Molecular epidemiology and antimicrobial resistance of group A streptococcus recovered from patients in Children

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Research article

Keywords: Group A streptococcus, GAS, Streptococcal M protein, emm types, Superantigens, antimicrobial resistance, scarlet fever, Children

Posted Date: January 29th, 2020

DOI: https://doi.org/10.21203/rs.2.22135/v1

Version of Record: A version of this preprint was published on July 13th, 2020. See the published version at https://doi.org/10.1186/s12879-020-05241-x.

Abstract

Background: Group A streptococcus (GAS) is an important human pathogen responsible for a broad range of infections. Epidemiological surveillance has been crucial to detect changes in the geographical and temporal variation of the disease pattern. The objective of this study was to investigate the molecular epidemiological characteristics and antimicrobial resistance of GAS isolates from patients in Children's Hospital in Beijing.

Methods: From 2016 to 2017, pharyngeal swab samples were collected from the outpatients in Children's Hospital, Capital Institute of Pediatrics, who were diagnosed as scarlet fever. Antimicrobial susceptibility test was performed according to the distribution of common antibiotics and Clinical and Laboratory Standards Institute (CLSI) recommendations. The distribution of the macrolide-resistance genes (ermB, ermA, mefA), emm (M protein-coding gene) typing, and superantigens (SAg) gene profiling were examined by polymerase chain reaction (PCR).

Results: A total of 297 GAS strains were collected. The sensitivity of the strains to penicillin, ceftriaxone, and levofloxacin was 100%. The rate of antimicrobial resistance to erythromycin and clindamycin were 98.3% and 96.6%, respectively. The dominant emm types were emm12 (65.32%), emm1 (27.61%), emm75 (2.69%), and emm89 (1.35%). Of the 297 isolates, 290 (97.64%) carried the ermB gene, and 5 (1.68%) carried the mefA gene, while none carried the ermA gene. The most common superantigen genes identified from GAS isolates were smeZ (96.97%) , speC (92.59%), speG (91.58%), and ssa (85.52%), speI (54.55%), speH (52.19%), and speA (34.34%). Isolates with the genotype emm 1 possessed speA, speC, speG, speJ, speM, ssa, and smeZ, while emm 12 possessed speC, speG, speH, speI, speM, ssa, and smeZ superantigens.

Conclusions: The prevalent strain of GAS isolates in Beijing has a high antimicrobial resistance rate to macrolides, while penicillin can still be used as the preferred treatment choice. The primary mechanism of resistance to erythromycin is related to the expression of ermB . The common emm types were e mm 12 and emm 1. A correlation was established between emm and the superantigen gene. Thus, long-term monitoring and investigation of the emm types and superantigen genes of GAS prevalence is imperative.

Background

Streptococcus pyogenes (Lancefield group A streptococcus; GAS) is a major pathogen causing infectious diseases in children. It causes suppurative and non-suppurative diseases, such as erysipelas, suppurative tonsillitis, scarlet fever, rheumatic fever, and glomerulonephritis [1]. Globally, there are about 616 million cases of GAS pharyngitis every year, among which, 17,800 cases are new infections, and about 517,000 patients with severe GAS are deceased every year[2]. Recently, the positive rate of GAS was estimated 21.2% in the pharyngeal culture of patients diagnosed as "streptococcal infection/tonsillitis/angina" [3]. Moreover, the incidence of streptococcal pharyngitis is common in children aged 0–14 years. From 2012 to 2014, the average number of positive cases of streptococcal culture was 2685.1/100,000 children in Beijing, including 1652.7 outpatient visits[4] In 2011, scarlet fever broke out in mainland China and Hong Kong, with a sharp increase in incidence[5, 6]. Penicillin is the preferred clinical treatment of GAS infection, while erythromycin is the first alternative antibiotic for patients allergic to penicillin, followed by clindamycin. The drug resistance rate to macrolides, used as alternative antibiotics, is gradually increasing, which might be related to the overuse of such antibiotics [7, 8].

