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Original Article

Comparison of group B streptococci colonization in vaginal and rectal specimens by culture method and polymerase chain reaction technique

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Abstract

Background: *Streptococcus agalactiae* (group B streptococci, GBS) is a colonizing microorganism in pregnant women and without causing symptoms. Colonization of GBS in the rectovaginal region in late of pregnancy is a risk factor for newborn diseases. GBS infection in newborn babies is acquired by the aspiration of infected amniotic fluid or vertical transmission during delivery through the birth canal. The aim of this study was determination of GBS prevalence among vaginal and anorectal specimens at gestation females by polymerase chain reaction (PCR) and culture-based methods.

Methods: In this study, 137 rectal and vaginal swabs were separately collected from women with gestational age 35−37 weeks from July 2013 to March 2014 at the teaching hospital of Razi, Ahvaz, Iran. All samples were enrichment in selective culture media Todd−Hewitt broth for 24 hours and recognized by standard culture using blood agar, phenotypic tests, and amplification of the *CFB* gene.

Results: Age range was 16−45 years (mean, 28.34 ± 0.7 years). Of rectal samples, 42 (30.7%) were positive based on culture method and 57 (41.6%) samples were positive by PCR. Of 137 vaginal samples, 38 (27.7%) were positive by culture and 60 (43.8%) samples were positive by PCR. The chance of colonization with GBS was increased in women with a history of urinary tract infection.

Conclusion: The frequency of GBS culture from rectal samples was higher than vaginal samples. However, the detection percentage of GBS using PCR from vaginal samples was higher than rectal samples. By contrast, the culture is a time-consuming method requiring at least 48 hours for GBS fully identification but PCR is a sensitive and rapid technique in detection of GBS, with the result was acquired during 3 hours.

Keywords: culture; group B streptococci; polymerase chain reaction; rectal; vaginal

1. Introduction

Streptococcus agalactiae (group B streptococci, GBS) is a colonizing asymptotic microorganism in pregnant women.[1] The lower gastrointestinal tract is considered to be the main habitat of the bacterium. The anatomical location of this bacterium is caused by transfer of GBS from the gastrointestinal region to the genital tract.[2] In women, genitourinary tract vaginal colonization usually occurs in late adolescence rather than childhood. Women of childbearing age carry GBS at variable frequencies of 4.6−31.3% with similar figures in both developing and developed countries.[3] This bacterium is colonized in pregnant women transiently, intermittently, or persistently.[4] However, the rate of GBS colonization among pregnant women usually remains stable over time.[5] Additionally, colonization during the end of pregnancy is a risk factor for newborn diseases.[4] GBS infection in newborn babies arises by the aspiration of infected amniotic fluid or vertical transmission during delivery through the birth canal.[6] About

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50% of those babies infected at birth acquire GBS from colonized mothers during vertical transmission.[7] According to clinical syndromes, the neonatal patients are classified into two groups: those who become ill in the 1st week of life, referred to as early onset disease (EOD), and those affected in the end of the neonatal period, referred to as late onset disease (LOD).[6] EOD typically presents with fulminant pneumonia, sepsis or less commonly meningitis at birth or within the first 24 hours. However, many GBS *colonized* newborns are asymptomatic and never become infected. LOD are presented with bacteremia and/or meningitis. The peak of incidence in infants presenting with LOD is around 1 month after birth. In addition to bacteremia and meningitis, cellulitis and osteoarticular infections may occur but are relatively rare in LOD.[7] Late-onset infections occur in the perinatal period from mother to infant or from hospital or community sources.[1] The incidence of GBS EOD is from one to three per 1000 live births in industrialized countries but higher in developing countries.[8,9] In the late 1970s to mid-1990s, the mortality rate among babies born with GBS was 40%.[10] Due to the high prevalence of neonatal morbidity and mortality of GBS colonization, the Centers for Disease Control and Prevention recommend a universal *intrapartum* antimicrobial prophylaxis in women at high risk at 35−37 weeks of gestation. Nowadays, the incidence at newborn infections have decreased.[4] Unfortunately, the strategies of *intrapartum* antimicrobial prophylaxis for *at risk* pregnant women have no effect on GBS LOD.[11] According to a USA consensus statement, screening of anorectal and vaginal specimens at 35−37 weeks of pregnancy is recommended.[12] There are two strategies in screening these specimens: (1) culture-based methods; and (2) non-culture methods such as polymerase chain reaction (PCR).[13] In this study, we investigated the prevalence of GBS in vaginal and anorectal specimens in pregnant women by PCR and culture-based methods.

