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NOTE

Isolation of Quinupristin/Dalfopristin-Resistant *Streptococcus agalactiae* from Asymptomatic Korean Women

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Seven *Streptococcus agalactiae* isolates were obtained from the vagina of 80 asymptomatic women. Three of these isolates showed multi-drug resistant (MDR) phenotypes: two isolates were resistant to clarithromycin, clindamycin, erythromycin, and tetracycline; and one isolate was resistant to clarithromycin, clindamycin, erythromycin, tetracycline, and quinupristin/dalfopristin. There was no clonal relationship among the MDR isolates. This is the first report of quinupristin/dalfopristin-resistant *S. agalactiae*.

***Keywords*:** *Streptococcus agalactiae*, quinupristin/dalfopristin, antimicrobial resistance, vagina, multi-drug resistance

Streptococcus agalactiae, a β-hemolytic group B streptococcus (GBS), is an important pathogen that causes maternal infections and neonatal infections characterized by sepsis and meningitis (Glaser *et al*., 2002). However, the epidemiological characteristics of *Streptococcus agalactiae* infections are changing to include non-pregnant women and elderly adults (Farley, 2001; Henning *et al.,* 2001), and these infections are now associated with bacteremia, endocarditis, skin and tissue infections, and osteomyelitis (Wu *et al.,* 1997). Most of these patients have significant underlying illnesses and reside in long-term care facilities (Manson *et al.,* 2003). Infections caused by antimicrobial-resistant GBS strains are challenging to treat. Antimicrobial-resistant GBS strains make it especially difficult to adequately treat genitourinary infections in menopausal women, who are vulnerable to genitourinary pathogens due to a lack of normal vaginal microbiota (Redondo-Lopez *et al.,* 1990; Onderdonk and Wissemann, 1993; Ruoff *et al.,* 1999; Simonsen *et al.,* 2004). In this study, GBS was isolated from the vagina of asymptomatic women during routine physical examinations, and their minimal inhibitory concentrations (MICs) to various antimicrobial agents were determined.

Mid-vaginal swabs from 80 non-pregnant women between the ages of 20 and 60 years were obtained during routine physical examinations at a primary care clinic in Seoul, Korea. The clinical findings and lack of self-reported complaints or abnormalities indicated that all of the women tested were in good health. The bacterial cells retrieved from the swabs were inoculated into transport media (OTS Transport Medium, Yuhan Lab. Tech., Korea) and transferred to the laboratory. The bacterial cells on each swab were suspended in sterile saline and inoculated on blood agar (BD, USA). After incubation at 35°C for 24 h, a single well-isolated colony was selected from each plate and observed under a light microscope after Gram-staining. The remainder of the suspension was mixed with 20% glycerol (final concentration) and stored at -70°C until use. Each isolate was identified according to the method described in Bergey's Manual of Systematic Bacteriology (Hardie, 1986) using an API 20 STREP kit (Bio-Merieux, France). Identification was confirmed by sequencing of the 16S rRNA gene. The 16S rRNA genes were amplified by PCR using the primer set: 27F; 5'-AGAGTTTGATCCTGGCTCAG-3' and 1088R; 5'-GCTCGTTGCGGGACTTAACC-3' designed by Suzuki and Giovannoni (1996). PCR was carried out as follows: 30 cycles of denaturation at 95°C for 30 sec, followed by annealing at 57°C for 30 sec, and polymerization at 72°C for 45 sec. The PCR products were electrophoresed on a 1% agarose gel, and DNA fragments were extracted and purified from the gel using a gel extraction kit (QIAGEN, USA). The fragments were then sequenced according to Sanger's method using an API prism 310 Genetic Analyzer (PE Applied BioSystems, USA). The DNA sequences obtained were compared with sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/). Antimicrobial susceptibilities to ampicillin, cefotaxime, chloramphenicol, clindamycin, erythromycin, levofloxacin, linezolid, ofloxacin, penicillin, tetracycline, and vancomycin were determined using the disk diffusion method, performed according to the guidelines established by the Clinical Laboratory Standards Institute (CLSI, 2004). The MICs to clarithromycin, clindamycin, erythromycin, quinupristin/dalfopristin (QD, Synercid®, a gift from SK Chemicals), and tetracycline were determined using the standard agar dilution method (CLSI, 2003) in Mueller-Hinton medium (BBL, USA) sup-

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plemented with 5% horse blood. All antimicrobial agents and chemicals were purchased from Sigma- Aldrich (USA) unless otherwise stated. Chromosomal DNA was extracted in agarose plugs and treated with *Sma*I restriction endonuclease. The resulting DNA fragments were analyzed by pulsed-field gel electrophoresis (PFGE) in a CHEF-DR III system (Bio-Rad) at 6 V/cm with increasing pulse times from 2 to 22 sec for 19 h and 0.2 to 5.1 sec for 2 h at 14℃ as previously

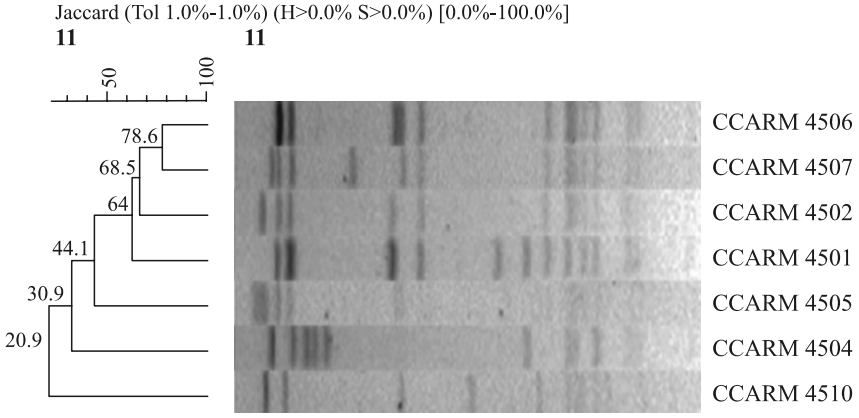

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA of *Streptococcus agalactiae* isolated from the vagina of healthy women.