Our previous study has shown high frequency of resistance to erythromycin, clindamycin, and tetracycline in GAS [9].

The M protein encoded by the emm gene is the main pathogenic factor of GAS, and different types vary in pathogenicity. Therefore, emm typing is often used to track the outbreak and routinely monitor the GAS diseases. From 2011 to 2013, the proportion of emm12.0 in children with GAS infection in Xicheng District of Beijing decreased gradually, and emm1.0 increased every year [10]. The correlation between GAS diseases and emm types, superantigen gene, as well as antimicrobial resistance, needs further investigation [11, 12]. The resistance of GAS to macrolides is related to the mechanism underlying target modification mediated by ermA and ermB and pumping mechanism mediated by mefA; the primary mechanism of resistance of various epidemic strains is different [13]. The superantigen gene is the main virulence factor and closely related to the pathogenicity of GAS. Hitherto, 11 superantigen genes have been found, including speA, speC, speG, speH, speI, speJ, speK, speL, speM, smeZ, and ssa [14, 15]. Previous investigation on the emm typing and superantigen of GAS strains from different regions of mainland China indicated that the typing and antimicrobial resistance were slightly different[16, 17]. In this study, we isolated the GAS strains from patients with pharyngitis/scarlet fever and other GAS related diseases in Children's Hospital, Capital Institute of Pediatrics from 2016 to 2017, and conducted antimicrobial susceptibility test, emm genotype analysis,, and combined analysis of superantigen to assess the molecular epidemiological characteristics and antimicrobial resistance mechanisms of GAS strains.

Methods

Strain collection

A total of 297 cases of GAS strains in this study were recovered from pediatric patients presenting with scarlet fever in the Children's Hospital, Capital Institute of Pediatrics, from January 2016 to December 2017. Throat swabs were obtained from patients by two physicians for routine microbiologic analysis.

Bacterial identification

The throat swab samples were inoculated on a Colombian blood plate (BD, USA) and cultured in a CO_2 incubator at 37 °C for 24–36 h. A single round colony with the transparent hemolytic ring was selected, cultured and evaluated by Gram staining. The Streptococcus typing reagents (Oxoid, Basingstoke, UK) were used to classify the suspicious colonies.

Antimicrobial susceptibility testing

According to the distribution of common antibiotics and recommendation of Clinical and Laboratory Standards Institute (CLSI), paper diffusion method (K-B method) was used to test the sensitivity of ten antibiotics to the isolated *Streptococcus pyogenes*. The distance between each piece of paper was > 24 mm, and the distance between the center of the paper and edge of the dish was > 15 mm. The drug-sensitive paper was placed in the plate and incubated in a 37 °C incubator. Only two plates were stacked together. After incubation for 18–24 h, the diameter of the bacteriostatic ring was measured with a Vernier caliper. The edge of the bacteriostatic ring was limited, as the bacteria cannot be observed by the naked eye. The sensitivity of bacteria was determined by the diameter of the bacteriostatic ring and CLSI standard.

DNA extraction of GAS genome

According to the recommended method on the CDC (Center for Disease Control and Prevention) website (http://www.cdc.gov/ncidod/biotechnology/stream/protocol_emm-type.htm), one ring of GAS was suspended in 300 μL saline and incubated in the water at 70 °C for 15 min. After centrifugation, the precipitate was mixed with 50 μL TE (pH 8.0), 10 μL mutanolysin (3000 U/mL), and 2 μL hyaluronidase (30 mg/mL) in a water bath at 37 °C for 30 min, boiled at 100 °C for 10 min, and centrifuged to obtain genomic DNA.

Emm genotyping

According to the recommendation of the CDC (USA), the genomic DNA of the strain and *emm* typing primers (*emm*F and *emm*R) were used in the reaction system. The data were uploaded to the emm typing database (http://www.cdc.gov/ncidod/biotechnology/strep/strepblast.htm) for comparison.

