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## ORIGINAL PAPER / GYNECOLOGY

### **Comparison of Polymerase Chain Reaction Method with Culture Method in Antenatal Group B Streptococcus Screening**

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#### **ABSTRACT**

**Objectives:** The aim of this study was to investigate the prevalence of *Group B Streptococcus* (GBS) colonization in pregnancies between 35 and 37 weeks of gestation and to compare the

effectiveness of polymerase chain reaction (PCR) method with gold standard technique of culture in antenatal GBS screening.

**Material and methods:** Vaginal and rectal swabs of a total of 106 pregnant women between 35<sup>th</sup> and 37<sup>th</sup> weeks of gestation, who were admitted to our clinic between January 2022 and August 2022, were evaluated using culture and PCR method. The prevalence of GBS was estimated. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the PCR method were analyzed.

**Results:** The prevalence of GBS was 10.4% and 21.69% using the culture and PCR method, respectively. Compared to the culture, the sensitivity, specificity, PPV, NPV and accuracy of PCR were found to be 100%, 87%, 47%, 100%, and 88%, respectively.

**Conclusions:** This study results suggest that the PCR method is a simple, effective and fast method with high sensitivity, specificity, PPV, and NPV in antenatal GBS screening.

**Keywords:** *Group B Streptococcus*; antenatal screening; culture; polymerase chain reaction

## INTRODUCTION

*Streptococcus agalactiae*, also known as *Group B Streptococcus* (GBS), is a facultative gram-positive bacterium. This microorganism naturally resides in the gastrointestinal and vaginal microbiome of some women. However, the potential pathogenic properties of GBS can lead to serious infections, especially during pregnancy and the neonatal period. This demonstrates that GBS is both a commensal and a pathogenic microorganism [1]. Maternal colonization is the primary risk factor for GBS infection in neonates and young infants and GBS infection in neonates and young infants is classified as early, late, and very late onset. The early onset may be due to rupture of membranes, intraamniotic infection, or vaginal transmission in labor, occurring within 24 hours to 6 days postpartum and leading to generalized sepsis, pneumonia, meningitis, or pulmonary hypertension. Late onset, usually 7 to 89 days, presents as bacteremia, resulting in meningitis, pneumonia, septic arthritis and osteomyelitis. A very late onset is usually seen in infants older than 90 days [2].

In a meta-analysis including 37 countries in 2016, the prevalence of GBS was reported varied between 6.8 and 26.7% [3]. GBS screening for pregnant women is recommended between 36

0/7 and 37 6/7 weeks of gestation [4, 5] or 3 to 5 weeks before the expected delivery date [6]. Thanks to screening strategies, the incidence of infant GBS has decreased from 1.7 cases per 1000 live births to 0.5 cases per 1000 live births in (within) the last 15 years [7].

After 18 to 24 hours of incubation on blood agar at 37°C, a narrow zone of hemolysis forms large, oval-shaped, mucoid, gray-white colonies [8], which is evaluated after 24 hours of incubation and re-evaluated for another 24-hour incubation, if no growth occurs [9]. Rectovaginal swab specimens taken for screening in pregnant women should be cultivated in selective broth media, as they can be only directly identified with sheep blood agar, when there is extensive colonization [10]. The Centers for Disease Control and Prevention (CDC) recommends the use of Todd-Hewitt broth supplemented with either colistin, nalidixic acid, or gentamicin + nalidixic acid to suppress normal flora elements [11]. Techniques such as indirect immunofluorescence, reverse immunoelectrophoresis, staphylococcal coagglutination, and enzyme-linked immunosorbent assay (ELISA), most commonly latex agglutination test, are used to identify antigenic structures specific to GBS [12]. Molecular methods, such as real-time polymerase chain reaction (PCR) are used for rapid identification of GBSs and are commercially available [13]. Thus, genome sequencing, serotype, and antimicrobial resistance can be determined easily [14].

