Annals of Clinical Microbiology

www.acm.or.kr

Original Article

emm Types and Clusters of Group A Streptococcus Causing Acute Pharyngitis in Changwon Korea, 2018–2019

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2018-2019년 창원 지역 인두염 환자에서 분리된 A군 사슬알균의 *emm* 유전자형 및 클러스터

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ABSTRACT

Background: Group A streptococcus (GAS) is the most common cause of bacterial pharyngitis. This study aimed to characterize the molecular epidemiology of GAS infection using an *emm*-typing and *emm*-clustering approach.

Methods: A total of 372 patients from Changwon who showed pharyngitis symptoms were recruited during the sampling period of 2018–2019 and throat cultures were obtained from them. *emm* typing was performed using polymerase chain reaction (PCR) and direct sequencing. *emm* genotypes and GAS clusters were classified based on a web-based database.

Results: Of the 372 throat swab specimens, 101 (27.2%) were positive for GAS. *emm* typing analysis was performed on 59 GAS isolates. The most prevalent *emm* type was *emm*89 (20.3%), followed by *emm*12 (16.9%). Seven *emm* clusters were identified: E4 (*emm*89/*emm*28, 32.2%), A-C4 (*emm*12, 16.9%), E1 (*emm*4, 13.6%), A-C5 (*emm*3, 10.2%), E6 (*emm*75, 8.5%), M6 (*emm*6, 8.5%), and A-C3 (*emm*1, 6.8%).

Conclusion: Diverse and temporal changes were observed in the distribution of *emm* types and clusters of GAS. Continuous surveillance based on *emm* genotyping is needed to monitor the epidemiological characteristics of GAS pharyngitis.

Keywords: Epidemiological surveillance, Group A streptococcus, *Streptococcus pyogenes*, Pharyngitis

pISSN : 2288-0585
eISSN : 2288-6850

Ann Clin Microbiol 2021 December, 24(4): 127-134
https://doi.org/10.5145/ACM.2021.24.4.3

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Received: May 07, 2021
Revised: July 28, 2021
Accepted: September 09, 2021

INTRODUCTION

Group A streptococcus (GAS) causes a wide spectrum of diseases, ranging from mild infections of the throat and skin, such as pharyngitis and impetigo, to severe infections including necrotizing fasciitis,

bacteremia, and toxic shock-like syndrome. The mortality rate due to toxic shock-like syndrome and necrotizing fasciitis has reached 15-25% [1-3]. In addition, it involves the immune mediated sequelae, such as acute rheumatic fever, rheumatic heart disease and post streptococcal glomerulonephritis [4,5]. Annually, about 600 million patients are infected by GAS and over 500,000 die of GAS infections or sequelae [4,6].

Because of high GAS disease burden, epidemiologic surveillance is important to detect changes in the disease pattern in various region or population. A safe and effective vaccine has not been available at the medical field yet [7]. Recently, molecular genetic methods have been used for the epidemiological investigation of GAS infections [8,9]. Many epidemiological studies have focused on GAS characteristics by *emm* types and clusters [10]. The *emm* gene of GAS, which is approximately 500-1500 bp in size, encodes the M protein [10]. The M protein of GAS is the major virulence factor that has anti-phagocytic effects towards white blood cells and anti-complement effects to the immune system [11,12]. Classical serotyping methods based on the different surface antigens of M protein have been replaced by sequence typing of the genetically variable N-terminal part of the *emm* gene [13,14]. *emm* genotyping is most widely used for epidemiological surveillance of GAS pharyngitis or invasive disease of GAS [15-17]. As *emm* types are too diverse and complex, *emm* cluster typing system has been proposed [18,19]. This system classifies most of the 250 different *emm* types into 48 functional clusters, containing closely related M proteins that share their structural properties [10,18]. The advantage of *emm* cluster system is that they help to predict the virulence potential and vaccine efficacy by ascribing M protein binding attributes to *emm* types belonging to the same cluster [18].

In this study, we performed the molecular genetic analysis on GAS isolated from the patients of pharyngitis. Additionally, this study aimed to identify the temporal changes in the *emm* genotype and cluster, and to investigate the microbiological characteristics and antibiotic resistance of GAS isolated.