Erythromycin-resistance gene detection

The extracted DNA was detected by *mef*A, *erm*B, and *erm*A. Primer sequences are listed in Table 1. The reaction system consisted of 25 μL, 1 μL 2 m < DNA template, 1 μL (10 mm) for each primer, 0.2 μL Taq DNA polymerase (5 U/μL), 2 μL of 2.5 mM dNTPs, 2.5 μL of 10XTaq buffer (2.5 mM $MgCl_2$ plus; Takara Biotechnology Co.), and 17.3 μL water. The initial denaturation was performed for 1 min at 94 °C, followed by denaturation for 30 s at 94 °C annealing for 30 s at 54 °C, extended for 30 s at 72 °C for 29 cycles and final extension for 3 min at 72 °C.

Table1. Primer sequences for erythromycin-resistance genes

Gene	Primer direction	Primer sequence	Amplicon size (bp)
ermB	Forward	5’-ATTGGAACAGGTAAAGGGC-3’	639
	Reverse	5’-GAACATCTGTGGTATGGCG-3’	
ermA	Forward	5’-AACTTGTGGAAATGAGTCAACGG-3’	530
	Reverse	5’-CAGAATCTACATTAGGCTTAGGG-3’	
mefA	Forward	5’-AGTATCATTAATCACTAGTGC-3’	348
	Reverse	5’-TTCTTCTGGTACTAAAAGTGG-3	

Superantigen detection

The genomic DNA extracted from *emm* serotype was used to amplify 11 superantigen genes (*speA*, *speC*, *speG*, *speH*, *spel*, *speJ*, *speK*, *speL*, *speM*, *ssa*, and *smez*) by PCR. The primers for amplification are listed in Table 2. The product comparison predicted the size of the positive fragment and detected the superantigen carried by GAS.

Table 2. Primers for PCR of virulence and superantigen genes

Gene	Primer direction	Primer sequence	Annealing Temperature (°C)	Amplicon Size (bp)
speA	Forward	5’-ATGGAAAACAATAAAAAAGTATTG-3’	52	765
	Reverse	5’-TTACTTGGTTGTTAGGTAGACTTC-3’		
spec	Forward	5’-AATTTCGATTCTGCCGCTTA-3’	52	400
	Reverse	5’-GCAGGGTAAATTTTTCAACGAC-3’		
speG	Forward	5’-TCATGTGTTTTTAGCTATGGAAGTC-3’	52	590
	Reverse	5’- ACTGTCTCGACTTAAAAGCTTATCA-3’		
speH	Forward	5’-AGATTGGATATCACAGG-3’	52	416
	Reverse	5’- CTATTCTCTCGTTATTGG-3’		
speI	Forward	5’- AATGAAGGTCCGCCATTTTC-3’	52	516
	Reverse	5’-TCTCTCTGTCACCATGTCCTG-3’		
speJ	Forward	5’-GATAGTGAAAATATTAAAGACG-3’	52	630
	Reverse	5’- TTATTTAGTCCAAAGGTAAATATC-3’		
speK	Forward	5’-GTGTGTCTAATGCCACCGTCT-3’	52	564
	Reverse	5’-GGAACATATATGCTCCTAGAT-3’		
speL	Forward	5’-CAGCACCTTCCTCTTTCTCG-3’	52	459
	Reverse	5’-GGAAAAAGAGGGACGCAAG-3’		
speM	Forward	5’-GGATGAGTGAATAAATCGGTAAAC-3’	55	425
	Reverse	5’-AGTCTGGGACGATGATAA-3’		
ssa	Forward	5’-TGATCAAATATTGCTCCAGGTG-3’	52	502
	Reverse	5’-TCCACAGGTCAGCTTTTACAG-3’		
smeZ	Forward	5’-CTTCAATATTCATTGCAATAATTTC-3’	52	430
	Reverse	5’-TGTAACTGTTGTTTTGTTAGTTGAT-3’		

Results

Antimicrobial susceptibility testing results

292/297 cases of GAS isolates were resistant to erythromycin at a rate of 98.3%. The resistance rate of clindamycin was 96.6% (287/297). Erythromycin-resistance isolates presented a cross-resistance to clindamycin at a rate of 96.3%. The resistance rate to tetracycline was 90.23% (268/297). All strains were sensitive to penicillin, ceftriaxone, cefotaxime, cefepime, vancomycin, and levofloxacin (Table 3).

