Comparison of culture and PCR methods for diagnosis of group B *Streptococcus* in women

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Group B Streptococcus (GBS), as a risk factor for newborns, is one of the most important causes of meningitis and septicaemia in newborns, intrauterine infections in women and colonization in the vaginal region in late gestation. We evaluate GBS prevalence and infection among pregnant women through culture and PCR methods, and then compare these methods. To this end, vaginal and urine swabs were separately collected from 246 women at the Women's Hospital of Sari, Iran. Next, the samples were enriched in selective culture media Todd–Hewitt broth for 24 h at 37°C, recognized using blood agar media, and finally were amplified STREP gene by PCR technique. The indicated that the frequency of GBS in samples collected from urine and vaginal cultures and PCR method was positive. In addition, no significant relationship was found among the positive results of culture, maternal age, gestational age, a history of abortion and infection. With regard to the cultivation method as a standard technique, the sensitivity of PCR test was 100% and specificity was 96%. Moreover, it was found that the colonization rate of GBS in women was significant in Sari. Therefore, PCR is recommended as a reliable and rapid method for detection of GBS.

Keywords: Culture, detection, group B *Streptococcus*, polymerase chain reaction, women, pregnancy.

STREPTOCOCCUS AGALACTIAE or group B *Streptococcus* (GBS) are Gram-positive, beta-hemolytic bacteria that live in many bacteriological environments. They can colonize in the human digestive system and genital region of women. It is noteworthy that the existence of such colonization in the obstetric canal during late pregnancy may cause serious infections in both the newborns and mothers[1]. GBS in the first month of life may occur as fulminant sepsis, meningitis or respiratory distress syndrome in the developed countries[2,3]. The bacteria can colonize almost 10–40% of the digestive system and genital region in women and can result in various problems such as premature delivery, premature rupture of membrane and postpartum fever[4,5]. GBS infection in non-pregnant adults is increasing; however, the rate of infection among pregnant women generally remains stable over time[6]. Two rapidly growing populations, including the elderly and immunocompromised patients, are at risk for invasive more than others. Thus, detection of GBS colonization in women can be vital for neonatal GBS infection. Nonetheless, predisposing factors for GBS infection include diabetes mellitus, cancer, ageing, hepatic cirrhosis, consuming corticosteroids and HIV infection. The bacteria in pregnant women could be due to asymptomatic bacteriuria, indicating contamination of the upper part of the genital tract[2,3,7]. The important diseases caused by this bacterial infection include early onset neonatal infection, late onset neonatal infection and infection in women[8]. The association between maternal genital tract colonization during delivery and early neonatal invasive infection has been well documented. Needless to say that three clinical manifestations (symptoms) of the infection include septicaemia, pneumonia and neonatal meningitis[9,10]. Given the intensity and spread of GBS infection and the importance of protecting both the mother and the newborn against infection after delivery, CDC instructions were revised in 2002 and bacteriological screening was made compulsory for all pregnant women at 35–37 weeks of gestation[11–13]. Also, the prevalence of bacterial vaginitis could have different outcomes due to geographical and ethnicity conditions. Therefore, it is recommended that its relationship with preterm delivery be studied separately in each country[14,15]. A routine procedure to detect infection from the bacteria includes cultivation from vaginal secretions, urine and anus. PCR as a valuable and rapid method can identify bacterial infections in a short time. Furthermore, this molecular method could be used for timely treatment, thus preventing unnecessary treatment, and no colonization cases. The aim of this study was to detect GBS carriers in women through PCR and standard microbiological cultures in Sari, Iran.

This study was conducted at the Women's Hospital of Sari, Iran and approved by the Research Ethics Committee, Mazandaran University of Medical Sciences, Sari, Iran. In this study, 246 women residents of Sari have been examined casually after obtaining their consent and filling out a questionnaire regarding gestational history, abortion, infection history, current status of pregnancy and preterm delivery for seven months. Simultaneous sampling for testing culture and PCR was performed using sterilized swabs. The first two swabs – one of them containing vaginal and the other urine – were applied to

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directly checking the specimens. Then, the swabs containing the specimens were transferred rapidly to the Microbiology Laboratory of Mazandaran University of Medical Sciences through transport culture medium. Two swabs were placed in PBS buffer for testing PCR[16]. All the swabs were enriched in selective culture media Todd–Hewitt broth for 24 h at 37°C. Then the samples were cultured on a blood agar of culture medium. Subsequently, they were incubated at 37°C for 24–48 h to obtain isolated colonies[17,18]. After a designated time, tiny colonies containing hemolysis B were removed and a Gram-staining was done following preparation of slides containing the samples. It was observed that the bacteria were predominantly positive and belonged to two types of genera – *Streptococcus* and *Staphylococcus*. Thereafter, catalase testing was conducted on Gram-positive bacteria, and *Streptococcus* was reported provided to being negative catalase. In this study, Gram-positive bacteria that were negative catalase have been selected from primary cultures of blood agar and specified tests have been performed to distinguish different species of *Streptococcus*.

