The Prevalence of Rectovaginal Colonization and Antibiotic Susceptibility Pattern of *Streptococcus agalactiae* in Pregnant Women in Al-Zahra Hospital, Rasht, Iran

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Background: Maternal rectovaginal colonization with group B streptococcus (GBS) is a main risk factor for vertical transmission of GBS to newborns and life-threatening neonatal invasive diseases. The aim of this study was investigation of the prevalence of anorectal and vaginal colonization with GBS in late of pregnancy by culture-based and polymerase chain reaction (PCR) methods and antimicrobial susceptibility patterns of the GBS isolates in Rasht, Iran.

Methods: We analyzed 245 anorectal and vaginal swab samples separately from pregnant women at 35 to 37 weeks of gestation. All samples were cultured after enrichment in a selective Todd-Hewitt broth and then assayed by phenotypic characterizations and PCR method for *cfb* conserved gene. Antimicrobial susceptibility was performed using the Kirby–Bauer method.

Results: In total of 245 vaginal samples, 19 (7.8%) were positive based on culture method and 28 (11.4%) by PCR method. Among 245 rectal samples, 24 (9.8%) were positive by culture and 29 (11.8%) samples were positive by PCR. Of 245 pregnant women studied were found to have 9.7% GBS rectovaginal by culture and 15.9% by PCR methods. All GBS isolates were sensitive to ampicillin (77.2%) and vancomycin (72.2%) and were resistant to Penicillin (88.6%), ceftriaxone (75%), clindamycin (95.4%), azithromycin (86.3%), tetracycline (61.3%), erythromycin (47.7%), and levofloxacin (27.2%).

Conclusions: The results of this study indicate that the frequency of GBS isolation from rectal samples was higher than vaginal samples by both culture and PCR. Our study recommended intrapartum antibiotic prophylaxis against GBS infections based on ampicillin or vancomycin for GBS carriers in Rasht.

Key Words: *Streptococcus agalactiae*, pregnant women, antibiotic susceptibility

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Streptococcus agalactiae, frequently as group B streptococcus (GBS), may be detected asymptomatically in 6.5% to 40% of healthy women in industrialized and developing countries.[1,2] The lower gastrointestinal tract is considered as the primary habitat of the GBS with a secondary spread to the genital tract that can be transient, intermittent, or persistent during pregnancy.[3] Group B streptococcus is one of the important causes of invasive disease most frequently found in infants, pregnant, or postpartum women. It has been established that maternal rectovaginal colonization with GBS is the main risk factor for early-onset life-threatening sepsis, meningitis, and pneumonia in newborns in the first week of life and late-onset infections in newborns with bacteremia and/or meningitis in the first 3 months of life.[3,4] Pregnant women who are GBS genital tract carriers have potential for transmission of GBS infection to their neonate by the aspiration of infected amniotic fluid after the beginning of labor or rupture of membranes and by vertical transmission during passage through the birth canal. Approximately 50% of infected newborns acquire GBS during vertical transmission.[3,5]

For the prevention of early-onset GBS disease, revised guidelines from the Centers for Disease Control and Prevention (CDC) in 2002 recommend universal culture-based screening of all pregnant women for rectovaginal GBS colonization, as the gold standard method for GBS detection, at 35th and 37th weeks of pregnancy to receive intrapartum antibiotic prophylaxis to carriers.[6] As a result of prevention efforts, striking reduction in neonatal transmission of GBS and neonatal GBS disease occurred.[7,8]

According to the CDC guidelines, it is essential to identify the GBS carries to prevent invasive GBS diseases in pregnant women and infants. Also, to optimize intrapartum antibiotic prophylaxis, understanding local patterns of antibiotic resistance in GBS is needed.[3] To our knowledge, there is no information about GBS epidemiology characteristics and antibiotic resistance profiles in Rasht, Iran. Therefore, this study aimed to investigate the prevalence of anorectal and vaginal colonization with GBS of pregnant women at 35th and 37th weeks of gestation in teaching Al-Zahra hospital in Rasht by culture-based and polymerase chain reaction (PCR) methods. In addition, we explored GBS susceptibility to 9 antibiotics to guide intrapartum antibiotic prophylaxis for GBS carriers to reduce the invasive GBS diseases in neonates and their mothers.