Table 3. Antimicrobial susceptibility test of 297 strains of GAS from Children's Hospital between 2016 and 2017

Antibiotic	Susceptibility (n/%)		
	Susceptible	Intermediate	Resistant
Penicillin C	297/100	0	0
Ceftriaxone	297/100	0	0
Clindamycin	8/2.69	2/0.67	287/96.6
Erythromycin	3/1.01	2/0.67	292/98.3
Tetracycline	16/5.39	13/4.38	268/90.23
Vancomycin	297/100	0	0
Chloramphenicol	283/95.29	12/4.04	2/0.67
cefepime	297/100	0	0
cefotaxime	297/100	0	0
Levofloxacin	297/100	0	0

Distribution of emm types

Overall, 9 emm types were detected in GAS strains, including 28 subtypes from 2016 to 2017. The majority of the cases were *emm12* (65.32%, 194/297), *emm1* (27.61%, 82/297), *emm75* (2.69%, 8/297), and *emm89* (1.35%, 4/297). *Emm12*.0 and *emm1*.0 are the most prevalent subtypes, accounted for 46.8% and 26.26%, respectively. A variant subtype (stg485.0) was also detected. The distribution of the emm types are shown in Table 4.

Table 4. Distribution of the *emm* genotypes *S. pyogenes* strains from Children's Hospital between 2016 and 2017

emm types	*emm* subtypes	Count (n)
emm1	*emm1.0*	78
	emm1.25	1
	emm1.3	1
	emm1.33	2
emm12	*emm12.0*	139
	emm12.12	1
	emm12.13	1
	emm12.19	27
	emm12.20	1
	emm12.21	2
	emm12.30	1
	emm12.36	4
	emm12.37	8
	emm12.40	2
	emm12.66	2
	emm12.69	2
	emm12.70	1
	emm12.72	2
	emm12.76	1
emm6	*emm6.19*	1
	emm6.4	1
	emm6.89	1
emm75	*emm75.0*	8
emm89	*emm89.0*	4
stg485	*stg485.0*	1
emm225	*emm225*	1
emm3	*emm3.1*	3
emm4	*emm4.0*	1
Total	28	297

Emm types and erythromycin-resistance genes

Among the 297 isolated GAS strains, 290 (97.64%) carried *ermB*, while 5 (1.68%) carried mefA and none carried *ermA* (Table 5). Three erythromycin sensitive strains were found among the isolates, distributed in subtype *emm12.0* and *emm3.1*. None of the three isolates showed the presence of *ermA*, *ermB*, and *mefA*. Clindamycin-sensitive strains were distributed in *emm12.0* and *emm3.1* subtypes (data not shown). The positive rates of *ermB, ermA, and mefA* in *emm*12 and *emm*1 strains were 45.79%, 0%, 0.34% and 26.3%, 0%, 0.67%, respectively.

Table 5. Results of 297 *emm* types and macrolide resistance genes in Children's Hospital from 2016 to 2017

Emm Type	*ermB* (n/%)	*ermA* (n/%)	*mefA* (n/%)
emm1.0	78/26.3	0/0	2/0.67
emm12.0	136/45.79	0/0	1/0.34
emm3.1	1/0.34	0/0	1/0.34
emm4.0	2/0.67	0/0	0/0
emm6.19	1/0.34	0/0	0/0
emm6.4	1/0.34	0/0	0/0
emm6.89	1/0.34	0/0	0/0
emm75.0	8/2.69	0/0	0/0
emm89.0	4/1.35	0/0	0/0
emm225	1/0.34	0/0	0/0
STG485.0	1/0.34	0/0	0/0
emm1.25	1/0.34	0/0	0/0
emm1.3	1/0.34	0/0	0/0
emm1.33	2/0.67	0/0	0/0
emm12.12	1/0.34	0/0	0/0
emm12.13	1/0.34	0/0	0/0
emm12.19	27/9.09	0/0	0/0
emm12.20	1/0.34	0/0	1/0.34
emm12.21	2/0.67	0/0	0/0
emm12.30	1/0.34	0/0	0/0
emm12.36	4/1.35	0/0	0/0
emm12.37	6/2.02	0/0	0/0
emm12.40	2/0.67	0/0	0/0
emm12.66	2/0.67	0/0	0/0
emm12.69	2/0.67	0/0	0/0
emm12.7	1/0.34	0/0	0/0
emm12.72	2/0.67	0/0	0/0
emm12.76	1/0.34	0/0	0/0