At first the swabs placed in PBS buffer were transferred to the laboratory, and maintained at –70°C until further use. After purification of genomic DNA (Bioneer Kit, South Korea), the considered samples, standard DNA and also in any RUN, a number of samples of positive control (ATCC 13813) and negative control were used for authenticity of the test. The primer sequences used are as follows: with forward primer 5′-TGAAGTGCTGCTTGTAATGT-3′, and reverse primer 5′-GGAACTCTAGTGGCTGGT-3′, which amplified a 722 bp fragment from the GBS *STREP* gene. The primers were purchased from Takapuzist Co, Tehran, Iran. The PCR reaction mixture with a final volume of 25 ml included: 200 µmol dNTPs, 10 pmol of each primer, 1.5 mmol/l $MgCl_2$, 0.5 unit of enzyme Tag, and 50 ng of DNA samples. The cycling program was: one cycle at 94°C for 5 min; 35 cycles at 94°C for 30 sec; 61°C for 60 sec; 72°C for 60 sec; and final extension cycle at 72°C for 5 min. Study of fragments (pieces) was carried out through agarose gel electrophoresis 1% stained with ethidium bromide.

The mean and standard deviation for continuous variables and the frequency of reporting for qualitative variables were determined. Sensitivity and specificity were calculated for the PCR method by comparing with the culture method, which is considered as the gold standard. The concordance between assays was determined using the Kappa coefficient. In order to test the association between qualitative variables, chi-square and Fisher's exact test were used. Statistical analysis was performed using SPSS version 16.00, significant at the 0.05 level.

Out of the 246 patients, 74 were less than 25 years, 51 were 25–29 years and 121 patients were more than 30 years. The frequency of GBS was 15 (6.1%) and 24 patients (9.7%) using culture and PCR methods respectively. All the cases that were positive by PCR method were positive using the culture method too. Using the obtained results of PCR, 9 persons were carrier of group B *Streptococcus* which using culture method have been reported negative (Table 1, Figure 1). The results obtained on the basis of PCR and culture methods are associated with both urine and vaginal samples. It should be noted that of the total 146 samples included 111 samples from vagina and 135 samples from urine, 12 cases (80%) of culture-positive in vaginal samples and only 3 cases (20%) were observed in urine samples. However, using PCR method, 83% of vaginal and 17% of urine samples were reported positive. It should be noted that the most positive cases in either culture or PCR technique were associated with those who had a history of infection – 14 cases using culture method and 17 cases using PCR technique were reported positive. Moreover, 10 cases (66.6%) of culture-positive samples related to those who had a history of pregnancy which by PCR technique, it was reported 12 cases. The agreement between PCR and culture methods for two samples of urine and vagina using Kappa coefficient was 75.1%, which was statistically significant at 0.05 level ($P = 0.001$). Chi-square test results

Table 1. Colonization frequency of Group B *Streptococcus* in specimens based on PCR and culture results

	Culture		
PCR	Positive	Negative	Total
Positive	15	9	24
Negative	0	222	222
Total	15	231	246

$$\text{Sensitivity} = \frac{15}{15} = 100\% \quad \text{PPV} = \frac{15}{24} = 62.5\%$$

$$\text{Specificity} = \frac{222}{231} = 96\% \quad \text{NPV} = \frac{222}{222} = 100\%$$

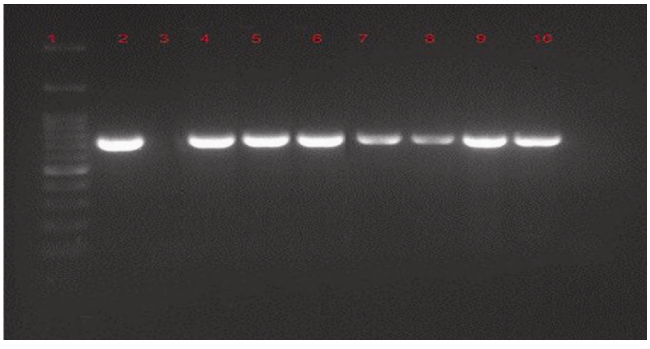

Figure 1. Amplification results of *STREP* gene in group B *Streptococcus* isolates. Lane 1, 50 base pair ladder (Takapu, Tehran, Iran); lane 2, Positive control strain (*Streptococcus agalactiae* ATCC 13813); lane 3, Negative control and lanes 4–10, GBS isolates from clinical samples.

