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Decreased Activity of Erythromycin against *Streptococcus pyogenes* in Taiwan

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A total of 78 clinical isolates of *Streptococcus pyogenes* were collected from January 1992 through December 1993 from patients in southern Taiwan. The in vitro activities of 10 antimicrobial agents were determined by the agar dilution method. Penicillin, cephalothin, cefotaxime, vancomycin, and ofloxacin were shown to be active against *S. pyogenes* isolates, with MICs at which 90% of isolates are inhibited (MIC_{90}s) being ≤0.03, ≤0.13, ≤0.13, ≤0.13, and ≤0.25 μg/ml, respectively. Erythromycin and azithromycin both had poor activities (MIC_{50}s, 16 and >128 μg/ml, respectively; MIC_{90}s, >128 and >128 μg/ml, respectively). The activities of tetracycline, clindamycin, and chloramphenicol against a significant number of these isolates were also limited. As the MICs of clindamycin and chloramphenicol for the isolates increased, the MICs of the two macrolides also increased. Clindamycin, chloramphenicol, and the two macrolides were less potent against isolates recovered from throat swab samples than against those from blood or other sources. Isolates of the T12 and T1 serotypes accounted for 53.8% of all isolates. The majority (87.5%) of the isolates recovered from throat swab samples were of the T12 serotype, whereas 19.2% of the isolates recovered from blood were of the T12 serotype. In contrast, 66.7% of the isolates of the T1 serotype were derived from blood but none were derived from throat swab samples. Of the 33 T12 serotype isolates, erythromycin MICs for 78.8% of the isolates were >128 μg/ml. Because of the poor activities of erythromycin and azithromycin against *S. pyogenes* isolates from patients in southern Taiwan, these drugs should no longer be considered the drugs of choice for the management of group A streptococcal infections among patients who live in this area.

Penicillin is uniformly active against *Streptococcus pyogenes* and has remained the drug of choice for the treatment of infections caused by this organism. Erythromycin or clindamycin has been recommended as an alternative treatment for patients who are allergic to penicillin (5, 11). In 1959, Lowburry and Hurst (14) first reported the decreased potency of erythromycin against *S. pyogenes* isolates. Since then, similar observations have been reported in many parts of the world (3, 10, 12, 15, 19, 20–22, 24). In most countries, erythromycin shows limited activity against only a few clinical isolates of *S. pyogenes*. A low level of potency of erythromycin against a large proportion of isolates has been reported in Australia (22), Finland (20), the United Kingdom (12), and Japan (15). It is possible that the widespread use of erythromycin is related to the emergence of the isolates for which MICs are elevated (12, 15, 20, 22). In Japan isolates for which erythromycin MICs are elevated were characterized as (i) commonly of the T12 serotype, (ii) less susceptible to tetracycline, clindamycin, and chloramphenicol simultaneously, and (iii) in the majority of cases, recovered from cultures of throat swab specimens (15, 16, 23). However, these results were not in agreement with those of other investigators (6, 10, 20, 22).

The purpose of the present study was (i) to evaluate the in vitro activities of 10 commonly used antimicrobial agents against *S. pyogenes* isolates recovered from patients in southern Taiwan, (ii) to determine the T-protein patterns of these isolates, and (iii) to analyze the association among the distributions of T-protein patterns, the in vitro activity of erythromycin, and the sources of these isolates.

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MATERIALS AND METHODS

Bacterial strains. A total of 78 isolates of *S. pyogenes* recovered from 78 consecutive patients were collected from three major microbiological laboratories in southern Taiwan from January 1992 to December 1993. Throat swab samples provided 24 isolates, 26 were derived from blood, and 28 were from other sources, including pus from cutaneous lesions (n = 22), sputum (n = 3), ascites (n = 1), and urine (n = 2). All isolates were identified by colony morphology, bacitracin susceptibility, and the pyrrolidonyl arylamidase test (8) and were confirmed by commercial latex agglutination (Oxoid, Basingstoke, United Kingdom). All isolates were stored at −70°C in Todd-Hewitt medium (Difco Laboratories, Detroit, Mich.) with 15% glycerol until testing.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing of the isolates was done by the agar dilution method (17). Mueller-Hinton agar supplemented with 5% sheep blood was used. For susceptibility testing of macrolides, the Mueller-Hinton agar medium was adjusted to pH 7.3. All of the antimicrobial agents except azithromycin and ofloxacin were obtained as standard reference powders of known potency for laboratory use from Sigma Chemical Co. (St. Louis, Mo.); azithromycin was obtained from Pfizer Inc. (New York, N.Y.), and ofloxacin was obtained from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). The drugs were incorporated into the agar in serial twofold concentrations as follows: penicillin, 0.03 to 0.5 μg/ml; cephalothin, cefotaxime, and vancomycin, 0.13 to 2.0 μg/ml; erythromycin and azithromycin, 0.03 to 128 μg/ml; clindamycin, 0.03 to 32 μg/ml; tetracycline, 0.5 to 128 μg/ml; chloramphenicol, 0.5 to 128 μg/ml; and ofloxacin, 0.25 to 8.0 μg/ml. The bacterial inocula were prepared according to the guidelines of the National Committee for Clinical Laboratory Standards (17). The final inocula, containing approximately 1×10^4 to 3×10^4 CFU, were applied onto the plates with a Steers replicator (17). The plates were incubated at 35°C in ambient air for 24 h. The MIC of each antibiotic was defined as the lowest concentration which inhibited visible growth of the organism. *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922,