MATERIALS AND METHODS

1. Study subjects

A total of 372 patients who showed pharyngitis symptoms or signs from August 2018 to December 2019 in five pediatric clinics in Changwon, were recruited for this study.

2. Research ethics and consent

Prior to proceeding, the study was approved by the Institutional Review Board (IRB) of Gyeongsang National University Changwon Hospital (IRB No. 2018-01-008).

3. Bacteria isolation

Both tonsils were rubbed with a sterilized cotton swab to ensure that bacteria were sufficiently adhering to the cotton swabs. The swab was immediately placed in the transport media (Asan Pharmaceutical, Giheung,

Korea), stored at room temperature, and transported to Gyeongsang National University Changwon Hospital for culture. Cotton swabs were inoculated on a blood agar plate (BAP, Asan Pharmaceutical), placed in a general incubator, and incubated for 16-18 hours at 35°C. The next day, small, grayish-white colonies showing complete hemolysis were retrieved from the BAP and identified by bacitracin disk (0.04 U), latex agglutination test (Seroiden Strepto Kit, Eiken, Tokyo, Japan), and/or VITEK mass spectrometry (bioMerieux, Marcy-l'Étoile, France).

4. Antimicrobial susceptibility test

Susceptibility tests were performed for all isolates by disk diffusion method according to the guidelines and interpretative criteria of the Clinical Laboratory and Standards Institute (CLSI) [20]. The following antimicrobial disks of BD BBL Sensi-Disk (Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA) were used: erythromycin (ERY; 15 μg), clindamycin (CLI; 2 μg), tetracycline (TET; 30 μg), and ofloxacin (OFX; 5 μg). *Streptococcus pneumoniae* ATCC 49619 was used as the control strain. Three resistance phenotypes, such as $cMLS_B$, $iMLS_B$, and M phenotypes, and three resistance genes, such as *mef*A, *erm*A, and *erm*B genes were investigated.

5. Genetic analysis of *emm* gene

1) *emm* gene amplification

DNA was directly isolated from colonies grown on BAP using DNase Blood & Tissue Kits (Qiagen, Hilden, Germany). The base sequence of the forward primer was 5'-TAT TCG CTT AGA AAA TTA A-3' and that of the reverse primer was 5'-GCA AGT TCT TCA GCT TGT TT-3'. All protocols and assignments of *emm* types and subtypes were as described for the GAS database (http://www.cdc.gov/streplab/groupa-strep/emm-typing-protocol.htm).

2) Sequencing

Direct sequencing was performed using the amplified *emm* gene product at Macrogen (Seoul, Korea). Gene sequencing was performed using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster city, CA, USA) and MJ Research PTC-225 Peltier Thermal cycler (MJ Research Inc., Hercules, CA, USA). Bidirectional sequencing was performed and a MOPC (Macrogen Oligonucleotide Purification Cartridge, Macrogen) purified primer (5'-TAT TCG CTT AGA AAA TTA AAA ACA GG-3') and emmseq2 (5'-TAT TCG CTT AGA AAA TTA AAA ACA GG-3'). The fluorescently labeled fragment was purified by the BigDye XTerminator Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA), then loaded into an ABI PRISM 3730XL Analyzer (Applied Biosystems). The nucleotide sequence was compared with the database (https://www2.cdc.gov/vaccines/biotech/strepblast.asp) of the National Center for Biotechnology Information (NCBI) and was determined as the corresponding genotype of the homology was at least 95% . The size of the analyzed nucleotide sequence was approximately 150-200 bp. *emm* cluster was obtained at the same website above of the NCBI together with *emm* genotype.

RESULTS

1. Bacterial culture

Of 372 patients of acute pharyngitis, GAS was isolated from 101 patients (27.2%), with 57.4% being male. The age distribution of the patients ranged from 1 to 61 years, with an average age of 9.9 years (95% confidence interval [CI], 8.5-11.2 years). The distribution of positive rates by the sex and age is shown in Table 1. In terms of age, six to eight-year-old showed the highest positive rates. Patients under two years of age did not grow GAS.

