Risk Factors Associated with Group B Streptococcus Resistant to Clindamycin and Erythromycin in Pregnant Korean Women

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Background: The prevalence of group B streptococcus (GBS) among pregnant women and neonates in the Republic of Korea has increased. In addition, rates of resistance to antibiotics recommended for pregnant women allergic to penicillin, such as clindamycin and erythromycin, have increased. The aim of this study was to evaluate subject characteristics associated with GBS resistance to clindamycin and erythromycin.

Materials and Methods: A total of 418 clinical isolates from pregnant women in Korea were screened for antibiotic resistance from January 2006 to December 2011. Sociodemographic information, medical and obstetric history, and details of events during the previous 2 weeks were recorded using a standardized questionnaire.

Results: The resistance rates were 39.5% for clindamycin and 23.0% for erythromycin. In multiple logistic regression analysis, the subject characteristic significantly associated with resistance to both antibiotics was a history of symptomatic sore throat in the 2 weeks before obtaining the specimen (erythromycin: odds ratio [OR]: 2.13, 95% confidence interval [CI]: 1.10 to 4.13; clindamycin: OR: 2.31, 95% CI: 1.21, 4.42). Premature rupture of membranes (PROM) had an association of borderline significance.

Conclusions: In the urgent treatment of GBS-colonized pregnant women, the subject’s history of previous sore throat and PROM should be considered when choosing appropriate antibiotics.

Key Words: Antibiotic resistance, Clindamycin, Erythromycin, Risk factors, Streptococcus agalactiae

Introduction

Streptococcus agalactiae (group B streptococcus, GBS) is a significant cause of perinatal and neonatal infections worldwide. The maternal genital tract is the usual source of GBS, and GBS from this source can cause early-onset neonatal in-
fection in the first week of life [1]. The first case of GBS neonatal infection in Korea was described in 1984. Since then, the number of reported cases of neonatal GBS disease has increased steadily in Korea [2]. Risk of disease is affected by GBS serotype, neonatal birthweight, and the immune status of neonate and mother during pregnancy, but the prevalence of neonatal GBS infection depends mainly on the GBS colonization rate of pregnant women [3].

Asymptomatic colonization with GBS is common worldwide, with estimates from vaginal and rectal sampling ranging from 15% to 30% depending on the population [4]. Screening for colonization is not a standard procedure in all Korean hospitals; a few published reports have suggested that GBS colonization rates are considerably lower in Korea than elsewhere, ranging from 0.3% to 5.9% [5-7]. However, a recent study has reported the prevalence of GBS in pregnant women to be 8% (range, from 4.6% to 10.5%) in Korean hospitals [8].

In an attempt to prevent GBS infection in neonates, the USA and several European countries have introduced screening programs and intrapartum antibiotic prophylaxis [9, 10], but these have not yet been approved as standard procedures for antenatal care in Korea. Penicillin is the intrapartum prophylactic antibiotic of choice for the prevention of GBS-induced neonatal sepsis. In pregnant women with penicillin hypersensitivity, clindamycin or erythromycin is recommended [4]. In the past, GBS was generally susceptible to erythromycin and clindamycin. However, recent studies have revealed substantial changes in the susceptibility of GBS to erythromycin and clindamycin, although resistance rates to these agents differ across geographical regions and studies [11]. Publications from the USA and Canada have reported rates of GBS resistance to clindamycin ranging from 3% to 21% and to erythromycin ranging from 5% to 29% [12-15]. In Korea, resistance rates to these 2 antibiotics have increased from 35.0% to 49.4% for clindamycin and from 30.0% to 35.1% for erythromycin [16, 17].

Since August 2000, the policy of “Separation in prescribing and dispensing of medications” has been practiced in Korea in order to decrease the number of antibiotic prescriptions and reduce the acquisition of antimicrobial resistance due to selection pressure. However, to reduce antimicrobial resistance rates, research on risk factors, including behavioral factors associated with resistance, is needed.

We investigated subject factors associated with erythromycin or clindamycin resistance in GBS-colonized pregnant Korean women.

