Scientific Research Publishing

Journal of Biosciences and Medicines, 2018, 6, 89-97
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Investigation of *Streptococcus pyogenes* Carriage in Population Vulnerable to Scarlet Fever during 2015-2017 in Shanghai, China

Mingliang Chen[1,2], Chi Zhang[1], Dechuan Kong[1], Hao Pan[1], Xi Zhang[1], Min Chen[1*]

[1]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China
[2]Shanghai Institutes of Preventive Medicine, Shanghai, China

Email: *chenmin@scdc.sh.cn

How to cite this paper: Chen, M.L., Zhang, C., Kong, D.C., Pan, H., Zhang, X. and Chen, M. (2018) Investigation of *Streptococcus pyogenes* Carriage in Population Vulnerable to Scarlet Fever during 2015-2017 in Shanghai, China. *Journal of Biosciences and Medicines*, **6**, 89-97.
https://doi.org/10.4236/jbm.2018.611009

Received: October 26, 2018
Accepted: November 16, 2018
Published: November 19, 2018

Abstract

This study aimed to investigate the carriage of *Streptococcus pyogenes* in population vulnerable to scarlet fever and to compare their genotypic characterization between different age groups. Pharyngeal swabs were collected from 120 - 150 students in each of the three districts in Shanghai in May and December during 2015 to 2017, while *emm* typing and detection of 12 superantigen genes were performed to characterize the isolates. During 2015-2017, the average carriage rate in students was 5.7% (135/2,371), without significant difference between different years or districts. The carriage rate was significantly different between children from the three age groups, with 2.4% in 3 - 4 years, 5.4% in 5 - 9 years, and 9.1% in 10 - 14 years. Eight *emm* types were found, including *emm* 1, *emm* 4, *emm* 12, *emm* 22, *emm* 75, *emm* 89, *emm* 70 and *emm* 241, among which *emm* 12 accounted for 60%, and *emm* 1 27.5%. The predominance of *emm* 12 was found in each year, but the proportion of *emm* 12 was lower in 10 - 14 years (43.3%) than in 3 - 4 years (86.7%) and in 5 - 9 years (73.3%) ($P = 0.002$ and 0.003). Superantigen genes of *speB*, *speC*, *speG*, *ssa* and *smeZ* were found in almost all the isolates. The average carriage of *S. pyogenes* in population vulnerable to scarlet fever was 5.7% in Shanghai, highest in 10 - 14 years (9.1%), while *emm* 12 was the predominant type.

Keywords

Scarlet Fever, Carriage Rate, Child, *Emm* type, Superantigen

1. Introduction

Scarlet fever, one of the class B notifiable diseases in China [1], is caused by

Streptococcus pyogenes. Since 2011, epidemics of scarlet fever have been prevalent in China, with annual reported cases ranging from 34,207 to 68,249 [2]. The epidemics peaked in April to June and November to the next January, and mainly affected children (aged < 15 years) in kindergarten and school [3]. That is why we supposed school-age children as "population vulnerable to scarlet fever". However, little is known about the *S. pyogenes* carriage in this population. To bridge the gap, this study investigated and analyzed the carriage of *S. pyogenes* in population vulnerable to scarlet fever in Shanghai during 2015 and 2017, which can provide data for preventing and controlling the scarlet fever outbreaks.

2. Materials and Methods

2.1. Carriage Surveys of *S. pyogenes* in Schools

In May and December, the peak months of scarlet fever epidemics, the carriage surveys were conducted in schools of three districts, including one urban district (Xuhui), one suburban district (Minhang), and one rural district (Fengxian) during 2015 and 2017. The carriage surveys were performed in two schools without scarlet fever clusters within recent one month, and classes without cases were chosen to cover three age groups (3 - 4 years, 5 - 9 years, and 10 - 14 years) each with 40 - 50 students. After the participates' parent(s)/guardian(s) gave informed consent, posterior pharyngeal swab samples were obtained by the trained personnel and were sent to Shanghai CDC laboratory in four hours for further test. The study was approved by Shanghai CDC Ethical Review Committee (No.: 2016-4).

