The investigation of antibiotic resistance and rapid detection of group B *Streptococcus* (Bca) from vaginal specimens of pregnant women by colony PCR method

Aazam Khani Daramroodi[1], Fatemeh Keshavarzi[2*], Farshid Raissi[3]

1. Department of Biology, Kurdistan Science and Research Branch, Islamic Azad University, Sanandaj, Iran
2. Department of Genetics, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
3. Department of Pathology, Faculty of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran

***Corresponding author:**Tel: +98 87 33287652 Fax: +98 8733233831
Address: Department of Genetics, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
E-mail: fkeshavarzi@iausdj.ac.ir
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Abstract

Introduction: Group B *Streptococcus* (GBS) is one of the most causes of neonatal infections. The bacterium colonizes genitourinary tracts of pregnant women and transmits to infants. The aim of this study was investigating colony PCR and culture methods to detection of GBS in pregnant women.

Materials and methods: Hundred pregnant women, at the 35th and 37th weeks of pregnancy, were selected from the Obstetrics and Gynecology Unit of the Moatazedi and Shahid Chamran Hospitals in Kermanshah province. Specimens were collected from vaginal introitus and investigated by selective culture and colony PCR methods. Then, antibiotic resistance tests were performed according to the latest guidelines of Clinical and Laboratory Standards Institute (CLSI).

Results: Prevalence of GBS colonization was shown to be 5% and 6% by the culture and colony PCR methods, respectively. Also, resistance rate to erythromycin, penicillin, vancomycin, and the clindamycin were determined to be 50%, 16.66%, 16.66% and 33.33 %, respectively. Moreover, the highest resistance was for erythromycin and the appropriate antibiotics were penicillin and then vancomycin.

Conclusion: A higher prevalence of GBS colonization in pregnant women in the Kermanshah city of Iran was detected using colony PCR method compared to culture method.

Keywords: Antibiotic Resistance, BCA gene, Colony-PCR, GBS

Introduction

Streptococci are spherical and gram-positive bacteria, which are the natural flora of the pharynx, skin, and intestines of humans, and if they enter the blood or tissues, cause disease (1). Streptococcus agalactia (*S. agalactia*), the second group of streptococcal bacteria was recognized as a cause of breast infection in cows since 1970 (2). They are similar to other streptococci in terms of morphology and metabolism. But, they create larger colonies and narrow zone of beta-hemolysis in culture media. Some of them are non-hemolytic or have alpha-hemolytic

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strains (3). A specific polysaccharide antigen, composed of rhamnose, n-acetyl Glucosamine and galactose, is in their cell wall. *S. agalactia* is a pathogenic bacterium that causes diseases such as bacteremia, meningitis and early death in newborn. *S. agalactica* also prominent veterinary pathogens because they are can cause mastitis disease in cows (breast inflammation in dairy cows). Bca gene encodes alpha-C protein. The removal of the gene (bca) causes to reduce the pathogenicity of GBS in the immature mouse model (4-7). The previous investigation demonstrates that 10-40% of pregnant women have a bacterium colonized and 70-80% of those bacteria transmitted to infants that are colonization and subsequent diseases in the infant which could occur in the uterus at birth or in the first months of life. The incidence of this disease in the 1990s has been significantly reduced due to the use of antibiotics during childbirth (3). A number of *S. agalactia* has been resistant at least in one of the antibiotics erythromycin, penicillin, vancomycin, and clindamycin (4). Risk factors included prolonged rupture of the embryonic curtain (less than 18 hours), preterm delivery (less than 37 weeks), maternal fever during childbirth (more than 38 degrees), urinary tract infection during pregnancy, history of the complications of GBS infections. In the screening program, GBS culture from a vagina and rectum occur at 35-37 weeks of Pregnancy (5). While, in developed countries, in pregnant women sepsis is one of the causes of death in infants (6 - 8). The bacterium in vaginal or rectum colonize of the pregnant women in Southeast Asia is estimated and shows about 20-30% (9-12), In the United States, the Center of Disease Control (CDC) states that pregnant women of 35-37 weeks are screened to identify the bacterium carriers (13) which are treated by antibiotic prophylaxis. In fact, a recognizing and diagnosis, effective drug against this bacterium is important in protecting the health of pregnant women (15-14). Carrier diagnostic methods of this bacterium are different. Therefore, the aim of the present study was screening for GBS colonization of pregnant women by culture and Colony-PCR methods for BCA gene.

