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Macrolide Resistance in β-Hemolytic Streptococci: Changes after the Implementation of the Separation of Prescribing and Dispensing of Medications Policy in Korea

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This study evaluated the antimicrobial susceptibilities and macrolide resistance mechanisms of beta-hemolytic streptococci (BHS), and an additional objective was to assess the effects of 'the separation of prescribing and dispensing (SPD) of medications' on bacterial resistance rate and distribution of phenotypes and genotypes of erythromycin-resistant BHS by comparing the antimicrobial susceptibility data before (1990-2000) and after the implementation of SPD at one tertiary care hospital in South Korea. Between the period of January 2001 and December 2002, the minimal inhibitory concentrations of six antimicrobials were determined for 249 clinical isolates of BHS. Resistance mechanisms of erythromycin-resistant (intermediate and resistant) isolates were studied by using the double disk test and PCR. Overall, the resistance rates to tetracycline, erythromycin, and clindamycin were 75.5%, 32.9%, and 32.5%, respectively. Sixty-seven (81.7%) of 82 erythromycin-resistant isolates expressed constitutive resistance to macrolide-lincosamide-streptogramin B antibiotics (a constitutive MLS_B phenotype); 11 isolates (13.4%) expressed an M phenotype; and four isolates (4.9%) had an inducible MLS_B resistance phenotype. *erm*(A) was found in isolates with constitutive/inducible MLS_B phenotypes, *erm*(B) with the constitutive/inducible MLS_B phenotype, and *mef*(A) with the M phenotype. We found that resistance rates to erythromycin and clindamycin among *S. agalactiae*, *S. pyogenes*, and group C streptococci isolates were still high after the implementation of the SPD policy in Korea, and that the constitutive MLS_B resistance phenotype was dominant among erythromycin-resistant BHS in this Korean hospital.

Key Words: Beta-hemolytic streptococci, erythromycin resistance, MLS_B phenotype, *erm*(A), *erm*(B), *mef*(A)

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INTRODUCTION

BHS are responsible for acute and chronic diseases, such as respiratory tract infections, endocarditis, sepsis, meningitis, bone and joint infections, pyelonephritis, necrotizing fasciitis, and delayed sequelae including glomerulonephritis, rheumatic fever, and permanent neurological problems.[1] Current practice guidelines for the management of pharyngitis caused by *Streptococcus pyogenes* include the use of erythromycin as an alternative to penicillin when indicated and clindamycin for persons with multiple, recurrent episodes.[2] Macrolide or lincosamide therapy is also a recommended treatment option for *Streptococcus agalactiae* infection or for prophylaxis when streptococcal colonization among pregnant women is suspected.[3] However, recent studies have shown that changes in the susceptibility of BHS to erythromycin and clindamycin have been substantial, although differences in resistance rates to these agents have existed according to geographical variation and investigators.[4-8] The high transmissibility of BHS, which include resistant clones and the association of increased macrolide usage, may play a significant role in the variable resistance rates that have been reported during the last decade.[9-11]

Two major mechanisms account for erythromycin resistance in many gram-positive bacteria: target site modification and active efflux.[12-14] Target site modification is mediated by erythromycin resistance methylase, which is encoded by the *erm* class genes. Methylases cause a conformational change in the prokaryocytic ribosome,

leading to the reduced binding of MLS_B (macrolide-lincosamide-streptogramin$_B$) antibiotics to the target site in the 50S ribosomal subunit. The phenotype expression of MLS_B resistance in streptococci can be either constitutive or inducible.[12-15] Macrolide efflux, which is effected by a membrane protein encoded by the *mef* class genes, has recently emerged among *S. pyogenes* and *Streptococcus pneumoniae* in many countries.[10,14-16] It has been well documented that there is considerable variation between countries in terms of the frequency of MLS_B resistance phenotypes among streptococci.[4,8,10,17-19]

In many Asian countries, physicians both prescribe and dispense drugs. This practice is hypothesized to have caused high drug expenditure and widespread prescription of antibiotics in Asia. In Korea, resistant bacteria are more prevalent than in other industrialized countries.[20] On July 1, 2000, the Korean government instituted the new health policy, 'the separation of prescribing and dispensing (SPD) of medications', to provide greater differentiation between the roles of physicians and pharmacists than had historically existed in South Korea.