2. Methods

2.1. Study design

This study was conducted from July 2013 to March 2014 at the teaching hospital of Razi, Ahvaz, Iran, and approved by the Research Ethics Committee, Jundishapur University of Medical Sciences, Ahvaz, Iran. All participating women completed written informed consent. In this study, 274 samples (1 sample from the rectum and a later sample from the vagina) were taken from 137 women at 35−37 weeks of pregnancy as recommended by the Center for Disease Control.[4] The pregnant women involved in this study were not taking any antibiotics or *intrapartum* chemoprophylaxis for GBS at the time of this study.

2.2. GBS isolation by culture method

Vaginal and rectal swabs were taken from each woman and were transferred to the microbiology laboratory of Jundishapur University of Medical Sciences within 4 hours. All of the swabs were placed in selective enrichment culture media (Todd−Hewitt broth; Conda, Pronasida, Spain) with gentamicin (8 mg/L) and nalidixic acid (15 mg/L), and were incubated for 1−2 days under microaerophilic condition (8% CO_2) at 37°C. Then, a full loop from the broth medium with sufficient turbidity was cultured on nonselective blood agar and incubated again for 1−2 days under microaerophilic condition (8% CO_2) at 37°C. The broths without turbidity were incubated for another 24 hours. Identification of GBS was performed based on colony morphology, β-hemolytic reaction, Gram-stain, hippurate hydrolysis, and cAMP factor.

2.3. GBS detection by PCR

We used 500 μL of Todd−Hewitt broth containing vaginal or rectal samples from the previous steps and taken in 1.5 mL microtubes. DNA was extracted using High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany) according to the manufacturer's recommendations. In order to identify GBS, amplification of the *CFB* gene (encoding the CAMP factor that is conserved in all GBS isolates) was performed. The primers used for PCR were as follows, with forward primer 5′TTTCACCAGCTGTATTAGAATA-3′, and reverse primer 5′- GTTCCCTGAACATTATCTTTGAT-3′, which amplified a 153 bp fragment from the GBS *CFB* gene.[13] The volume of reaction was 25 μL and was prepared as follows: 12.5 μL 2× master mix (Ampliqon, Odense, Denmark), 0.4μM of each primer and 50ng of DNA sample. Amplification was carried out in a thermal cycler (*Eppendorf, Hamburg,* Germany). The cycling program was: one cycle at 94°C for 5 minutes; 35 cycles at 94°C for 45 seconds, 49°C for 45 seconds, and 72°C for 45 seconds; and a final extension cycle at 72°C for 5 minutes. The amplicon was visualized on a 1.5% agarose gel stained with ethidium bromide.

2.4. Statistical analysis

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were calculated for the PCR technique using culture as a "gold standard test". The descriptive statistics, chi-square and logistics regression tests were performed in SPSS version 16.00, and a level of significance of $p < 0.05$ was used.

3. Results

In this study 274 samples (1 sample from the rectum and 1 sample from the vagina) were taken from 137 pregnant women. Their ages ranged from 16 years to 40 years (mean, 28.34 ± 0.7 years). The mean ages of GBS-carriers and non GBS-carriers were 28.17 ± 0.5 years and 28.34 ± 0.2 years, respectively. The distribution of GBS colonization based on age ranges is shown in Table 1. Among them, 30 women had a history of abortion and 40 women had a history of urinary tract infection (UTI). Molecular identification of GBS was performed by amplification of the *CFB* gene as shown in Fig. 1. Out of 137

Table 1
The distribution of GBS colonization based on age ranges.