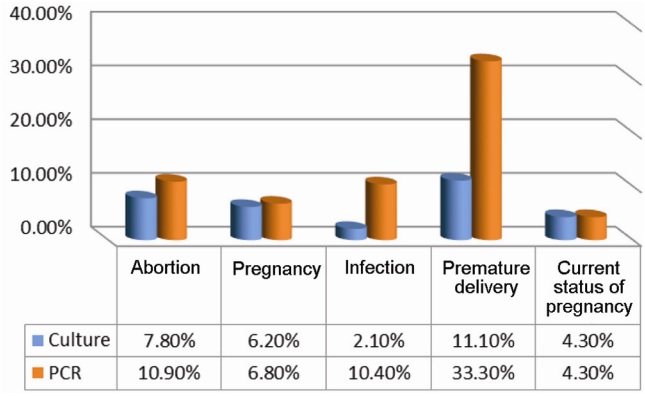

Figure 2. Infection in terms of different variables on the basis of both culture and PCR methods.

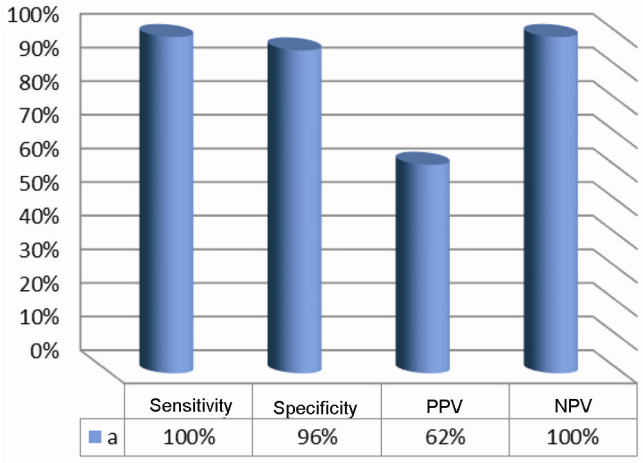

Figure 3. Sensitivity, specificity, PPV and NPV values of PCR compared to culture method.

showed that there was no significant relationship between test results (positive or negative) and age, marital status, pregnancy history, infection and history of abortion ($P > 0.05$), except for the type of sample, where a significant relationship was found with the test results ($P = 0.005$) (Figure 2). Considering the cultivation method as standard, the sensitivity of PCR technique was 100% and its specificity was 96%; also the PPV of PCR test was 62% and its NPV was 100% (Figure 3).

S. agalactiae is one of the main causes of early onset neonatal infection in the form of pneumonia, meningitis and bacteraemia, last onset neonatal infection and infections in women. So all pregnant women should undergo diagnosis in the early weeks of pregnancy for bacterial infection, prevention and timely treatment[7,8,19]. In the present study, we examined the prevalence of GBS through culture and PCR methods. PCR is a rapid and precise method for diagnosis of bacterial colonization. The obtained results confirm the agreement between the two methods in determining the amount of GBS, but due to the difference in the results of two methods, it shows that the power of isolation and detection of bacteria using the mentioned methods may be varied. Therefore, if it was decided that the detection of bacterial infections is based only on culture, then the positive cases that were positive by PCR will be lost. Epidemiological studies showed that GBS colonization among Iranian women was 1.9–26.7% (refs 20–23). A wide variation in the percentage of GBS carriers in Iran may arise due to the use of different sites for sample collection, bacterial load in swab samples, gestational age at the time of sampling and also differences in diagnostic methods[24,25]. Therefore, comparing the two techniques to detect GBS in this study, and given the importance of detection in a very short time, we can conclude that molecular methods like PCR will be useful for diagnosis. Furthermore, applying PCR technique in its colonization caused the results to be increased from 6.1% to 9.7%. In this study, nine samples that were positive by PCR, were not detected by the culture; this indicates the high sensitivity and specificity of the PCR technique. Statistical analysis showed that the sensitivity of the PCR technique in the cultivation was 100% and its specificity was 96%. A similar study was conducted in Turkey by Eren *et al.*[26] on 500 pregnant women, and the rate of infection was reported to be 2.9%; however, the findings of the present study are more consistent. Uhi *et al.*[27] examined samples taken from the rectum and vagina using two methods of real-time PCR to determine the carriers of GBS. Their results indicated sensitivity of 100% and specificity of 97%, which are consistent with the present study.

A study of GBS frequency in the birth (delivery) canal was performed in different parts of the Netherlands. Here, 1102 pregnant women (35–37 weeks) were sampled, and age, economic and parity conditions were examined. The results revealed that 21% of women were infected. According to the study, infection rate compared to African women (29%) was lower while compared to Asian women (13%) as well as the results of the present study was higher[28]. Motlová *et al.*[29] examined the prevalence of GBS in pregnant women in the Czech Republic and reported the rate of colonization of the bacteria to be 3.29% which is statistically very high compared to the present study[29]. In another study conducted at the Gilan University of Medical Sciences in Iran, on 100 pregnant women, it was found that 15 of them were colonized with GBS; their colonization has been reported as 15%, which is consistent with the present study[30].

Thus, it is recommended that more detailed studies are necessary using different methods of culture and PCR for the detection of GBS. The culture method is time-consuming, while the PCR method shows high sensitivity and specificity. Therefore, the latter is recommended as a suitable method for separation of GBS.

Conflict of interest: The authors declare no conflict of interest.

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