Jpn. J. Infect. Dis., 59, 192-194, 2006

Short Communication

Characterization of Erythromycin Resistance of *Streptococcus pyogenes* Isolated from Pharyngitis Patients in Korea

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(Received October 11, 2005. Accepted April 21, 2006)

SUMMARY: Six hundred fifteen isolates of *Streptococcus pyogenes* were collected over a 6-year period from patients with pharyngitis in Korea. All isolates were characterized in terms of their antibiotic resistance, the phenotypes of erythromycin resistance, the frequencies of *erm*(B), *erm*(A), and *mef*(A) genes, and the *emm* genotype. The prevalent *emm* genotypes were *emm*12 and *emm*4. Moreover, the *emm*12 genotype was found to be the most resistant strain to erythromycin. Among the 126 strains demonstrating resistance to erythromycin, those with *erm*(B) were the most prevalent, accounting for 64.3% of the total. In summary, it is suggested that the *S. pyogenes* pathogen isolated from pharyngitis patients in Korea developed resistant gene acquisition, as well as a resistant phenotype, according to the annual prevailing *emm* type. It is also suggested that the *emm* genotype distribution of erythromycin-resistant strains is correlated to the acquisition of resistant genes.

Streptococcus pyogenes is the most common cause of bacterial pharyngitis in school-age children. The M protein is a major surface protein and a virulence factor of *S. pyogenes*. In the absence of M type-specific antibodies, the bacteria are able to resist phagocytosis by the host's polymorphonuclear leukocytes. M proteins have been classified by more than 120 serotypes, according to their antigenicity; and each M protein type is involved with a specific disease. It has been reported that the epidemiological change of an infection caused by this antigen is correlated with the change in the M protein distribution (1,2). Moreover, the prevalence of erythromycin-resistant *S. pyogenes* has been observed in many countries, with the reported resistance percentage varying among different countries and also from year to year (3).

We analyzed the *emm* genotype of 615 *S. pyogenes* isolates from patients that had been diagnosed with pharyngitis. These isolates were obtained from provincial health institutes and clinical centers in the 6-year period from 1998 to 2003.

Emm genotyping was performed with the PCR-ELISA method (4). Antibiotic resistance phenotypes were determined by using the double-disc method with erythromycin (diffusible content 15 μg) and clindamycin (diffusible content 2 μg) disc in accordance with NCCLS guidelines (5). Resistance to both erythromycin and clindamycin indicated the presence of a constitutive MLS_B phenotype, and susceptibility to clindamycin with blunting and resistance to erythromycin indicated an inducible MLS_B phenotype. Susceptibility to clindamycin with no blunting indicated an M phenotype. The *erm*(B), *mef*(A), and *erm*(A) genes were detected by PCR using previously published primers (6).

The prevalent genotypes in 1998 and 1999 were *emm*12 and *emm*4. The resistance rate to erythromycin was 44.7% in 1998 and 26.9% in 1999, the latter of which demonstrated a decreased resistant rate, but which still remained comparatively high. In 2000 and 2001, the prevalent *emm* genotype distribution of isolated strains included *emm*1, *emm*22, and *emm*2. The resistance rate to erythromycin was 20.2% in 2000 and 18.5% in 2001. In 2002, the prevalent *emm* genotype strains were *emm*12 and *emm*22. The resistance rate to erythromycin increased to 21.7%. In 2003, the prevalent *emm* genotype strains were *emm*1 and *emm*4. The resistance rate to erythromycin decreased to 10.3% (Table 1). We concluded that the resistance rate to erythromycin increased when the prevalent *emm* genotype of isolated strains was the *emm*12 gene type.

