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ORIGINAL ARTICLE

Prevalence of Group B *Streptococcus* Colonization in Pregnant Women at a University Hospital in Korea

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SUMMARY

Background: **Group B *Streptococcus* (GBS) colonization in pregnant women is a risk factor for causing infection in neonates; therefore, GBS screening tests are performed on them. Culture methods and molecular diagnostics are mainly performed for GBS detection; however, culture methods differ in the detection rate for GBS depending on the procedure of culture. The authors intended to confirm the difference in GBS colonization rate in the conventional culture method, enrichment culture method, and molecular genetic test as screening tests for GBS.**
Methods: **Duplicate vagino-rectal swabs were collected from 371 pregnant women between the 35th and 37th week of gestation; one was used for conventional culture method and the other was frozen at -80℃, followed by enrichment culture method and molecular genetic test.**
Results: **The prevalence of GBS colonization identified by conventional culture, enrichment culture, and molecular genetic test was 4.35% (17/391), 8.95% (35/391), and 22.25% (87/391), respectively. The detection rate by enrichment culture method was 2.06 times higher (17/391 vs. 35/391) than that by conventional culture method. It was identified that there was a significant difference in the detection rates of GBS between the two methods ($p < 0.001$). The detection rate identified in molecular genetic test was much higher at 22.25% (87/391). The concordance rate of the results from three detection methods for GBS was 80.05% (313/391). All pregnant women colonized with GBS were given intrapartum antibiotic prophylaxis using cefazolin and their neonates were confirmed not to be infected with GBS.**
Conclusions: **Prevalence of GBS colonization in pregnant women is shown to vary depending on detection method. Particularly, it differs greatly depending on the use of enrichment media in the culture method. Therefore, it is necessary that the microbiological laboratory implements the culture method with supplementary procedures such as selective or enrichment media in order to improve the detection rate of GBS.**
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KEY WORDS

group B *Streptococcus*, pregnant women, prevalence, GBS colonization, enrichment culture

INTRODUCTION

Group B *Streptococcus* (GBS), also called as *Streptococcus agalactiae*, is a kind of Gram-positive cocci and belongs to a species of pyogenic streptococci [1].
GBS is still an important pathogen in high-risk groups,

including the elderly, pregnant women, neonates, and immunocompromised patients [2,3].

GBS colonizes genitourinary and gastrointestinal tracts of pregnant women without causing any clinical symptom [3,4]. Globally, the rates of asymptomatic rectovaginal colonization in pregnant women are known to vary widely, from 5% to 30%, depending on the region and population [2,4-7]. In addition, the incidence of the invasive diseases caused by GBS in pregnant women was reported to be twice as that of non-pregnant women [8]. In neonates, early-onset GBS disease is one of the infections caused by vertical transmission of GBS in colonized pregnant women, and is related to high morbidity [3]. Therefore, the screening tests for GBS in pregnant women by culture method or molecular diagnostics are currently a recommended practice by the revised CDC guidelines [9]. The incidence of neonatal GBS infection has been reported to be reduced prominently through intrapartum antibiotic prophylaxis of pregnant women when GBS had been detected [2-4].

The colonization of GBS can be intermittent or fluctuate depending on the trimester of pregnancy, hence, screening tests for GBS are usually conducted at a fixed period, between 35 and 37 weeks of gestation [2,3,9-11]. In addition, the GBS colonization rate was also reported to be affected by the sample type. It has been reported to be preferable to perform both vaginal and rectal swabs at the same time than to perform a vaginal swab only [3, 4,11].

Conventionally, the culture method has been used as a screening test for GBS. To increase sensitivity, supplementary methods that can increase detection rates of GBS such as selective or enrichment media have been used. Now, as molecular techniques have been developed, they also have been implemented as screening tests for GBS. The culture method using enrichment broth has been proposed as the gold standard method of GBS screening test in pregnant women. However, the authors' laboratory has not performed GBS screening by using selective media or enrichment broth due to the high cost of the test. In 2018 and 2019, it was confirmed that the prevalence of GBS in pregnant women at this laboratory was 5.81% (10/172). Furthermore, it was noted that the prevalence reported from culture methods using supplementing methods in Korea were 11.6 - 19.5% [12,13], which were higher than that of this laboratory.

Therefore, the authors intended to confirm the difference in GBS colonization rate in the conventional culture method, enrichment culture method, and molecular genetic test as screening tests for GBS.

MATERIALS AND METHODS

Antepartum vagino-rectal swab specimens

Women between the 35th and 37th week of pregnancy were included in this study for screening of maternal GBS colonization. Approval from the Institutional Review Board of Kyungpook National University Chilgok Hospital was obtained prior to the beginning of the study. Written informed consent including permission for use of the specimens and review of medical records for research purposes were also obtained from all subjects.

