

Original Article

Decrease of Erythromycin Resistance in Group A Streptococci by Change of *emm* Distribution

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SUMMARY: With the increasing resistance of group A streptococci (GAS) to macrolides in some countries including Korea, throat cultures were taken from healthy elementary school children to isolate GAS in 2004. Antibiotic susceptibility, macrolide-resistant phenotypes and genotypes as well as *emm* genotyping were studied and compared with previous data in 2002. In the present study, resistance rates to erythromycin and clindamycin in 2004 decreased to 9.8 and 8.8%, respectively, compared with 51.0 and 33.7%, respectively, in 2002. While *emm*44/61 increased from 0 to 29.3%, *emm*12 decreased from 34.4 to 6.4% during the 2002-2004 period. All *emm*44/61 strains were susceptible to erythromycin, while 81.0% of *emm*12 strains were resistant to erythromycin. The dramatic decrease of erythromycin resistance during this short period might be related to a change in the distribution of *emm* types in the community.

INTRODUCTION

Group A streptococci (GAS) are the major pathogen associated with bacterial pharyngitis in children. This infection is also related to other invasive and noninvasive diseases, as well as the potentially fatal postinfectious sequelae rheumatic fever and poststreptococcal glomerulonephritis (1).

Penicillin remains the drug of choice for the treatment of streptococcal pharyngitis. However, an increasing failure rate attributable to coinfection with β -lactamase-producing microorganisms has been reported (2). In these cases and in patients allergic to penicillin, erythromycin or other macrolides have been commonly used (2). Recently, the proportion of erythromycin-resistant GAS has been rising in many countries, including Korea (3-5). The phenotypic expression of macrolide resistance is classified as constitutive resistance, inducible resistance, or M phenotype. Macrolide, lincosamide, and streptogramin B (MLS_B) have a similar antibacterial spectrum and resistance mechanism. The gene *emm* is the encoding gene of M protein, which is the major virulent determinant of GAS. The *emm* gene has highly variable 5'-ends, and *emm* genotyping performed by using polymerase chain reaction (PCR) followed by automatic sequencing has become essential for the epidemiologic study of GAS infections.

We determined the antimicrobial resistance rates, mechanisms of macrolide resistance and distribution of *emm* genotypes for GAS isolated from elementary school children in 2004. Unexpectedly, we observed a dramatic decrease of macrolide resistance compared to the rates determined in the past (1995, 2002). We investigated the reason for the decline of macrolide resistance in this study.

MATERIALS AND METHODS

Sample collection and isolation of bacteria: Throat swab specimens were taken from 2,351 healthy elementary school children (7-12 years old) in Jinju during the period of October through December 2004. Jinju is located in the southern part of Korea and has a population of 300,000. Written informed consent was obtained from each child's guardian. Only one isolate per student was used. The students did not have symptoms or signs of bacterial pharyngitis. Throat swab specimens were cultured on 5% defibrinated sheep blood agar plates and were incubated at 37°C for 16-18 h in ambient air. A small colony showing beta-hemolysis was identified with a bacitracin disk (0.04 U) and latex agglutination test (Seroiden Strepto Kit; Eiken, Tokyo, Japan).

Susceptibility tests: Antimicrobial susceptibility testing by the agar dilution method was performed against erythromycin and clindamycin according to the guidelines of the Clinical and Laboratory Standards Institute (6). Antibiotics were tested at final concentrations ranging from 0.06 μ g/mL to 128 μ g/mL. *Streptococcus pneumoniae* ATCC 49619 was used as a control strain.

Erythromycin resistance phenotypes: The phenotype of resistance to erythromycin was evaluated with a double disk diffusion test with erythromycin (15 μ g) and clindamycin (2 μ g) disks placed 15 mm (edge to edge) apart on 5% defibrinated sheep blood agar. After overnight incubation at 37°C, the presence of blunting in the zone of inhibition of the clindamycin disk was recorded. If the clindamycin inhibition zone was blunted toward the erythromycin disk, the strain was interpreted as clindamycin-inducible. The resultant phenotype patterns were clindamycin-sensitive strains (M phenotype) and clindamycin-resistant (cMLS_B) or clindamycin-inducible (iMLS_B) strains.

Erythromycin resistance genes: All macrolide-resistant isolates were screened for the causative resistance genes. The *erm*(B), *erm*(A), and *mef*(A) genes were detected by PCR amplification using specific primers (5).

