Macrolide Resistance in *Streptococcus pyogenes* and Its Correlation with T-type

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Erythromycin is accepted as the second drug of choice for streptococcal infections. The incidence of macrolide resistance in streptococcal strains has been low and the previously reported macrolide-resistant strains were almost all serotype T-12.

In a 2-year survey conducted from 1979 to 1980, the strains isolated from a wide variety of geographic locations throughout Japan were studied for their macrolide-resistance. The resistance to erythromycin was correlated with the T-type of these strains and its minimum inhibitory concentrations were determined. Serotype T-12 strains isolated during this period were highly resistant to erythromycin. In addition, resistant strains were found in T-4 and T-1, the two other frequently isolated serotypes. Two point eight percent strains were moderately resistant with MICs of 1.56 to 12.5 μg per ml and 25.5% of the strains were highly resistant with 100 μg MIC or more.

MACROLIDE resistance in group A beta hemolytic streptococci (*Streptococcus pyogenes*) has been documented in various reports.¹⁻⁵ The prevalence of macrolide resistant streptococci is still low in the USA^{6,7} and other countries⁸ except Japan^{9,10} where erythromycin is generally regarded as the antibiotic of choice in treating infections by *Streptococcus pyogenes* in patients for whom penicillin is contraindicated.¹¹

In Japan, since 1972, clinical isolates of highly resistant strains to both erythromycin and lincosamin⁸⁻¹⁰ have increased. Among serotypes of group A streptococcus, T-types 1, 4 and 12 are still epidemic strains in Japan.^{9,10} However, only T-type 12 strains were reported to be resistant to erythromycin by 1978.^{9,10,12}

On the basis of these findings, we evaluated antibiotic susceptibility of group A streptococcus strains recently isolated from patients living in various districts of Japan.

MATERIALS AND METHODS

Bacterial isolates: All strains used in this study were isolated based on clinical materials from a wide variety of geographic locations throughout Japan. The cultures were preserved by freezing at -70°C .

Culture media: Trypticase soy agar supplemented with 5% sheep blood was used for subculturing the stock cultures of the isolates. Heart infusion broth was used to prepare the standard suspension. Minimum inhibitory concentration assay was performed using Trypticase soy agar supplemented with 5% sheep blood.

Antimicrobial agents: The antimicrobial agents were supplied as follows: penicillin-G (Meiji Seika Co., Ltd., Japan), tetracycline

Key Words:

Streptococcus pyogenes
Macrolide resistance
T-type

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TABLE I MICs DISTRIBUTION FREQUENCY OF 389 ISOLATES OF GROUP A STREPTOCOCCI FROM 1979 TO 1980 IN JAPAN

MIC $\mu\text{g/ml}$	Antibiotic Agents			
	Penicillin G	Tetracycline	Chloramphenicol	Erythromycin
0.006 $\geq$	361			14
0.013	20			8
0.025	7			96
0.05	1			160
0.1		2		1
0.2		49	1	
0.39		71		
0.78		16	12	
1.56		5	230	2
3.13		1	32	3
6.25		2	1	4
12.5		3	5	2
25		59	75	
50		147	33	
100 $\leq$		34		99

TABLE II RELATIONSHIP BETWEEN ANTIMICROBIAL RESISTANCE PATTERNS AND T-TYPES OF GROUP A STREPTOCOCCUS IN JAPAN (1979-1980)

Resistant pattern	Number of strain	T-type of Group A Streptococcus							
		T-1	T-4	T-6	T-12	T-13	T-28	B3264	Others
Sensitive to all antibiotics	145	39	3	1	9		18	9	66
TC	110	5	29		44	2	1	2	27
TC, CP	24			2	1			1	20
EM	4	4							
TC, EM	22		10		11	1			
TC, CP, EM	84		2		82				
Total	389	48	44	3	147	3	19	12	113

TC = tetracycline, CP = chloramphenicol, EM = erythromycin

(Lederle, Japan), chloramphenicol (Sankyo Seiyaku Co., Ltd., Japan), erythromycin (Nippon Abott Co., Ltd., Japan).