According to the ACOG and ASM guidelines, nucleic acid amplification testing (NAAT) can be also used as a rapid test for the detection of GBS with equivalent detection rates to culture-based screening [4, 5, 15]. For further detection, it is recommended that all vaginal and rectal swabs should be inoculated into selective enrichment broth medium and incubated for 18 to 24 hours at 35 to 37°C, 5% carbon dioxide (CO<sub>2</sub>) conditions. Intrapartum NAAT without enrichment has a high false-negative rate ranging from 6.3 to 22%. Therefore, the use of intrapartum NAAT without enrichment is not recommended to rule out the need for prophylaxis. Vaginal and rectal specimens should be collected using a flocked swab and placed in a liquid-based transport medium such as Amies transport medium. Vaginal and rectal specimens should be transported to the testing laboratory within 24 hours [5].

In the literature, although it is recommended to perform screening with culture, it has certain drawbacks, such as yielding results within 24 to 48 hours and producing false-negative results in low colony counts [16]. In the present study, authors hypothesized that PCR, a rapid test, could be routinely used to screen for antenatal GBS. Therefore, aimed to investigate the prevalence of GBS between 35 and 37 weeks of gestation and to compare the effectiveness of culture and PCR method in antenatal GBS screening.

## **MATERIAL AND METHODS**

### **Study design and study population**

This single-center prospective study was conducted at the Department of Obstetrics and Gynecology of a tertiary care center between January 2022 and August 2022. A written informed consent was obtained from each participant. The study protocol was approved by the institutional Ethics Committee (No: 340 and Date: 26/05/2021). The study was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 106 antenatal pregnant women between the 35<sup>th</sup> and the 37<sup>th</sup> weeks of gestation were included in this study. The mean age of the patients was  $29.14 \pm 5.4$  (range 17 to 40) years. The mean gestational age was  $35.96 \pm 0.62$  (range 35.0 to 37.0) weeks. Among all women who applied to our clinic for a routine pregnancy control between January 2022 and August 2022, those with a pregnancy less than 35 weeks or older than 37 weeks, those who received antibiotherapy in the last month, had bleeding or refused to participate in the study were excluded. Data including demographic and clinical characteristics of the patients, gravidity, parity, number of abortions, previous cesarean-section (C/S) delivery and vaginal delivery, week of gestation, the presence of comorbidities such as gestational hypertension (GHT), gestational diabetes (GDM), and type 2 diabetes, smoking and alcohol use, education status and employment status were recorded. Demographic and clinical characteristics of the patients at the time of vaginal and rectal sampling are shown in Table 1.

### **Sample collection method**

Vaginal and rectal swabs were collected without speculum. A single swab was used to obtain the specimen first from the lower vagina and then, from the rectum. The specimen first from the vagina (near the introitus) was collected by inserting the swab about 1.5 to 2 cm and then, from the rectum by inserting the same swab 1 cm through the anal sphincter. The vaginal and rectal specimens in a single medium (Stuart's transport medium) were transported to the testing laboratory immediately. The specimens were analyzed using both the culture and PCR method separately.

### **Culture method**

All vaginal and rectal swabs were inoculated into the sheep blood agar (BD, Heidelberg, Germany) and incubated at 35 to 37°C in 5% CO<sub>2</sub> conditions.

The culture plates were examined at 24 and 48 hours. The Columbia agar has a high starch content and, thus, beta-hemolytic streptococci may show alpha rather than beta-hemolytic reactions or may exhibit weak hemolytic reactions on media based on this formulation [9]. Therefore, the culture plates were assessed at 24 and 48 hours after incubation and large, gray, translucent colonies with or without narrow beta-hemolysis were examined. In suspected cases, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Biotyper®, USA) was used. After MALDI-TOF MS testing, colonies with GBS results were considered culture-positive.

### **BD™ MAX™ GBS PCR testing**

After inoculation, the swabs were placed in selective LIM medium (Todd-Hewitt broth supplemented with 10 µg/mL of colistin and 15 µg/mL of nalidixic acid). The LIM medium (BD GmbH, Germany) was incubated at 37°C in 5% CO<sub>2</sub> conditions for minimum 18 hours.

### **Sample preparation**

After incubation, the specimens in the LIM medium were vortexed. Using a pipette and a long pipette tip, 15 µL of the specimen was aspirated from the LIM medium and mixed with the sample preparation reagent included in the BD MAX GBS assay kit (BD GmbH, MD, Germany). A homogeneous mixture was obtained by pipetting several times. A GBS Master Mix, a GBS extraction reagent, and a BD MAX GBS unitized reagent strip included in the assay were used for each sample to be tested. The samples placed on racks in accordance with the manufacturer's recommendations were placed in the BD MAX device (BD GmbH, Germany). The results were recorded, and the device was operated.