Table 1. Antimicrobial susceptibility of *Streptococcus agalactiae* from healthy women according to disk diffusion

Antimicrobial agent	Growth inhibition zone (mm)*							
	CCARM 4501	CCARM 4502	CCARM 4504	CCARM 4505	CCARM 4506	CCARM 4507	CCARM 4510	*S. pneumoniae* ATCC 49619
Ampicillin	28	28	27	30	29	27	27	36
Cefotaxime	30	28	29	31	30	29	28	39
Chroamphenicol	**12**	19	19	22	20	20	21	27
Clindamycin	**6**	18	**6**	21	**6**	**6**	18	25
Erythromycin	**6**	24	**6**	24	24	19	28	30
Levofloxacin	18	17	17	18	19	17	18	25
Linezolid	24	21	23	25	23	22	23	38
Ofloxacin	16	16	16	17	18	16	17	21
Penicillin	30	28	27	30	28	27	27	30
Tetracycline	**10**	**7**	**6**	**8**	**8**	**7**	**7**	29
Vancomycin	18	18	18	19	18	17	17	28

* Growth inhibition zones ranged to the resistance were presented in bold.

Table 2. Antimicrobial susceptibility of *Streptococcus agalactiae* from healthy women according to agar dilution

Isolate No.	MIC (μg/ml)*				
	Clarithromycin	Clindamycin	Erythromycin	Quinupristin/Dalfopristin	Tetracycline
CCARM 4501	**>512**	**512**	**>1024**	**16**	**32**
CCARM 4502	≤0.125	≤0.125	≤0.125	2	32
CCARM 4504	**>512**	**512**	**>1024**	2	64
CCARM 4505	≤0.125	≤0.125	≤0.125	2	16
CCARM 4506	≤0.125	**4**	≤0.125	2	16
CCARM 4507	**2**	**4**	**2**	2	16
CCARM 4510	**1**	≤0.125	**2**	0.5	32
S. pneumoniae ATCC 49619	≤0.125	≤0.125	≤0.125	0.5	0.25

* MICs ranged to resistance were presented in bold.

described (Manson *et al.,* 2003). The gel was stained with ethidium bromide and then photographed under UV light. Analysis of the *Sma*I restriction profiles was carried out using a Gelcompar II system (Applied Maths, Kortrijk, Belgium).

Seven *S. agalactiae* isolates were obtained from the vagina of 80 women. All GBS isolates were susceptible to ampicillin, cefotaxime, chloramphenicol, penicillin, quinolone, and vacomycin. Four (57%), two (29%), and seven (100%) isolates were resistant to clindamycin, erythromycin, and tetracycline, respectively, when assayed with the disk diffusion method (Table 1). MICs were determined using the agar dilution method, and the results showed that four (57%) isolates were resistant to clarithromycin, four (57%) were resistant to clindamycin, four (57%) were resistant to erythromycin, one (14%) was resistant to QD, and seven (100%) were resistant to tetracycline (Table 2). These antimicrobial resistance rates are higher than those reported from other countries (Murdoch and Reller, 2001; Diekema *et al.,* 2003; Heelan *et al.,* 2004); however, they are similar to those reported by another study conducted in Korea (Uh *et al*., 2007). Antimicrobial susceptibility studies of GBS in other countries have shown that the prevalence of erythromycin (14∼25%) and clindamycin (9∼17%) resistance is increasing. In Korea, the resistance rates of *S. agalactiae* to erythromycin, clindamycin, and tetracycline were 37%, 43%, and 95%, respectively (Uh *et al.,* 2007). These results showed that the resistance profiles of *S. agalactiae* in Korea are quite different from those in other countries, with the exception of a few cases. Malbruny *et al.* (2004) recently reported the characterization of a new phenotype of resistance to lincosamide and streptogramin A-type antibiotics in isolate CCARM No. 4506, which showed low-level resistance to clindamycin (MIC, 4 mg/L) and susceptibility to erythromycin (MIC, ≤0.125 mg/L) in the present study.

Four of the seven isolates were multi-drug resistant (MDR) GBS. Two isolates (CCARM 4504 and 4507) showed resistance to clarithromycin, clindamycin, erythromycin, and tetracycline. One isolate (CCARM 4510) was resistant to clarithromycin, erythromycin, and tetracycline. Another isolate (CCARM 4501) showed resistance to clarithromycin, clindamycin, erythromycin, Q/D, and tetracycline. QD is a new injectable streptogramin antimicrobial agent proposed for the treatment of severe antimicrobial infections, and it has been shown to be active against MDR Gram-positive cocci, with excellent inhibitory activity against GBS reported worldwide (Pechere, 1992; Dowzicky *et al.,* 1998; Marchese *et al.,* 1999). The seven isolates, including the four MDR isolates, were not clonally related to each other as revealed by PFGE, suggesting that the resistance observed in vaginal *S. agalactiae* is not due to clonal spread, as observed in Spain (Culebras *et al.,* 2002).

To the best of our knowledge, this is the first detection of a QD-resistant GBS. The genetic background of resistance in MDR *S. Agalactiae* isolates obtained from the vagina is currently under investigation.

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