Materials and Methods

1. Study collection

GBS isolates were collected between January 2006 and December 2011 from pregnant women who had routine antenatal testing at 35–37 weeks of gestation in Daejeon at the Eulji University Hospital or the Mote Obstetrics and Gynecology (OBGY) Clinic, or in Seoul at the Eulji General Hospital or Cheil Women’s Hospital. Among the collected GBS isolates, 410 isolates from pregnant women with a single serotype were counted as group 1. Three hundreds eighteen isolates were duplicated samples, among them, 2 isolates with different serotypes, 5 isolates with different antimicrobial resistance, and 1 isolate with both different serotype and different antimicrobial resistance were counted as independent isolate samples. All isolates were tested for antimicrobial resistance in either the Departments of Laboratory Medicine of the Eulji hospitals in Seoul and Daejeon or in the Seoul Clinical Laboratory for samples obtained at Cheil Hospital. The Institutional Review Boards at the Eulji (06-25 and 11-031) and Cheil (SCH-IRB-2005-24 and CGH-IRB-2010-1) hospitals approved the study protocol. Written informed consent permitting the use of the sample materials and medical records for research purposes was obtained from each study participant.

2. GBS isolates

1) GBS collection

Vaginal mucus or discharge was collected with a swab from the vaginal introitus without inserting a speculum, and placed in Stuart’s transport medium. A swab was inserted through the anal sphincter, rotated 2 or 3 times, and placed into a separate container of transport medium. Urine samples were self-collected specimens of the first 20 mL of urine. All participating laboratories used the same protocols for GBS incubation and identification.

2) GBS culture

To repress the growth of microorganisms other than GBS, Todd-Hewitt broth supplemented either with gentamicin (8 μg/mL) + nalidixic acid (15 μg/mL) or with colistin (10 μg/mL) + nalidixic acid (15 μg/mL) was used. Urine samples were centrifuged, and 1 mL of the sedimtented sample was inoculated on the selective medium. Rectal and vaginal swabs were used to inoculate the selective broth medium. Cultures were shaken 3 or 4 times to ensure adequate mixing of the analyte. The lids of the culture tubes were closed loosely, and the
cultures were incubated along with a negative control for 18–25 h at 35–37°C in ambient air containing 5% CO₂. If the medium in the tubes remained clear after 18–25 h, the cultures were re-incubated and re-inspected at 48 h. Specimens with evident bacterial growth were subcultured on plates containing sheep blood agar, i.e., tryptic soy agar with 5% defibrinated sheep blood (TSAII; KOMED Co., Sungnam, Korea).

3) GBS identification

We used a catalase test followed by a latex agglutination assay (Streptex; Murex Biotech Ltd, Dartford, UK) to confirm that each isolate was GBS.

4) Antimicrobial resistance

GBS-positive samples were tested for antibiotic resistance by culturing samples of the bacteria on disks containing erythromycin and clindamycin (Sigma Chemical Co., St Louis, MO, USA) in Mueller–Hinton agar (Mueller-BAP; KOMED Co.). The size of the inhibitory zone was observed after 18–36 h of incubation. Guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS)[18] were used to interpret the disk diffusion test results.

3. Questionnaire and medical records

Participants completed a self-reported questionnaire, which included questions on general characteristics (such as education level, household monthly income, health status, smoking history, alcohol intake during pregnancy, and weight and height before and after pregnancy), obstetric characteristics (such as the number of antenatal examinations), presence of symptoms in the 2 weeks prior to the test, and disease history. Antibiotic intake during the last 2 weeks was also recorded. Information on gravidity, complications during pregnancy, delivery type, presence of ruptured membranes, and duration of membrane rupture were obtained from the medical records reviewed following delivery.

4. Statistical analysis

All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The relationships between GBS antimicrobial resistance and various risk factors were tested for statistical significance using the Chi-square test, Fisher’s exact test, and logistic regression models. To measure adjusted odds ratios, multiple logistic regression models were used: using the stepwise backward method, the final model showed statistically significant risk factors. All P-values were 2-tailed and P < 0.05 was considered to be statistically significant.