Materials and methods

Samples: Vaginal secretion samples were collected from100 pregnant women during visits to the Obstetrics and Gynecology Unit of the Moatazedi and ShahidChamran Hospitals in Kermanshah Province. Samples were collected using sterile swabs without using a speculum, according to CDC guidelines, during physical examination of the women between the 35^{th} and 37^{th} weeks of pregnancy. All patients provided written informed consent prior to inclusion in the study.

GBS culture: the swabs were transferred to Todd-Hewith-Broth medium and incubated at 33-37°C for 18 to 24h. Then, Samples were cultured on 5% sheep blood agar plates and incubated at 33-37°C for 18 to 24h in a 5% CO_2 atmosphere. β-hemolytic and non-β-hemolytic colonies were subculture in Todd-Hewitt broth and subjected to CAMP (creating a flash head at the intersection of Staph and Strep bacteria) test and latex agglutination analyses to confirm that they were GBS.

Antibiogram tests: Antibiotic resistance was evaluated using a diffusion method according to the standard CLSI instructions and the use of the antibiotic discs of erythromycin, penicillin, vancomycin, and clindamycin, and concentration, resistance, and sensitivity of these antibiotics were determined.

Colony PCR assay: In this study, a specific fragment of the BCA Streptococcus gene of B group was proliferated at 205 bp.The colony PCR was performed to a volume of 25µL containing 1.5 U of Taq DNA polymerase (sinagene,Iran); 0.4µM each GBS-specific primers bca F(5′-GCAACTGAGAAACATCCCCA-3′) and

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bca R (5′- GCTGCATCTACAA TTCCAGGG -3′); 2.5µL of 10× buffer containing 15mM MgCl2; 2.5 µL of dNTP with 0.2mM each nucleotide; and some bacterial colonies. The conditions for the PCR were as follows: 94°C for 1 min, followed by 35 cycles divided into denaturation (94°C, 1 min), annealing (55°C, 45 sec), and extension (72°C, 1 min). Subsequently, the material was maintained at 72°C for 10 min, and the amplified product was stored at 4°C until analysis. The electrophoresis was performed using 1.5% agarose gel. The amplification products were detected using under ultraviolet light. A ladder with fragments of known molecular weight was used as a marker (100pb ladder).

Results

Of all specimens, colony-PCR analysis detected GBS in a higher number of patient samples (6%) than the culture method (5%) (Table 1).

Table 1. Prevalence of Group B *Streptococcus* (GBS) colonization in 100 pregnant women, as determined by culture and PCR-based detection methods.

	Colonization*		
	Positive	Negative	Samples
Method	Number (%)	Number (%)	Number
Culture	5 (5)	95(95)	100
Colony PCR	6 (6)	94(94)	100)

As all the culture-positive samples were also positive by the PCR analysis, the sensitivity and specificity of the applied colony PCR method was 100% and 95.6%, respectively. Of the 95 culture-negative specimens, one tested positive for GBS by colony PCR.

Also, the results of antibiotic resistance test showed that resistance to erythromycin, penicillin, vancomycin and clindamycin were determined as 50%, 16.66%, 16.66% and 33.33 %, respectively. In addition, the highest resistance was against erythromycin and the appropriate antibiotics were penicillin and then vancomycin (Table 2). Based on the results of 6 known isolates from *S. agalactia*, some of them showed resistance to at least one of the antibiotics erythromycin, penicillin, vancomycin, and clindamycin. The isolates showed more sensitivity to vancomycin. Data analysis also showed that some of the isolates were resistant to two antibiotics at the same time. Resistance to erythromycin and clindamycin shown to be increased.