***emm* genotyping:** The AccuPower DNA Extraction Kit

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(Bioneer, Chungwon, Korea) was used to isolate bacterial DNA according to the manufacturer's instructions. The PCR was carried out using the AccuPower PCR PreMix Kit (Bioneer) and *emm* primers (7). The amplification products were purified by the AccuPrep PCR Purification Kit (Bioneer), and their nucleotide sequences were determined using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif., USA) and the BLAST program (<http://ncbi.nlm.nih.gov>) of the NCBI (National Center for Biotechnology Information).

Statistical analysis: The isolation rates of GAS, antibiotic resistance rates, distribution of erythromycin resistance phenotypes, and *emm* genotypes were compared by using a χ^2 -test. A *P* value of <0.05 indicated statistical significance.

RESULTS

Isolation of GAS: Of the 2,351 samples received, 328 (14.0%) were GAS positive. There was no significant difference in the isolation rate between boys (14.6%) and girls (13.1%) or between ages.

Macrolide-resistant phenotypes and genotypes: Thirty-two strains (9.8%) and 29 strains (8.8%) were resistant to erythromycin and clindamycin, respectively. Among 29 strains resistant to both erythromycin and clindamycin, constitutive resistance (cMLS_B) was observed in 28 strains (87.5%) and inducible resistance (iMLS_B) in only one strain (3.1%). Three

strains (9.4%) showed the M phenotype (Table 1). The *erm(B)* gene was present in 30 strains (90.6%), and the strain with the iMLS_B phenotype was positive for the *erm(B)* gene. The *erm(A)* gene was not detected. None of the erythromycin-resistant strains had two or more resistance genes. All of the strains with cMLS_B phenotypes had the *erm(B)* gene and MIC ranges of ≥ 128 $\mu\text{g/mL}$ for erythromycin and 32 - ≥ 128 $\mu\text{g/mL}$ for clindamycin (Table 2). One isolate with the iMLS_B phenotype had the *erm(B)* gene and MICs of 4 $\mu\text{g/mL}$ for erythromycin and 0.12 $\mu\text{g/mL}$ for clindamycin. Three isolates (9.4%) with the M phenotype had the *mef(A)* gene and MIC ranges of 8-16 $\mu\text{g/mL}$ for erythromycin and ≤ 0.06 $\mu\text{g/mL}$ for clindamycin.

Comparison with the past results and distribution of *emm* genotypes: We had previously determined erythromycin resistance rates and conducted *emm* genotyping or M typing of GAS isolated from 85 children in 1995 and from 98 children in 2002 in the same region (Table 1). The resistance rates to erythromycin and clindamycin, 29.4 and 10.1%, respectively, in 1995, increased to 51.0 and 33.7%, respectively, in 2002 (8); however they declined significantly to 9.8 and 8.7%, respectively, in 2004 ($P < 0.05$). While *emm12* had been the most common type in the past (Fig. 1), *emm44/61* was the most prevalent in 2004. *emm44/61* had not been present in the past.

The *emm44/61* genotype accounted for 29.3%, while *emm6* and *emm1* were the next most common at 11.6 and 9.8%, respectively (Table 2). The strains with *emm12* and *emm77* showed high erythromycin and clindamycin resistance rates, such as 81 and 60%, respectively, with high MIC levels. Most of the other *emm* types (*emm1*, 3, 5, 6, 22, 44/61, and 75) were erythromycin- and clindamycin-susceptible.

DISCUSSION

For patients infected by GAS who are allergic to penicillin, macrolides are the therapy of choice (2). However, in Korea, macrolides have been prescribed too often for the treatment of respiratory infections without identifying the etiological organisms. Increasing resistance of GAS to macrolides has been observed in some European countries, especially Spain

Table 1. Comparison of antibiotic resistance rates and macrolide resistance phenotypes of group A streptococci (GAS) in Jinju, Korea between 1995-2004

	1995	2002	2004
No. of GAS	85	98	328
Resistance rates (%)			
Erythromycin	29.4	51.0	9.8
Clindamycin	10.1	33.7	8.8
Resistance phenotypes (%)			
cMLS _B	36.0	61.2	87.5
M	64.0	36.7	9.4
iMLS _B	0	2.0	3.1

