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Recent Epidemiological Changes in Group B *Streptococcus* Among Pregnant Korean Women

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Background: Although group B *Streptococcus* (GBS) colonization rate among pregnant Korean women is lower than that among women from many Western countries, recent data show an upward trend. We investigated recent epidemiological changes in GBS among pregnant Korean women in terms of colonization rate, antimicrobial susceptibility, sero-type, and resistance genotype.

Methods: Vaginal and anorectal swab specimens from 379 pregnant Korean women were cultured on Strep B Carrot Broth with GBS Detect (Hardy Diagnostics, USA), selective Todd-Hewitt broth (Becton Dickinson, USA), and Granada agar plate medium (Becton Dickinson). The antimicrobial susceptibility, serotypes, and macrolide-lincosamide-streptogramin B (MLS_B) resistance genes of the GBS isolates were tested.

Results: The GBS colonization rate among pregnant Korean women was 19.8% (75/379). Colonization rates using Strep B Carrot Broth with GBS Detect, selective Todd-Hewitt broth, and Granada agar plate medium cultures were 19.5%, 19.3%, and 15.0%, respectively. Six pregnant women were colonized by non-beta-hemolytic GBS and were detected only in Strep B Carrot Broth with GBS Detect. Resistance rates of GBS to clindamycin, erythromycin, and tetracycline were 16.0%, 28.0%, and 42.7%, respectively. The most common GBS serotypes were V (22.7%), VIII (20.0%), and III (20.0%). The frequency of MLS_B resistance genes *erm*(B) and *erm*(TR) were 63.6% and 36.4%, respectively.

Conclusions: The GBS colonization rate among pregnant Korean women has risen to levels observed in Western countries. To accurately evaluate GBS epidemiology among pregnant Korean women, periodic studies in multiple centers, including primary clinics, are necessary.

Key Words: Group B *Streptococcus*, Pregnant Korean women, Clindamycin, Erythromycin, Tetracycline, Serotype, Genotype

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INTRODUCTION

In the absence of effective prevention measures, group B *Streptococcus* (GBS) is the main cause of neonatal bacterial infections, including sepsis, pneumonia, and meningitis, which can lead to death or have long-term effects [1, 2]. GBS infections can present from birth to day 6 (early-onset disease [EOD]) or from day 7 to day 89 (late-onset disease). EOD is the result of

vertical transmission (at delivery or shortly before) from a mother with GBS colonization in the anorectal and vaginal sites [3, 4]. GBS serotypes II and III have the highest pathogenicity, and serotype III is most frequently isolated from neonatal GBS infections. In some regions, serotype V isolation has been rapidly increasing [5-8]. Moreover, serotypes III and V have shown multidrug resistance to clindamycin, erythromycin, and tetracycline, thus narrowing the choice of therapeutic agents available against infections caused by these serotypes [7].

Factors influencing EOD incidence include strain virulence, inoculum size, premature or prolonged membrane rupture, preterm delivery, maternal bacteriuria, and serum concentrations of immunoglobulin G specific for the colonizing capsular polysaccharide type [9]. GBS presence in the maternal genital tract at delivery is critical for EOD [9]. The GBS colonization rate among pregnant Korean women is known to be lower than that among pregnant women from Western countries; however, an increase in this rate has been reported recently [10]. According to our previous studies, the GBS colonization rate among pregnant women was 3.9% in 1993 [11], 5.9% in 1995 [12], and 11.5% in 2008–2009 [8]. The most common GBS serotype also changed from Ib in 1995 to III in 2008–2009 [8, 12]. As the colonization rates of multi-drug-resistant GBS serotypes, such as III and V, have increased, their resistance rates to clindamycin and erythromycin have also increased from 13.3% and 5% in 1995 [12] to 44.4% and 33.3% in 2008–2009 [8], respectively.

In Korea, nationwide epidemiological studies on neonatal GBS infections are very rare [13], and there are no microbiological screening guidelines for preventing neonatal GBS infections or accurate data on the epidemiological changes in GBS among pregnant women. As a basic study to investigate the cause of the upward trend in GBS colonization rate among pregnant Korean women, we analyzed the colonization rate, antimicrobial susceptibility, serotype, and the macrolide-lincosamide-streptogramin B (MLS_B) resistance genotype.