TABLE 1. In vitro susceptibilities of 78 isolates of *S. pyogenes* to 10 antimicrobial agents

Antibiotic	Total (n = 78)[a]			Blood (n = 26)		Throat swab (n = 24)		Others (n = 28)[b]	
	MIC (μg/ml)			MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)	
	Range	50%	90%	50%	90%	50%	90%	50%	90%
Penicillin	≤0.03–0.06	≤0.03	≤0.03	≤0.03	<0.03	≤0.03	≤0.03	≤0.03	≤0.03
Cephalothin	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.03	≤0.03	≤0.13	≤0.13
Cefotaxime	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13
Erythromycin	≤0.03–>128	16	>128	2	>128	>128	>128	0.13	>128
Azithromycin	≤0.03–>128	>128	>128	16	>128	>128	>128	0.5	>128
Tetracycline	≤0.5–64	32	32	32	32	16	32	32	32
Clindamycin	≤0.03–>32	0.13	>32	0.13	>32	>32	>32	0.13	0.13
Chloramphenicol	2–64	4	32	4	32	32	32	2	32
Vancomycin	≤0.13–0.5	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13	≤0.13
Ofloxacin	≤0.25–1	1	1	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1.0

[a] Numbers in parentheses indicates the number of isolates recovered from the indicated specimens.
[b] Isolates were recovered from pus (n = 22), sputum (n = 3), ascites (n = 1), and urine (n = 2).

Enterococcus faecalis ATCC 29212, and *S. pyogenes* ATCC 10389 were used in each run as controls for susceptibility testing.

T-protein typing. The T-protein pattern of each isolate was identified by slide agglutination of the bacterial suspension in the presence of type-specific antisera (Institute of Sera and Vaccine, Prague, Czech Republic) (7, 13). The bacteria were inoculated onto 5% sheep blood agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). The plates were incubated overnight at 35°C with 5% CO_2. Fresh colonies were inoculated into normal saline, and the turbidity of the bacterial suspension was adjusted to match that of a McFarland no. 2 nephelometer standard. Digestion of the bacterial suspension with trypsin was performed according to the manufacturer's instructions.

RESULTS

Susceptibility testing. The MIC ranges and the MICs at which 50% (MIC_{50}s) and 90% (MIC_{90}s) of the 78 isolates of *S. pyogenes* are inhibited are given in Table 1. All three β-lactam antibiotics had good in vitro activities (MIC_{90}s, ≤0.13 μg of cephalothin and cefotaxime per ml and ≤0.03 μg of penicillin per ml). Compared with β-lactam antibiotics, tetracycline (MIC_{90}, 32 μg/ml), clindamycin (MIC_{90}, >32 μg/ml), and chloramphenicol (MIC_{90}, 32 μg/ml) were clearly less active. Erythromycin and azithromycin both had poor activities (MIC_{50}s, 16 and >128 μg/ml, respectively; MIC_{90}s, >128 and >128 μg/ml, respectively). In general, the MICs of azithromycin were 4- to 32-fold greater than those of erythromycin. As the MICs of clindamycin and chloramphenicol increased, the MICs of the two macrolides also increased. All isolates were inhibited by vancomycin at a concentration of ≤0.13 μg/ml and ofloxacin at a concentration of 1 μg/ml.

When the data were analyzed with regard to the sources of the isolates, the MIC ranges of most antimicrobial agents were identical or differed among the three subgroups by at most 1 dilution step. Remarkable differences were detectable for the macrolides, clindamycin, and chloramphenicol, which had relatively poorer activities against isolates recovered from throat swab samples than those recovered from blood or other sources (Table 1).

Serotype distribution. As indicated in Table 2, 22 different T-protein patterns and one nontypeable isolate were identified. More than half of all isolates belonged to serotypes T12 (42.3%) and T1 (11.5%).

