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Erythromycin Resistance Genes in *Streptococcus pyogenes* Isolates in Kanagawa, Japan

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***Abstract*: The susceptibility of 224 *Streptococcus pyogenes* isolates obtained from children in Japan from 1981 to 1997 to treatment with erythromycin was determined by the agar dilution method. A total of 17 isolates belonging to serotype M12T12 were resistant (MICs>1 μg/ml). Fourteen of the 17 resistant strains obtained from 1982 to 1985 harbored *ermB* and showed an identical pulsed-field gel electrophoresis pattern, indicating the spread of a single clone. Two *ermTR*-containing isolates were obtained in 1983. *mefA* gene was found in a strain obtained in 1994 in the present study, although this gene is predominantly associated with recent erythromycin resistance among *S. pyogenes* strains in many countries.**

Key words*: Erythromycin, Susceptibility, *Streptococcus pyogenes

A high rate of erythromycin resistance among *Streptococcus pyogenes* isolates has been demonstrated in Europe (2, 11), California (17), and South Africa (7). It has also been shown that a number of these isolates have the macrolide efflux (*mefA*) gene (2, 6, 11, 17). On the other hand, a significant decline in erythromycin resistance in *S. pyogenes* was reported after reduction of the use of macrolides in Finland (13). In Japan, the frequency of erythromycin resistance among *S. pyogenes* increased to approximately 80% during the 1970s (5, 8) and the resistance rate of isolates showed a marked decrease during the 1980s (1, 4), although the relationship between the use of macrolides and resistance to them has not been published. Erythromycin resistance determinants present among Japanese isolates have not been investigated. Recently, primers designed to identify different erythromycin resistance genes were described (14, 16). In the present study, we characterized erythromycin-resistant strains of *S. pyogenes* isolated in Kanagawa, Japan, on the basis of resistance determinant encoded, strain serotype and pulsed-field gel electrophoresis (PFGE) pattern.

One-hundred-and-sixty-five isolates of *S. pyogenes* obtained from pediatric patients (3–9 years old) with pharyngitis living in Kanagawa Prefecture, Japan, who attended sentinel clinics of the National Epidemiological Surveillance of Infectious Diseases from 1981 to 1997, and 59 isolates from asymptomatic healthy children (5–10 years) living in different geographical regions of Kanagawa Prefecture from 1981 to 1985 were used. Susceptibility to erythromycin was determined by the agar dilution method (10). A total of 17 isolates, 14 from patients with pharyngitis and 3 from healthy subjects, showed MICs of >1 μg/ml and were interpreted as resistant (10). These strains were subjected to further analysis. The MICs of clindamycin, clarithromycin, roxythromycin, spiranomycin, josamycin, cephalothin, tetracycline, ciprofloxacin, chloramphenicol, penicillin, and vancomycin were also determined by the agar dilution method. Breakpoints described elsewhere (6, 10) were used for the interpretation of resistance to these agents. For the identification of erythromycin resistance genes, the DNAs of the strains were amplified with primers specific for the *ermA* (16), *ermB* (16), *ermC* (16), *mefA* (16), and *ermTR* (6) genes. PFGE patterns of the *Sma*I-digested chromosomal DNA of the strains were analyzed (9, 15). The strains were also examined for T-protein serotyping using slide agglutination with commercial rabbit antisera (Denka Seiken Co., Ltd., Tokyo) (3, 9) and M-protein serotypes by microdiffusion with acid extracts and rabbit antisera as described (9, 12), and were found to express T12 and M12 proteins.

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Abbreviations: MIC, minimum inhibitory concentration; PFGE, pulsed-field gel electrophoresis.

Table 1. Detection of erythromycin-resistant genes and MICs of antibiotics for *S. pyogenes* serotype M12T12 isolates tested

	Erythromycin-resistant gene							
MIC of:	*ermB*	(14[a])	*ermTR*	(2)	*mefA*	(1)	Negative	(50)
Erythromyin	>64	(14[b])	4–8	(2)	16	(1)	0.063–0.25	(0)
Clindamycin	>64	(14)	0.063	(0)	0.125	(0)	ND[c]	
Clarithromycin	>64	(14)	2–4	(2)	4	(1)	ND	
Roxythromycin	>64	(14)	16–64	(2)	64	(1)	ND	
Spiramycin	>64	(14)	8–32	(2)	1	(0)	ND	
Josamycin	>64	(14)	1	(0)	0.25	(0)	ND	
Cephalothin	0.125	(0)	0.125	(0)	0.125	(0)	ND	
Tetracycline	0.125–32	(13)	32	(2)	64	(1)	0.125–>64	(4)
Chloramphenicol	4–32	(9)	4	(0)	2	(0)	2–32	(1)
Vancomycin	0.5–1	(0)	0.25–0.5	(0)	0.5	(0)	ND	
Penicillin	0.008–0.016	(0)	0.016	(0)	0.016	(0)	0.008–0.063	(0)
Ciprofloxacin	0.125–0.25	(0)	0.125–0.25	(0)	0.25	(0)	ND	
Isolation year	1982–1985		1983		1994		1985–1997	

[a] Number of isolates.
[b] Number of isolates resistant to each of the antimicrobials.
[c] Not done.