The objectives of the present study were to investigate the incidence in susceptibility among the BHS that was isolated from the clinical specimens in a Korean hospital and to clarify the phenotypes and genotypes of erythromycin-resistant isolates. Additional objectives were to assess the effects of SPD on bacterial resistance rate and distribution of phenotypes and genotypes of erythromycin-resistant BHS isolates by comparing the antimicrobial susceptibility data before and after the implementation of SPD in this hospital.

MATERIALS AND METHODS

Bacterial isolates

A total of 249 strains of BHS were stocked from various clinical specimens between January 2001 and December 2002 at Wonju Christian Hospital. Multiple isolates from the same patient were avoided. The isolates were identified using standard criteria on the basis of hemolytic patterns on 5% sheep blood agar, colony morphology, Gram stain, catalase reaction, CAMP reaction, and latex agglutination assay (Streptex; Murex Biotech Limited, Dartford, England). Beta-hemolytic strains with group F antigens were excluded because they are considered part of the viridans group streptococci. The strains were stored in brain heart infusion broth plus 20% glycerol at -70℃ until studied. The frozen isolates of BHS were thawed, inoculated onto a 5% sheep blood agar plate, and incubated at 35℃ overnight. Pure isolates of BHS obtained from three consecutive subcultures were tested for susceptibility and PCR.

Determination of MICs

Susceptibility to penicillin G, erythromycin, clindamycin, tetracycline, ceftriaxone (Sigma Chemical Co, St Louis, MO, USA), and vancomycin (Daewoong Lilly, Korea) was tested through the agar dilution method.[21] Mueller-Hinton agar with 5% defibrinated sheep blood was used for the agar dilution test. Inocula were prepared by suspending colonies in tryptic soy broth to form approximately 10^4 colonies when inoculated with a Steers replicator (Craft Machine Inc, Chester, PA, USA). The minimal inhibitory concentration was determined after 24 hours of incubation. *S. pneumoniae* ATCC 49619 was used as the control in the MIC determinations. The MIC was defined as the lowest concentration of an agent that yielded no growth or a marked change in the appearance of growth when compared to the growth control plate.

Determination of resistance phenotypes

The resistance phenotypes of erythromycin-resistant isolates were determined by the double-disk test with erythromycin (15 μg) and clindamycin (2 μg) disks. After the plates had been inoculated with a swab from a bacterial suspension with a turbidity equal to that of a 0.5 McFarland standard, the disks were placed near each other (distance 10 mm) on Mueller-Hinton agar plates that contained 5% sheep blood. After 18 - 24 hours of incubation at 35℃ in 5% CO_2, blunting of the clindamycin zone of inhibition proximal to the erythromycin disk indicated an $iMLS_B$ resistance phenotype. Resistance to both erythromycin and clindamycin indicated a

$cMLS_B$ resistance phenotype, and the M phenotype was defined as those showing susceptibility to clindamycin with no blunting of the zone of inhibition around the clindamycin disk.[22]

Detection of resistance genotypes

The genomic DNA extractions were carried out with the Easy-DNA kit (Invitrogen Carlsbad, CA, USA) according to the manufacturer's instructions. The presence of genes encoding MLS_B resistance due to alteration of the ribosome target site was determined by PCR amplification of the erm genes using previously described primers[16,23] specific for *erm*(A), *erm*(B), *erm*(C), and *erm*(A) subclass *erm*(TR). Additionally, the presence of the gene that was involved in the macrolide efflux system was determined by PCR with the primer for *mef*(A).[23] The PCR mixture and the PCR conditions were the same as described previously.[16,23] After the PCR amplification with the DNA thermal cycler (Mastercycler 5330, Eppendorf, Hamburg, Germany), 10 μL of the reaction mixture was run in a 2% agarose gel. A 100-bp ladder (Invitrogen Carlsbad, CA, USA) was used in each gel as the DNA fragment size marker. After staining with ethidium bromide, PCR products were photographed on a film in UV light.