Table 1. Frequency and percentage of resistance and sensitivity measured from 6 isolates of *S. agalactia*.

	Resistance and sensitivity rates		
Antibiotic	Sensitive	Medium	Resistance
Erythromycin	2(33.33)	1(16.66)	3(50)
Penicillin	3(50)	2(33.33)	1(16.66)
Vancomycin	5(83.33)	0(0)	1(16.66)
Clindamycin	4(66.33)	0(0)	2(33.33)

Data are shown as number (percent).

Discussions

Detection of GBS colonization during pregnancy and prenatal care recommend by Medical Guidelines. In other countries, for example, European countries, such guidelines have been used for more decades (15-14). GBS that colonizes and infect the genital and urinary tract are causes of GBS neonatal disease. Therefore, this study and previous studies can help to set out guidelines for the prevalence indexes for GBS, according to geographic location, sociodemographic and clinical characteristics in different rejoin of Iran. Prevalence of GBS colonization was 5% and 6% by the culture and colony PCR methods, respectively. In this study, the GBS isolation rates increased to 6% when used by the colony PCR method. Sampling should be from vaginal and perianal, but only used vaginal swabbing. Also, in this study the results of antibiogram test showed resistance rates to erythromycin, penicillin, vancomycin and clindamycin were determined as 50%, 16.66%, 16.66% and 33.33 %, respectively. In addition, the highest resistance was to erythromycin and the appropriate antibiotic was first

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evaluated for penicillin and then vancomycin. The results indicate that resistance to erythromycin and penicillin and other antibiotics are increasing. Widespread use of antibiotics to prevent infections caused by this bacterium has raised concerns about the appearance of microbial resistance in group B streptococci. The most commonly used antibiotics are penicillin (14-17). In cases of allergy to beta-lactam, erythromycin and clindamycin are replaced; if clindamycin is susceptible or unavailable, vancomycin is used (15). Resistance to any of these drugs has been reported in numerous studies from around the world. *S. agalactia*, as an opportunistic organism, is able to produce various infections of all ages. This bacterium tends to be colonized in pregnant women and can be caused preterm delivery as well as infections dangerous (including meningitis, pneumonia, and septicemia) (14). Approximately 40 to 10 percent of pregnant women with GBS bacteria in both the rectum and the vagina carrying bacteria is colonized, and 80-70 percent of these women transmit GBS to their infants; therefore, the occurrence of carriers groups B streptococcus in pregnant women can be considered as a serious risk factor for pregnancy (16). According to the CDC proposal, all pregnant women between 37-35 weeks of pregnancy should be considered for the being carrier of GBS and treated with antibiotics (17). In the other study on 605 samples, they were obtained 16 percent in a culture method and a 28 percent in colony-PCR method on GBS vector (18). Also, in another study conducted in Iran, the GBS carriers in 250 pregnant women, 8.4 percent in culture, and molecular methods of 6.9 percent were reported (19). For study the resistance level of group B resistance, the frequency of the bca gene was reported to be 5.1 percent was reported (20). The results of this study are closed to the results above. In a study done in Brazil by Costa, the antibiotic resistance of group B streptococci, clindamycin, 25.4 and erythromycin 23.4 percent was reported (10). Also, Shahram Habibzadeh et al (2009) performed a study on the antibiotic resistance of group B streptococci, the erythromycin resistance of 1.6 and clindamycin 17 percent reported (20). In another study, the antibiotic resistance of group B streptococci to erythromycin and clindamycin, 15.57 and 92.2 percent respectively was reported (21).The results of isolated strains on this study were matched with some of the above and were not match with some another ones. This may be due to the difference in the exposure of bacteria to the antibiotics used for other purposes. Although, the suitable use of antibiotics leads to rapid treatment of streptococcal infections, inappropriate administration and change of bacterial properties lead to the drug resistance in this bacterium.

Conclusion

Based on the results, the rate of GBS prevalence in Kermanshah women is increasing. In addition, a higher prevalence of GBS colonization in pregnant women in Kermanshah was detected using the colony PCR method.

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