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Decline in Erythromycin Resistance in Group A Streptococci from Acute Pharyngitis due to Changes in the *emm* Genotypes Rather Than Restriction of Antibiotic Use

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Background: Group A streptococcus (GAS) is the most common cause of bacterial pharyngitis in children. Antibiotic resistance rates and *emm* genotypes of GAS isolated from patients with acute pharyngitis were studied in 2009.

Methods: Throat cultures were taken from 499 children with acute pharyngitis in Jinju, Korea, in 2008-2009. A total of 174 strains (34.9%) of GAS were isolated, and antimicrobial susceptibility testing was performed using the disk diffusion method. The phenotypes of macrolide resistance and macrolide resistance genes were determined. The *emm* genotypes were identified using PCR and sequencing. The data were compared with those acquired in 2002 in the same region. Data on the annual macrolide production were collected between 1999 and 2008.

Results: The resistance rates of GAS to erythromycin, clindamycin, and tetracycline were 4.6%, 2.9%, and 2.3%, respectively. The constitutive resistance rate was 62.5% for the *erm*(B) gene and 37.5% for the M phenotype of the *mef*(A) gene. *emm*4 was most frequently detected (28.2%), followed by *emm*89 (20.1%). Most of the erythromycin resistant strains had the *emm*28 genotype. We noted a gradual increase in macrolide production during the study period.

Conclusions: The erythromycin resistance rate of GAS isolated from children with acute pharyngitis was significantly lower in 2009 (4.6%) than in 2002 (44.8%). We observed a remarkable change in the distribution of *emm* genotypes during the 7-yr period. The significant decline in erythromycin resistance in 2009 might be associated with a prominent decrease in the resistant genotype *emm*12 (3.4% in 2009 vs. 28.0% in 2002) rather than restriction of macrolide use. *(Korean J Lab Med 2010; 30:485-90)*

Key Words : *Group A Streptococci, Streptococcus pyogenes, Acute pharyngitis, Erythromycin resistance, emm genotype*

INTRODUCTION

Group A streptococcus (GAS) is the most common cause of bacterial pharyngitis in children. Although GAS pharyngitis is a mild disease, it can cause immunological sequelae, including rheumatic fever, rheumatic heart disease, and acute poststreptococcal glomerulonephritis [1]. Recently, severe cases of necrotizing fasciitis or toxic shock-like syndrome due to GAS have been reported in developed countries [2, 3]. Penicillin is the drug of choice for the treatment of streptococcal pharyngitis, but erythromycin (ERY) or other macrolides are used for patients who are allergic to β-lactams [4]. In recent years, increasing rates of macrolide resistance to GAS have been described in many countries [5-8]. The ERY

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resistance rate was recently reported as almost 100% in China [9]. The high resistance rate of GAS to macrolides in some countries, including Korea [9-11], is a concern when selecting the best treatment regimen for GAS pharyngitis.

Typing of the *emm* gene, which encodes the M protein, is more useful for epidemiologic studies of GAS than direct typing of M, which requires multiple kinds of anti-M sera. As more and more clinical microbiology laboratories incorporate PCR and DNA sequencing, the performance of *emm* genotyping has become more available [12]. Genotyping of *emm* has become a standard method for the study of GAS epidemiology, thereby replacing the ambiguous reactions of T typing, time-consuming M typing, or the limited resources of opacity factor (OF) typing, which are conventional serological typing methods for GAS. The distribution of *emm* genotypes during a defined period can be used to understand the dynamic changes in GAS strains in the region. Several reports have been published on the correlation between *emm* genotypes and antibiotic resistance, but the distributions of antibiotic-resistant *emm* genotypes vary geographically [13, 14]. In general, antibiotic resistance is closely associated with the consumption of drugs. As it was too difficult to acquire drug consumption data, we used the production cost data from the pharmaceutical companies instead.

The aim of this study was to investigate the epidemiology of GAS isolated from patients with acute pharyngitis in 2009 in Jinju, Korea, using *emm* genotypes, macrolide resistance phenotypes, and resistance determinants. We also compared our results with data from 2002 to identify any change in the epidemiology of GAS causing acute pharyngitis in the same geographic region.