Results

Of the 5,095 pregnant women who agreed to participate in the study, 410 women had GBS colonization. A total of 728 isolates from 410 pregnant women who submitted specimens at different sites and were colonized with GBS strains were included in the analysis. Excluding duplicated isolates, a total of 418 isolates were analyzed.

Among these 418 isolates, antimicrobial resistance rates were 39.5% for clindamycin and 23.0% for erythromycin.

The sociodemographic characteristics of the study participants are shown in Table 1. The characteristics of a lower monthly household income and a lower education level were significantly associated with clindamycin resistance among the GBS isolates. Women who had higher body mass index (BMI) were more likely to be colonized with GBS strains resistant to clindamycin, although the association was not statistically significant. There were no significant differences between the general characteristics of pregnant women with erythromycin resistant strains and those without resistant strains.

The obstetric characteristics of the study participants were evaluated (Table 2). There was no association between resistance to clindamycin or erythromycin and gravidity, parity, number of previous abortions, delivery mode, antibiotic use in the 2 weeks before GBS screening, and the number of vaginal examinations or vaginal sonograms performed before the GBS screening.

Premature rupture of membranes (PROM) during the current pregnancy was related to resistance (P = 0.086 for clindamycin and P = 0.029 for erythromycin).

The events, symptoms, antibiotic use during the 2 weeks before obtaining the specimen, and history of diseases during each subject’s pregnancy and lifetime were evaluated (Table 3). Symptomatic sore throat during the previous 2 weeks was significantly associated with resistance to both clindamycin (P = 0.007) and erythromycin (P = 0.022). Influenza-like illness during the previous 2 weeks showed an association of borderline significance to clindamycin resistance (P = 0.090).

Statistically significant variables in the univariate analysis were included in multiple logistic regression models. A history of symptomatic sore throat was significantly associated with an increased risk of clindamycin or erythromycin resistance (clindamycin: odds ratio [OR]: 2.31, 95% confidence interval [CI]: 1.21 to 4.42; P = 0.011; erythromycin: OR: 2.13, 95% CI: 1.10 to 4.13; P = 0.025); education level was also significantly related to clindamycin resistance. PROM during the current pregnancy
### Table 1. Clindamycin and erythromycin resistance in *Streptococcus agalactiae* according to the general characteristics of pregnant women (35–37 weeks' gestation) in Korea (2006–2011)