2.2. Strain Collection and Identification

After culture for 18 - 24 h on Columbia sheep blood agar at 36˚C with 5% carbon dioxide, β-hemolytic Gram-positive cocci were further tested by latex-agglutination with the Diagnostic Streptococcal Grouping Kit (Oxoid, Hampshire, United Kingdom) and were identified by Vitek 2 system (bioMe'rieux, Marcy l'Etoile, France).

2.3. Molecular Typing for *S. pyogenes*

According to the protocol recommended by CDC [4], chromosomal DNA was extracted, *emm* gene was amplified and sequenced. Nucleotide sequence was blast in the website (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp), and the *emm* type and subtype were determined. Twelve superantigen genes were screened by PCR as previously reported [5], including *speA*, *speB*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa*, and *smeZ*.

2.4. Statistical Analysis

Statistical analysis was performed using OpenEpi (Version 3.01) [6]. Statistical significance was assessed at $P < 0.05$. Figures were produced using Microsoft

Excel 2010.

3. Results

3.1. Carriage Surveys during 2015 and 2017

A total of 2371 pharyngeal swabs were collected during 2015 and 2017, and 135 (5.7%) were culture-positive. When analyzed by year, the carriage rates were 6.3% in 2015, 5.7% in 2016, and 5.1% in 2017, respectively (**Table 1**), but the difference showed no statistical significance ($P = 0.3 - 0.6$). When analyzed by geographical location, the rates were 4.6% in urban distinct, 5.6% in suburban district, and 6.7% in rural distinct, respectively (**Table 1**), without statistically significant difference ($P = 0.08 - 0.4$). When analyzed by age, the rates were 2.4% in 3 - 4 years group, 5.4% in 5 - 9 years group, and 9.1% in 10 - 14 years group, respectively (**Table 2**), with statistically significant difference ($P < 0.05$).

3.2. *emm* Typs of *S. pyogenes*

A total of 120 isolates were available for *emm* typing. There were eight *emm* types, including *emm* 1, *emm* 4, *emm* 12, *emm* 22, *emm* 75, *emm* 89, *emm* 170, and *emm* 241. Isolates of *emm* 12 were predominant (60%), followed by *emm* 1 (27.5%), while isolates of other types accounted for 12.5%, including *emm* 4 (n = 4), *emm* 89 (n = 4), *emm* 22 (n = 3), *emm* 75 (n = 2), *emm* 170 (n = 1), and *emm* 241 (n = 1).

Table 1. *S. pyogenes* carriage in population vulnerable to scarlet fever in three districts of Shanghai, China during 2015 and 2017.

District	2015	2016	2017	Average rate
Urban distinct (Xuhui)	5% (12/240)	4.6% (11/241)	4.2% (10/240)	4.6% (33/721)
Suburban district (Minhang)	10.2% (26/256)	0.8% (2/248)	5.7% (14/246)	5.6% (42/750)
Rural distinct (Fengxian)	4% (12/300)	10.7% (32/300)	5.3% (16/300)	6.7% (60/900)
In total	6.3% (50/796)	5.7% (45/789)	5.1% (40/786)	5.7% (135/2,371)

Table 2. *S. pyogenes* carriage in population vulnerable to scarlet fever in three age groups in Shanghai, China during 2015 and 2017.

Age group (year)	2015	2016	2017	Average rate	Proportion of *emm* 12
3 - 4	3.2% (7/221)	2.6% (7/266)	1.3% (3/234)	2.4% (17/721)	86.7% (13/15)
5 - 9	5.1% (16/315)	4.9% (13/264)	6.2% (18/292)	5.4% (47/871)	73.3% (33/45)
10 - 14	10.4% (27/260)	9.7% (25/259)	7.3% (19/260)	9.1% (71/779)	43.3% (26/60)

In each year during 2015 and 2017, *emm* 12 was the predominant type, and the proportion ranged from 44.4% to 80% (**Figure 1**), with statistically significant difference found between the proportion in 2014 (44.4%) and that in either of other two years ($P = 0.002$ and 0.03). The proportion of *emm* 12 varied in different age groups, with difference found between the proportion in 10 - 14 years group (43.3%) and that in other two age groups ($P = 0.002$ and 0.003).