MATERIALS AND METHODS

1. Sample collection and isolation of bacteria

Throat swab specimens were taken from 499 children (age, 2-18 yr) in 3 pediatric clinics in Jinju from September 2008 to February 2009. The patients visited the clinics because of symptoms or signs of bacterial pharyngitis, such as sore throat, cervical lymphadenopathy, high fever, abdominal pain, or headache. Throat swab specimens placed in transport media were stored in the refrigerator and sent to the clinical microbiology laboratory of the Gyeongsang National University Hospital every other day. The cotton swabs were inoculated on 5% sheep blood agar plates (Asan Pharmaceutical Co., Seoul, Korea) and incubated at 37℃ for 16-18 hr in ambient air. Of the 499 samples received, 174 (34.9%) grew GAS which was identified by their susceptibility to 0.04 U bacitracin and latex agglutination with group A-specific antisera (Seroiden Strepto Kit, Eiken, Tokyo, Japan). One isolate per patient was stored at −70℃ for further evaluation.

2. ERY resistance phenotypes

The phenotypes of ERY resistance were evaluated using the previously described double disk synergy test [15]. Among the ERY resistant strains, resistant phenotype patterns were classified as clindamycin (CLI)-susceptible (M phenotype), -resistant (constitutive phenotype, $cMLS_B$), or -inducible (inducible phenotype, $iMLS_B$).

3. ERY resistance genes

All ERY-resistant isolates were screened for causative resistance determinants. The *erm*(B), *erm*(A), and *mef*(A) genes were detected using PCR amplification with specific primers [11]. The PCR products were analyzed using 1.5% agarose gel electrophoresis in TBE (Tris/borate/EDTA) buffer (pH 8.0).

4. *emm* genotyping

The AccuPower DNA Extraction Kit (Bioneer, Chungwon, Korea) was used to extract bacterial DNA from the colonies on agar plates. The PCR was carried out using the AccuPower PCR PreMix Kit (Bioneer) and known *emm* primers in a thermal cycler (GeneAmp 9700, Perkin-

Elmer Co., Foster City, CA, USA). Once the amplification products were confirmed by gel electrophoresis, they were purified with the AccuPrep PCR Purification Kit (Bioneer) and sequenced using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA) and the BLAST program (http://ncbi.nlm.nih.gov) provided by the National Center for Biotechnology Information (NCBI).

5. Macrolide production

Data on the annual macrolide production were collected from the reports of the Korean Pharmaceutical Manufacturers Association between 1999 and 2008 to observe the change in the production of each macrolide, such as ERY, clarithromycin, roxithromycin, and midecamycin. These drugs were sold domestically rather than exported.

6. Statistical analysis

The isolation rates of GAS, antibiotic resistance rates, and the distribution of ERY-resistant phenotypes and *emm* genotypes between 2002 and 2009 were compared using Chi-square tests. A P value of <0.05 indicated statistical significance.

RESULTS

1. Macrolide-resistant phenotypes and genotypes

Out of 174 GAS isolates, the resistance rates to ERY, CLI, and tetracycline were 4.6%, 2.9%, and 2.3%, respectively. Among 8 ERY-resistant strains, 62.5% had the $cMLS_B$ phenotype and 37.5% had the M phenotype. No cases of $iMLS_B$ were observed (Table 1). All 5 $cMLS_B$ strains harbored the *erm*(B) gene and were typed as *emm*28, while 3 strains with the M phenotype were positive for the *mef*(A) gene and were identified as *emm*28 (2 strains) and *emm*89 (1 strain). None of the ERY-resistant strains had more than 1 resistance gene.

2. Distribution of *emm* genotypes

The most frequent genotype was *emm*4, accounting for 28.2% followed by *emm*89 (20.1%) and *emm*6 (12.1%) (Table 2). Strains with *emm*28 showed a high ERY resistance rate (58.3%), while most of the other *emm* types were ERY-susceptible (Fig. 1).

3. Comparison with data from 2002

We conducted antimicrobial susceptibility testing and *emm* genotyping of 125 GAS isolates from patients with acute pharyngitis in 2002 in the same region. The resistance rates to ERY, CLI, and tetracycline were as high as 44.8%, 19.2%, and 23.2%, respectively, in 2002 [16]. The most common genotype was *emm*12, accounting for

Table 1. Comparison of antibiotic resistance rates and macrolide resistance phenotypes of group A streptococci between 2002 and 2009 in Jinju, Korea

	2002	2009
N of group A streptococci	125	174
N (%) of resistant strains		
Erythromycin	56 (44.8)	8 (4.6)
Clindamycin	24 (19.2)	5 (2.9)
N (%) of resistance phenotypes		
$cMLS_B$	24 (42.9)	5 (62.5)
M	32 (57.1)	3 (37.5)
$iMLS_B$	0 (0.0)	0 (0.0)