Emm type and superantigen distribution

In 297 strains, the most common superantigen genes identified from *S. pyogenes* were *smeZ* (96.97%), *speC* (92.59%), *speG* (91.58%), and *ssa* (85.52%), while the expression rate of other superantigens was slightly lower: *speI* (54.55%), *speH* (52.19%), *speA* (34.34%), *speM* (24.57%), *speJ* (22.22%), *speL* (5.05%), and *speK* (2.02%). Emm1 tended to harbor speA, speC, speG, speJ, speM, ssa, and smeZ, but less speI, speK, and speL. Emm12 tended to harbor *speC*, *speG*, *speH*, *speI*, *speM*, *ssa*, and *smeZ*, with little or no *speJ*, *speK*, *speL*. Variant *stg485* did not express any superantigens. The details of superantigen distribution are shown in Table 6.

Table 6. Distribution of *emm* type and superantigen in 297 GAS strains at Children's Hospital in 2016-2017

emm types	Distribution of superantigens										
	speA	*speC*	*speG*	*speH*	*speI*	*speJ*	*speK*	*speL*	*speM*	*ssa*	*smeZ*
emm1.0	70	76	74	3	5	55	1	2	13	66	76
emm12.0	11	125	126	123	124	6	0	3	40	130	134
emm3.1	2	2	3	0	1	0	3	0	1	1	3
emm4.0	0	1	1	0	0	0	0	0	0	1	1
emm6.19	1	1	1	0	0	0	1	0	1	0	1
emm6.4	1	1	1	0	0	0	1	0	1	0	1
emm6.89	1	1	1	0	0	0	0	0	0	0	1
emm75.0	1	8	7	6	8	1	0	8	6	0	8
emm89.0	0	4	4	0	0	0	0	0	1	0	4
emm255.0	1	1	1	0	0	0	0	0	0	1	1
stg485.0	0	0	0	0	0	0	0	0	0	0	0
emm1.25	1	1	1	0	0	1	0	0	0	1	1
emm1.3	1	1	1	0	0	1	0	0	0	1	1
emm1.33	2	2	2	0	0	2	0	0	0	2	2
emm12.12	0	1	1	1	1	0	0	0	0	1	1
emm12.13	0	0	1	1	1	0	0	1	1	1	1
emm12.19	4	26	25	3	3	0	0	1	4	27	27
emm12.20	1	1	1	1	1	0	0	0	0	1	1
emm12.21	0	1	1	1	2	0	0	0	0	1	1
emm12.30	0	0	1	1	1	0	0	0	1	0	1
emm12.36	2	4	4	3	3	0	0	0	0	4	4
emm12.37	2	8	7	7	7	0	0	0	2	8	8
emm12.40	0	2	1	2	2	0	0	0	1	2	2
emm12.66	0	2	2	2	2	0	0	0	1	2	2
emm12.69	1	2	1	0	0	0	0	0	0	2	2
emm12.7	0	1	1	0	0	0	0	0	0	0	1
emm12.72	0	2	2	0	0	0	0	0	0	2	2
emm12.76	0	1	1	1	1	0	0	0	0	0	1
Percentage	34.34	92.59	91.58	52.19	54.55	22.22	2.02	5.05	24.58	85.52	96.97

(%)

