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ORIGINAL ARTICLE

Antibiotic susceptibility pattern and erythromycin resistance mechanisms in beta-hemolytic group G *Streptococcus dysgalactiae* subspecies *equisimilis* isolates from central Taiwan



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Background/Purpose: Information concerning antibiotics susceptibilities of beta-hemolytic group G *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) clinical isolates in central Taiwan was limited.

Methods: Totally, 246 SDSE isolates were collected from mainly five regional hospitals, from February 2007 to August 2011. Disk diffusion method, broth microdilution method, and clindamycin induction test (D test) were respectively performed according to the guidelines of the Clinical and Laboratory Standards Institute. Polymerase chain reaction was used to detect the corresponding erythromycin resistance genes.

Results: All isolates were susceptible to penicillin, cefotaxime, and vancomycin. The rate of erythromycin resistance was 24.0% (59/246), whereas that of clindamycin resistance was 12.2% (30/246). The resistance rates of isolates from different hospitals varied from 15.0% to 45.5% for erythromycin and from 7.1% to 36.4% for clindamycin. For erythromycin-resistant SDSE isolates, three different phenotypes with resistance to macrolides (M), lincosamides (L), and type B streptogramins (S_B) were observed: M (49.2%), constitutive MLS_B (cMLS_B, 35.6%), and inducible MLS_B (iMLS_B, 15.3%). All M phenotypic isolates carried *mefA*. The most prevalent genotypes among cMLS_B and iMLS_B phenotypic isolates were *ermB*, followed by *ermTR*. One

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isolate with cMLS_B phenotype carried both *ermB* and *ermTR*, whereas one isolate with iMLS_B phenotype carried both *ermB* and *ermC*.

Conclusion: This is the first trial investigating the antimicrobial susceptibility pattern and erythromycin resistance mechanisms of beta-hemolytic group G SDSE isolates in central Taiwan. The resistance rates for both erythromycin and clindamycin varied significantly among hospitals located in this area and should be monitored continuously in the future.

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Introduction

The clinical importance of group G streptococci (GGS) is increasing with incidence, with the occurrence of infection caused by GGS being higher than that of either group A *Streptococcus* or group B *Streptococcus* infection among beta-hemolytic streptococci.^{1,2} Human GGS mainly include the *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) and the *Streptococcus anginosus* group.³ Generally, SDSE represents the most predominant species.⁴ Recently, in a survey of beta-hemolytic GGS isolated in central Taiwan, we found that 90.1% of GGS isolates belong to SDSE.⁵ A broad spectrum of human diseases is associated with SDSE, ranging from wild pharyngitis¹ to life-threatening streptococcal toxic shock syndrome.⁶

Penicillin is generally used to treat SDSE infection. In addition to penicillin allergy, however, penicillin tolerance [minimum bactericidal concentration/minimum inhibitory concentration (MBC/MIC) ratio ≥ 32] in SDSE has been reported.⁷ Meanwhile, disease recurrence is another problem encountered while treating SDSE infection.⁸ In such circumstances, erythromycin can be used instead of beta-lactam antibiotics. Considering that SDSE isolates may display different susceptibility patterns, antibiotic susceptibility tests should be performed to determine the proper medical regimen.

Two major mechanisms of erythromycin resistance are recognized in streptococci, including SDSE.^{9,10} Erythromycin resistance methylase (encoded by *erm*) modifies 23S rRNA, leading to the coresistance toward lincosamide and streptogramin B, which is defined as MLS_B phenotype. Two expression patterns can be observed: inducible and constitutive MLS_B (iMLS_B and cMLS_B). Isolates with cMLS_B phenotype constitutively express rRNA methylase and can easily be detected by routine disk diffusion method. Isolates with iMLS_B phenotype produce methylase only in the presence of an inducing agent (such as erythromycin), and display erythromycin resistance and clindamycin susceptibility, as evident from routine disk diffusion test. For the detection of iMLS_B phenotype, a special disk diffusion method, the clindamycin induction test (D test), should be used.¹¹ The second mechanism, represented as M type, involves an efflux pump (encoded by *mef*) and shows no cross-resistance to lincosamide and streptogramin B. The difference in macrolide resistance mechanisms among streptococci can affect the therapeutic strategy.¹² For example, *Streptococcus pneumoniae* with *mefE* genotype generally has an erythromycin MIC of 2–16 mg/L¹³;

however, isolates with the *erm* genotype have an MIC higher than 128 mg/L.¹⁴ As azithromycin can be concentrated at the infection site, this antibiotic may treat *mefE*-genotypic *S. pneumoniae* infection, but not *erm*-genotypic bacterial infection. Studies on antibiotic susceptibility patterns and resistance mechanisms may not only be useful for providing information on proper treatment, but also be helpful in understanding the regional diversity in resistance rates and the spread of resistance-associated genes.

Several surveys concerning antimicrobial resistance in GGS have been conducted in northern Taiwan,^{15,16} but they were rarely focused on SDSE or performed in central Taiwan. Owing to the clinical significance of SDSE among GGS, we examined 246 consecutive nonduplicate beta-hemolytic SDSE isolates that were collected mainly from five regional hospitals in central Taiwan. Antibiotic susceptibility pattern and erythromycin resistance mechanisms were investigated to unclosethis issue in this area.