Age range (y)	Carriers with GBS
16−20	25.56 (4/16)
21−24	20 (4/20)
25−28	33.33 (11/33)
29−32	31.42 (11/35)
33−36	23.80 (5/21)
37−40	18.18 (2/11)

Data are presented as % (*n*/*N*).

rectal samples, 42 (30.7%) were positive based on culture method and 57 (41.6%) by PCR technique. The rate of sensitivity and specificity of PCR to culture was 92.85% and 81.1%, respectively. Among 137 vaginal samples, 38 (27.7%) were positive by culture and 60 (43.8%) by PCR. Rates of sensitivity and specificity of PCR to culture were 81.6% and 70.7%, respectively. NPV of rectal swabs was 96% whereas that of vaginal swabs was 91%. PPV of rectal swabs was 68% and that of vaginal swabs was 52%. It was observed that the colonization frequencies of GBS in rectal and vaginal samples were based on culture and PCR results, respectively (Tables 2 and 3). In this study, a history of abortion and UTI was reported in eight of 30 (26.66%) and 15 out of 40 (37.5%) women, respectively. The relationship between GBS carriers with abortion and UTI histories was calculated by Chi-square test. There was not any statistically significant relationship between an abortion history with carrying GBS ($p > 0.05$), but carrying GBS was associated with UTI history. Moreover, females with UTI history had an increased chance of colonization with GBS (odds ratio = 2.5, 95% confidence interval 1.1−3.9).

4. Discussion

Streptococcus agalactiae is a leading cause of neonatal bacterial sepsis and meningitis and is increasingly associated with invasive disease in adults.[5] Infants often begin showing symptoms of disease several hours after birth approximately 1% of the infants who colonized with GBS develop sepsis during the first week of life.[14] The risk of colonization in newborns rises if the mother is heavily colonized with this bacterium. However, GBS-invasive diseases develop in a few neonates.[15] In our study, the prevalence of GBS based on culture method was 30.7% in rectal and 27.7% in vaginal samples. According to our results, it would appear that rectal samples culture is more effective than vaginal swabs in detecting GBS. By contrast, Orrett et al found that GBS was isolated more often from vaginal samples than rectal samples by culture method (65.2% vs. 53.2%).[3] This difference may be due to the type of culture medium used. Kadanali et al isolated GBS from 48 patients including 25 (16.7%) involving both vaginal and rectal swabs, 16 (10.7%) cases only from the vagina, and 7 (4.7%) only from rectal swabs.[15] It seems that, to increase the chance of isolation of GBS more effectively, the cultures of both vaginal and rectal specimens are needed. The prevalence of this bacterium in other regions of Iran was reported to be variable, including 9.5% in Busher,[2] 9.1% in Shiraz,[17] and 22.76% in Tehran.[18] Several other countries had rates comparable to our study, including 32% in Turkey,[16] 32.9% in Trinidad,[3] and 25.4% in Sweden.[19] In our study only vaginal and rectal samples of pregnant females were surveyed. However, GBS is a relativity common cause of asymptomatic bacteriuria in pregnant women, which contributes to maternal pyelonephritis and preterm birth. The prevalence of GBS in the urine of pregnant women varies from 0.4% to 5%.[20] maternal colonization with GBS in the genital tract increases the possibility of bacteriuria. Additionally, it causes vertical transmission of GBS from mother to fetus and is associated with a high risk for EOD.[21,22] In our study, GBS was detected with greater frequency by PCR of the *CFB* gene than by the culture method. Consistent with our study, other research has compared culture and PCR of GBS (Table 4).[23−27] In another study, Rallu and colleagues[27] compared

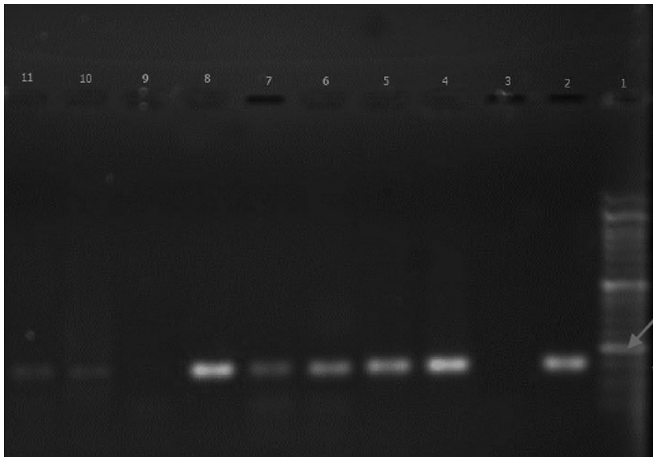

Fig. 1. Amplification results of *CFB* gene in group B *Streptococcus* (GBS) isolates. Lane 1, 50 base pair ladder (Cinnagen, Tehran, Iran); Lane 2, positive control strain (*Streptococcus agalactiae* ATCC 12386); Lane 3, negative control (water); Lanes 4−8,10, and 11, GBS isolates from clinical samples; and Lane 9, a clinical sample lacking GBS.