Results of antimicrobial susceptibility tests are shown in Table 1. All 17 erythromycin-resistant strains were also resistant to clarithromycin and roxythromycin. Fourteen of the 17 strains obtained during 1982 to 1985, including 3 from healthy subjects, had the *ermB* gene and were resistant to the other macrolide compounds tested. Two of the 17 strains obtained from patients with pharyngitis in 1983 were positive with the primer specific for the *ermTR* gene. The one remaining strain, that was isolated from a patient in 1994, harbored the *mefA* gene. The *ermTR* and *mefA*-positive strains were susceptible to clindamycin and josamycin. The *mefA*-positive strain was also susceptible to spiramycin. Of the erythromycin-resistant strains, 16 were resistant to tetracycline. All 9 chloramphenicol-resistant strains were also resistant to tetracycline.

The genetic relationship of erythromycin-resistant and erythromycin-susceptible *S. pyogenes* isolates with serotype M12T12 was analyzed by PFGE. The patterns obtained were distinguished by more than one-band difference between each of two isolates (Fig. 1). Twelve of the 14 *ermB*-positive strains showed an identical PFGE pattern, which was arbitrarily designated as pattern 1 (Table 2). These results indicate that the *ermB*-positive strains were of one clonal origin. Remarkably, erythromycin-resistant strains of *S. pyogenes* increased in accordance with the prevalence of serotype T12 during the 1970s in Japan (5). PFGE patterns of the erythromycin-resistant strains having the *ermTR* or *mefA* gene were different from those of erythromycin-susceptible strains (Table 2). The *ermTR* gene was recently described, and was widespread among *S. pyogenes* isolates in Finland during 1994 and 1995 (14). In the present study, however, the *ermTR* gene was identified in the strains from patients with pharyngitis in 1983. Although *mefA* is predominant among erythromycin-resistant *S. pyogenes* strains isolated in recent years (2, 6, 11, 17), it was found in only one strain in this study. These observations indicate that the strain harboring the *ermTR* and *mefA* genes has not spread in Kanagawa, Japan.

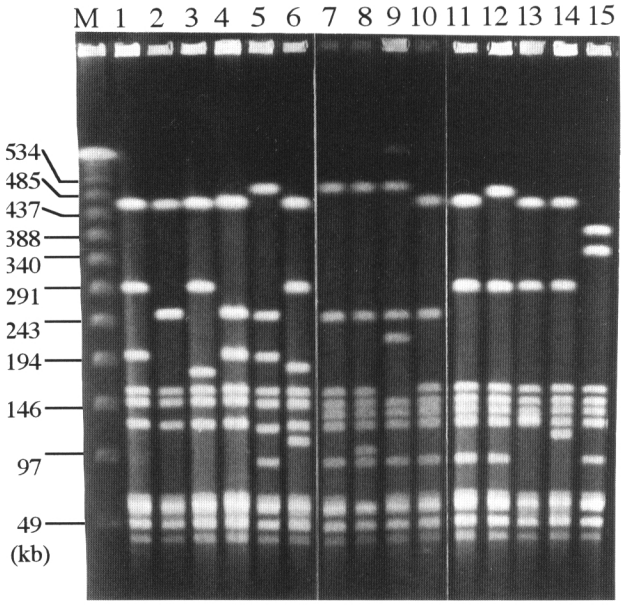

Fig. 1. Representative PFGE patterns of *Sma*I-digested chromosomal DNA of *S. pyogenes* serotype M12T12 isolates. The number above each lane refers to the PFGE pattern. Lane M, lambda ladder. The sizes of the markers are indicated to the left in kilobase pairs.

Table 2. PFGE patterns of *S. pyogenes* serotype M12T12 isolates tested

PFGE pattern	No. of isolates	Presence of the following gene:		
		ermB	*ermTR*	*mefA*
1	12	+	−	−
1	2	−	−	−
2	2	−	+	−
3	1	−	−	−
4	1	+	−	−
5	1	−	−	−
6	1	−	−	−
7	15	−	−	−
8	1	−	−	−
9	1	−	−	+
10	4	−	−	−
11	21	−	−	−
12	1	+	−	−
13	1	−	−	−
14	2	−	−	−
15	1	−	−	−

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