Discussion

S. pyogenes or GAS is a major pathogen causing infectious diseases in children. The GAS infection manifests as mild non-invasive diseases, such as acute pharyngitis or life-threatening invasive diseases, such as sepsis and toxic shock syndrome [15]. Penicillin has always been the preferred treatment for the GAS infection. Reportedly, the minimum inhibitory concentration (MIC) value of penicillin is increasing. Also, azithromycin and other macrolides have become common antibiotics s for the treatment of pharyngitis; however, the drug resistance rate of macrolides has also been increasingly gradually [7]. Of the GAS strains isolated from the pharyngeal swabs of children with pharyngitis, 15% were not sensitive to clindamycin or erythromycin, and 12% induced resistance (D test positive) [18].

Nonetheless, the antimicrobial susceptibility results showed that all GAS strains isolated in this experiment were highly sensitive to penicillin, cephalosporin, levofloxacin, and vancomycin and highly resistant to tetracycline and macrolides, with resistance rate over 90% (Table 3). From 2016 to 2017, no significant fluctuation was detected in the resistance rate of GAS strains to antibiotics (data not shown). These findings were consistent with those from previous studies [9, 19, 20]. S. pyogenes is highly sensitive to penicillin, and can still be used as the first option in the clinical treatment of streptococcal infections. However, it exhibits high resistance to macrolides, such as erythromycin and clindamycin, and hence, might not be appropriate to use these antibiotics, especially macrolides, as an alternative treatment for penicillin-allergic patients. Thus, careful selection of these antibiotics is imperative.

Emm genotyping of GAS showed that the distribution of emm genotypes varied according to the countries, regions, and periods. Emm1 is the most popular type in Germany, consistent with that in the USA, Australia, and Japan; the prevalent types were emm1 (31.8%), emm28 (15.4%), and emm 89 (14.5%) [14, 21]. Presently, the most popular emm types in China are emm12 and emm1. In 2011, two patients with scarlet fever died in Hong Kong; the GAS pathogens were emm1 and emm12[22]. In Chaoyang district, Beijing, in 2011, the main GAS epidemic strain of scarlet fever in children was emm12.0 [23]. In this study, 297 GAS strains were isolated from patients with GAS infection at the Children's Hospital from 2016 to 2017. The study revealed 9 emm types in 297 strains, including 28 subtypes, of which, emm12 (65.32%, 194/297) and emm1 (27.61%, 82/297) were the main types (Table 4). Eight types of emm were found in 155 strains of GAS isolated from the pharynx of children with scarlet fever, pharyngeal tonsillitis, as well as healthy children in Beijing. Emm1.0 and emm12.0 were the main types of scarlet fever and pharyngeal tonsillitis. Stg485, emm18.0, emm1.0, and emm12.0 were the main types carried by healthy children[24]. From 2009 to 2016, the main emm types of GAS strains were emm12 (42.9–62.2%) and emm1 (30.7–35.0%) [25, 26]]. Interestingly, the proportion of emm12 and emm1 in this experiment was similar to that reported previously. These results showed that the emm genotypes of GAS isolates changed significantly in recent years as compared to those identified in the 1990s. The most common emm genotypes in 1993–1994 were emm3.1, emm1.0, emm4.0, emm12.0, st1815.0, emm6.0, and emm18.0 [27].

A total of 35 emm types in 1282 strains isolated from GAS infection in children in Greece from 2007 to 2013, including emm1 (16.7%), emm12 (13.6%), emm77 (10.9%), emm6 (6.8%), and emm89 (6.6%)[1]. Among 1122 invasive isolates from Finland during 2008–2013, 72 emm types were identified, of which emm28 (26%), emm89 (12%), and emm1 (12%) were the most common types [28]. The main emm types of iGAS strains in Portugal

from 2010 to 2015 were emm1 (28%), emm89 (11%), emm3 (9%), emm12 (8%), and emm6 (7%) [29]. Furthermore, the isolates of emm60.1 and emm63.0 genotypes were prevalent in the children from the villages of Guizhou Province in China, which led to the outbreak of acute glomerulonephritis in 2005[30]. In 2012, many people suddenly had a fever, sore throat and/or fatigue, headache, and other similar symptoms within 24 h in Beijing. The isolated GAS strain had the same genotype (emm 89), which was first discovered to cause tonsillar pharyngitis in Beijing, China[31], and also detected in this study. Between January 2016 and May 2017, a rare emm66.0 outbreak of GAS occurred in England and Welsh [32]. Therefore, continuous monitoring of streptococcal infection is required.