Table 2. Comparison of *emm* types of group A streptococci isolated from children with acute pharyngitis between 2002 and 2009

emm types	2002	2009
	N (%)	N (%)
1	5 (4.0)	9 (5.2)
2	15 (12.0)	0 (0.0)
3	9 (7.2)	0 (0.0)
4	2 (1.6)	49 (28.2)
6	2 (1.6)	21 (12.1)
12	35 (28.0)	6 (3.4)
18	10 (8.0)	1 (0.6)
22	16 (12.8)	13 (7.5)
28	0 (0.0)	12 (6.9)
75	23 (18.4)	18 (10.3)
89	0 (0.0)	35 (20.1)
Others	8 (6.4)	10 (5.7)
Total	125 (100.0)	174 (100.0)

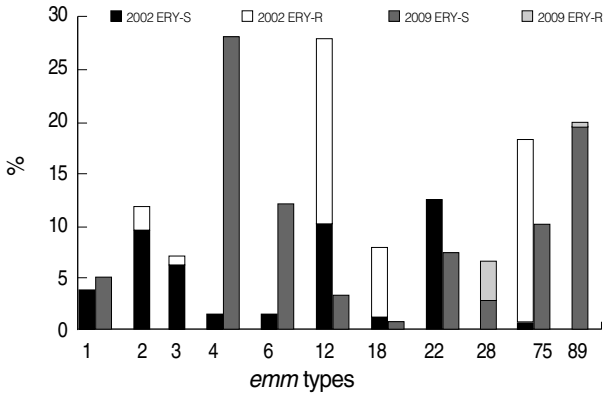

Fig. 1. Distribution of *emm* types of ERY-resistant and ERY-susceptible strains in 2002 and 2009. $P<0.05$.
Abbreviations: 2002 ERY-S and 2002 ERY-R, erythromycin-susceptible and -resistant in 2002; 2009 ERY-S and 2009 ERY-R, erythromycin-susceptible and -resistant in 2009.

28.0%, followed by *emm*75 (18.4%). In contrast, the *emm*12 genotype (3.4%) was rarely found in 2009. The numbers of strains with *emm*18 or *emm*75 genotypes were also markedly lower in 2009 (Table 2). The difference in the distribution of the *emm* genotypes between 2002 and 2009 was significant ($P<0.05$). In 2002, about 60% of the strains with the *emm*12 genotype were $cMLS_B$, while over 80% of the *emm*18 or *emm*75 genotypes had an M phenotype, suggesting that these *emm* genotypes are associated with macrolide resistance.

4. Changes in macrolide production

The analysis of the data on the annual macrolide production revealed a remarkable increase in the total macrolide production in 2005 and 2008 compared to 1999 and 2002 (Table 3). Although the proportion of each macrolide differed every year, a decrease in the erythromycin and midecamycin production as well as a markedly increased production of clarithromycin and roxithromycin were noted during the study period.

DISCUSSION

The increase in macrolide resistance among GAS is a concern, because macrolides are the common regimen for the treatment of pharyngitis caused by GAS as well as other pathogens, such as chlamydia and mycoplasma. In the present study, ERY resistance was observed only in 8 strains among 174 isolates (4.6%), which represents a significant decrease compared to the resistance rate in 2002 (44.8%) [16]. The ERY resistance rate of GAS isolated from patients with acute pharyngitis was reported to be 20.5% using data obtained over a 6-yr period (1998-2003) from provincial health institutes and clinical centers in our country [17]. Another group reported an ERY resistance rate of 23%, which was based on data collected over a 5-yr period (1997-2003) in Seoul and Masan [10]. Changes in the prescription pattern of antibiotics or macrolide consumption could theoretically have an impact on the resistance rates to ERY for GAS [18, 19], but these parameters are difficult to estimate without the respective data. If we assume that the production of drugs is closely correlated with consumption, we could utilize the production data to estimate their consumption. Recently, a remarkable increase in the production of new macrolides has been noted (Table 3), which indicated that the use of macrolides is not restricted. It is also important to know whether the currently observed decrease in ERY resistance rate is restricted to our region or is a nationwide phenomenon.