Erythromycin susceptibility. The association among the in vitro activity of erythromycin, the T-protein patterns, and the sources of the isolates is shown in Fig. 1. Eighty-eight percent (21 isolates) of the isolates recovered from throat swab samples were serotype T12, whereas 19.2% (5 isolates) of isolates from blood were serotype T12. In contrast, 66.7% (six isolates) of the serotype T1 isolates were derived from blood but none were derived from throat swab samples. Erythromycin was more active against isolates of the T1 serotype than those of the T12 serotype. Of the nine isolates of the T1 serotype, erythromycin MICs for two (22.2%) of the isolates were 32 μg/ml and MICs were ≤0.03 μg/ml for seven (77.8%) of the isolates. However, the majority (78.8%) of the isolates of the T12 serotype were not inhibited by erythromycin at a concentration of 128 μg/ml. Of the 28 isolates for which erythromycin MICs were >128 μg/ml, 26 (92.9%) were of the T12 serotype and 19 (67.9%) were recovered from throat swab samples.

DISCUSSION

The decreased activity of erythromycin against *S. pyogenes* isolates is a serious problem in Taiwan. Our study showed that both erythromycin and azithromycin have poor in vitro activities against isolates collected from patients in southern Taiwan from 1992 through 1993. A study conducted in the same period by investigators at National Taiwan University Hospital, a

TABLE 2. T-protein patterns of 78 isolates of *S. pyogenes*

Pattern	No. (%)
12	33 (42.3)
1	9 (11.5)
8/25/Imp. 19	4 (5.1)
8/25	3 (3.8)
27	3 (3.8)
5/27/13/B3264	3 (3.8)
4	3 (3.8)
9/23	2 (2.6)
8	2 (2.6)
8/9	2 (2.6)
28	2 (2.6)
2	1 (1.3)
3	1 (1.3)
5	1 (1.3)
6	1 (1.3)
9	1 (1.3)
23	1 (1.3)
5/9	1 (1.3)
9/22	1 (1.3)
8/9/25	1 (1.3)
3/13/B3264	1 (1.3)
5/27/28/9	1 (1.3)
Nontypeable	1 (1.3)
Total	78 (100)

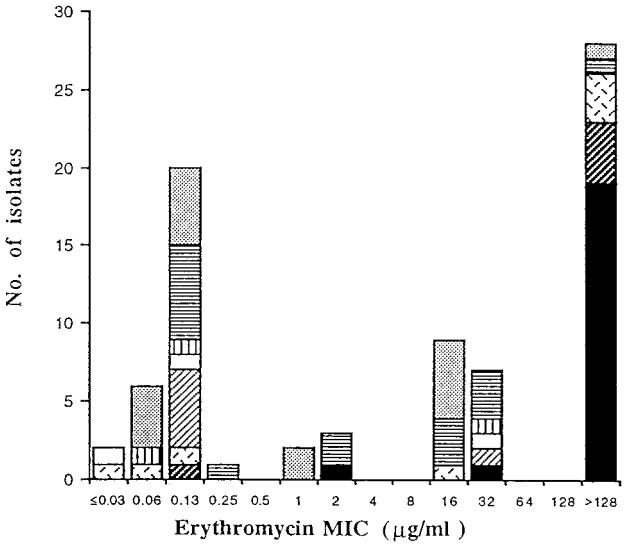

FIG. 1. Association among in vitro activity of erythromycin, T-protein patterns, and sources of the 78 isolates of *S. pyogenes*. ■, serotype T12 isolates from throat swabs (21 isolates); ▨, serotype T12 isolates from blood (5 isolates); stippled box, serotype T12 isolates from other sources (7 isolates); ▨, serotype T1 isolates from blood (6 isolates); □, serotype T1 isolates from other sources (3 isolates); ◫, serotypes of other patterns from throat swabs (3 isolates); ▤, serotypes of other patterns from blood (16 isolates); ▤, serotypes of other patterns from other sources (17 isolates).

medical center in northern Taiwan, also demonstrated similar results (4). There did not appear to have been any clustering of cases in particular parts of Taiwan, nor were we aware of any *S. pyogenes* outbreaks at schools or hospitals during this time period.

Appropriate MIC breakpoints for defining the susceptibility and resistance of *S. pyogenes* isolates to erythromycin and other antimicrobial agents have not been approved by the National Committee for Clinical Laboratory Standards (17). However, several investigators have assigned MIC breakpoints for resistance and have reported the incidence of erythromycin-resistant isolates of *S. pyogenes* in their own countries (2, 3, 5, 10–12, 14, 15, 19–24). An increasing trend toward erythromycin resistance (MICs, ≥32 μg/ml) was well established and was reported two decades ago in Japan (15, 23). Studies in Japan reported that the frequency of erythromycin resistance in *S. pyogenes* increased to 83.1% and then decreased dramatically to less than 1% after the use of erythromycin was reduced (2, 9, 15, 23). In Finland, however, Seppälä et al. (20) revealed that variations in the prevalence of erythromycin resistance (MICs, ≥2 μg/ml) did not correlate well with differences in the amount of erythromycin use at the community level. In Taiwan, erythromycin is commonly used in primary-care clinics and is readily available over the counter at drugstores, without a physician's prescription. Furthermore, this drug is prescribed frequently as a first-line antibiotic for patients with upper respiratory tract infections at hospitals. The widespread overuse of erythromycin may be a contributory factor to its poor activity against *S. pyogenes* in Taiwan.