MIC assay: The MICs of 4 antimicrobial agents were determined by the agar dilution method.¹³ Two-fold dilutions of the antimicrobial agents ranging in concentration from 0.006 to 100 μg per ml were used in each agar plate. Cultures to be tested were grown over night at 37°C on Trypticase soy agar and standard suspensions of approximately 10^8 CFU per ml were prepared in heart infusion broth. One loopful of the suspension approximately 10^6 CFU was

inoculated onto the antimicrobial-containing agar plate using Multipurpose Typing Apparatus (Muto Machine Works, Tokyo, Japan). Agar plates were incubated over night at 37°C. The MIC was defined as the least amount of antimicrobial which inhibited growth as observed by the naked eye.

Serological grouping and T-typing: Serological grouping of the isolated strains was conducted by using group specific antisera (A, B, C and G). T-typing of group A streptococci was performed by mono specific T-antisera,¹⁴ Toshiba Kagaku Co., Ltd., Japan.

RESULTS

All 389 isolates were susceptible to penicillin G at an MIC of 0.005 μg or less per ml. Chloramphenicol and tetracycline MICs showed a bimodal distribution. Sixty-two point one percent of the isolated strains were resistant with MIC values of 12.5 μg or more of tetracycline per ml. Twenty-seven point seven percent of the strains were resistant to 12.5 μg or more of chloramphenicol per ml. MICs for erythromycin showed three different distributions. Two point eight percent of strains were moderately resistant with MIC values of 1.56 to 12.5 μg per ml of erythromycin, and 25.5% of the strains were highly resistant with 100 μg MIC or more of erythromycin per ml. (Table I).

Serotype distribution and resistance patterns are shown in Table II. Thirty-seven point eight percent of isolates were T-type 12, 11.3% isolates were T-type 4 and 12.3% were T-type 1. One hundred and forty-five strains out of 389 total group A streptococcus were susceptible to all antibiotic agents tested. Strains resistant to chloramphenicol alone were not isolated. Serotype of all the strains resistant to erythromycin alone was T-type 1 and the MICs of these strains ranged from 1.56 to 12.5 μg per ml. Strains resistant to tetracycline alone were scattered among various serotypes. Six point two percent of strains were doubly resistant to tetracycline and chloramphenicol, and 5.7% were resistant to both tetracycline and erythromycin. Triply resistant strains to tetracycline, chloramphenicol and erythromycin were 21.6% of all isolates. Sixty-three point three percent of T-type 12 strains, 27.3% of T-type 4 strains and 8.3% of T-type 1 were resistant to erythromycin.

DISCUSSION

Reports on the macrolide-resistance in strains of group A *Streptococcus* are few, although multiple antimicrobial resistance to tetracycline, chloramphenicol, erythromycin and lincomycin has been described in some strains, especially in those isolated from Japan. The previously reported macrolide-resistant strains were almost all serotype T-12. Strains of T-types 4 and 1, which were comparable to those of T-type 12 in their isolation frequency from clinical material, were rarely resistant to macrolides.

The 2-year survey reported in this investigation was conducted from 1979 to 1980. Serotype

T-12 strains isolated during this period were highly resistant to macrolides and this observation was similar to our previous reports^{8,10}. In our latest survey, the frequently isolated strains belonging to T-types 4 and 1 also showed a significant resistance to erythromycin, although the percentage of resistant strains was less than that in T-type 12. Our results demonstrate the significance and the frequency of macrolide-resistant strains in clinical isolates of *Streptococcus pyogenes*.

Erythromycin is conventionally used as the second drug of choice in Streptococcal infections.¹¹ The results reported in this study warrant a careful observation of the antimicrobial susceptibility of clinical isolates of *Streptococcus pyogenes*. These findings draw our attention to an increasing incidence of macrolide resistance and also to the detection of erythromycin resistant strains in serotypes other than T-type 12. The continuous use of erythromycin as the second drug of choice, often performed in Japan, has the potential of selecting out resistant strains. It would be worthwhile to study both the mechanism of this resistance as well as other antimicrobial agents as a substitute for erythromycin.

Acknowledgement

We thank Nancy Khardori, M.D. for many helpful discussions and Masaji Shitara for his skillful assistance.

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