RESULTS

Antimicrobial susceptibilities

The overall resistance rates of BHS were found to be 75.5% to tetracycline, 32.9% to erythromycin, and 32.5% to clindamycin, whereas all isolates were susceptible to penicillin G, ceftriaxone, and vancomycin. Tetracycline was inactive against *S. agalactiae* (95.7% resistant) compared to *S. pyogenes*, group C streptococci, and group G streptococci (47.1-77.8% resistant). The resistance rates to erythromycin were, in decreasing order, *S. agalactiae* (41.3%), group C streptococci (33.3%), *S. pyogenes* (25.7%), and group G streptococci (12.5%). *S. agalactiae* had the highest rate of clindamycin resistance (48.6%), followed by *S. pyogenes* (15.8%), group C streptococci (11.1%), and group G streptococci (6.3%). With *S. pyogenes*, the resistant rates to erythromycin, clindamycin, and tetracycline in 2002 were higher than those of 2001. With *S. agalactiae*, there was no significant difference in the resistance rates of erythromycin, clindamycin, and tetracycline during the study period. With group G streptococci, the resistant rates to erythromycin and clindamycin in 2001 were 20.0% and 10.0%, respectively. However, neither erythromycin-resistant nor clindamycin-resistant strains existed in 2002 (Table 1).

Of 82 erythromycin-resistant BHS, 81.7% of them illustrated the $cMLS_B$ phenotype (constitutive resistance to MLS_B), 13.4% illustrated the M phenotype, and 4.9% illustrated the $iMLS_B$ phenotype (inducible resistance to MLS_B). The proportion of $cMLS_B$ phenotypes of *S. agalactiae* and *S. pyogenes* were 87.7% and 77.8%, respectively. Group C streptococcus isolates showed an equal distribution of MLS_B phenotypes. Half (50%) of group G streptococci had the M phenotypes. Of *S. pyogenes* isolates, *erm*(B) and *erm*(A) genes

Table 1. Antimicrobial Susceptibilities of BHS Isolates between 2001 and 2002

Serogroup (No. of tested)	Erythromycin		Clindamycin		Tetracycline	
	$MIC_{50/90}$*	%R (2001/2002)	$MIC_{50/90}$	%R (2001/2002)	$MIC_{50/90}$	%R (2001/2002)
A (70)	0.06/4	25.7 (22.7/30.8)	0.06/2	15.8 (13.6/19.2)	0.5/32	47.1 (43.2/53.8)
B (138)	0.06/256	41.3 (41.7/40.7)	0.12/256	48.6 (48.8/48.1)	32/64	95.7 (95.2/96.3)
C (9)	0.06/256	33.3 (33.3/33.3)	0.12/128	11.1 (0/16.7)	16/32	77.8 (100/66.7)
G (32)	0.06/0.5	12.5 (20.0/0)	0.06/0.25	6.3 (10.0/0)	2/32	50.1 (50.0/50.0)
Total (249)	0.06/256	32.9 (33.1/32.7)	0.12/256	32.5 (32.4/32.7)	32/64	75.5 (74.2/77.6)

%R, percent resistance (intermediate and resistant).

*$MIC_{50/90}$, MIC (μg/mL) for 50 and 90% of strains tested.

were detected in both $cMLS_B$ and $iMLS_B$ phenotypes. The *mef*(A) genes were exclusively detected in the M phenotype. In six isolates (three with $cMLS_B$ phenotypes, two with M phenotypes and one with $iMLS_B$ phenotype) among the 82 erythromycin-resistant isolates, none of the *erm* (A), *erm*(B), *erm*(C), or *mef*(A) genes were detected by PCR (Table 2).