GAS infection patients with penicillin allergy are commonly treated with macrolide antibiotics. In the late 1990s, the drug resistance rate of GAS isolates to erythromycin in most regions of China was < 50%. Around 2008, the drug resistance rate of GAS to erythromycin was 95–100%, while that for the isolates in Taiwan decreased from 53.1% in 2000 to 0% in 2010, but rapidly increased to 65% in 2011. The genes involved in erythromycin resistance were mefA (53.1%), ermB (35.9%), and ermtr (10.9%), and emm12 was the main serotype resistant to macrolides[33]. In this study, the rate of drug resistance rate to erythromycin was 98.3%, much higher than that detected in North America and some European countries (9.6–35.8%). In the mechanism of drug resistance, 290/297 strains (97.64%) GAS strains and 5 (1.68%) mefA strains were found to carry ermA. This phenomenon differed from that in the USA, Italy, Chile, and Canada where erythromycin-resistant strains of GAS are mainly m-resistant phenotypes mediated by mefA. The target modification mechanism mediated by ermB is the main resistance mechanism of GAS in China. The pattern of antibiotic resistance fluctuates worldwide. In a study in India, 51.4% of the GAS strains were resistant to erythromycin, of which, 65.1% had ermB and 32.5% had mefA as the only genes resistant to macrolides, while 2.2% had both ermB and mefA [8]. The drug resistance rate of erythromycin and clindamycin in Korea decreased from 51.0% and 33.7% in 2002 to 9.8% and 8.8% in 2004, respectively. The sharp decline in erythromycin resistance in a short period may be related to the change in emm type distribution in the community [34]. In Portugal, the drug resistance rates of erythromycin and clindamycin were 14% (carrying the erm(B) gene) and 9% (harbouring the erm(TR) gene) in 2010–2015, respectively [29] .Thus, it could be deduced that the high resistance rate of macrolides in China was related to the distribution of emm types.

GAS superantigens, except speG, speJ, and smeZ, were encoded by chromosome, and the remaining (speA, speC, speH, spel, speK, speL, speM, and ssa) are encoded by phage, which is the main driving force for pathogenic strains to obtain pathogenic factors through horizontal transfer. The horizontal transfer and mutation of genes can produce highly pathogenic GAS strains, which affect the epidemic situation of the GAS disease, resulting in different distributions of the S. pyogenes superantigen gene spectrum in different periods and geographical areas. A study from Portugal showed that smeZ (96.0%) and speG (86.9%) were common in GAS, followed by speC, ssa, speJ, speA, speK, and spel [35]. A multicenter study in China has proved that 31.1% of the strains contain speA, while 58.6% contain speC [17]. The GAS strains isolated from pediatric patients in China during 1993–1994 and 2005–2006 primarily consisted of emm1 and emm12. The GAS strains carrying six or more superantigen genes increased from 46.53% in 1993–1994 to 78.39% in 2005–2006. The ssA, speH, and speJ genes increased and speA decreased. The gene spectrum of superantigen is related to the type of emm, but the same emm type strains occasionally carry different superantigen genes in the two periods. Intriguingly, no significant difference was detected in the distribution of emm types and SAg gene spectrum among different disease isolates [27]. In this study, 11 superantigens, including speC, speG, and smeZ of GAS strains were detected. Emm1 harbored speA, speC, speG, speJ, speM, ssa, and smeZ, but spel, speK, speL was less. Emm12