The macrolides have a bacteriostatic mechanism of action, which blocks bacterial RNA- dependent protein synthesis, by reversibly combining with the 50S subunit on the ribosome. Changes in the target ribosome demonstrated simultaneous resistance to both macrolides and lincosamides, and these changes were observed in the MLS_B (the macrolide, the lincosamide and the streptogramin B), where the *erm* gene encoded the change. Through the process of efflux, bacteria demonstrated resistance to a few antibiotics and also demonstrated that the M-resistant phenotype is encoded in the *mef*(A) gene. In Spain, Chile, Germany, and Italy, the phenotypes of isolates resistant to erythromycin, which were reported from 1997 to 2002, demonstrated that the M type accounted for 60 - 90%, which surpassed the MLS_B type. In our case, the phenotype of isolated strains with resistance to erythromycin from 1998 to 1999 demonstrated that the constitutive MLS_B type showed 89.3%, and the M type showed a lower frequency of 10.7%. However, in 2000 the M type increased to 66.7%, while the constitutive MLS_B decreased to 33.3%; and in 2001, the M type was still increased at 58.6%, while the constitutive MLS_B remained decreased at 37.9%. In 2002, strains with constitutive MLS_B continued to decrease to 23.1%, while the M phenotype increased to 41.0%; and in 2003, strains with constitutive MLS_B decreased further to 16.7%, while the M phenotype remained at 75.0%. This result demonstrated that the phenotypes of isolated strains with resistance to erythromycin have gradually changed to

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Table 1. Distribution of *emm* genotypes and erythromycin resistant strains of *S. pyogenes* isolated from pharyngitis patients by year of isolation (1998 to 2003)

emm genotype	No. of isolates						Total	Resistant number (%)
	1998	1999	2000	2001	2002	2003		
*emm*1	0	3	27	7	17	40	94	5 (5.3)
*emm*2	0	0	6	34	18	1	59	9 (15.3)
*emm*3	1	1	13	11	16	3	45	0
*emm*4	12	5	14	14	28	41	114	10 (8.8)
*emm*5	1	0	0	1	0	0	2	0
*emm*6	1	0	5	8	8	11	33	0
*emm*11	0	0	0	0	5	0	5	0
*emm*12	25	8	5	25	41	7	111	81 (73.0)
*emm*22	2	5	15	42	29	3	96	6 (6.3)
*emm*28	1	1	0	1	4	3	10	2 (20.0)
*emm*75	0	0	0	14	8	6	28	4 (14.3)
Non typeable	4	3	4	0	6	1	18	9
Total	47	26	89	157	180	116	615	126 (20.5)

Table 2. Genotype and phenotype of 126 macrolide-resistant strains of *S. pyogenes* isolates

Genotype	No. of isolates	Phenotype		
		$cMLS_B$	$iMLS_B$	M
erm(B)	81	48	11	22
mef(A)	25	1	1	23
erm(A)	4	0	0	4
erm(B), *erm*(A)	2	2	0	0
erm(B), *mef*(A)	2	0	1	1
mef(A), *erm*(A)	1	0	1	0
ND[1)]	11	2	2	7
Total (%)	126	53 (42.1)	16 (12.7)	57 (45.2)

[1)]: Not determined, because no amplification was obtained with PCR primers for *erm*(B), *mef*(A), *erm*(A).
$cMLS_B$, constitutive macrolide-lincosamide-streptogramin B resistance; $iMLS_B$, inducible macrolide-lincosamide-streptogramin B resistance.

Table 3. Distribution of erythromycin resistant genotypes according to the *emm* genotypes of *S. pyogenes* isolates

emm genotype	No. of isolates	Erythromycin resistance genotype			
		erm(B)	*mef*(A)	*erm*(A)	ND[1)]
*emm*1	5	3	0	1	1
*emm*2	9	1	7[3)]	1	1
*emm*4	10	1	4	2	3
*emm*12	81	71	4	1[2)]	6
*emm*22	6	3	3	0	0
*emm*28	2	2	0	0	0
*emm*75	4	0	4	0	0
Non typeable	9	4	6[3)]	2[2)]	0
Total	126	85	26	7	11

[1)]: Not determined, because no amplification was obtained with PCR primers for *erm*(B), *mef*(A), *erm*(A).
[2)]: Including one strain harboring both *erm*(B) and *erm*(A).
[3)]: Including each one strain harboring *erm*(B), *mef*(A) and *erm*(A) and *mef*(A).