There were 21 *emm* subtypes, including 4 *emm* 1 subtypes and 8 *emm* 12 subtypes. The subtypes *emm* 12.0 (40%, 48/120) and *emm* 1.0 (20%, 24/120) were prevalent, while other subtypes accounted for 40% (48/120). The profile of *emm* subtypes was not the same in either year during 2015 and 2017, with the number ranging from eight to fourteen (**Table 3**).

3.3. Superantigen Profile of *S. pyogenes*

Among the 120 isolates, all possessed *speB*, *ssa*, *and smeZ*, the majority harbored *speC* (99.2%) and *speG* (98.3%), almost half possessed *speH* (54.2%) and *speI* (59.2%), and a few had *speA* (29.2%), *speJ* (12.5%), *speL* (2.5%), and *speM* (2.5%), while none had *speK* (**Table 4**). In *emm* 12 isolates, the superantigen profile of *speB-speC-speG-speH-speI-ssa-smeZ* was predominant (84.7%, 61/72), while in *emm* 1 isolates, *speA-speB-speC-speG-speJ-ssa-smeZ* (39.4%, 13/33) and *speA-speB-speC-speG-ssa-smeZ* (33.3%, 11/33) were much common.

4. Discussion

China has been undergoing scarlet fever epidemics these years [7]. Based on continuous surveillance on children in school, this study discovered the carriage rate of *S. pyogenes* in population vulnerable to scarlet fever was 5.7% in peak seasons of scarlet fever, with *emm* 12 as the predominant type and without difference in geographical districts. Due to no carriage data from other countries with scarlet

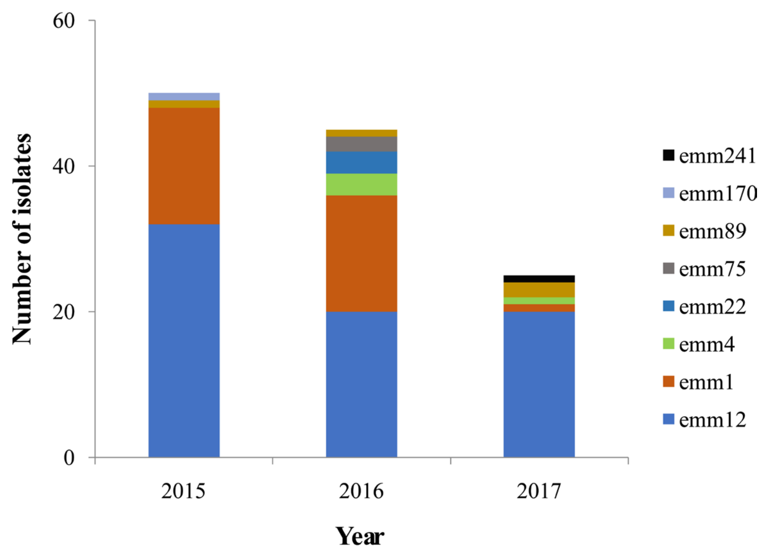

Figure 1. Proportion of *emm* types of isolates from carriage surveys in Shanghai, China during 2015 and 2017.

Table 3. Distribution of *emm* types and subtypes of *S. pyogenes* from population vulnerable to scarlet fever in Shanghai, China, during 2015 and 2017.