MATERIALS AND METHODS

Study population

From May 2017 to May 2019, data from 379 pregnant Korean women visiting Wonju Severance Christian Hospital, Wonju, Korea, were prospectively collected and analyzed. Of these, 90% were between 26 and 40 years of age (median, 34.0; range, 17–45 years). Further, 235 women visited the hospital for antenatal care, including GBS screening at \geq 35 weeks of gestation, and 144 women visited the hospital owing to preterm labor. The Institutional Review Board of Yonsei University Wonju Severance Christian Hospital (approval number CR319119) approved this study on October 22, 2019, and waived the requirement for written informed consent.

Culture method

Specimens were collected in three cotton swabs each from the vagina and anorectal area. All swabs were immediately placed



in Stuart's transport medium (Becton Dickinson, Sparks, MD, USA). The swabs were used to inoculate Strep B Carrot Broth (Hardy Diagnostics, Santa Maria, CA, USA), selective Todd-Hewitt broth (Becton Dickinson) with 8 mg/L gentamicin and 15 mg/L nalidixic acid, and Granada agar plate medium (Becton Dickinson). Inoculated Strep B Carrot Broth was incubated aerobically overnight at 35°C. In the absence of visual color production, the inoculated broth was used for subculture in GBS Detect (Hardy Diagnostics) plate medium, which was then incubated aerobically overnight at 35°C. The inoculated selective Todd-Hewitt broth was incubated aerobically overnight at 35°C and then subcultured on 5% defibrinated sheep blood agar (Becton Dickinson). The inoculated Granada agar plate medium was incubated anaerobically for 18-24 hours at 35°C; in the absence of growth, plates were incubated for an additional 18-24 hours. For GBS detection, plausible colonies on each medium (colonies causing beta hemolysis on 5% defibrinated sheep blood agar and orange colonies on Granada agar plate medium, Strep B Carrot Broth, or GBS Detect) were tested for Christie-Atkins-Munch-Peterson reaction and agglutination using the Streptex group B Streptococcus reagent (Wellcome Diagnostics, Dartford, England). When GBS was detected in any of the three culture methods, the case was defined as that of a pregnant woman carrying GBS, and the colonization ratio was analyzed according to the culture method and specimen type. GBS isolates obtained from 75 pregnant women were kept frozen until testing for antimicrobial susceptibility, serotype, and resistance genotype by PCR.

Antimicrobial susceptibility test

The GBS isolates were tested for antimicrobial susceptibility to ampicillin, penicillin, cefotaxime, ceftriaxone, cefepime, meropenem, levofloxacin, clindamycin, erythromycin, tetracycline, chloramphenicol, and vancomycin using the MICroSTREP plus antimicrobial panel (Beckman Coulter, Brea, CA, USA). For 23 clindamycin non-susceptible or erythromycin non-susceptible GBS isolates, the minimum inhibitory concentrations (MICs) of clindamycin (Sigma Chemical Company, St. Louis, MO, USA) and erythromycin (Sigma Chemical Company) were determined using the CLSI-recommended broth microdilution method employing lysed horse blood-supplemented cation-adjusted Mueller-Hinton broth [14]. The MICs of clindamycin and erythromycin for *Streptococcus pneumoniae* ATCC 49619 (Microbiologics, Inc., St. Cloud, USA) were within acceptable quality control ranges.

Serotyping

Serotyping was conducted using the Strep-B-Latex Kit (SSI Di-

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agnostica, Hillerød, Denmark). The typing sera used in this study were Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX. One droplet (10 μ L) of a bacterial suspension cultured overnight in Todd-Hewitt broth and one droplet (10 μ L) of latex suspension were mixed on a plastic slide. Agglutination appearing within 10 secs was interpreted as a positive reaction.