### **Interpretation of results**

Test results were automatically interpreted by the BD MAX System software as NEG (-), POS (+) or IND (indeterminate). Tests with positive results were interpreted as GBS DNA detected, negative results as GBS DNA not detected and IND results as PCR reaction, reagent failure or no sample process control amplification. Samples with IND results were re-run by applying the sample preparation procedure. Again, the examples that resulted in IND were indicated.

### **Statistical analysis**

Statistical analysis was performed using the SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD),

median and interquartile range (IQR) or number and frequency, where applicable. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the PCR method were analyzed.

## **RESULTS**

The vaginal and rectal swabs were obtained from the patients and were analyzed using both the culture and PCR method, separately. The results are presented in Table 2. Accordingly, the prevalence of GBS was 21.69% (n = 23) using the PCR method.

Compared to the culture, the sensitivity, specificity, PPV, NPV and accuracy of the PCR are given in Table 3. Accordingly, these values were found to be 100%, 87%, 47%, 100% and 88%, respectively.

The records of the patients who underwent GBS screening were examined after delivery and those were called by phone to complete missing data and none of the infants were diagnosed with early-onset GBS.

## **DISCUSSION**

The colonization of GBS varies depending on race, geographical region, and sociocultural factors with a rate ranging from 4 to 40% in the literature [17]. The carriage rate has been reported as ranging from 0.4 to 32% in Türkiye [18, 19].

The gold-standard method for GBS screening is the culture method and many studies have compared the culture method with the PCR method. In a study, during the third trimester of pregnancy, the culture result was positive in 18.4% vaginal samples and positive in 18.1% rectal samples, while PCR yielded positive results in 22.6% vaginal samples and positive in 21.2% rectal samples [20]. The authors concluded that PCR was able to identify more colonized pregnant women than culture and was a fast and useful screening method with a shorter detection time.

In the light of these data, in this study, the authors compared the culture method with the PCR method, which can yield faster results and can also work with low colony counts. The prevalence of GBS was 10.4% and 21.69% using the culture and PCR method, respectively. The samples obtained were enriched with the LIM broth. Although this allowed to obtain

results with higher sensitivity in both culture and PCR methods, time to enrichment limited the ability to obtain rapid results.

In a study including 204 pregnant women, the rate of GBS was found to be 26% with the PCR method and 22% with the culture method using vaginal and perianal swabs, and the sensitivity, specificity, PPV, and NPV were reported as 100%, 95.6%, 86.8% and 100%, respectively [21]. A high NPV, fast results, and high sensitivity are desirable characteristics of a screening test. In this study, the sensitivity of the PCR method was found to be 100%, specificity 87%, PPV 47%, NPV 100%, and accuracy 88%. Even if it is used with enrichment, its main advantages are that it yields results within 12 to 24 hours, compared to culture method and can detect smaller colonies. A PPV of 47% in this study can be attributed relatively low prevalence compared to previous studies.

In addition, although we consider the culture method as the gold standard, sensitivity may decrease due to reasons such as the need for living bacteria in the sample obtained and the overgrowth of microbiota-derived organisms that can inhibit the growth of GBS in the presence of a small number of living bacteria. The prevalence of GBS colonization may have been higher by the PCR than the culture method, as PCR can only detect bacterial genes and not viable bacterial colonies and, therefore, the culture method cannot detect them.

In the literature, there are several studies in which rectal and vaginal swabs are obtained separately or combined. In most studies comparing individual swabs, the sensitivity and specificity of both culture and PCR are reduced, particularly in vaginal specimens [16, 22]. According to these results, rectal sample culture seems to be more effective than vaginal culture [16]. In other words, there is a need for vaginal-rectal sampling to increase the chance of GBS isolation more effectively. In this study, vaginal and rectal sampling was performed.

The culture technique is a time-consuming method requiring at least 48 hours for the complete identification of GBS, whereas PCR is a sensitive and accelerated technique for detecting GBS with results available within 3 hours [16]. Compared to the culture method, PCR can be a fast and effective screening and diagnostic method with high sensitivity and NPV, and the ability to identify even low numbers of colonies, as it focuses on genetic material.