MATERIALS AND METHODS

Study Design

This study was implemented over the period from June 2017 to February 2018 in teaching Al-Zahra hospital in Rasht, Iran, and approved by the Research Ethics Committee, Guilan University of Medical Sciences, Rasht, Iran. All pregnant women at 35 to 37 weeks of gestation that were referred to the Al-Zahra hospital and do not have any health problem completed a written informed consent before inclusion in the study. In this study, a total of 490 samples (1 swab from the lower vagina and another from the rectum) were obtained from 245 pregnant women according to the recommendation from the CDC.[6] All pregnant women included in this study were not taking any intrapartum antibiotic prophylaxis for GBS at the time of study.

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Collecting and Processing Samples for GBS Culture

The lower vagina and rectal samples were obtained from each pregnant woman by sterile cotton swabs. Vaginal and rectal swabs were placed separately into Amie's transport medium (Conda, Pronasida, Spain) and then transferred to department of Microbiology located at school of medicine, Guilan University of Medical Sciences. Swabs then were inoculated separately into a selective broth medium (Todd-Hewitt broth; Conda, Pronasida, Spain) supplemented with gentamicin (8 μg/mL) and nalidixic acid (15 μg/mL) and were incubated at least 24 hours at 35°C to 37°C in a 5% to 10% CO_2 atmosphere. After incubation period, loopful from the broth medium was subcultured to a 5% sheep blood agar and incubated for 24 hours at 35°C to 37°C in a 5% to 10% CO_2 atmosphere. Identification of GBS was performed based on colony morphology, gram-positive cocci, negative catalase reaction, β-hemolysis in 5% sheep blood agar, positive for sodium hippurate hydrolysis and CAMP factor.[9]

GBS Detection by PCR Assay

For DNA extraction, 500 mL of vaginal and rectal Todd-Hewitt broth cultures were harvested by centrifugation. DNA was then extracted by thermal lysis as described previously[10] with minor modifications. Briefly, two 1.5-mL aliquots of vaginal and rectal Todd-Hewitt broth cultures were centrifuged at 13,000 rpm for 10 minutes at room temperature. The resulting precipitates were resuspended in 1× Phosphate-buffered saline solution, centrifuged at 8000 rpm for 5 minutes at 4°C, and washed twice with the same buffer. The cell pellets were resuspended in 300-μL Tris-EDTA buffer (10 mM Tris-HCl [pH 7.5], 0.1 mM ethylenediaminetetraacetic acid) and heated at 95°C for 20 minutes and placed at room temperature for 5 minutes. The suspension was then placed at −20°C for 10 minutes and after centrifugation at 14,000 rpm for 10 minutes at 4°C, were stored at −20°C until use.

Conventional PCR was used to confirm presence of GBS in vaginal and rectal samples, by amplification of the *cfb* gene (CAMP factor) which is present in all of GBS strains and is well conserved within this species.[11] The primers used for PCR were as follows: Sag59: 5′-TTTCACCAGCTGTATTAGAAGTA-3′ and Sag190: 5′-GTTCCCTGAACATTATCTTTGAT-3′ which amplified a 153-bp fragment from the GBS *cfb* gene.[12] In each assay, the final volume of each reaction was 25 μL which contained 12.5-mL 2× master mix (Ampliqon, Odense, Denmark), 1 μL of each primer (10 pmol/μL), and 5 μL of each DNA sample. DNA was amplified in a Mastercycler Eppendorf (Eppendorf, Germany) under the following conditions: initial denaturation for 3 minutes at 94°C followed by 35 cycles at 94°C for 30 seconds, at specific annealing temperature for 55 seconds, then at 72°C for 45 seconds. A final extension was performed for 5 minutes at 72°C. Amplicons were separated by electrophoresis on a 1.5% agarose gel.