The vagino-rectal swab specimens were collected according to the CDC recommendations [9]. Two swab specimens were collected from each subject, and each was put in a Liquid Stuart Transport Medium (Becton Dickinson, Sparks, MD, USA). One was used right after collecting it for conventional culture method and the other was cryopreserved at -80℃ and used for enrichment culture method and molecular genetic test later.

Conventional culture method

The swab specimen in a Liquid Stuart Transport Medium (Becton Dickinson) was inoculated directly onto 5% sheep blood agar plate (BAP, ASAN PHARM. CO., LTD., Seoul, South Korea) and incubated aerobically overnight at 37℃. The suspected β-hemolytic or non-hemolytic colonies were sub-cultured onto BAP and incubated under the same conditions as above. After that, the isolate was suspected as GBS based on Gram stain and catalase test. Finally, it was confirmed using the Vitek2 Gram-positive identification system (bioMérieux, Marcy l'Etoile, France) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Billerica, MA, USA). In addition, when necessary, we also performed 16S rRNA sequencing by ABI 3730*xl* DNA analyzer (Applied Biosystems, Foster City, CA, USA) to confirm the exact bacteria.

Enrichment culture method

The swab specimens cryopreserved at -80℃ were thawed and inoculated in a LIM broth enrichment medium (Becton Dickinson, Franklin Lakes, NJ, USA). The media tubes with loosened caps were incubated at 37℃ in an aerobic atmosphere for 18 - 24 hours, and then the broth was sub-cultured onto 5% BAP and incubated under the same conditions of the conventional culture. The rest of the process was identical to the conventional culture method.

Molecular genetic test

The BD MAX GBS assay (Becton Dickinson) was performed through real-time polymerase chain reaction for GBS. It was conducted automatically from nucleic acid extraction to the final report of the result according to the manufacturer's instructions using 15 μL aliquot of LIM broth enrichment medium (Becton Dickinson) after appropriate incubation as mentioned in enrichment culture method.

Statistical analysis

Data were analyzed using Microsoft Excel (2013) (Microsoft Corporation, Redmond, WA, USA) with Analyse-it Ver. 5.50 (Analyse-it Software Ltd., Leeds, UK).

Table 1. Prevalence of Group B *Streptococcus* colonization in pregnant women according to the detection methods.

Result	Conventional culture	Enrichment culture	Molecular genetic test
Positive	**17 (4.35%)**	**37 (9.46%)**	**87 (22.25%)**
Negative	**374 (95.65%)**	**354 (90.54%)**	**304 (77.75%)**
Total	**391**		

Table 2. Comparison between conventional culture and enrichment culture methods.

		Conventional culture		
		Positive	**Negative**	**Total**
Enrichment culture	**Positive**	**16**	**21**	**37**
	Negative	**1**	**353**	**354**
	Total	**17**	**374**	**391**

The Fisher's exact test showed a statistically significant difference with a p-value of < 0.001.

Table 3. Comparison between conventional culture method and molecular genetic test.

		Conventional culture		
		Positive	**Negative**	**Total**
Molecular genetic test	**Positive**	**13**	**74**	**87**
	Negative	**4**	**300**	**304**
	Total	**17**	**374**	**391**

The Fisher's exact test showed a statistically significant difference with a p-value of < 0.001.

Table 4. Comparison between enrichment culture method and molecular genetic test.

		Enrichment culture		
		Positive	**Negative**	**Total**
Molecular genetic test	**Positive**	**34**	**53**	**87**
	Negative	**1**	**303**	**304**
	Total	**35**	**356**	**391**

The Fisher's exact test showed a statistically significant difference with a p-value of < 0.001.

It was also analyzed using the Fisher's exact test and chi-squared test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 391 women aged from 16 to 44 (mean ± SD, 32.43 ± 4.21) years old were examined for the methods used in screening of GBS colonization.

The prevalence of GBS colonization identified by each detection method is shown in Table 1. It was 4.35%

(17/391) by conventional culture method. On the other hand, it was 8.95% (35/391) by LIM broth enrichment culture method. It was noticed that there was a significant difference in the detection rates of GBS between the two methods ($p < 0.001$). The detection rate by enrichment culture method was 2.06 times higher (35/391 vs. 17/391) than that by conventional culture method. The comparison results between the conventional culture and the enrichment culture methods are shown in Table 2. The discrepancy between the two methods was found to be 5.63% (22/391).

However, the detection rate identified in the molecular genetic test was much higher at 22.25% (87/391). It was also found that there were significant differences in the detection rates between conventional culture method and molecular genetic test and between enrichment culture method and molecular genetic test, respectively ($p < 0.001$ and $p < 0.001$) (Table 3 & 4). The concordance rate of the results from three detection methods for GBS was 80.05% (313/391).