Methods

Bacterial collection and molecular identification

We collected and stocked 246 consecutive nonduplicate beta-hemolytic group G SDSE isolates mainly from facilities located in central Taiwan, including the Central Laboratory of the Central Region Hospital Alliance (Taichung, Taiwan) and the Clinical Bacteriology Laboratory of Lin Shin Hospital (Taichung, Taiwan), between February 2007 and August 2011, using previously described procedures.⁵ Bacteria were identified using 16S rRNA gene sequencing.⁵ Among the 246 SDSE isolates, 66 were from invasive infection sites, including blood and other sterile body fluids, whereas 180 were from noninvasive infection sites, including sputum, pus, urine, throat, vaginal discharge, and the gastrointestinal tract.

Antibiotic susceptibility tests

We used the disk diffusion method to determine the susceptibility of SDSE isolates toward penicillin, vancomycin, erythromycin, clindamycin, levofloxacin, and cefotaxime, in accordance with the Clinical and Laboratory Standards Institute guidelines.¹⁷ In addition, broth microdilution method was applied to determine MIC of these antibiotics among erythromycin-resistant isolates.¹⁷ The clindamycin induction test (D test) was then used to detect isolates with erythromycin-resistant and clindamycin-susceptible

Table 1 Antibiotic susceptibility patterns of 246 SDSE isolates

Antibiotics	No. of isolates (%)		
	Resistant	Intermediate	Susceptible
Penicillin	0 (0)	0 (0)	246 (100.0)
Vancomycin	0 (0)	0 (0)	246 (100.0)
Erythromycin	59 (24.0)	6 (2.3)	181 (73.6)
Clindamycin	30 (12.2) ^a	2 (0.8)	214 (87.0)
Cefotaxime	— ^b	— ^b	246 (100.0)
Levofloxacin	3 (1.2)	3 (1.2)	240 (97.6)

^a Confirmed by D test.^b No interpretive criteria other than "susceptible".SDSE = *Streptococcus dysgalactiae* subspecies *equisimilis*.**Table 2** Erythromycin and clindamycin resistance rates in SDSE isolates from different hospitals

Source (no.)	No. of resistant isolates (%)	
	Erythromycin	Clindamycin
Taichung Hospital (45)	14 (31.1)	5 (11.1)
Fong Yuan Hospital (40)	13 (32.5)	9 (22.5)
Chang Hua Hospital (11)	5 (45.5)	4 (36.4)
Nantou Hospital (60)	9 (15.0)	6 (10.0)
Lin Shin Hospital (85)	17 (20.0)	6 (7.1)
Others ^a (5)	1 (20.0)	0 (0.0)
Total	59 (24.0)	30 (12.2)

^a Patient samples from some central Taiwan clinics.SDSE = *Streptococcus dysgalactiae* subspecies *equisimilis*.

phenotypes.^{9,11} Briefly, Müller-Hinton agar supplemented with sheep blood (5% v/v) was used to inoculate bacterial suspension (adjusted to McFarland 0.5 turbidity). The 15- μ g erythromycin disk and 2- μ g clindamycin disk were spaced 12 mm apart, and then the plate was placed in a 5% CO₂ incubator at 37°C for 20–24 hours. Isolates with iMLS_B phenotype have the feature of flattening of the inhibition zone adjacent to the erythromycin disk and should be regarded as resistant to clindamycin. For genotyping, polymerase chain reaction (PCR) was used to detect the corresponding *mef* and *erm* genes.^{6,18}

Results

Antibiotic susceptibility patterns among SDSE isolates

The antibiogram of 246 SDSE isolates for the six antibiotics examined is presented in Table 1. All isolates were susceptible to penicillin, cefotaxime, and vancomycin, whereas three were resistant to levofloxacin. Rates of erythromycin and clindamycin resistance reached 24.0% and 12.2%, respectively. Resistance rates of the isolates from five hospitals were then compared. We observed a range from 15.0% to 45.5% for erythromycin, and from 7.1% to 36.4% for clindamycin (Table 2). The MIC range, MIC₅₀, and MIC₉₀ of erythromycin-resistant isolates were further analyzed (Table 3).

Phenotypes and genotypes of erythromycin resistance

The phenotypes and genotypes of 59 erythromycin-resistant SDSE isolates were assessed. There were 29 (49.2%), 21 (35.6%), and nine (15.3%) isolates with M, cMLS_B, and iMLS_B phenotypes, respectively (Table 4). All M-type isolates carried *mefA*. Among the 21 cMLS_B isolates, 16 and four isolates carried *ermB* and *ermTR*, respectively, whereas the remaining one possessed both *ermB* and *ermTR*. For the nine iMLS_B isolates, five and three isolates carried *ermB* and *ermTR*, respectively, and the remaining one carried both *ermB* and *ermC*. Thus, *ermB* was found to be the most common genotype among MLS_B-type isolates in central Taiwan.