Sequencing of PCR Product

Sequencing Service from Microsynth Corporation in Switzerland was used for the sequencing of amplified product from 1 isolate. Then, the sequence related to the amplified product from *cfb* gene was entered into a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the accuracy of PCR results.

Antimicrobial Susceptibility Testing

The GBS isolates were tested against penicillin, ampicillin, ceftriaxone, levofloxacin, clindamycin, erythromycin, azithromycin, vancomycin, and tetracycline by the Kirby-Bauer disk diffusion (Oxoid Limited, United Kingdom) method according to Clinical and Laboratory Standards Institute 2018 guidelines.[13] The criteria for GBS antimicrobial sensitivity patterns are listed in Table 1.

Statistical Analysis

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were calculated for the PCR technique in comparison with culture as a "gold standard test." The concordance between assays was determined using the Kappa coefficient. All statistical calculations were done using SPSS for windows version 21.

RESULTS

A total of 245 pregnant women at 35 to 37 weeks of gestation were enrolled in our study. In total, 490 vaginal and anorectal swab samples separately were investigated for GBS by culture and PCR methods. As shown in Figure 1, the results of DNA amplification by the PCR method based on the specific primers used in this study showed the presence of a 153-bp fragment for the *cfb* gene in the DNA preparations obtained from GBS isolates.

In total of 245 vaginal samples, 19 (7.8%) were positive based on culture method and 28 (11.4%) by PCR method. The rate of sensitivity and specificity of PCR to culture was 100% and 96%, respectively. Among 245 rectal samples, 24 (9.8%) were positive by culture and 29 (11.8%) by PCR. Rates of sensitivity and specificity of PCR to culture was 95.8% and 87.8%,

TABLE 1. Criteria for GBS Antimicrobial Sensitivity Patterns

		Diameter of the Inhibition Zone, mm		
Antibiotics	**Drug Concentration, μg**	**Sensitive**	**Intermediate**	**Resistant**
Penicillin	10	≥ 24	—	—
Ampicillin	10	≥ 24	—	—
Ceftriaxone	30	≥ 24	—	—
Levofloxacin	5	≥ 17	14–16	≤13
Clindamycin	2	≥ 19	16–18	≤15
Erythromycin	15	≥ 21	16–20	≤15
Azithromycin	15	≥ 18	14–17	≤ 3
Vancomycin	30	≥ 17	—	—
Tetracycline	30	≥ 23	19–22	≤18

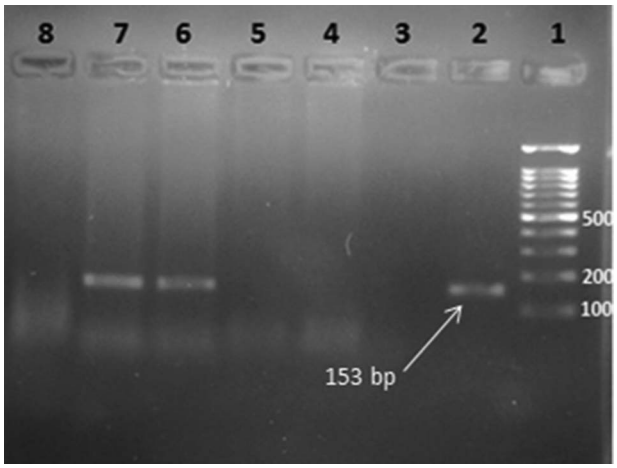

FIGURE 1. DNA amplification results of representative GBS isolates in 1.5% agarose gel. Lane 1, 100 base pair DNA ladder; Lane 2, positive control strain (*Streptococcus agalactiae* ATCC 12386); lane 3, negative control strain (*Staphylococcus aureus* ATCC 25923); lanes 4, 5, 8, clinical sample lacking GBS and lanes 4 and 5, GBS isolates from clinical samples.

respectively. The NPV of vaginal swabs was 100%, whereas that of rectal swabs was 99.5%. The PPV of vaginal swabs was 67.9% and that of rectal swabs was 70.3%. Colonization frequencies of GBS in vaginal and rectal samples based on culture and PCR results are shown in Tables 2 and 3, respectively. Of 245 pregnant women studied, 9.7% were found to have GBS rectovaginal colonization by culture and 15.9% by PCR methods. The agreement beyond chance (kappa) between the techniques was 0.85, indicating substantial agreement.

