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Change during an 8-Year Period in *Streptococcus Pyogenes emm* Types in Pharyngeal Isolates from Children with Noninvasive Infections

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Background: *Streptococcus pyogenes*, or group A streptococcus (GAS), is one of the most common bacterial pathogens in children. GAS can cause such nonserious and noninvasive diseases as pharyngitis and skin infection, as well as serious, invasive diseases like streptococcal toxic shock syndrome. One factor that makes GAS pathogenic is the type-specific M protein on its cell surface. To identify *emm* types and their characteristics, we previously examined GAS strains isolated from children with noninvasive infections at our hospital. The present study was conducted 8 years later, for comparison.

Methods: The 23 participants were inpatients and outpatients at Nippon Medical School Tama Nagayama Hospital during 2016 and 2017. A pharyngeal swab specimen was obtained from each child, and genes encoding M proteins were amplified by polymerase chain reaction.

Results: *emm* type analysis identified *emm*1 in 11 of the 23 strains and *emm*12 in 4. Three group G streptococcus (GGS) strains carried M-like protein genes.

Conclusions: The predominant *emm* type was *emm*12 in our previous report and *emm*1 in this study. This study also identified 3 GGS strains among the isolates, which carried either the *stg*245, *stg*6795, or *stg*840 M-like protein gene. One GAS strain carried *stg*485, a gene associated with GGS rather than GAS. (J Nippon Med Sch 2020; 87: 211–214)

Key words: *Streptococcus pyogenes*, dominant *emm*, pharyngitis, children

Introduction

Streptococcus pyogenes, or group A streptococcus (GAS), is a species of pathogenic bacterium commonly associated with pediatric pharyngitis and tonsillitis. GAS can also cause skin infections and sometimes leads to sequelae such as acute glomerulonephritis and rheumatic fever[1]. Some strains of GAS and group G streptococcus (GGS) also cause streptococcal toxic shock syndrome. Many current GAS antigen test kits also detect GGS. The type-specific M protein found on the GAS cell surface is resistant to the phagocytic activities of polymorphonuclear leukocytes, which makes it an important pathogen[2].

In the current study, pharyngeal swab specimens from pediatric patients were used to isolate GAS strains and analyze their M protein gene (*emm*) types and type specificity. The results were then compared with those from our earlier study[3].

Materials and Methods

The 23 participants were 11 boys and 12 girls who were inpatients and outpatients at the Nippon Medical School Tama Nagayama Hospital during 2016-2017. A pharyngeal swab specimen was obtained from each child (**Table 1**) and tested for GAS and GGS antigens with a rapid testing kit (Denka Seiken, Tokyo, Japan). Samples were also smeared on 5% sheep blood agar plates (Eiken, To-

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Table 1 Diseases, Treatments, and Underlying Diseases

	Age	Sex	Sample	Treatment	Disease	Underlying Disease
1	4Y1M	F	Pharynx	ABPC	URI	
2	5Y1M	F	Pharynx	SBT/ABPC	URI	Allergic Purpura
3	6Y0M	F	Pharynx	AMPC	URI	
4	8Y9M	M	Pharynx	CTX	URI	
5	2Y10M	M	Pharynx	ABPC	URI	Convulsion
6	12Y8M	M	Pharynx	AMPC	URI	Acute glomerulonephritis
7	3M	F	Pharynx	CAM	URI	
8	6Y3M	M	Pharynx	AMPC	URI	
9	6Y9M	F	Pharynx	CTRX	URI	
10	4Y5M	M	Pharynx	SBT/ABPC	URI	Acute glomerulonephritis
11	5Y11M	M	Pharynx	AZM	URI	
12	9Y8M	F	Pharynx	MINO	URI	
13	2Y3M	M	Pharynx	CTX, CDTR	URI	
14	4Y0M	M	Pharynx	ABPC/SBT	URI	
15	8Y2M	M	Pharynx	ABPC	URI	
16	1Y4M	F	Pharynx	ABPC	URI	
17	1Y6M	F	Pharynx	CCL	URI	Kawasaki disease
18	6M	M	Pharynx	CTX	URI	Kawasaki disease
19	5Y0M	F	Pharynx	CTX	URI	
20	8Y0M	F	Pharynx	AMPC	URI	
21	2Y0M	F	Pharynx	AMPC	URI	
22	7Y3M	F	Pharynx	AMPC	URI	
23	7Y7M	M	Pharynx	ABPC	URI	

URI: upper respiratory infection, ABPC: ampicillin, AMPC: amoxicillin, SBT/ABPC: sulbactam/ampicillin, CTX: cefotaxime, CAM: clarithromycin, CTRX: ceftriaxone, AZM: azithromycin, MINO: minocycline, CDTR: cefditoren, CCL: cefaclor

kyo, Japan) and cultured at 37.5°C. Beta hemolytic colonies were then subcultured to identify GAS and GGS strains by group-specific latex agglutination. Cultures were preserved at −80°C for further testing. Genes encoding M proteins (*emm* genes) were amplified by polymerase chain reaction in accordance with the procedure reported by Beall et al[4]. The US Centers for Disease Control and Prevention database was used to identify the base sequences. The *emm* type data obtained from patients in 2016-2017 (11 boys, 12 girls; mean age 4.9 years) were then compared with those obtained in our previous study performed in 2008 (16 boys, 11 girls; mean age 5.7 years). Penicillins are now more commonly used to treat GAS[5] and were therefore used in the present study. Change in the proportions of *emm*1 within GAS strains was statistically analyzed with the Fisher exact test.

