Letter to the Editor

Molecular Epidemiological Analysis of *Group A Streptococci* Isolated from Children in Chaoyang District of Beijing, 2011: *emm* Types, Virulence Factor Genes and Erythromycin Resistant Genes

WANG Hai Bin[1,§], SONG Yan Yan[1,§], YOU Yuan Hai[2,3,§], WANG Heng Wei[1], HAN Qin Hua[1], ZHAO Jian Hong[1], and ZHANG Xiao Xi[1,#]

Group A streptococcus (GAS) causes a wide range of diseases in the human population. GAS diseases are more common in children than in adults, with clinical manifestations ranging from pharyngitis and impetigo to invasive infections and post streptococcal sequelae, such as acute rheumatic fever and acute post-streptococcal glomerulonephritis[1]. GAS harbors a host of virulence factors that contribute to its complex pathogenicity and differences in the disease severity and frequency. M protein, one of the major virulence factors, is encoded by the *emm* gene induces a type of specific host immune response and confers antiphagocytic properties. Sequence analysis of the *emm* gene has become an important surveillance tool for investigating the dynamics of GAS infections. Among the virulence factors that regulate GAS pathogenicity, the well-known extracellular proteins, including superantigen toxins, streptolysin O (SLO) and speF toxin, are principal contributors. Superantigens are bacterial toxins that are characterized by their ability to induce overproduction of inflammatory cytokines. All GAS isolates virtually harbor SLO to destroy erythrocytes and leukocytes. The speF toxin, initially described as superantigen, has been shown to be a deoxyribonuclease recently. Penicillin is the first choice for the treatment of infections caused by GAS. Erythromycin is recommended as an alternative medicine for patients who are allergic to penicillin. Unfortunately, the development of erythromycin resistance in China may occasionally limit the use of this antibiotic. Resistance to erythromycin in streptococci is mediated by three major resistance genes, including *ermA*, *ermB, and mefA*.

Molecular epidemiological analysis of GAS isolates including virulence factor genes, *emm* types, and resistance genes are of basic importance for the determination of their potential to cause disease and the development of vaccines. Therefore, this paper described a study on molecular characteristics of GAS isolates collected during a short period within Chaoyang. District, one metropolitan area of Beijing Municipality. Seventy one isolates were *emm*-typed and tested for ten virulence factor genes, including *speA, speI, ssa*, *speC, speJ, speG, speH, smeZ, speF,* and *slo*. Three erythromycin resistance-associated genes were also determined for their presence. The prevalence of virulence factor genes and the relationship between virulence factor genes, *emm* types and the diseases caused by GAS were investigated to give a detailed description of the molecular epidemiological characteristics of GAS in this district in 2011.

For the ten virulence factor genes, *speC*, *smeZ,* and *slo* were detected in all GAS isolates. The other seven genes *speF*, *ssa*, *speG*, *speH*, *speI*, *speA,* and *speJ* were found in 97.2% (69/71), 97.2% (69/71), 95.8% (68/71), 80.3% (57/71), 80.3% (57/71), 15.5% (11/71), and 12.7% (9/71) of GAS isolates, respectively. The *speG, smeZ,* and *speJ* are genome-carried superantigen genes. The *speG* and *smeZ* genes had high detection rates which were consistent with previous studies[2]. In contrast, the *speJ* gene had a low detection rate in the present study. This result was consistent with the previous research in Chongqing Municipality of China[3]. In other studies, the detection rate of *speJ* gene ranged from 24% to 100%[4]. Most of the superantigen genes in GAS were carried by prophages. The reported prevalent *speA* and *speC* are the most variable prophage-associated genes. The prevalence of *speA* in the present study was obviously lower than that in

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1. Laboratory of Microbiology, Chaoyang District Center for Disease Control and Prevention, Beijing 100021, China; 2. Department of Diagnosis, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 3. State Key Laboratory for Infectious Disease Prevention and Control, Beijing 102206, China

previous studies reported by the Beijing Capital Medical University[5-6]. The *speC* gene had high prevalence in China, which was similar to that found in other study[7]. The *ssa, speH,* and *speI* were shared by most of the 71 GAS isolates as reported by other Chinese studies, but the prevalence of these three genes were low in other countries[4]. The *speF* and *slo* genes were common among GAS, which had been reported as the basic virulence factors. We also identified that the *speH* and *speI* were co-detected at a high rate (63/71, 88.7%), but found no such correlation among other superantigen genes. This was consistent with the results of Commons R. and his colleagues[4]. These observations are in agreement with the adjacent locations of *speH* and *speI* pairs on each of their prophages.

In total, 4 *emm* types were identified from the 71 isolates (Table 1), of which *emm*12.0 (62/71, 87.4%) was the most prevalent, followed by *emm*1.0 (7/71, 9.8%), *emm*22.0 (1/71, 1.4%), and *emm*75.0 (1/71, 1.4%). Our finding was consistent with almost all previous studies undertaken in different regions of China[3,5-6]. Since the specific N-terminus area of the M protein encoded by the *emm* gene is the component of GAS vaccine, identification of the popular *emm* type in one region for different periods or in one period for different regions is extremely important for vaccine development. Studies on the *emm* type in a long period can also give valuable information for the prediction of future persistence or emerging *emm* clones.