Table 1. Isolation rate (%) of group A streptococci (GAS) according to age and sex

Age (yr)	Male			Female			Total		
	No.	GAS	%	No.	GAS	%	No.	GAS	%
1	5	0	0	1	0	0	6	0	0
2	7	0	0	10	0	0	17	0	0
3	11	1	9.1	10	1	10.0	21	2	9.5
4	16	4	25.0	13	1	7.7	29	5	17.2
5	26	6	23.1	24	6	25.0	50	12	24.0
6	26	10	38.5	24	9	37.5	50	19	38.0
7	24	13	54.2	20	5	25.0	44	18	40.9
8	18	11	61.1	20	9	45.0	38	20	52.6
9	17	6	35.3	14	3	21.4	31	9	29.0
10	6	3	50.0	7	1	14.3	13	4	30.8
11	4	2	50.0	10	1	10.0	14	3	21.4
12	3	1	33.3	9	2	22.2	12	3	25.0
13	5	1	20.0	2	0	0	7	1	14.3
14	5	0	0	5	1	20.0	10	1	10.0
15-18	1	0	0	5	2	40.0	6	2	33.3
> 18	3	0	0	21	2	9.5	24	2	8.3
Total	177	58	32.8	195	43	22.1	372	101	27.2

2. Antibiotic resistance and its mechanism

The results of the antimicrobial susceptibility test showed that only one (1.7%) of 59 GAS, successfully grown in subculture, was resistant to ERY, CLI, and OFX, at the same time. All isolates were susceptible to TET. One isolate resistant to ERY possessed the *erm*B gene with the $cMLS_B$ phenotype.

3. Molecular typing

DNA was extracted for the *emm* genotyping of 59 GAS, successfully grown in subculture. Among the 59 isolates, the most common *emm* genotypes were *emm*89 (20.3 %), followed by *emm*12 (16.9 %), *emm*4 (13.6 %), *emm*28 (11.9 %), *emm*3 (10.2 %) and *emm*75/6 (8.5/8.5 %) (Table 2). The most common *emm* clusters were E4 (*emm*89/28, 32.2%), followed by A-C4 (*emm*12, 16.9%), E1 (*emm*4, 13.6%), A-C5 (*emm*3, 10.2%), E6 (*emm*75, 8.5 %) and M6 (*emm*6, 8.5 %). One strain (1.7 %) did not belong to any of the above clusters (Table 3).

Table 2. Comparison of distribution (%) of *emm* types in four studies in Korea

emm types	Period and regions studied			
	2014–2018	2008–2015	2017	2018–2019
	All regions [24]	Gwangju [25]	Changwon [22]	Changwon*
*emm*89	14.9	2.3	12.6	20.3
*emm*12	12.8	5.2	3.2	16.9
*emm*4	14.2	35.6	53.2	13.6
*emm*28	19.1	14.8	11.6	11.9
*emm*3	10.6		0.5	10.2
*emm*75		5.7	2.6	8.5
*emm*6		2.8		8.5
*emm*1	11.5	14.5	10.0	6.8
*emm*90				1.7
stG485				1.7
*emm*128			1.6	
*emm*228			1.1	
*emm*131			1.1	
*emm*161			1.1	
*emm*245			0.5	
*emm*255			0.5	
stG643			0.5	
*emm*5		0.3		
Unknown	16.9	18.8		

*This study.

Table 3. Distribution of *emm* clusters and *emm* genotypes

emm clusters	*emm* types	No. (%)
E4	*emm*89	12 (20.3)
	*emm*28	7 (11.9)
A-C4	*emm*12	10 (16.9)
E1	*emm*4	8 (13.6)
A-C5	*emm*3	6 (10.2)
E6	*emm*75	5 (8.5)
M6	*emm*6	5 (8.5)
A-C3	*emm*1	4 (6.8)
E2	stG485	1 (1.7)
NA		1 (1.7)
Total		59 (100.0)

Abbreviation: NA, not available.