PCR

Genomic DNA was extracted using the Easy-DNA kit (Invitrogen Carlsbad, CA, USA), according to the manufacturer's instructions. The presence of *erm* and *mef* class MLS_B resistance genes was determined by PCR amplification, using previously described primers specific for *erm*(A), *erm*(B), *erm*(C), *erm*(TR), and *mef*(A) [15, 16]. The PCR mixture and the PCR conditions were the same as described previously [8]. After the PCR amplification in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems Inc., CA, USA), 10 μ L of the reaction mixture was run in a 2% agarose gel. A 100-bp DNA ladder (Gibco/BRL, LifeTechnologies Inc., Gaithersburg, MD., USA) was used in each gel as the size marker.

RESULTS

Colonization rate

The GBS colonization rate was 19.8% (75/379). The GBS colonization rate by culture method using Strep B Carrot Broth with

GBS Detect, selective Todd-Hewitt broth, and Granada agar plate medium was 19.5% (74/379), 19.3% (73/379), and 15.0% (57/379), respectively. The GBS colonization rate by culture site was 17.9% (68/379) and 9.5% (36/379) for the anorectal and vaginal areas, respectively. Of the 75 GBS-colonized pregnant women, 48.0% (36), 44.0% (33), and 8.0% (6) were positive for GBS colonization at both sites, only the anorectal area, and only the vagina, respectively. Furthermore, of the 75 GBS isolates, 8% (6) showed colonization with non-beta-hemolytic GBS, as identified using GBS Detect, and four showed positive reactions 48 hours after incubation on Granada agar plate medium (Table 1).

Antimicrobial susceptibility

All 75 GBS isolates were susceptible to ampicillin, penicillin, cefotaxime, ceftriaxone, cefepime, meropenem, and vancomycin; however, 20.0%, 16.0%, 28.0%, 42.7%, and 4.0% of these isolates were resistant to levofloxacin, clindamycin, erythromycin, tetracycline, and chloramphenicol, respectively. One GBS serotype V isolate showed clindamycin-resistant erythromycinsusceptible phenotype (Table 2).

Distribution of serotypes and MLS_B resistance genes

The most common GBS serotypes were V (22.7%), VIII (20.0%), and III (20.0%). The total proportion of serotypes VI–IX was 34.7%. The erythromycin resistance rate according to serotype was

 Table 1. GBS colonization rate by culture method and specimen type among 379 pregnant Korean women

Culture methods	(Total N ($^{\circ}$) of positivo		
Culture methods	Vagina only, N (%)	Anorectus only, N (%)	Vagina and anorectus, N (%)	Total N (%) of positive
Strep B Carrot Broth with GBS Detect	6 (1.6)	39 (10.3)	29 (7.7)	74 (19.5)
Selective Todd-Hewitt broth	3 (0.8)	39 (10.3)	31 (8.2)	73 (19.3)
Granada agar plate medium	6 (1.6)	30 (7.9)	21 (5.5)	57 (15.0)

Abbreviation: GBS, group B Streptococcus.

Table 2. Antimicrobial susceptibilities and serotypes of 75 GBS isolates from 379 pregnant Korean women

Antimicrobials	MIC (µg/mL)			Susceptibility results by serotype (N of S/I/R)										
	Range	MIC ₅₀	MIC ₉₀	la (N=5)	lb (N=8)	 (N=4)	III (N=15)	IV (N = 0)	V (N=17)	VI (N=3)	VII (N = 4)	VIII (N = 15)	IX (N=4)	% of S/I/R
Chloramphenicol	1−≥32	4	4	5/0/0	8/0/0	4/0/0	14/1/0	-	14/3/0	3/0/0	4/0/0	15/0/0	4/0/0	94.7/1.3/4.0
Clindamycin	0.03-≥256	0.06	≥256	5/0/0	8/0/0	4/0/0	10/0/5	-	10/0/7	3/0/0	1/0/3	15/0/0	4/0/0	84.0/0/16.0
Erythromycin	0.06-≥256	0.06	128	5/0/0	8/0/0	4/0/0	5/0/10	-	9/0/8	3/0/0	1/0/3	15/0/0	3/1/0	70.7/1.3/28.0
Levofloxacin	0.05-≥8	1	≥ 8	4/0/1	3/1/4	4/0/0	13/0/2	-	13/0/4	2/0/1	4/0/0	14/0/1	2/0/2	78.7/1.3/20.0
Tetracycline	0.12−≥8	0.5	≥8	2/0/3	7/0/1	4/0/0	6/0/9	-	6/0/11	3/0/0	1/0/3	12/0/3	2/0/2	57.3/0/42.7

Abbreviations: GBS, group B Streptococcus; MIC, minimum inhibitory concentration; S, susceptible; I, intermediate; R, resistant.