type tended to contain speC, speG, speH, speI, speM, ssa, and smeZ, with little or no speJ, speK, and speL. A German study showed that the most common superantigen genes in GAS were speG (92.1%), speJ (50.9%), and speC (42.0%). Simultaneously, a correlation was established between emm type or superantigen gene and clinical complications [14]. In a rare infection outbreak in multiple trauma treatment centers, emm58 type GAS produced streptococcal exotoxins, SPEB, SPEC, SPEG, SPEF, and SMEZ; it was also termed as a macrolide- and tetracycline-resistant strain [36]. From 2009 to 2016, all strains from infected patients in 10 general tertiary hospitals in 7 provinces (cities) of China, whether invasive or not, contained superantigen genes, SpeB and SLO. Other superantigen genes, such as smeZ, speF, and speC, accounted for 96.4%, 91.4%, and 87.1%, respectively. All strains were sensitive to penicillin, ampicillin, cefotaxime, and vancomycin, while the resistance rates to erythromycin, clindamycin, and tetracycline were 93.5%, 94.2%, and 86.4% respectively, indicating high genotype diversity and macrolide resistance rate of S. pyogenes in clinical isolates in China [25]. In the previous studies of strains isolated from children, 30.5% and 57.2% of the strains contained superantigen genes speA and speC, respectively. 88.8% of emm1.0 genotype strains contained the speA gene, while 69.6% of the emm12.0 genotype strains contained the speA gene [17]. In Taiwan, strains with emm1.0, emm4.0, and emm12.0 genotype are the main causes of non-invasive diseases. Only a few strains show emm1.0 genotype containing the speC and SpeH genes, and a few with emm12.0 genotype contain speJ and smeZ genes [37]. In Spain, the emm1.0 strain of S. pyogenes associated with pharyngitis consists of speA, speG, and speJ genes, but does not have speC, speH, speI, or ssa genes [38], indicating the time- and location-dependent distribution of the emm genotypes and the superantigen gene spectrum.

Conclusions

Classification of pathogenic microorganisms is a critical method for epidemiological research. In this study, all GAS strains isolated from the Children's Hospital were sensitive to penicillin, ceftriaxone, and vancomycin, and highly resistant to erythromycin and clindamycin. Nevertheless, penicillin can still be used as the first option for the treatment of streptococcal infections. Emm gene sequence typing shows that emm12 and emm1 are the most prevalent subtypes, carrying ermB gene is the mechanism underlying resistance to macrolides, and speC, speG, and smeZ are the most common superantigens of GAS. The long-term monitoring of the emm type and superantigen gene analysis of GAS is crucial for understanding the variations in the GAS M protein antigen, generation of new bacterial type, epidemiological research, and vaccine preparation.

Abbreviations

GAS
Group A streptococcus ; emm:encoding mature M Protein gene; CLSI:Clinical and Laboratory Standards Institute; MIC:minimum inhibitory concentration; CDC:Center for Disease Control and Prevention; PCR:polymerase chain reaction;

Declarations

Funding

This work was supported by Youth Fund Project of the Capital Institute of Pediatrics (NO.QN-2016-09), and Cultivation Plan Fund of the Capital Institute of Pediatrics (NO.PY-2020-05). The study sponsors had no role in

study design; collection, analysis, and interpretation of data; writing the report; or the decision to submit the report for publication.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

Hongxin Li, Lin Zhou, Yong Zhao designed the study; Hongxin Li, lin Zhou, Xiaoyan Liu, , Jin Hu, Lijuan Ma performed the data collection; Xiaoyan Liu, Jin Hu, Linjuan Ma, coordinated and supervised the data collection; Yong Zhao, Hongxin Li, Lin Zhou participated in the analysis of data; Hongxin Li, Lin Zhou participated in the interpretation of data; Hongxin Li, Yong Zhao drafted the initial manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of work.

Competing interests

The authors have indicated they have no competing interests.

Ethics approval and consent to participate

This study was approved by ethics committee of the Capital Institute of Pediatrics. Written consent forms were obtained from the participants' guardians before collecting the pharyngeal swab samples, and anonymity of the participants was guaranteed.

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