DISCUSSION

We isolated GAS from 27.2% for patients with acute pharyngitis who visited a small-to-medium-sized pediatric clinics in Changwon in 2018-2019. In the antimicrobial susceptibility test of 59 isolates, one strain (1.7%) was resistant to each of the ERY, CLI, and OFX. That is lower resistance rate compared to 3.2% ERY, 2.6% CLI, 1.1% TET and 2.6% OFX in Changwon a year ago [21], and is much lower than 10.3% ERY, in Seoul reported in 2015 [22]. The difference in GAS resistance rate might be due to the different subjects, disease severity, antibiotic consumption and circulating strains. Judging from this low resistant rate result, ERY may be useful to treat pharyngitis at least in our region.

From the analysis of *emm* genotypes, *emm*89 (20.3%) was the most prevalent, followed by *emm*12 (16.9%) and *emm*4 (13.6%). The data collected in 2014-2018 reported by the Korea Centers for Disease Control and Prevention (KCDC), common *emm* genotypes were *emm*28 (19.1%), *emm*89 (14.9%), *emm*4 (14.2%), *emm*12 (12.8%), *emm*1 (11.5%) and *emm*3 (10.6%) [23]. The investigation of Jinju region nearby Changwon analyzing the *emm* genotype of GAS five times from 1995 to 2009 showed the change in *emm* types over a few decades. Previously, *emm*12, *emm*22, and *emm*44/61 were prevalent, but recently, *emm*4 and *emm*89 being more common [21]. By analyzing four representative papers that studied *emm* genotypes in Korea, temporal changes are observed [24] (Table 2). Also, the cluster shows more pronounced change, with a high level change from E1 to E4 [25,26]. Fluctuation in *emm* type and cluster distribution have been attributed to change of circulating *emm* type strains. It is known that the *emm* cluster A-C originated from pharyngeal samples, *emm* cluster D from skin samples, and *emm* cluster E from both pharyngeal and skin samples [12], which is in line with our results of pharyngeal strains. It is important to know the association between the *emm* types and the diverse GAS infections. Recently the data on the *emm* type distribution of population-based GAS surveillance have been proposed for the development of GAS vaccine [27].

Limitations of this study include low numbers of isolates analyzed for *emm* genotypes. In addition, we included only GAS isolated from pharynx, not from invasive diseases.

In conclusion, we found a continuation of very low antibiotic resistance level of GAS and a marked fluctuation of *emm* types even during a short period of time in Changwon.

요약

배경: A군 사슬알균(group A streptococcus, GAS)은 세균성 인두염의 가장 흔한 원인 균이다. 인두염 환자에서 배양된 GAS를 대상으로 *emm* 유전자형과 클러스터를 확인하여 최근 유행한 GAS의 분자유전학적 특징을 조사하였다.

방법: 2018-2019년 사이에 경남 창원 지역에서 인두염이 의심되는 372명의 환자를 대상으로 인두 검체를 채취하여 인두배양 검사를 시행하였다. 배양된 균주를 대상으로 polymerase chain reaction (PCR) 및 직접 염기서열분석법으로 *emm* 유전자형을 분석하고 *emm* 클러스터를 조사하였다.

결과: 총 372명의 인두 검체 중 배양검사 양성은 101명(27.2%) 였다. 배양 양성 검체 중 계대배양에 성공한 59개 균주를 대상으로 유전자 분석을 하였는데, 흔한 *emm* 유전자형은 *emm*89 (20.3%)와 *emm*12 (16.9%) 였다. *emm* 클러스터는 E4 (*emm*89/*emm*28, 32.2%), A-C4 (*emm*12, 16.9%), E1 (*emm*4, 13.6%), A-C5 (*emm*3, 10.2%), E6 (*emm*75, 8.5 %), M6 (*emm*6, 8.5 %), 그리고 A-C3 (*emm*1, 6.8%) 등 7가지로 분류되었다.

결론: GAS는 지역과 시기에 따라 다양한 *emm* 유전자형 및 클러스터가 나타나며, 이러한 분자생물학적 연구는 GAS 감염의 역학적 데이터로 유용하게 사용될 수 있다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

FUNDING

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant No. H19C0047).

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