Table 3. Distribution of MLS_B genes and serotypes among 75 GBS isolates from 379 pregnant Korean women

Susceptibility to				Serotype, N (%)									- Total
MLS_B gene	Erythromycin	Clindamycin	n la lb 8 5 (6.7) (10.7)	ll 4 (5.3)	III 15 (20.0)	IV 0 (0)	V 17 (22.7)	VI 3 (4.0)		VIII 15 (20.0)	IX 4 (5.3)	N (%)	
erm(B)	Resistant	Resistant	0 (0)	0 (0)	0 (0)	5 (35.7)	-	6* (42.9)	0 (0)	3 (21.4)	0 (0)	0 (0)	14 (18.7)
erm(TR)	Resistant	Susceptible	0 (0)	0 (0)	0 (0)	5 (71.4)	-	2 (28.6)	0 (0)	0 (0)	0 (0)	0 (0)	7 (9.4)
	Intermediate	Susceptible	0 (0)	0 (0)	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (1.3)
None	Susceptible	Susceptible	5 (9.6)	8 [†] (15.4)	4 (7.7)	5† (9.6)	-	8 [†] (15.4)	3 (5.8)	1 (1.9)	15 (28.8)	3 (5.8)	52 (69.3)
	Susceptible	Resistant	0 (0)	0 (0)	0 (0)	0 (0)	-	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)

*Three GBS isolates were non-beta-hemolytic; [†]One GBS isolate of each serotype was non-beta-hemolytic. Abbreviation: GBS, group B *Streptococcus*.

Abbreviation: GBS, group B Streptococcus.

75.0% (3/4) for VII, 66.7% (10/15) for III, and 47.1% (8/17) for V. Of the six non-beta-hemolytic GBS isolates, four, one, and one corresponded to serotypes V, Ib, and III, respectively (Table 3).

DISCUSSION

The Centers for Disease Control and Prevention of the United States initially recommended using one of the two strategies (risk-based intrapartum antibiotic prophylaxis [IAP] or IAP after microbiological screening) to prevent perinatal GBS infection [17]. Routine microbiological screening of pregnant women is performed in most developed countries, although there is currently no international consensus regarding whether IAP is best achieved through microbiological screening or the identification of clinical risk factors [18]. Several countries, such as the United Kingdom and the Netherlands, have introduced IAP polices based on the presence of clinical risk factors [18], as routine microbiological screening is deemed non-cost-effective and clinical risk factor-based IAP strategies may expose fewer women to the potential risks associated with widespread antibiotic use [18].

The GBS colonization rate among pregnant Korean women in our study is 19.8%, which is significantly increased compared with the rate of 11.5% observed in 2008–2009 [8]. Although the exact cause of this significant and sustained increase is unknown, possible factors may include socioeconomic status and dietary and lifestyle changes. Consequently, it is necessary to include GBS microbiological screening at 35–37 weeks of pregnancy in the prenatal screening guidelines in Korea.

To effectively identify pregnant women with GBS, both the sampling site and culture methods are crucial. In this study, the GBS detection rate was higher in the anorectal area than in the vagina. Dillon, *et al.* [19] suggested that the intestinal tract is a primary reservoir for GBS and is the likely source of vaginal or