This study was approved by the ethics committee of Nippon Medical School Nagayama Hospital (No. 444).

Results

The characteristics of the patients and results of *emm* type analysis are shown in **Table 1, 2**. Twenty-three strains of streptococcus bacteria were isolated from the 23 children, namely, 20 GAS strains (87.0%) and 3 GGS strains (13.0%). Analysis of *emm* type identified *emm*1 in 11 of the 20 GAS strains (55%) and *emm*12 in 4 (20%). Three strains carried *emm*89 and 1 strain carried *emm*48. The 3 GGS strains carried either the *stg*245, *stg*6795, or *stg*840 M-like protein gene. One GAS strain carried *stg* 485, a gene normally associated with GGS. In our 2008 study, *emm*1 was found in 7 of 26 GAS strains (26.9%), *emm*12 in 12 strains (46.2%), and others in 7 strains (26.9%).

In the present study, the distribution of pharyngitis and tonsillitis cases in relation to *emm* type showed a marked change from that in our 2008 study (**Fig. 1**), although the difference in the *emm*1 proportion between studies bordered on statistical significance (P=0.05). Our previous study identified only 1 GGS strain (3%) from pharyngeal isolates, whereas the current study found 3 (13.0%). In addition, a GAS strain from 1 patient carried a gene normally associated with GGS.

Discussion

Our 2008 study[3] identified *emm*12 as the predominant M protein gene type in GAS strains, followed by *emm*1. This

Table 2 Lancefield group and *emm* type of streptococci isolated from pharynges

	Lancefield type	*emm* type
1	Group A	*emm*1
2	Group A	*emm*12
3	Group A	*emm*12
4	Group A	*emm*1
5	Group A	*emm*1
6	Group A	*emm*1
7	Group A	*emm*1
8	Group A	*emm*48
9	Group A	*emm*12
10	Group A	*emm*1
11	Group G	*stg*245
12	Group A	*emm*1
13	Group G	*stg*6795
14	Group G	*stg*840
15	Group A	*emm*1
16	Group A	*stg*485*
17	Group A	*emm*1
18	Group A	*emm*1
19	Group A	*emm*89
20	Group A	*emm*89
21	Group A	*emm*1
22	Group A	*emm*12
23	Group A	*emm*89

* *emm* type normally associated with Group G strains

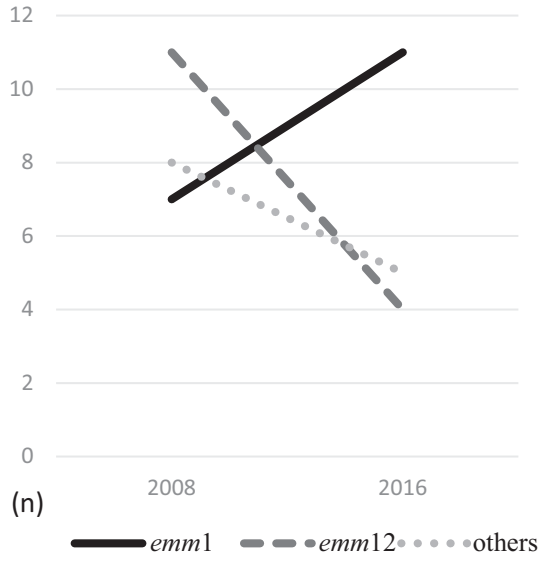

Fig. 1 Dominant GAS *emm* types in pharyngitis, 2008 and 2016

order was reversed in the present study[6,7]. Koutouzi et al.[8] reported a decline between 2007 and 2013 in GAS *emm* types 1 and 12 in pharyngeal isolates and found that other types were increasing. Although our sample size was smaller, the present study showed a decrease in *emm*12 and an increase in *emm*1.

Ikebe et al.[9] reported that *emm*1 had become more common in patients with streptococcal toxic shock-like syndrome. The present results indicate an increase in *emm*1 in patients with nonserious, noninvasive infections. In the 8 years separating our 2 studies, *emm*1 markedly increased (**Fig. 1**).

One isolate in the present study (No. 16) was classified as GAS by an antigen detection test, but its *emm* type was *stg*485, which is normally associated with GGS. Similar findings were reported by Katsukawa et al.[10] and might be a result of mutation in the GAS M protein genes, which could have given the strain an M protein-like gene carried in GGS. The prevalence of invasive *Haemophilus influenzae* disease recently decreased after introduction of the Hib vaccine[11]. A future study should evaluate an experimental multivalent *emm* vaccine of GAS, which would improve disease control[12].

Further studies will require larger sample sizes to determine if similar mutations in M protein gene types occur over time.

Conflict of Interest: The authors declare no conflict of interest.

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