Table 2
The colonization frequency of GBS in vaginal samples based on culture and PCR results.

PCR		Culture	
		Positive (%)	Negative (%)
Positive (%)	57 (41.6)	18 (18.9)	39 (92.9)
Negative (%)	80 (58.4)	3 (7.1)	77 (81.1)
Total (%)	137 (100)	42 (100)	95 (100)

Table 3
The colonization frequency of GBS in rectal samples based on culture and PCR results.

PCR		Culture	
		Positive (%)	Negative (%)
Positive (%)	60 (43.8)	29 (29.9)	31 (89.1)
Negative (%)	77 (56.2)	7 (18.4)	70 (70.7)
Total (%)	137 (100)	38 (100)	99 (100)

Table 4
Comparison of culture and PCR in other studies.

Culture	PCR	Sample	Reference
25/100 (25)	32/100 (32)	Vaginal	Bergh et al[22]
154/1226 (12.6)	192/1226 (15.7)	Vaginal	Morozumi et al[23]
42/263 (15.96)	71/263 (26.9)	Vaginal	de-Paris et al[24]
35/375 (9.3)	42/375 (11.2)	Vaginal and rectal	Bakhtiari et al[25]
15/203 (7.39)	19.7 (40/203)	Vaginal	Yousefi Mashouf et al[26]

Data are presented as *n*/*N* (%).

the culture method with two PCR assays for *cfb* and *scpB* genes and GBS antigen detection. According to their results, out of 605 vaginal samples, the culture identified 96 positive samples. The GBS antigen detection test recognized 132 positive samples (22%), *CFB* PCR identified 171 positive samples (28%), and *scpB* PCR identified 226 positive samples (37%). In our study, amplification of the *CFB* gene was performed. However, it seems that amplification of the *scpB* gene in detection of GBS is more effective than by the *CFB* gene. In addition, the culture method had the lowest efficacy as compared with two other techniques. In our study, the frequency of GBS identification in vaginal samples was higher than rectal using samples PCR. However, unlike our results, Farhadifar et al found a higher rates of GBS detection from rectal rather than vaginal samples by PCR (75% vs. 61.5%).[13] This contrast may be explained by differences in gestational ages, a change in the GBS distribution over time, or the existence of different ethnic groups in our region. In our study, the sensitivity of PCR to culture was 92.8% and 81.6% for rectal and vaginal specimens, respectively. The differences may be attributed to higher GBS loads in rectal than vaginal specimens or possibly the type of mechanism used to process and analyze the specimens. Also the specificity rates of PCR to culture were 81.1% and 70.7% for rectal and vaginal specimens, respectively. This finding suggests that interpretation of rectal samples is more valuable than vaginal samples in GBS detection. However, culture is the gold standard of GBS diagnosis, but can provide a false negative result because other bacteria of the vaginal/genital tract can inhibit the growth of GBS even when using the selective broth media.[25] A high NPV, rapid result, and high sensitivity are desirable characters of a screening test. In our study, NPV of rectal swabs was 96% whereas that of vaginal swabs was 91%. This finding indicates that almost all of the samples with negative results are truly negative and prophylaxis for the mother is not necessary.

Table 5
Comparison of sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) in our study with other studies.

Sensitivity	Specificity	PPV	NPV	Reference
96.1	95.9	77	99.4	Morozumi et al[23]
100	82.6	59	100	de-Paris et al[24]
100	98	100	100	Bakhtiari et al[25]
88.2	87.4	35.7	98.9	Yousefi Mashouf et al[26]

Data are presented as %.

Consistent with our study, other research also has reported amounts of sensitivity, specificity, NPV, and PPV (Table 5).The culture method may not be absolutely effective in the identification of GBS. However, use of the PCR technique provides a rapid and accurate result in the detection of GBS and it seems to be more effective than culture.

In conclusion, we have detected GBS by use of both the culture and PCR methods from vaginal and rectal samples. According to the results of our study, the frequency of GBS isolation by using culture from rectal samples was higher than by using vaginal samples. However, the detection percent of GBS by using PCR from vaginal samples was higher than rectal samples. By contrast, culture is a time-consuming method requiring at least 48 hours for GBS fully identified, whereas PCR is a sensitive and expedited technique used to detect GBS where results can be acquired within 3 hours.

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