When the molecular genetic test was used as the gold standard, positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of the conventional culture method were 76.47% (13/17), 80.21% (300/374), 14.94% (13/87), and 98.68% (300/304), respectively (Table 3). For the enrichment culture method, they were 97.14% (34/35), 85.11% (303/356), 39.08% (34/87), and 99.67% (303/304), respectively (Table 4).

All pregnant women colonized with GBS were given intrapartum antibiotic prophylaxis using cefazolin and their neonates were confirmed not to be infected with GBS.

DISCUSSION

The capabilities to detect GBS and its prevalence in pregnant women were reported to vary depending on the time of specimen collection, the specimen type, the sampling techniques, the implemented detection method, and performing urine culture for GBS at the same time [10,11,14-17]. GBS bacteriuria in pregnancy is known to be an indicator for heavy vagino-rectal GBS colonization [10]. In addition, it was also reported that GBS colonization in pregnant women can be intermittent or transitory [18]. At present, many medical institutions perform GBS screening tests in pregnant women to reduce GBS infections in neonates according to the CDC recommendation [9]. In other words, vagino-rectal swab samples are routinely being collected from pregnant women at 35th - 37th weeks of gestation for GBS screening following the recommended guidelines.

However, our laboratory has only been conducting conventional culture method without using enrichment broth or selective agar media due to the high cost of testing. The prevalence of GBS in pregnant women in this hospital was shown to be low at 5.81% (9/176) compared to those of other reports in Korea (11.6% and 19.8%) [12,13]; therefore, the enrichment culture was additionally conducted to identify the difference according to the culture methods.

It was confirmed that the detection rate by enrichment culture method was 2.06 times higher (35/391 vs. 17/391) than that by conventional culture. When the molecular genetic test was used as the gold standard method, it was also confirmed that the enrichment culture method showed higher PPV, NPV, sensitivity, and specificity than the conventional culture method. Therefore, it was determined that the GBS detection by culture method needs to be supplemented with other procedures such as the enrichment media and the use of selective media.

Culture method for GBS detection usually takes two to three days, and it also requires trained medical staff who can select the proper colonies and perform the bacterial identification test. In addition, it differs according to the use of the selective or enrichment media, the proficiency of the examiner, and the variation of the sample. Due to the disadvantages of the culture method, the molecular genetic test has recently been used as a GBS screening test. Compared to the culture method, molecular genetic tests are reported to have significantly higher detection rates and the short turn-around time (TAT) [1,11,19-21].

Molecular diagnostic tests including the BD MAX GBS assay (Becton Dickinson) and loop-mediated isothermal amplification are efficient methods to detect GBS through shortening TAT and personnel labor [1,22]. CDC recommends that the molecular diagnostic tests for GBS screening should be conducted following overnight incubation of inoculated enrichment broth to increase the sensitivity for GBS detection [9]. In this study, the detection rates of GBS by the molecular genetic test were found to be 5.1 and 2.5 times higher than those of the conventional culture and the enrichment culture, respectively.

We encountered four samples which showed a discrepancy between the positive result in the culture test and the negative result in the molecular genetic test. Among these, the two samples stored in a frozen state were cultured repeatedly. As a result, GBS was detected in one of them, and the identification test for the isolate was performed with 16S rRNA sequencing method, to confirm that it was GBS. In other words, the sample, for which sequencing analysis was performed, showed positive results in both of the two culture methods, but negative in molecular genetic test. This result was different from the report that the positive cases in the culture test also showed 100% positive results in the molecular genetic test using the BD MAX GBS assay (Becton Dickinson) [20]. This may be partially due to variation in the samples as this procedure was conducted separately after the two swab samples were collected from one subject. However, considering that the sensitivity of the molecular genetic testing is much higher than that of the culture test, it is difficult to easily explain the reason for the discrepancy.

CONCLUSION

Our laboratory has been conducting GBS screening tests in pregnant women to reduce GBS infections in neonates. However, only the conventional culture method was performed to detect GBS in vagino-rectal swabs. The comparison analyses were conducted to confirm the difference in GBS colonization rate in the conventional culture method, enrichment culture method, and molecular genetic test as screening tests for GBS. The results showed significant differences between the conventional culture and the enrichment culture methods, and those compared with the molecular genetic test showed greater difference. Therefore, the prevalence of GBS colonization in pregnant women was found to vary depending on detection method.

In conclusion, since GBS screening test in pregnant women is known to reduce early-onset neonatal disease, the microbiological laboratory needs to implement the culture method using selective or enrichment media in order to improve detection rate of GBS.

Ethical Approval:
This study was approved by the Institutional Review Board of Kyungpook National University Chilgok Hospital, Daegu, Korea (IRB File No.: KNUCH 2018-01-007, KNUCH 2020-09-001).

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Declaration of Interest:
None.

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