emm type and subtype	2015 (n = 50)	2016 (n = 45)	2017 (n = 25)	In total (n = 120)
emm 1				
1.0	10 (20%)	14 (31.1%)	0	24 (20%)
1.16	0	1 (2.2%)	0	0
1.25	4 (8%)	0	0	4 (3.3%)
1.65	2 (4%)	1 (2.2%)	1 (4%)	4 (3.3%)
emm 12				
12.0	21 (42%)	15 (33.3%)	12 (48%)	48 (40%)
12.18	0	1 (2.2%)	0	1 (0.8%)
12.19	1 (2%)	1 (2.2%)	1 (4%)	3 (2.5%)
12.37	1 (2%)	2 (4.4%)	0	3 (2.5%)
12.71	1 (2%)	0	0	1 (0.8%)
12.76	8 (16%)	1 (2.2%)	0	9 (7.5%)
12.8	0	0	6 (24%)	6 (5%)
12.95	0	0	1 (4%)	1 (0.8%)
emm 4				
4.0	0	0	1 (4%)	1 (0.8%)
4.14	0	3 (6.7%)	0	3 (2.5%)
emm 22				
22.0	0	1 (2.2%)	0	1 (0.8%)
22.1	0	2 (4.4%)	0	2 (1.7%)
emm 75				
75.0	0	1 (2.2%)	0	1 (0.8%)
75.3	0	1 (2.2%)	0	1 (0.8%)
emm 89				
89.0	1 (2%)	1 (2.2%)	2 (8%)	4 (3.3%)
emm 170				
170.0	1 (2%)	0	0	1 (0.8%)
emm 241				
241.1	0	0	1 (4%)	1 (0.8%)

fever epidemics were available, such as United Kingdom, South Korea, and Poland [3] [8] [9] [10] [11], we could not compare the carriage rates with them. While comparing with other provinces in China, the carriage rate in this study was higher than another study in Shandong Province (2.4%, 6/253), but the latter without information of age groups [12].

Analyzed by age, the carriage rate was highest in 10 - 14 years group, followed by 5 - 9 years group, then 3 - 4 years, which was not parallel with the scarlet fever incidences in these age groups. Studies on scarlet fever cases in Hong Kong,

Table 4. Distribution of superantigen genes in *S. pyogenes* from population vulnerable to scarlet fever in Shanghai, China, during 2015 and 2017.

emm subtype	Total Number	*speA*	*speB*	*speC*	*speG*	*speH*	*speI*	*speJ*	*speK*	*speL*	*speM*	*ssa*	*smeZ*
emm 12 (n = 72)	61	-	+	+	+	+	+	-	-	-	-	+	+
	8	-	+	+	+	-	-	-	-	-	-	+	+
	2	+	+	+	+	-	-	-	-	-	-	+	+
	1	+	+	+	+	+	+	-	-	-	-	+	+
emm 1 (n = 33)	13	+	+	+	+	-	-	+	-	-	-	+	+
	11	+	+	+	+	-	-	-	-	-	-	+	+
	5	+	+	+	+	-	+	-	-	-	-	+	+
	1	+	+	-	+	-	-	+	-	-	-	+	+
	3	-	+	+	+	-	-	-	-	-	-	+	+
emm 170	1	-	+	+	+	+	+	-	-	+	+	+	+
emm 22	3	-	+	+	+	-	-	-	-	-	-	+	+
emm 241	1	+	+	+	+	-	-	+	-	-	-	+	+
emm 4 (n = 4)	2	-	+	+	+	-	-	-	-	-	-	+	+
	2	-	+	+	-	-	-	-	-	-	-	+	+
emm 75	2	-	+	+	+	+	+	-	-	+	+	+	+
emm 89 (n = 4)	3	-	+	+	+	-	-	-	-	-	-	+	+
	1	+	+	+	+	-	-	-	-	-	-	+	+
In total*	120	35 (29.2)	120 (100)	119 (99.2)	118 (98.3)	65 (54.2)	71 (59.2)	15 (12.5)	0 (0)	3 (2.5)	3 (2.5)	120 (100)	120 (100)

*the number in bracket stands for the percentage.

Beijing, Jiangsu, and Shanghai showed that scarlet fever mainly affected children younger than 10 years old [3] [5] [8] [13]. This disparity could be explained with factors including bacteria, host, and environment. Firstly, the *S. pyogenes* isolates carried by children younger than 10 years old might be different from those by 10 - 14 years old, in which the proportion of *emm* 12 isolates was found much lower than that in the 3 - 4 years and the 5 - 9 years groups in this study (**Table 2**). Moreover, *emm* 12 *S. pyogenes* was responsible for the scarlet fever epidemics in China since 2011 [2]. Secondly, the immune system of children younger than 10 years old might be not so mature as that of 10 - 14-year-old children, which makes the former much more vulnerable than the latter when facing the same type of *S. pyogenes* strain. Thirdly, we also thought about the social environment of these groups, but this factor seemed not so important, for children younger than 10 years old and 10 - 14 years old are both in school.