Amplified product of *cfb* gene underwent bidirectional sequencing by the ABI 3730XL DNA Analyzer (Applied Biosystems, USA). The sequence of the *cfb* gene were entered into a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were performed with the MEGA (version 4). The sequence of *cfb* gene PCR product confirmed the accuracy of the PCR results.

The results of the antibiotic susceptibility patterns for GBS isolates by the Kirby-Bauer method and according to the recommendations of the Clinical and Laboratory Standards Institute are shown in Table 4. In total, in 43 of the GBS isolates, 88.6% were resistant to penicillin, 75% were resistant to ceftriaxone, 95.4% were resistant to clindamycin, 86.3% were resistant to azithromycin, and 61.3% were resistant to tetracycline. A total of 77.2% of 43 isolates were sensitive to ampicillin and 72.2% were sensitive to vancomycin under study. Relatively, GBS showed low resistance to levofloxacin (27.2%) and high resistance to erythromycin with 47.7%. There was no statistically significant difference in antibiotic resistance pattern between GBS vaginal and rectal isolates.

TABLE 2. The Colonization Frequency of GBS in Vaginal Samples Based on Culture and PCR Results

PCR	Culture Positive (%)	Culture Negative (%)
Positive (%)		
28 (11.4)	19 (67.9)	9 (32.1)
Negative (%)		
217 (88.5)	0 (0.0)	217 (100)
Total (%)		
245 (100)	19 (7.8)	226 (92.2)

TABLE 3. The Colonization Frequency of GBS in Rectal Samples Based on Culture and PCR Results

PCR	Culture Positive (%)	Culture Negative (%)
Positive (%)		
29 (11.8)	23 (79.3)	6 (20.7)
Negative (%)		
216 (88.1)	1 (0.5)	215 (99.5)
Total (%)		
245 (100)	24 (9.8)	221 (90.2)

DISCUSSION

For the prevention of life-threatening sepsis and meningitis in newborns and invasive diseases in postpartum women, CDC recommends universal GBS screening of all pregnant women for rectovaginal GBS colonization in late of pregnancy.[6,14] Intrapartum antibiotic prophylaxis in GBS carriers will be most effective in reduction of the incidence of GBS infections in neonates and their mothers.[15] Although some of screening studies have been performed in several parts of Iran, there is no any GBS screening information and epidemiology characteristics in Rasht, Iran. In the current study, GBS colonization rates were 9.7% using the culture and 15.9% using PCR. Furthermore, our results have shown that the rate of rectal carries with GBS were slightly higher (9.7%) than vaginal carries (7.2%) by culture method. In agreement with our study, Mengist et al[16] reported that GBS rectal recovery was significantly greater than vagina. By contrast, Orrett[17] and Wollheim et al[18] found that GBS was isolated more often from vaginal samples than rectal. This difference rate of GBS isolation by culture method may be due to the selective broth media, variety of GBS strains, and condition of their growth. One main reason for these differences is the false-negative results due to the inhibition of the growth of GBS by other bacteria in genital and gastrointestinal tracts even when using selective broth media.[19]

Rectal colonization with GBS is a major risk factor for vaginal colonization especially in pregnancy. Therefore, cultures of both vaginal and rectal specimens are necessary. Even in cases of rectal carriage, intrapartum antibiotic prophylaxis is more effective to prevention of GBS vaginal colonization.[3]

TABLE 4. Antimicrobial Susceptibility Pattern of GBS Isolated (N = 43) From Pregnant Women