Because there was only 1 isolate in *emm*22.0 and *emm*75.0, respectively, isolates in *emm*12.0 and *emm*1.0 were only used to analyze the relationship between virulence factor genes, *emm* types and disease caused by GAS. Significant differences in the distribution of superantigen genes were found between *emm*12.0 and *emm*1.0 types ($P<0.05$) (Table 1). In the present study, the *speA* and *speJ* genes were present in 85.7% (6/7) and 71.4% (5/7) of *emm*1.0 isolates, respectively. The *speI* and *speH* genes were identified in 85.4% (53/62) and 88.7% (55/62) of *emm*12.0 isolates, respectively. Two previous studies have suggested that the majority of *emm*1.0 isolates contain *speA, speG, speJ,* and *smeZ*, but do not possess *speC, ssa,* or *speH*[4, 8], which indicate that the low level of *speJ* in our study may be linked to the present *emm* type distribution. Then, we identified that *speI* and *speH* genes were positively associated with the disease types caused by GAS ($P<0.05$) (Table 2), while there was no association between *emm* type and diseases caused by GAS (Table 3). The *speI* and *speH* genes had the same detection rates, 74% (37/50) in the scarlet fever group and 95.2% (20/21) in the tonsillitis group. Lintges[1] also reported that the superantigen gene profile was more important for the clinical outcome of GAS infections. Based on our results, we suggest that superantigen genes could be used as an additional epidemiological tool to explore genomic heterogeneity.

All isolates were erythromycin resistant and sensitive to ampicillin. The *ermA*, *ermB*, and *mefA* genes were present in 5.6% (4/71), 90.1% (64/71), and 4.2% (3/71) of the tested strains respectively. The high rate of resistance that we found is probably a reflection of the high level of antibiotic usage which may be incorrect or abusive around Chaoyang District. There is significant geographic variation in the prevalence of erythromycin resistant genes. In some countries around Europe and America, *mefA* gene which confer low-level resistance is the main contributor to erythromycin resistance[9], whereas the

Table 1. Distribution of Virulence Factor Genes among *Emm* Types

Genes	*Emm*12.0 (62 strains)		*Emm*1.0 (7 strains)		*Emm*22.0 (1 strain)		*Emm*75.0 (1 strain)		*P*
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	
speA	5	57	6	1	0	1	0	1	<0.05
speI	53	9	2	5	1	0	1	0	<0.05
ssa	61	1	7	0	1	0	0	1	0.64
speC	62	0	7	0	1	0	1	0	
speJ	4	58	5	2	0	1	0	1	<0.05
speG	59	3	7	0	1	0	1	0	1.00
speH	55	7	1	6	0	1	1	0	<0.05
SMEZ	62	0	7	0	1	0	1	0	
speF	60	2	7	0	1	0	1	0	1.00
slo	62	0	7	0	1	0	1	0	

Table 2. Distribution of Virulence Factor Genes among Different Diseases Caused by GAS

Genes	Scarlet Fever (50 strains)		Tonsillitis (21 strains)		Total (71 strains)		*P*
	Positive	Negative	Positive	Negative	Positive	Negative	
speA	10	40	1	20	11	60	0.105
speI	37	13	20	1	57	14	0.040
ssa	50	0	19	2	69	2	0.085
speC	50	0	21	0	71	0	-
speJ	8	42	1	20	9	62	0.194
speG	47	3	21	0	68	3	0.550
speH	37	13	20	1	57	14	0.040
smeZ	50	0	21	0	71	0	-
speF	48	2	21	0	69	2	1.000
slo	50	0	21	0	71	0	-

Table 3. The *Emm* Types among Different Diseases Caused By GAS

Emm Types	Scarlet Fever (50 strains)	Tonsillitis (21 strains)	Total (71 strains)	*P*
*emm*12.0	44	18	62	0.792
*emm*1.0	5	2	7	0.951
*emm*22.0	1	0	1	-
*emm*75.0	0	1	1	-
total	50	21	71	-

rates of resistance associated with *mefA* accounted for 4.2% ,6.8%, and 0% in the present study and other two Chinese studies[3,10]. The high positive rate of *ermB* gene which confer high-level resistance and low positive rate of *mefA* in China reflect the serious situation of erythromycin resistance. Fortunately, the GAS strains in this study were still highly susceptible to ampicillin. Our findings revealed that the presence of the *ermA, ermB*, and *mefA* genes was completely consistent with the results of the antimicrobial susceptibility testing.

This molecular epidemiological study on GAS has demonstrated different *emm* types, virulence factor genes and erythromyein resistance genes in Chaoyang District of Beijing in 2011. The data may give important clues for vaccine construction, GAS pathogenesis and correct drug usages. This study has also analyzed the relationships between virulence factors, *emm* types and the diseases caused by GAS. The importance and relevance of these relationships need further investigation to clarify their role in the outcome of GAS infections .

[#]Correspondence should be addressed to ZHANG Xiao Xi. Tel: 86-10-67773661. E-mail: zhang_xx58@163.com

[§]WANG Hai Bin, SONG Yan Yan, and YOU Yuan Hai contributed equally to the work.

Biographical notes of the first authors: WANG Hai Bin, male, born in 1982, majoring in microbiology, E-mail: wanghb811@163.com; SONG Yan Yan, female, born in 1975, majoring in microbiology; YOU Yuan Hai, male, born in 1978, majoring in microbiology.

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