Besides *emm* typing, the superantigen genes were also studied in population vulnerable to scarlet fever. The genes of *speB*, *speC*, *speG*, *ssa*, and *smeZ* were found in almost all the *emm* types, and *spec* and *ssa* were supposed as the marker of scarlet fever outbreak strains in China [14]. The profile of superantigen genes, *speB-speC-speG-speH-speI-ssa-smeZ*, was shared by *emm* 12 isolates from carriers and scarlet fever patients [3], while the similar result was found in *emm* 1 isolates (*speA-speB-speC-speG-speJ-ssa-smeZ*), which suggested the scarlet fever epidemics were closely associated with the carriage of epidemic strains in population vulnerable to scarlet fever. Moreover, SpeA, encoded by *speA*, was supposed as an important virulence factor which facilitated the dissemination of *emm* 1 strains globally since 1980s due to its ability of enhancing the colonization of *S. pyogenes* in epithelial cells [15]. In previous reports, no *speA* gene was found harbored by *emm* 12 isolates, while in this study, we identify three *emm* 12 isolates possessing *speA* gene. This suggested the possibility that *emm* 12 isolates acquired *speA* gene from *emm* 1 isolates, which need more data to validate.

5. Conclusion

This study provided dynamic data of *S. pyogenes* carriage in school-age children in Shanghai and found the difference of carriage between children in various age groups, which are valuable for prevention and control of scarlet fever epidemics. More studies and surveillance on the carriage in population vulnerable to scarlet fever are needed all over the country.

Funding Statement

This study was supported by grants from National Natural Science Foundation of China (81601801), Shanghai Rising-Star Program (17QA1403100), a Municipal Human Resources Development Program for Outstanding Young Talents in Medical and Health Sciences in Shanghai (2017YQ039), Natural Science Foundation of Shanghai (16ZR1433300), the 4th Tree-year Action Plan for Public Health of Shanghai (GWTD2015S01) from Shanghai Municipal.

Commission of Health and Family Planning, and the 13th Five-Year Project of National Health and Family Planning Commission of the People's Republic of China (2017ZX10303405004 and 2017ZX10103009-003). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Data Availability Statement

The experimental data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors report no conflict of interests regarding the publication of this paper.

The authors alone are responsible for the content and writing of the paper.

References

[1] Zhang, X., Hou, F., Li, X., Zhou, L., Liu, Y. and Zhang, T. (2016) Study of Surveil-

lance Data for Class B Notifiable Disease in China from 2005 to 2014. *International Journal of Infectious Diseases*, **48**, 7-13. https://doi.org/10.1016/j.ijid.2016.04.010

[2] You, Y., Davies, M.R., Protani, M., McIntyre, L., Walker, M.J. and Zhang, J. (2018) Scarlet Fever Epidemic in China Caused by *Streptococcus pyogenes* Serotype M12: Epidemiologic and Molecular Analysis. *EBioMedicine*, **28**, 128-135. https://doi.org/10.1016/j.ebiom.2018.01.010

[3] Yang, P., Peng, X., Zhang, D., Wu, S., Liu, Y., Cui, S., Lu, G., Duan, W., Shi, W., Liu, S., Li, J. and Wang, Q. (2013) Characteristics of Group A *Streptococcus* Strains Circulating during Scarlet Fever Epidemic, Beijing, China, 2011. *Emerging Infectious Diseases*, **19**, 909-915. https://doi.org/10.3201/eid1906.121020

[4] Streptococcus Laboratory of Centers for Disease Control and Prevention (2017) Introduction to *emm* Typing: M Protein Gene (*emm*) Typing *Streptococcus pyogenes*. https://www.cdc.gov/streplab/groupa-strep/emm-background.html