In Türkiye, there is no antenatal and/or intrapartum GBS screening guide for pregnant women issued by the Republic of Türkiye, Ministry of Health. By virtue of its focus on genetic material, PCR serves as a rapid and reliable screening and diagnostic tool characterized by



high sensitivity and a strong negative predictive value (NPV). However, this test cannot determine antibiotic susceptibility. Within the population, the relatively high prevalence of penicillin intolerance, a mainstay of empiric therapy, may limit the test's applicability.

Two scenarios for employing the test can be considered:

1. Prenatal Screening: PCR may not be a cost-effective alternative for culture performance with positive results. This means that traditional culture tests might still be necessary for confirming positive results from PCR screening.

2. Intrapartum Testing: For patients who have not undergone prenatal screening, PCR can provide a rapid result. This can be particularly useful in emergency situations, where quick diagnostic results are needed. However, the relatively high cost of PCR limits the utilization of this screening test in our country.

### **Strengths and limitations**

The design of this study was prospective, but the number of cases was small and GBS typing was not performed.

### **CONCLUSIONS**

In conclusion, the prevalence of GBS detected by the culture and PCR methods in this study seems to be compatible with the national and global data. In Türkiye, there is no antenatal and/or intrapartum GBS screening guide for pregnant women issued by the Ministry of Health.

The PCR testing is a fast and effective method with high sensitivity, specificity, and NPV and can be used for GBS screening in pregnant women. However, enrichment with the LIM broth for PCR is a factor that limits obtaining rapid results. The authors believe that GBS screening in pregnant women would be faster and more effective with the use of molecular methods such as PCR, which can be studied directly from clinical samples with high sensitivity, specificity, and NPV, with faster results and lower cost, thanks to emerging technologies.

### **Article information and declarations**

#### **Data availability statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### **Ethics statement**

This study was approved by the Akdeniz University, Faculty of Medicine, Clinical Research Ethics Committee with the Approval No: 340 and Date: 26/05/2021.

### **Author contributions**

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Dođukan opur, zlem Koyuncu zyurt, and Hlyya Kandemir. The first draft of the manuscript was written by Hlyya Kandemir, Dođukan opur, and Prof. Dr. Dilara gn. Significant revisions were made by Prof. Dr. Dilara gn and İncanc Mendilciođlu. All authors read and approved the final manuscript.

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### **Conflict of interest**

The authors have no relevant financial or non-financial interests to disclose.

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**Table 1.** Demographic and clinical characteristics of patients at the time of vaginal and rectal sampling

Variable	
Age, year, median (IQR)	29 (8)
Body weight, kg median (IQR)	74 (17)
Height, cm median (IQR)	162 (9)
BMI, kg/m <sup>2</sup> , median (IQR)	28.45 (5.06)
Gravidity, n median (IQR)	2 (2)
Parity, n, median (IQR)	1 (2)
Abortion, n median (IQR)	0 (1)
Previous C/S delivery, median (IQR)	0 (1)
Previous vaginal delivery, n, median (IQR)	0 (1)
Gestational age, week, median (IQR)	36.05 (1.10)
GHT, n (%)	3 (2.8)
GDM, n (%)	9 (8.4)
T2DM, n (%)	1 (0.94)
Smoking, n (%)	16 (15.09)
Alcohol use, n (%)	1 (0.94)
Education up to high school, n (%)	74 (69.8)
Undergraduate education, n (%)	32 (30.1)
Employment with income, n (%)	40 (37.7)

IQR— Interquartile Range; BMI— body mass index; C/S — cesarean delivery, GHT — gestational hypertension; GDM — gestational diabetes; T2DM — type 2 diabetes mellitus

**Table 2.** Comparison of culture and PCR results of vaginal and rectal samples

		Culture method		Total (n)
		Positive (n)	Negative (n)	
PCR method	Positive (n)	11	12	23
	Negative (n)	0	83	83
Total (n)		11	95	106

PCR — polymerase chain reaction

**Table 3.** Characteristics of PCR method compared to gold standard of culture method

<b>Variable</b>	<b>%</b>
Sensitivity	100%
Specificity	87%
PPV	47%
NPV	100%
Accuracy	88%

PCR — polymerase chain reaction; PPV — positive predictive value; NPV — predictive value