Table 2. Distribution of *emm* genotypes and its relationship with erythromycin and clindamycin resistance rate and MICs of group A streptococci in 2004

<i>emm</i> type	No. (%) of isolates	Erythromycin			Clindamycin		
		Resistance	MIC ₅₀	MIC ₉₀	Resistance	MIC ₅₀	MIC ₉₀
		no. (%)	$\mu\text{g/mL}$	$\mu\text{g/mL}$	no. (%)	$\mu\text{g/mL}$	$\mu\text{g/mL}$
44/61	96 (29.3)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
6	38 (11.6)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
1	32 (9.8)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
22	27 (8.2)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
75	26 (7.9)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
12	21 (6.4)	17 (81.0)	≥ 128	≥ 128	17 (81.0)	32	≥ 128
5	13 (4.0)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
49	13 (4.0)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
77	10 (3.0)	6 (60.0)	64	≥ 128	6 (60.0)	64	≥ 128
71	9 (2.7)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
3	8 (2.4)	0	≤ 0.06	≤ 0.06	0	≤ 0.06	≤ 0.06
Others ¹⁾	32 (9.8)	9 (28.1)	≤ 0.06	≥ 128	6 (18.8)	≤ 0.06	128
Nontypeable	3 (0.9)	0	≤ 0.06	0.12	0	≤ 0.06	≤ 0.06
Total	328	32	(9.8)		29 (8.8)		

¹⁾ Others; *emm4*, 9, 11, 18, 24, 28, 50, 57, 74, 78, 86, 94, 110, and 123.

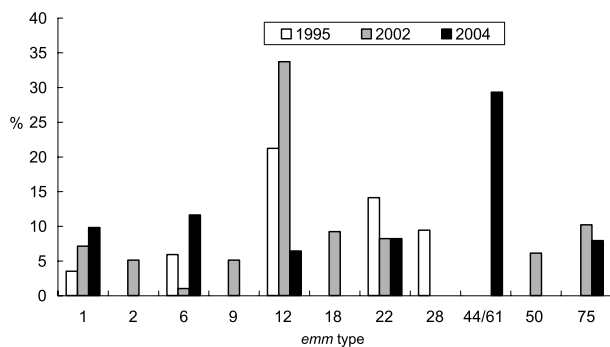


Fig. 1. Change of distribution of *emm* types of group A streptococci in Jinju, Korea between 1995-2004.

and Italy (9,10). The rate of resistance to erythromycin in GAS isolated from patients with pharyngitis in provincial health institutes and clinical centers in Korea was reported to be 20.5% over a 6-year period (1998-2003) in another study (11).

Although the erythromycin resistance rate decreased significantly, the proportion (87.5%) of cMLS_B increased gradually from 36.0% in 1995 to 61.2% in 2002 (8). The iMLS_B phenotype was rarely observed in the past, suggesting that it had not been common in Jinju. Among GAS isolated from patients with pharyngitis, the erythromycin resistance rate was reported to be 23% from 1997 through 2003 in Seoul; the iMLS_B phenotype was most common (51%), followed by the cMLS_B (31%) and M phenotypes (18%) (12). The rate of resistance to erythromycin and the distribution of macrolide phenotypes of GAS might be influenced by geographical variation in the same country. Although the M phenotype was the most prevalent in Spain (96%) (9) and Italy (63%) (10), the *erm*(B) gene, which might present cMLS_B, was more common in France (69%) (3).

In our previous studies in 1995 and 2002, there was no isolate of *emm*44/61, whereas *emm*12, 18, 22, and 75 were rather common (8) (Fig. 1). The prevalence of *emm*12 strains decreased significantly in 2004 compared with the previous results ($P < 0.05$). Not surprisingly, a significant decrease of *emm*12 strains among total isolates might have contributed to a rapid decline of erythromycin resistance in 2004. However the rates of the *emm*12 type among the total number of resistant isolates were 53% (17/32) in 2004, 57% (28/49) in 2002, and 48% (12/25) in 1995, showing that the rate of the *emm*12 type among resistant isolates was at an almost constant level during these years.