Isolates with the $cMLS_B$ phenotype had erythromycin MICs of 0.5 to ≥ 256 μg/mL (MIC50, ≥ 256 μg/mL) and had clindamycin MICs of 0.5 to ≥ 256 μg/mL (MIC50, ≥ 256 μg/mL). Isolates with the $iMLS_B$ phenotype had erythromycin MICs of 0.5 to 4 μg/mL and clindamycin MICs of 0.25 to 2 μg/mL. Isolates with the M phenotype had erythromycin MICs of 0.5 to 4 μg/mL and clindamycin MICs of 0.06 to 0.12 μg/mL (Table 3).

DISCUSSION

Although penicillin remains the drug of choice in the treatment for infections caused by BHS, drug tolerance and clinical therapeutic failures have been reported.[24] Macrolides and lincosamides have been frequently used to circumvent patients with a β-lactam allergy and have been also used as empiric and preventative therapies for the treatment of BHS. Until the 1980s, BHS was generally considered uniformly susceptible to erythromycin and clindamycin, but resistance spread rapidly in the 1990s. The prevalence of erythromycin-resistant BHS has been reported to be variable depending on the country,[4-8] selective pressure,[11,25] serogroup,[8] serotype,[7] age,[26,27] and season.[28] In this study, the overall resistance rate

Table 2. Distributions of Phenotype and Genotype of MLS_B Resistance Among 82 Isolates of Erythromycin-resistant BHS

Serogroup (No. of tested)	Phenotype (No.)	Genotype (No.)
A (18)	CR (14)	*erm*(B) (10), *erm*(A) (3), *erm*(A) + *erm*(B) (1)
	M (2)	ND (2)
	IR (2)	*erm*(A) (1), *erm*(A) + *erm*(B) (1)
B (57)	CR (50)	*erm*(B) (47), ND (3)
	M (6)	*mef*(A) (6)
	IR (1)	*erm*(A) (1)
C (3)	CR (1)	*erm*(B) (1)
	M (1)	*mef*(A) (1)
	IR (1)	ND (1)
G (4)	CR (2)	*erm*(B) (2)
	M (2)	*mef*(A) (2)

Abbreviations: CR, constitutive resistance; M, M phenotype; IR, inducible resistance; ND, not detected.

Table. 3. MIC Distributions by Mechanisms of MLS_B Resistance in 82 Isolates of Erythromycin-resistant BHS

Antimicrobial	MIC (μg/mL) for isolates with erythromycin non-susceptible phenotype*								
	cMLSB			$iMLS_B$			M		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Erythromycin	0.5-≥256	≥256	≥256	0.5-4	1	4	0.5-4	1	2
Clindamycin	0.25-≥256	≥256	≥256	0.25-2	0.25	2	0.06-0.12	0.06	0.12
Tetracycline	8-64	32	64	16-32	32	32	0.06-32	32	32

*Isolates with erythromycin MIC of ≥0.5 μg/mL.

among BHS was 32.9% (intermediate 2.0% and resistant 30.9%) for erythromycin and 32.5% (intermediate 0.8% and resistant 31.7%) for clindamycin during 2001 - 2002. The resistant rates to erythromycin and clindamycin in this study were higher than that the resistant rates reported in North America, Asia-Pacific, Europe, and Latin America.[17]