[5] Chen, M., Yao, W., Wang, X., Li, Y., Wang, G., Zhang, X., Pan, H., Hu, J. and Zeng, M. (2012) Outbreak of Scarlet Fever Associated with *emm* 12 Type Group A *Streptococcus* in 2011 in Shanghai, China. *The Pediatric Infectious Disease Journal*, **31**, e158-162. https://doi.org/10.1097/INF.0b013e31825874f3

[6] OpenEpi (2013) Open Source Epidemiologic Statistics for Public Health, Version 3.01. https://www.OpenEpi.com

[7] Liu, Y., Chan, T.C., Yap, L.W., Luo, Y., Xu, W., Qin, S., Zhao, N., Yu, Z., Geng, X. and Liu, S.L. (2018) Resurgence of Scarlet Fever in China: A 13-Year Population-Based Surveillance Study. *The Lancet Infectious Diseases*, **18**, 903-912. https://doi.org/10.1016/S1473-3099(18)30231-7

[8] Lau, E.H., Nishiura, H., Cowling, B.J., Ip, D.K. and Wu, J.T. (2012) Scarlet Fever Outbreak, Hong Kong, 2011. *Emerging Infectious Diseases*, **18**, 1700-1702. https://doi.org/10.3201/eid1810.120062

[9] Park, D.W., Kim, S.H., Park, J.W., Kim, M.J., Cho, S.J., Park, H.J., Jung, S.H., Seo, M.H., Lee, Y.S., Kim, B.H., Min, H., Lee, S.Y., Ha, D.R., Kim, E.S., Hong, Y. and Chung, J.K. (2017) Incidence and Characteristics of Scarlet Fever, South Korea, 2008-2015. *Emerging Infectious Diseases*, **23**, 658-661. https://doi.org/10.3201/eid2304.160773

[10] Turner, C.E., Pyzio, M., Song, B., Lamagni, T., Meltzer, M., Chow, J.Y., Efstratiou, A., Curtis, S. and Sriskandan, S. (2016) Scarlet Fever Upsurge in England and Molecular-Genetic Analysis in North-West London, 2014. *Emerging Infectious Diseases*, **22**, 1075-1078. https://doi.org/10.3201/eid2206.151726

[11] Staszewska, E., Kondej, B. and Czarkowski, M.P. (2014) Scarlet Fever in Poland in 2012. *Przeglad Epidemiologiczny*, **68**, 209-212, 329-231.

[12] Liu, Z., Fang, M., Hu, B., Bi, Z., Kou, Z., Ren, Y., Chen, B. and Bi, Z. (2014) Molecular Types of Group A Streptococcus Isolated from Scarlet Fever Patients and Asymptomatic Carriers in Shandong Province, 2013. *Zhonghua Liu Xing Bing Xue Za Zhi*, **35**, 1375-1378.

[13] Zhang, Q., Liu, W., Ma, W., Shi, Y., Wu, Y., Li, Y., Liang, S., Zhu, Y. and Zhou, M. (2017) Spatiotemporal Epidemiology of Scarlet Fever in Jiangsu Province, China, 2005-2015. *BMC Infectious Diseases*, **17**, 596. https://doi.org/10.1186/s12879-017-2681-5

[14] Davies, M.R., Holden, M.T., Coupland, P., Chen, J.H., Venturini, C., Barnett, T.C., Zakour, N.L., Tse, H., Dougan, G., Yuen, K.Y. and Walker, M.J. (2015) Emergence of Scarlet Fever *Streptococcus pyogenes* emm 12 Clones in Hong Kong Is Associated with Toxin Acquisition and Multidrug Resistance. *Nature Genetics*, **47**, 84-87.

https://doi.org/10.1038/ng.3147

[15] Aziz, R.K. and Kotb, M. (2008) Rise and Persistence of Global M1T1 Clone of *Streptococcus pyogenes*. *Emerging Infectious Diseases*, **14**, 1511-1517. https://doi.org/10.3201/eid1410.071660