| Subjects (N) | Clindamycin (N = 418) | Erythromycin (N = 418) |
|-------------|------------------------|------------------------|
|             | Resistance (N, %)      | P-value*               |
|             |                        |                       |
| Hospital    |                        |                        |
| Total       | 418                    | 165                    | 39.5 | 0.311 | 96 | 23.0 | 0.922 |
| Daejeon Eulji | 58                    | 25                    | 43.1 |      | 15 | 25.9 |      |
| Seoul Eulji  | 16                    | 5                     | 31.3 |      | 4  | 25.0 |      |
| Motae        | 41                    | 21                    | 51.2 |      | 10 | 24.4 |      |
| Cheil        | 303                   | 114                   | 37.6 |      | 67 | 22.1 |      |
| Age group    |                        |                        |      | 0.827 | 0.392 |      |
| < 25 yr      | 18                    | 8                     | 44.4 |      | 4  | 22.2 |      |
| 25–29 yr     | 89                    | 37                    | 41.6 |      | 16 | 18.0 |      |
| 30–34 yr     | 204                   | 76                    | 37.3 |      | 46 | 22.5 |      |
| ≥ 35 yr      | 102                   | 42                    | 41.2 |      | 29 | 28.4 |      |
| Missing      | 5                     | 2                     | 40.0 |      | 1  | 20.0 |      |
| BMI before pregnancy |          |                        |      | 0.195b | 0.263b |      |
| < 20 kg/m²   | 174                   | 63                    | 36.2 |      | 37 | 21.3 |      |
| 20–24 kg/m²  | 186                   | 75                    | 40.3 |      | 43 | 23.1 |      |
| ≥ 25 kg/m²   | 43                    | 20                    | 46.5 |      | 13 | 30.2 |      |
| Missing      | 15                    | 7                     | 46.7 |      | 3  | 20.0 |      |
| Household monthly income (×10,000\) | | | | 0.015 | | 0.374 |      |
| < 300        | 92                    | 38                    | 41.3 |      | 22 | 23.9 |      |
| 300–399      | 73                    | 39                    | 53.4 |      | 22 | 30.1 |      |
| 400–499      | 63                    | 20                    | 31.7 |      | 12 | 19.0 |      |
| ≥ 500        | 122                   | 39                    | 32.0 |      | 25 | 20.5 |      |
| Missing      | 68                    | 29                    | 42.6 |      | 15 | 22.1 |      |
| Education    |                        |                        |      | 0.009 | 0.363 |      |
| ≤ High school| 68                    | 35                    | 51.5 |      | 20 | 29.4 |      |
| College      | 66                    | 32                    | 48.5 |      | 17 | 25.8 |      |
| ≥ University | 255                   | 87                    | 34.1 |      | 55 | 21.6 |      |
| Missing      | 29                    | 11                    | 37.9 |      | 4  | 13.8 |      |
| Health status during pregnancy | | | | 0.612 | | 0.250 |      |
| Healthy      | 287                   | 114                   | 39.7 |      | 67 | 23.3 |      |
| Moderate     | 94                    | 34                    | 36.2 |      | 19 | 20.2 |      |
| Poor         | 12                    | 6                     | 50.0 |      | 5  | 41.7 |      |
| Missing      | 25                    | 11                    | 44.0 |      | 5  | 20.0 |      |
| Smoking during pregnancy | | | | 0.129c | | 0.376c |      |
| Never        | 302                   | 114                   | 37.7 |      | 74 | 24.5 |      |
| Second-hand  | 90                    | 38                    | 42.2 |      | 17 | 18.9 |      |
| Yes          | 5                     | 4                     | 80.0 |      | 2  | 40.0 |      |
| Missing      | 21                    | 9                     | 42.9 |      | 3  | 14.3 |      |
| Alcohol during pregnancy | | | | 0.207 | | 0.996 |      |
| Never        | 368                   | 141                   | 38.3 |      | 86 | 23.4 |      |
| Yes          | 30                    | 15                    | 50.0 |      | 7  | 23.3 |      |
| Missing      | 20                    | 9                     | 45.0 |      | 3  | 15.0 |      |

BMI, body mass index.

*P*-value obtained by the Chi-square test.

*P*-value obtained by the Chi-square test for trend.

*P*-value obtained by log-likelihood ratio test if the expected value was found to be small.
Table 2. Clindamycin and erythromycin resistance in *Streptococcus agalactiae* according to the obstetric characteristics of pregnant women (35–37 weeks’ gestation) in Korea (2006–2011)