Discussion

In this study, all SDSE isolates were found to be susceptible to penicillin, cefotaxime, and vancomycin, and most were also susceptible to levofloxacin. Resistance rates of the isolates from five hospitals varied significantly for both erythromycin and clindamycin. Thus, the discrepancy in resistance rates among hospitals located in central Taiwan desires continuous monitoring. We could only find literatures concerning antimicrobial susceptibility of GGS, but not SDSE, in Taiwan, which might partly be owing to the fact that clinical laboratories generally do not perform GGS speciation. Erythromycin resistance rates in streptococci, including group A

Table 3 MICs of six antibiotics among 59 erythromycin-resistant isolates

Antibiotics	MIC (μ g/mL)			Susceptible breakpoint (μ g/mL)	% Susceptible
	Range	50%	90%		
Penicillin	<0.016	<0.016	<0.016	\leq 0.12	100.0
Vancomycin	0.25–0.5	0.25	0.5	\leq 1.0	100.0
Erythromycin	2.0 to >16.0	8.0	>16.0	\leq 0.25	0.0
Clindamycin	<0.016 to >16.0	0.125	>16.0	\leq 0.25	51.0
Cefotaxime	<0.016 to 0.125	<0.016	<0.016	\leq 0.5	100.0
Levofloxacin	0.25–1.0	0.5	0.5	\leq 2.0	100.0

MIC = minimal inhibitory concentration.

Table 4 Phenotypes and genotypes of 59 erythromycin-resistant isolates

Phenotype	Genotype	No.	Total (%)
cMLS _B	<i>ermB</i>	16	21 (35.6)
	<i>ermTR</i>	4	
	<i>ermB</i> and <i>ermTR</i>	1	
iMLS _B	<i>ermB</i>	5	9 (15.3)
	<i>ermTR</i>	3	
	<i>ermB</i> and <i>ermC</i>	1	
M	<i>mefA</i>	29	29 (49.2)

Streptococcus, group B *Streptococcus*, and GGS, were considered to be abnormally high in Taiwan previously.¹⁶ After the implementation of a policy restricting erythromycin use in 2001, erythromycin resistance rate in group A *Streptococcus* has reduced significantly.¹⁹ The erythromycin resistance rate of GGS in northern Taiwan also declined from 32.6% in 1997 to 23.5% in 2008.^{15,16} In another report, the erythromycin resistance rates of non-A,B,D streptococci in a medical center in northern Taiwan also decreased from 31.7% to 17.9% between 1992 and 2005.²⁰ Recently, we reported that 18.5% of beta-hemolytic group G *S. anginosus* group isolates from central Taiwan are resistant to erythromycin.⁵ Thus, we should pay attention to the fairly high erythromycin resistance rate of SDSE reported in this study. Nevertheless, the lack of informative data on erythromycin resistance rates of SDSE in Taiwan prevents meaningful comparisons between our results and those from previous studies, and complicates measures that track changes in resistance trends in Taiwan.

Phenotypes of 59 erythromycin-resistant SDSE isolates were resolved, with M type (49.2%) being most dominant. All M-type isolates carried *mefA*. For both cMLS_B and iMLS_B isolates, the *ermB* genotype represented the most prevalent type, followed by *ermTR*. Two isolates were carrying more than one *erm* genes. Genes encoding erythromycin resistance determinants in streptococci generally exist in transposon, plasmid, or prophage.²¹ Interspecies mobilization of an *ermT*-carrying plasmid from SDSE has recently been reported.²² The present finding of two isolates with more than one *erm* genes may reflect the possible horizontal transfer of *erm* genes between streptococci in this area. Studies investigating the prevalence of erythromycin resistance rates of GGS in Europe revealed a diverse range of 3.5–33.3%.^{9,23,24} The most prevalent phenotype and genotype were iMLS_B and *ermTR*, respectively. Thus, the erythromycin-resistant trait among GGS in Europe displayed a considerably different character from SDSE investigated in this survey. In Asia, a study conducted in Japan focusing on SDSE reported an erythromycin resistance rate of 10.3% (15/145).²⁵ Three isolates displayed the iMLS_B phenotype with *ermA*, five displayed the cMLS_B phenotype with *ermB*, and the remaining seven displayed the M phenotype with *mefA*. Although the resistance rate of SDSE in Japan is much lower than that in this study, the rates of SDSE with iMLS_B (3/15, 20.0%), cMLS_B (5/15, 33.3%), and M (7/15, 46.7%) phenotypes are unexpectedly similar to our survey.

In conclusion, in this study, the antibiotic susceptibility pattern and erythromycin resistance mechanisms in beta-

hemolytic group G SDSE isolates in central Taiwan were first resolved. The observed erythromycin resistance rate of 24.0% is relatively high. Both erythromycin and clindamycin resistance rates of isolates from five hospitals varied significantly. Mechanisms of erythromycin resistance of SDSE display similar pattern to that of SDSE in Japan to a certain extent, but are different from GGS in Europe. The antibiotic susceptibility should be monitored continuously in central Taiwan to provide informative data for improving public health.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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