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Penicillin	11.3	—	88.6
Ampicillin	77.2	—	22.7
Ceftriaxone	22.7	2.2	75
Levofloxacin	47.7	25	27.2
Clindamycin	4.5	—	95.4
Erythromycin	25	27.2	47.7
Azithromycin	2.3	11.3	86.3
Vancomycin	72.7	—	27.2
Tetracycline	36.3	2.2	61.3

One meta-analysis study for investigation of the prevalence of GBS in pregnant women in Iran show that the prevalence of GBS colonization among Iranian pregnant women was 9.8%.[20] Indeed, the prevalence of GBS colonization could vary widely with geographic location, age, and socioeconomic status.[11]

Several other regions of world had GBS colonization rates comparable to our study, including de-Paris et al[10] (15.96% using the culture and 26.99% using PCR), Matani et al[21] (25.5%), Mengist et al[16] (19%).

In agreement with other studies, the PCR method in the present study was more effective for detection of GBS from vaginal and rectal specimens than by the culture method.[18] Also, our results showed that the frequency of GBS detection in rectal samples was higher than vaginal by using PCR.

In our study, the sensitivity of PCR to culture was 100% and 95.8% for vaginal and rectal samples, respectively. These results may be interpreted by the type of mechanism used to process and analyze the specimens. Also, the specificity of PCR to culture was 96% and 87.8% for vaginal and rectal samples, respectively. This finding suggests that the interpretation of vaginal samples is more valuable than rectal samples in GBS detection.

A high NPV, rapid results, and great sensitivity are desirable parameters of a screening test.[10] In our study, NPV of vaginal swabs was 100%, whereas that of rectal swabs was 91%. One hundred percent NPV for vaginal swab is important because it indicates that all samples with negative results are truly negative, and intrapartum antibiotic prophylaxis for pregnant women presenting PCR negative is not necessary. False-negative results in a screening test may lead to serious consequences for the pregnant women, because this test is determined to take a decision about antibiotic prophylaxis.

In this study, among the 9 studied antibiotics, GBS isolates showed higher susceptibility to ampicillin and vancomycin and resistance to penicillin (first-line choice), clindamycin, erythromycin, azithromycin, ceftriaxone, levofloxacin, and tetracycline. In agreement with our study, a few studies reported resistance to penicillin.[21,22] Our findings revealed that using not only penicillin but also macrolides, clindamycin, ceftriaxone, levofloxacin, and tetracycline in some communities such as our studied region is not effective as a standard prophylactic and therapeutic drug. Therefore, our study recommended treatment and prevention against GBS infections based on ampicillin or vancomycin in Rasht. Similar to other studies, our results showed susceptibility to ampicillin and vancomycin[1,16,23] and a high resistance to clindamycin, erythromycin, and levofloxacin.[1,16,21,23,24] Unlike the present study, some of studies reported sensitivity to penicillin.[1,16,23–25]

There are some limitations to this study. First, we did not identify the serotyping characteristics of GBS isolates because of the financial limitations to obtain the main and special antiserums of GBS. Second, we did not follow the newborns for GBS infections and their postpartum mothers who were GBS carries and received intrapartum antibiotic prophylaxis during pregnancy. Therefore, the results of this study will help clinicians and policy makers in the necessary performance of a uniform antibiotic prophylaxis program.

In conclusion, we have detected GBS using of both culture and PCR methods from vaginal and rectal specimens of pregnant women. The GBS colonization rates were 9.7% using the culture and 15.9% using PCR. According to our results, the frequency of GBS isolation from rectal samples was higher than vaginal samples by both culture and PCR. Although culture is the gold standard of GBS isolation, it is a time-consuming method. Polymerase chain reaction is a fast, sensitive, and reliable method for detection of GBS in rectovaginal samples and identification of GBS carriers to initiate intrapartum antibiotic prophylaxis. Our study recommended intrapartum antibiotic prophylaxis against GBS infections based on ampicillin or vancomycin antibiotics for GBS carriers in Rasht.

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