| Subjects (N) | Clindamycin (N = 418) | Erythromycin (N = 418) |
|-------------|-----------------------|------------------------|
|             | Resistance (N, %) | *P*-value | Resistance (N, %) | *P*-value |
| No. of live neonates | 0.880 | 0.901 |
| 0 | 277 | 110 | 39.7 | 64 | 23.1 |
| ≥ 1 | 131 | 51 | 38.9 | 31 | 23.7 |
| Missing | 10 | 4 | 40.0 | 1 | 10.0 |
| No. of abortions | 0.390 | 0.299 |
| 0 | 255 | 92 | 36.1 | 59 | 23.1 |
| 1 | 88 | 39 | 44.3 | 23 | 26.1 |
| ≥ 2 | 26 | 10 | 38.5 | 3 | 11.5 |
| Missing | 49 | 24 | 49.0 | 11 | 22.4 |
| Gravidity | 0.888 | 0.596 |
| 1 | 178 | 68 | 38.2 | 43 | 24.2 |
| 2–3 | 170 | 66 | 38.8 | 39 | 22.9 |
| ≥ 4 | 21 | 7 | 33.3 | 3 | 14.3 |
| Missing | 49 | 24 | 49.0 | 11 | 22.4 |
| Antibiotics 2 wk before test | 0.605<sup>b</sup> | 0.584<sup>b</sup> |
| No | 406 | 160 | 39.4 | 94 | 23.2 |
| Yes | 10 | 4 | 40.0 | 2 | 20.0 |
| Missing | 2 | 1 | 50.0 | 0 | |
| No. of prenatal vaginal exams | 0.461 | 0.808 |
| 0 | 43 | 13 | 30.2 | 8 | 18.6 |
| 1–2 | 112 | 46 | 41.1 | 26 | 23.2 |
| ≥ 3 | 39 | 15 | 38.5 | 8 | 20.5 |
| Missing | 224 | 91 | 40.6 | 54 | 24.1 |
| No. of prenatal vaginal sonograms | 0.324<sup>c</sup> | 0.905<sup>c</sup> |
| 0 | 6 | 2 | 33.3 | 2 | 33.3 |
| 1–2 | 99 | 42 | 42.4 | 23 | 23.2 |
| 3–4 | 70 | 25 | 35.7 | 16 | 22.9 |
| ≥ 5 | 45 | 12 | 26.7 | 9 | 20.0 |
| Missing | 198 | 84 | 42.4 | 46 | 23.2 |
| Delivery type | 0.513 | 0.781 |
| Vaginal | 284 | 114 | 40.1 | 65 | 22.9 |
| Cesarean section | 120 | 44 | 36.7 | 29 | 24.2 |
| Missing | 14 | 7 | 50.0 | 2 | 14.3 |
| PROM | 0.086 | 0.029 |
| No | 324 | 120 | 37.0 | 68 | 21.0 |
| Yes | 80 | 38 | 47.5 | 26 | 32.5 |
| Missing | 14 | 7 | 50.0 | 2 | 14.3 |
| Duration of PROM | 0.626 | 0.917 |
| < 18 h | 42 | 20 | 47.6 | 14 | 33.3 |
| ≥ 18 h | 28 | 15 | 53.6 | 9 | 32.1 |
| Missing | 10 | 3 | 30.0 | 3 | 30.0 |

PROM, premature rupture of membranes; 
*P*-values were obtained with the Chi-square test.
*P*-values were obtained with the Fisher’s exact test.
*P*-values were obtained with the log-likelihood ratio test if the expected value was found to be small. Missing data were not included when calculating statistics.
had an association of borderline significance to both clindamycin and erythromycin resistance (Table 4).

### Discussion

In the 15 years after the US Centers for Disease Control and Prevention (CDC) issued guidelines for the use of intrapartum antibiotic prophylaxis to prevent neonatal early-onset GBS disease [4, 19, 20], many investigators have reported an increase in the incidence of erythromycin and clindamycin resistance among both GBS, colonizing and invasive disease isolates. The aim of this study was to identify factors associated with antimicrobial resistance of GBS; thus, we assumed that these factors would be relevant to GBS strains isolated from individuals with similar characteristics. This study showed that antibiotic resistance rates to clinda-
mycin have remained high (35% in a previous study [16] vs. 39.5% in this study); however, the rate was lower than that of an earlier report from Korea (48.4%) [17]. Unlike reports from the US, Canada, and Germany, resistance to clindamycin exceeded that to erythromycin (39.5% vs. 23.0%) [21-23].

Many risk factors, including lower monthly household income, lower education level, symptomatic sore throat, influenza-like illness in the previous 2 weeks, and PROM, were found to be associated with resistance to clindamycin or erythromycin.