Table 3. The production cost of macrolides between 1999-2008*

Year	1999	2001	2005	2008
Erythromycin	34.7 (10.8)	34.8 (9.3)	23.1 (4.4)	18.2 (4.0)
Clarithromycin	54.3 (16.9)	52.1 (14.0)	226.4 (43.3)	134.8 (29.5)
Roxithromycin	80.0 (24.9)	214.5 (57.5)	183.2 (35.0)	271.4 (59.4)
Midecamycin	144.0 (44.8)	37.3 (10.0)	37.8 (7.2)	22.4 (4.9)
Other macrolides	8.7 (2.7)	34.1 (9.1)	59.5 (11.2)	9.9 (2.2)
Total	321.7	372.8	530.0	456.7

*Obtained from the Korean Pharmaceutical Manufacturers Association; 100 million won (%).

In general, high ERY resistance rates are associated with the MLS_B phenotype, whereas the M phenotype is found more frequently in countries with lower resistance rates [20]. Out of the 8 ERY-resistant strains, 5 $cMLS_B$ strains (62.5%) harbored the *erm*(B) gene, while 3 strains (37.5%) with the M phenotype were positive for the *mef*(A) gene. In 2002, the $cMLS_B$ phenotype constituted 42.1% of ERY-resistant isolates and the M phenotype accounted

for 57.9%. None of the ERY-resistant strains had more than 1 resistance gene in either 2002 or 2009. The iMLS$_B$ phenotype was not detected in 2002 or 2009, suggesting that it is very rare in our region. Although the ERY resistance rate dramatically decreased compared to that of 2002, a larger proportion of cMLS$_B$ strains was detected, thereby indicating that ERY-resistant isolates in our region exhibit high-level resistance to ERY.

A close association between the *emm* genotype and antibiotic resistance has been reported [5, 13]. We observed an ERY resistance rate of 4.6%, suggesting that almost all isolates were susceptible and that the ERY-resistant isolates belonged to a few limited *emm* types, such as *emm*28 and *emm*89. The strains with *emm*28 showed a high ERY resistance rate (58.3%), suggesting that strains with this *emm* genotype are highly resistant. All *erm*(B)-positive ERY-resistant GAS isolates had an *emm*28 genotype, which accounts for 50% and 70% of *erm*(B)-positive ERY-resistant GAS isolates in North America [21] and France [6], respectively. In our previous study (2002), the ERY resistance rate of *emm*12 strains was 63%, and most of them had the cMLS$_B$ phenotype, whereas *emm*18 and *emm*75 strains had the M phenotype. We did not detect *emm*89 isolates, whereas *emm*2, 3, 12, 18, 22, and 75 were rather common [16]. Among them, *emm*22 and *emm*75 strains were persistent in Jinju. The dramatic reduction in the number of *emm*12 strains harboring the *erm*(B) as well as the *emm*18-*mef*(A) determinant and the emergence of susceptible *emm* types, such as *emm*4, 6, and 89 in 2009, may be associated with the observed decrease in the macrolide resistance rate. Interestingly, almost all *emm*75 strains were susceptible to ERY in the present study, while they were resistant to ERY in 2002. The difference may be explained by the loss of the macrolide resistance gene, suggesting that *emm*75 strains in our region have changed genetically since 2002.

We recently reported decreases in ERY and CLI resistance rates of GAS in normal school children in our region [15]. In these children, the ERY and CLI resistance rates decreased from 51.0% and 33.7% in 2002 [22] to 9.8% and 8.8% in 2004, respectively. A dramatic drop in antibiotic resistance during such a short period seemed quite unusual and was difficult to explain. The *emm*4 and *emm*89 genotypes were found in almost half of the isolated GAS strains, and almost all of these genotypes were susceptible to ERY. Accordingly, the lower ERY resistance rate in 2004 appears to be due to expansion of these ERY-susceptible strains. These results are in agreement with our current observation of a dramatic decrease in antibiotic resistance in acute pharyngitis in 2009. The *emm* genotyping has shown a divergent feature of GAS epidemiology over a 7-yr span in the geographical region and this might affect the antibiotic resistance rate. This finding demonstrates the importance of continuous monitoring of the molecular epidemiology of GAS strains and comparison of these strains on a large scale for the appropriate selection of treatment agents and efforts for infection control.

In conclusion, the ERY resistance rate of GAS isolated from children with acute pharyngitis in 2009 decreased dramatically compared to that of 2002. This significant decrease in ERY resistance over a 7-yr span could be associated with changes in the distribution of the *emm* genotypes rather than restriction of antibiotic usage.

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