The emergence of *S. pyogenes* isolates for which erythromycin MICs are high should be of great concern in clinical settings, because there are only a few alternative antimicrobial agents that can be used to treat infections caused by this organism in patients who are allergic to penicillin or other β-lactam antibiotics (1). For the isolates in the United States and Europe for which erythromycin MICs were high the MICs of clindamycin were still low (3, 5, 6, 10, 11, 20–22, 24). Because of this, clindamycin has been recommended as a drug of choice secondary to erythromycin. Unfortunately, our study demonstrated that for most of the isolates for which erythromycin MICs are elevated, the MICs of clindamycin and chloramphenicol are concomitantly high, which limits the clinical usefulness of these drugs in southern Taiwan. It is of interest that for several isolates erythromycin MICs were elevated but the MICs of clindamycin and chloramphenicol were lower. A study of the inducibility of decreased activities between erythromycin and lincosamides, as well as the genes conferring resistance in our isolates, should be performed to elucidate this phenomenon. In addition, the poorer activity of azithromycin than that of erythromycin made for the disappointing clinical use of the new macrolides for treating infections caused by *S. pyogenes* (4). Tetracycline was the least potent of the antibiotics tested, indicating that tetracycline is not a treatment of choice. When faced with group A streptococcal infections, penicillin and cephalosporin remain the most active of the currently available antibiotics tested, while vancomycin and ofloxacin are potentially effective alternatives for the treatment of patients who are allergic to penicillin or β-lactam antibiotics.

The clonality of *S. pyogenes* isolates for which erythromycin MICs are high has been studied by analysis of DNA restriction profiles and the distribution of serotypes (18, 20). In Finland, researchers identified 14 different clones and eight serotypes among erythromycin-resistant isolates, with no single clone or serotype being predominant (20). Strains with erythromycin resistance (MICs, ≥1 μg/ml) in Australia (22) and Canada (6) also had a range of different serotypes. These results suggested clonal diversity among the isolates. In Japan (15) and Sweden (10), outbreaks of infection with erythromycin-resistant *S. pyogenes* (MICs, ≥1 μg/ml for Swedish isolates) were mainly caused by strains of the T12 serotype, but their antibiotic susceptibility patterns differed. It is also of interest that Japanese erythromycin-resistant *S. pyogenes* isolates were commonly derived from throat cultures, and tetracycline, chloramphenicol, and lincomycin had concomitantly poor activities against these isolates (15, 16). However, the majority of isolates from Finland (20), Australia (22), and other countries (6, 10) for which erythromycin MICs were elevated were recovered from blood and pus samples. The single serotype and identical resistance profile found among the isolates of erythromycin-resistant *S. pyogenes* in Japan suggested an outbreak caused by a single strain (18, 20). A similar scenario is unlikely for Taiwan. Although the distribution of the sources and the results of the in vitro activities of the antibiotics tested against our isolates were similar to those for the Japanese strains, we demonstrated that isolates for which erythromycin MICs were elevated represented several different serotypes, which was more in line with the results of Finnish, Australian, and Canadian investigators. In the present study, the MICs of erythromycin and other antibiotics for the *S. pyogenes* isolates of the T1 serotype were commonly lower compared with those for isolates of the T12 serotype. This is in agreement with the findings for isolates in Japan (15). More strains should be analyzed to elucidate the clinical significance of this phenomenon.

In conclusion, it can be seen that the in vitro activities of commonly used antibiotics against *S. pyogenes* isolates are changing both nationally and regionally with time. The increased frequency of *S. pyogenes* isolates for which erythromycin MICs are high is remarkable in Taiwan and should raise concern. For the treatment of infections caused by *S. pyogenes*, routine susceptibility testing of the organism remains unwar-

ranted when a penicillin or a cephalosporin is appropriate for treatment. However, in situations in which a penicillin or a cephalosporin cannot be used, susceptibility testing, particularly the E test, in view of its convenience, should be performed to verify the efficacy of the antibiotic chosen. It is also important that periodic surveys of the in vitro activities of erythromycin and other antimicrobial agents be carried out by clinical microbiology laboratories worldwide. Such studies are especially important in areas where macrolide antibiotics are frequently prescribed.

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