Macrolide resistance among *S. pyogenes* is an emerging concern. During the last decade, increased levels of erythromycin resistance of *S. pyogenes* have been reported in Europe and the Far East, especially in Finland (19.0%),[11] Spain (20.4 - 34.8%),[29,30] Italy (51%),[9] Greece (27.3%),[27] and Taiwan (54%).[31] Erythromycin resistance rate of *S. pyogenes* ranged from 14.3% to 23.8% between the years 1994 and 2000 in our laboratory,[32] and in 2001, it was 22.7% and 30.8% in 2002. Clindamycin-resistant *S. pyogenes* were isolated for the first time in 1995, and during the period of 1995 - 2000, the resistant rate ranged from 0% to 21.4%,[32] while in 2001 and 2002, the resistant rates were 13.6% and 19.2%, respectively. Macrolide resistance among *S. agalactiae* is also an emerging concern. Erythromycin resistance rate of *S. agalactiae* ranged from 26.0% to 40.0% during the period of 1996 - 2000,[32] and 41.7% in 2001 and 40.7% in 2002. Clindamycin resistance rate of *S. agalactiae* ranged from 22.0% to 36.8% during the period of 1994 - 2000,32 and in 2001 and 2002, they were 48.8% and 48.1%, respectively. These cumulative data showed that the resistant rates to erythromycin and clindamycin of *S. pyogenes* and *S. agalactiae* did not decrease, in this specific Korean hospital, after the implementation of the SPD policy. Group C and G BHS have been known to cause pharyngitis, including several outbreaks, and occasional invasive systemic diseases such as septicemia, endocarditis, and meningitis.[6] When the overall resistant rates to erythromycin and clindamycin of group C and G BHS in this study period were compared with our previous study period, the resistant rates to two antibiotics of group C and G streptococci increased, however, erythromycin-resistant or clindamycin-resistant group G streptococci did not exist in 2002.

The distribution of MLS_B phenotypes of BHS is influenced by geographical variation and changes with time.[4,10,11,18,19,31,32] In this study, the distribution of MLS_B phenotypes showed that $cMLS_B$ phenotype (81.7%) was an increasing trend, while M phenotype (13.4%) and $iMLS_B$ phenotype (4.9%) were on the decrease when compared with our previous report.[32] Regarding the frequency of the M phenotype of BHS, the results of this study were lower than those reported in western countries such as Europe, North America, Latin America (41.2 - 66.7%),[17] and in Taiwan (37%).[8] The increased distribution of $cMLS_B$ phenotype in this study was mainly due to the increase of $cMLS_B$ resistance phenotype of *S. pyogenes*.

We found that the resistance rates to erythromycin and clindamycin among *S. agalactiae*, *S. pyogenes*, and group C streptococci isolates were on an increasing trend after the implementation of the SPD policy in Korea, and that the $cMLS_B$ resistance phenotype of *S. pyogenes* was dominant among erythromycin-resistant BHS. These findings have suggested that a progressive increasing trend of resistance rates can be explained by the constant selective pressures exerted by the macrolides, and that the low prevalence of the M phenotype among erythromycin-resistant *S. agalactiae* and *S. pyogenes* isolates in our area raises the concern that neither clindamycin nor 16-membered macrolides are adequate alternatives. SPD was introduced in South Korea to facilitate the rational use of drugs. Consequently, antibiotic use and antimicrobial resistance were expected to decrease. However, the increase in antimicrobial resistance to commonly used antimicrobial agents to BHS was observed in this study. Although the exact causes of increasing resistance rates in this study are uncertain, an antibiotic prescription without organism identification and antimicrobial susceptibility testing in clinics would be one of the important factors. Routine susceptibility testing to macrolides and lincosamides for BHS isolates should be performed in Korea. Because macrolide antibiotics are also effective against infections caused by *Mycoplasma*, *Chlamydia*, and *Legionella*, physicians have a propensity for prescribing erythromycin. Thus, the Korean government and health policy-makers ought to be concerned about the nationwide antimicrobial surveillance network systems to prepare an effective strategy in successfully implementing the SPD. Continual moni-

toring of antimicrobial resistance among BHS is needed to provide the medical community with data regarding the resistance mechanisms that are most common to their local or regional environments.

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