Erythromycin resistance and *emm* genotypes seemed to be closely associated. The erythromycin resistance rates of *emm*12 and *emm*77 were 81 and 60%, respectively, with all of the strains showing cMLS_B, whereas the *emm*9 and *emm*18 strains showed the M phenotype (Table 2). The other *emm* genotypes were all susceptible to erythromycin. Yi, et al. (11) observed a similar finding in Korea; a high macrolide resistance rate in *emm*12 strains. Fourteen of the 17 erythromycin-resistant M12 strains harbored the *erm*(B) gene in Japan (13). However, *emm*4, st1815, and *emm*75 were the most prevalent *emm* sequences encountered in the GAS isolates resistant to erythromycin in Spain (14). The strains with *emm*28 and *emm*11 containing the *erm*(B) gene contributed to the

emergence of macrolide resistance in France (3).

One limitation of this study is the lack of macrolide consumption data in Jinju. Precise antibiotic consumption data in the community were not yet available in Korea. We obtained data showing that the total sales of macrolides had increased from 239 million dollars in 2003 to 262 million dollars in 2005. There was a remarkable increase in sales of new macrolides such as azithromycin and clarithromycin (data not shown). There has been no specific action or nationwide effort to reduce the usage of antibiotics during the last few years.

In conclusion, the rate of resistance to erythromycin decreased dramatically compared to past rates. Such a dramatic decrease in erythromycin resistance over a short period might be related to a change in the distribution of *emm* types.

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REFERENCES

- Cunningham, M.W. (2000): Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.*, 13, 470-511.
- Bass, J.W. (1991): Antibiotic management of group A streptococcal pharyngotonsillitis. *Pediatr. Infect. Dis. J.*, 10, S43-49.
- Bingen, E., Bidet, P., Mihaila-Amrouche, L., et al. (2004): Emergence of macrolide-resistant *Streptococcus pyogenes* strains in French children. *Antimicrob. Agents Chemother.*, 48, 3559-3562.
- Koo, H.K., Baek, S.C., Ma, S.H., et al. (2002): Trends of incidence of erythromycin-resistant group A streptococci in Korea from 1998 through 2002. *Infect. Chemother.*, 36, 75-82.
- Reinert, R.R., Luticken, R., Bryskier, A., et al. (2003): Macrolide-resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes* in the pediatric population in Germany during 2000-2001. *Antimicrob. Agents Chemother.*, 47, 489-493.
- Clinical and Laboratory Standards Institute (2003): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-6th edition; Approved Standard M7-A6. Clinical and Laboratory Standards Institute, Villanova, Pa, USA.
- Caparon, M.G. and Scott, J.R. (1987): Identification of a gene that regulates expression of M protein, the major virulence determinant of group A streptococci. *Proc. Natl. Acad. Sci. USA*, 84, 8677-8681.
- Kim, S. and Lee, N.Y. (2004): Epidemiology and antibiotic resistance of group A streptococci isolated from healthy schoolchildren in Korea. *J. Antimicrob. Chemother.*, 54, 447-450.
- Alos, J.I., Aracil, B., Oteo, J., et al. (2000): High prevalence of erythromycin-resistant, clindamycin/miocomycin-susceptible (M phenotype) *Streptococcus pyogenes*: results of a Spanish multicentre study in 1998. Spanish Group for the Study of Infection in the Primary Health Care Setting. *J. Antimicrob. Chemother.*, 45, 605-609.
- Bassetti, M., Manno, G., Collida, A., et al. (2000): Erythromycin resistance in *Streptococcus pyogenes* in Italy. *Emerg. Infect. Dis.*, 6, 180-183.
- Yi, Y.H., Choi, J.H., Lee, H.K., et al. (2006): Characterization of erythromycin resistance of *Streptococcus pyogenes* isolated from pharyngitis patients in Korea. *Jpn. J. Infect. Dis.*, 59, 192-194.
- Bae, S.Y., Kim, J.S., Kwon, J.A., et al. (2007): Phenotypes and genotypes of macrolide-resistant *Streptococcus pyogenes* isolated in Seoul, Korea. *J. Med. Microbiol.*, 56, 229-235.
- Murase, T., Suzuki, R., Watanabe, Y., et al. (2000): Erythromycin resistance genes in *Streptococcus pyogenes* isolates in Kanagawa, Japan. *Microbiol. Immunol.*, 44, 863-865.
- Alberti, S., Garcia-Rey, C., Dominguez, M.A., et al. (2003): Survey of *emm* gene sequences from pharyngeal *Streptococcus pyogenes* isolates collected in Spain and their relationship with erythromycin susceptibility. *J. Clin. Microbiol.*, 41, 2385-2390.