the M type in Korea, similar to those of other countries. In the case of macrolide-resistant genes, among the 126 strains with resistance to erythromycin, 64.3% of isolates harbored *erm*(B), 19.8% harbored *mef*(A), and 3.2% harbored the *erm*(A) gene. This study demonstrated that strains harboring the *erm*(B) gene are the most prevalent (Table 2). This result is in contrast to the acquisition of resistance observed in studies in other countries (3,5,7,8). The MIC_{50} of strains with *erm*(B) (>128 μg/ml) was 4-5 times higher than that of strains with *mef*(A) (>8 μg/ml) and *erm*(A) (> 4 μg/ml) (data was not shown). These are necessary to continue the investigation and maintain surveillance of those genes that provide resistance to erythromycin.

However, among the isolates harboring the resistant gene, the *emm*12 type had *erm*(B), which demonstrated the most resistance to erythromycin. This result was observed in 71 (87.7%) of 81 strains, suggesting that the extension of strains with resistance to erythromycin is possible when there is a prevalence of the *emm*12 genotype. Because the number of resistant strains was smaller, the other *emm* genotypes were difficult to interpret. However, *emm*1 and *emm*28 possessed *erm*(B), and genotypes *emm*2, *emm*4, and *emm*75 possessed *mef*(A), suggesting a correlation between a specific *emm* genotype and the acquisition of a resistant gene (Table 3).

In summary, it is suggested that *S. pyogenes* isolated from pharyngitis patients in Korea developed a change in antibiotic susceptibility, resistant gene acquisition, and resistant phenotype, according to the annual prevailing *emm* type. It is also suggested that the *emm* genotype distribution of strains with resistance to erythromycin is correlated with the acquisition of resistant genes.

REFERENCES

1. Hu, M. C., Walls, M. A., Stroop, S. D., Reddish, M. A., Beall, B. and Dale, J. B. (2002): Immunogenicity of a 26 valent group A streptococcus vaccine. Infect. Immun., 70, 2171-2177.
2. Bisno, A. L., Brito, M. O. and Collins, C. M. (2003): Molecular basis of group A streptococcal virulence. Lancet Infect. Dis., 3, 191-200.
3. AÍós, J. I., Aracil, B., Oteo, J. and Gómez-Garcés, J. L. (2003): Significant increase in the prevalence of erythromycin-resistant, clindamycin- and miocamycin-susceptible (M phenotype) *Streptococcus pyogenes* in Spain. J. Antimicrob. Chemother., 51, 333-337.
4. Saunders, N. A., Hallas, G., Gaworzewska, E. T., Metherell, L., Efstratiou, A., Hookey, J. V. and George, R. C. (1997): PCR-enzyme-linked immunosorbent assay

and sequencing as an alternative to serology for M-antigen typing of *Streptococcus pyogenes*. J. Clin. Microbiol., 35, 2689-2691.
5. Palavecino, E. L., Riedel, I., Berrios, X., Bajaksouzian, S., Johnson, D., Kaplan, E. and Jacobs, M. R. (2001): Prevalence and mechanisms of macrolide resistance in *Streptococcus pyogenes* in Santiago, Chile. Antimicrob. Agents Chemother., 45, 339-341.
6. Bingen, E., Fitoussi, F., Doit, C., Cohen, R., Tanna, A., George, R., Loukil, C., Brahimi, N., Thomas, I. and Deforche, D. (2000): Resistance to macrolides in *Streptococcus pyogenes* in France in pediatric patients. Antimicrob. Agents Chemother., 44, 1453-1457.
7. Zampaloni, C., Cappelletti, P., Prenna, M., Vitali, L. A. and Ripa, S. (2003): *Emm* gene distribution among erythromycin-resistant and susceptible Italian isolates of *Streptococcus pyogenes*. J. Clin. Microbiol., 41, 1307-1310.
8. Sauermann, R., Gattringer, R., Graninger, W., Buxbaum, A. and Georgopoulos, A. (2003): Phenotypes of macrolide resistance of group A streptococci isolated from out-patients in Bavaria and susceptibility to 16 antibiotics. J. Antimicrob. Chemother., 51, 53-57.