urogenital colonization in pregnant women. A primary medium based on Todd-Hewitt broth supplemented with nalidixic acid and either gentamicin or colistin is recommended for detecting pregnant GBS carriers [17]; however, Staphylococcus species often show growth in this medium, thus necessitating disadvantageous subculturing on a 5% sheep blood agar plate. In contrast, the Granada agar plate medium allows easy identification of GBS growth with the naked eye and presents no false positive results when used as a rapid screening method; thus, the use of this medium could facilitate prenatal culture processing at clinical laboratories with limited technical capacity [20]. However, as the ratio of non-beta-hemolytic GBS was relatively high (8.0%) in this study, the colonization rate determined using Granada agar plate medium was 15.0%, which is lower than that observed using the Strep B Carrot Broth with GBS Detect method (19.5%). In our previous study, the proportion of non-beta-hemolytic GBS, which mostly included serotype III GBS, obtained from clinical specimens was 4.7% [21]. PCR or GBS Detect can aid in detecting non-beta-hemolytic GBS when Granada agar plate medium or Strep B Carrot Broth produces a negative result, owing to the direct genetic linkage between orange pigment production and hemolysin production in GBS [22].

Although GBS with reduced penicillin susceptibility has been reported in Korea, Japan, and the United States [23, 24], intravenous penicillin remains the agent of choice for IAP, with intravenous ampicillin constituting an acceptable alternative. First-generation cephalosporins (i.e., cefazolin) are recommended for women whose reported penicillin allergy indicates a low anaphylaxis risk or is of uncertain severity [25]. For women with a high anaphylaxis risk, clindamycin is the recommended alternative to penicillin, but only if the GBS isolate is susceptible to clindamycin [25]. The clindamycin resistance rate of GBS has mainly been determined based on the distribution of MLS_B-resistance phenotypes. The resistance rates of GBS in pregnant Korean



women to clindamycin and erythromycin were 13.3% and 5.0% in 1995 [12], increased to 44.4% and 33.3% in 2008–2009 [8], and slightly decreased to 16.0% and 28.0% in this study (2017–2019), respectively. The reason for the clindamycin resistance rate being higher than the erythromycin resistance rate in 1995–2009 was thought to be the clonal spread of the clindamycin-resistant erythromycin-susceptible phenotype [26]. GBS with *erm*(TR)-mediated inducible MLS^B resistance showed resistance to erythromycin and susceptibility to clindamycin; however, GBS with inducible clindamycin resistance detected by disk diffusion using the D-zone test or broth microdilution should be reported as clindamycin resistant [14]. Therefore, documentation on the detection of inducible clindamycin resistance should be included in the Korean guidelines for the prevention of perinatal GBS infection.

In our study, the predominant GBS serotypes changed from Ib (48.3%), la (24.1%), and III (20.7%) in 1995 [12] to III (29.6%), V (22.2%), and VI (22.2%) in 2008–2009 [8] and to V (22.7%), VIII (20.0%), and III (20.0%) in 2017–2019. GBS serotype VIII is a novel serotype that has never been identified among pregnant Korean women. As GBS serotype VIII is rarely isolated outside of Japan, information about it is guite limited. In Japan, 26 (35.6%) and 18 (24.7%) of 73 vagina-colonizing GBS strains isolated from 441 pregnant women between May 1992 and June 1994 were serotype VIII and VI, respectively [27]. Analysis of the GBS serotype distribution among pregnant Japanese women from 2007 to 2008 showed that the distribution of serotypes VIII and VI decreased by 11.0% and 15.0%, respectively [28]. Although serotype VIII colonization is high among pregnant Japanese women, invasive serotype VIII infections are relatively rare among Japanese infants and pregnant and non-pregnant women [29]. Although serotype VIII is known to effectively colonize and invade human endothelial cells, it is less capable of inducing the secretion of inflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-10, compared with serotype III [29, 30].

In conclusion, by applying the same study method over a long period, we determined the epidemiological changes in GBS among pregnant Korean women. However, our study is limited in that it cannot represent all pregnant Korean women, as our data is based on a single center with limited geographical coverage. Therefore, to accurately evaluate GBS epidemiology among pregnant Korean women, periodic studies in multiple centers, including primary clinics, are necessary.

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AUTHOR CONTRIBUTIONS

Conceptualization: Choi SJ, Uh Y; Methodology: Uh Y; Formal analysis and Data curation: Kang J; Software and Validation: Choi SJ; Investigation: Choi SJ; Writing–original draft: Uh Y; Writing–review & editing: Choi SJ, Kang J; All authors approved the final manuscript.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article are reported.

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