In our multiple logistic regression model, the only factor that was identified as being predictive of resistance to both clindamycin and erythromycin was a history of sore throat in the previous 2 weeks. It is possible that pregnant women with a sore throat are more likely to seek medical care than they would be when not pregnant, and are thus more likely to be exposed to antibiotics. Even though the proportion of antibiotic use in sore throat group was higher than that of women without sore throat (4.3% vs. 2.2%), the association between sore throat and antibiotic use was not statistically significant (P = 0.317). However, only 10 people reported the use of antibiotics, substantially reducing the statistical power; this fact is a limitation of this study. There is general consensus that the increasing antibiotic pressure on the bacterial ecosystem—namely, previous exposure to antibiotics—is the most important factor in the emergence of antibiotic resistance [24-26].

Although we failed to find a correlation between symptomatic sore throat and antibiotic use, antibiotic use even for appropriate indications will continue to exert selective pressure, favoring drug-resistant strains. Therefore, antibiotics should be prescribed with caution and in a manner that minimizes the risk of the emergence of drug-resistant strains. However, whether the achievable reductions can have a measurable and durable impact on resistance rates remains uncertain.

The correlation between antibiotic use and resistance is not always straightforward, since multiple confounding factors can cause interference; the diversity of confounding factors requires more in-depth analysis. Thus, few studies have evaluated the relationship between antibiotic use or dosing regimens and the emergence of resistance in clinical studies.

The association between PROM and clindamycin and erythromycin resistance was also of borderline significance. In an earlier study, GBS colonization in pregnant women was not related to PROM [27]. Therefore, among GBS isolates, antimicrobial resistance among GBS may be a risk factor for PROM.

In conclusion, GBS isolates from GBS-colonized pregnant

Table 4. Risk of Streptococcus agalactiae antimicrobial resistance in pregnant women (35–37 weeks’ gestation) in Korea (2006–2011) using a multiple logistic regression model

| Variables of final model | Subjects (N) | Resistance (%) | Unadjusted OR | Adjusted OR* | 95% CI | P-value |
|--------------------------|-------------|----------------|--------------|--------------|--------|---------|
| **Clindamycin**          |             |                |              |              |        |         |
| Sore throat              |             |                |              |              |        |         |
| No                       | 367         | 37.1           | 1            | 1            |        |         |
| Yes                      | 47          | 57.4           | 2.29         | 2.31         | 1.21-4.42 | 0.011   |
| PROM                     |             |                |              |              |        |         |
| No                       | 324         | 37.0           | 1            | 1            |        |         |
| Yes                      | 80          | 47.5           | 1.54         | 1.64         | 0.97-2.76 | 0.065   |
| Education                |             |                |              |              |        |         |
| ≤ High school            | 68          | 51.5           | 2.05         | 2.14         | 1.22-3.77 | 0.008   |
| College                  | 66          | 48.5           | 1.82         | 1.78         | 1.01-3.16 | 0.047   |
| ≥ University             | 255         | 34.1           | 1            | 1            |        |         |
| **Erythromycin**         |             |                |              |              |        |         |
| Sore throat              |             |                |              |              |        |         |
| No                       | 367         | 21.3           | 1            | 1            |        |         |
| Yes                      | 47          | 36.2           | 2.10         | 2.13         | 1.10-4.13 | 0.025   |
| PROM                     |             |                |              |              |        |         |
| No                       | 324         | 21.0           | 1            | 1            |        |         |
| Yes                      | 80          | 32.5           | 1.81         | 1.63         | 0.93-2.85 | 0.089   |

PROM, premature rupture of membranes; OR, odds ratio; CI, confidence interval.

Full model included PROM, education, sore throat, influenza-like illness (ILI) and interaction term for sore throat and ILI.

*Backward stepwise method was used.
women showed high resistance rates to the second-line antibiotics, such as clindamycin and erythromycin. Furthermore, GBS resistance rates were higher in those with a history of sore throat in the previous 2 weeks and those with PROM in the current pregnancy. Therefore, when GBS-colonized pregnant women need urgent antibiotic treatment to prevent GBS-associated neonatal sepsis in circumstances where no information on antimicrobial resistance is available, the subject’s history of sore throat and PROM should be considered.

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Conflicts of Interest

No conflicts of interest.

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