with the national and regional surveillance data (Liu et al., 2018). The similar epidemiological features were also observed in Poland in 2012 (Staszewska et al., 2014). However, in England, only one peak season was seen from March to April during 2013–2016 (Lamagni et al., 2017), and in South Korea, the peak season was seen almost always in winter and only occasionally in summer during 2011–2015 (Park et al., 2017).

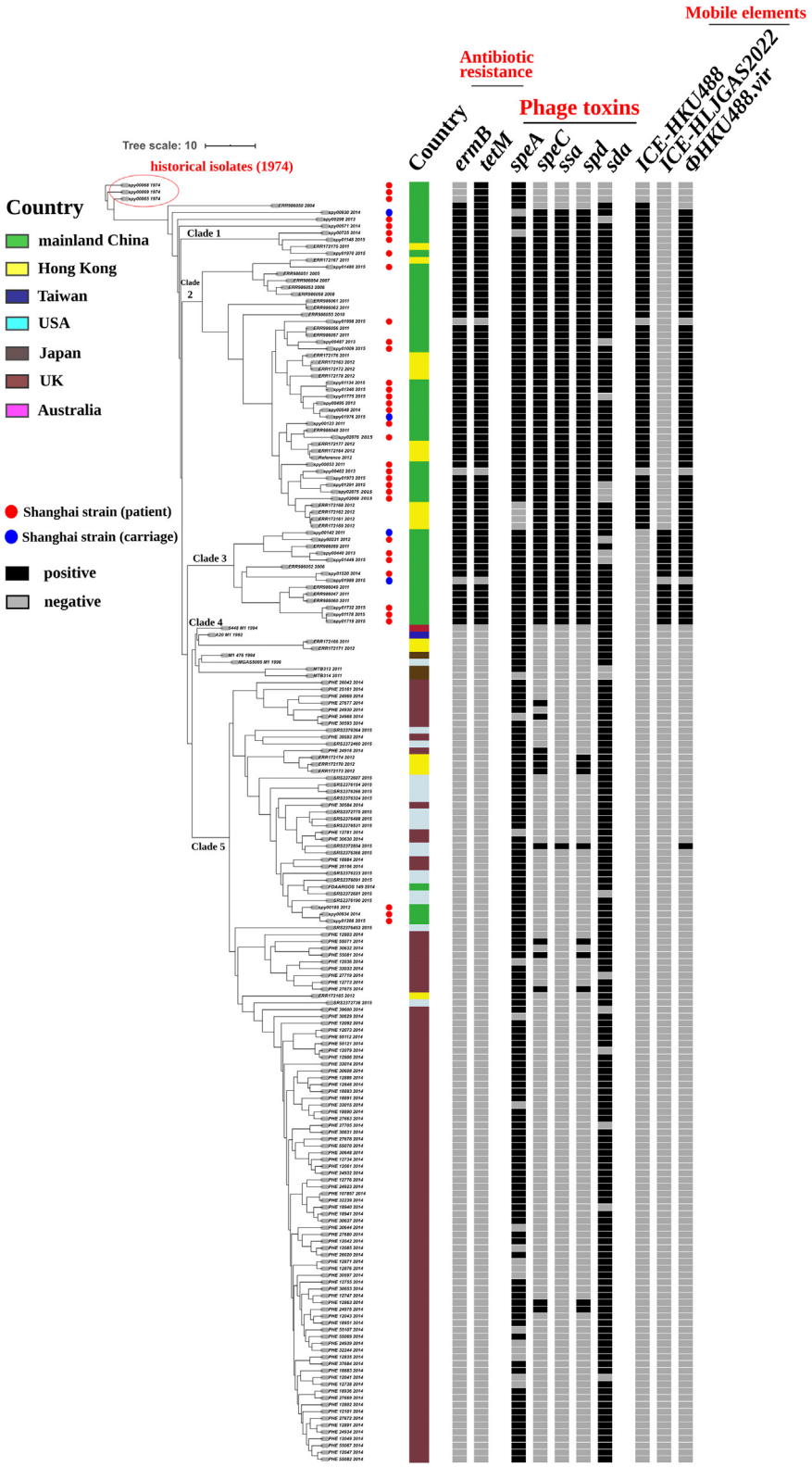

Figure 4. Phylogenetic analysis of *emm*1 GAS genomes using a maximum-likelihood tree based on single nucleotide polymorphisms (SNPs). The distribution of selected virulence genes, mobile elements, and antibiotic resistance genes is indicated. A total of 180,724 SNPs were identified from the 187 *emm*1 GAS genome alignment. Most *emm*1isolates from mainland China and Hong Kong were clustered together and constituted clades distinct from clades of other countries. The majority of *emm*1 Shanghai GAS isolates harbored the mobile genetic elements ΦHKU488.vir (n = 30; carrying *ssa*, *speC* and *spd*) and ICE-HKU488 (n = 22; carrying *tetM* and *ermB*).

The distinct seasonality of scarlet fever by country could be linked with bacterial, host, or environmental factors (Wong and Yuen, 2012). A few studies have suggested meteorological factors may contribute to the scarlet fever outbreak (Duan et al., 2016; Mahara et al., 2016; Duan et al., 2017); however, the consistent seasonal incidence makes the meteorological correlation conflicting. Thus far, there is still a gap in knowledge on the host's protective immunity role in the epidemics.

Conclusion

In conclusion, our study highlights the sustained burden and alarming regularity of annual scarlet fever epidemics in Shanghai. The increase of *emm*1 and the clonal transmission in institutionalized children could be related to the persistent outbreaks of scarlet fever in Shanghai. There is an unmet need to develop GAS vaccines in China to address the public health issue of the scarlet fever outbreak. Continuous clinical surveillance coupled with genomic epidemiology on scarlet fever epidemics is needed to track the fluctuating molecular, virulence, and antimicrobial resistance profiles of this substantial public health problem.

Author's contribution

MLC, MC, and MZ conceived and designed the experiments. JHC and CZ performed all the experiments. YFL and WLY collected clinical specimens. DCK, HP, and XZ collected epidemiological data. MLC, JHC, MRD, and MZ analyzed the data. MLC and MZ supervised the study and wrote the paper. MRD supervised the genome analysis and revised the paper. All authors have read and approved the final manuscript.

Conflicts of interest

All authors declare no competing financial interests. All authors declare no non-financial competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.06.053.

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