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A Study on the Frequency of 5 Virulence Genomes of Group B Streptococcus in Pregnant Women in Yasuj, Iran

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KEYWORDS

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A B S T R A C T

β Streptococci group B is a gram positive and CAMP positive bacterium which causes disease in humans and animals. It can be transferred to humans from dairy and animal products. *S.agalactiae* is the only species which carriers the group B antigen. The infection rate among pregnant women is higher than men. The bacteria can be transmitted through the placenta to the fetus and ultimately will lead to abortion in pregnant and septicemia in fetus. The aim of this study was to identify and treat pregnant women infected with the bacteria. A number of250 samples from pregnant woman at Imam Sajjad Hospital (Yasuj City) were prepared and cultured on culture media (Blood Agar). The cultures media were incubated for 24hours.Gram staining, CAMP, Catalase and Oxidase tests were used to detect the bacteria. Bacterial susceptibility testing was performed using McFarland Standard No 1. Out of 250 samples, 28 samples were contaminated with *β Streptococci Group* B (catalase and camp positive) that some of them had previous abortion. Meanwhile the rate of infected pregnant women 11.2 was reported. Among the patients the majority of them were susceptible to gentamycin (91.7%) and resistant to Erythromycin (94.3%).It also showed that the frequency of genome in this study were *scpb*(82.1%), *bca* (57.1%) *rib*(50%) *lmb*(32.1%) *cylE* (21.4%) respectively. According to the obtained results and complications of the infection, diagnosis and treatment of infections the disease can be significantly reduced.

Introduction

Group B Streptococcus (GBS) are capsulated gram-positive cocci and facultative anaerobic which colonize 10-40% of pregnant women's gastrointestinal and genital tract (1).Group B Streptococcus (GBS) remains a major cause of neonatal sepsis and is also associated with invasive and noninvasive infections in pregnant women and non-pregnant adults; elderly and patients with underlying medical conditions and vertical transmission from a colonized mother to her newborn during labor can result in life threatening infections. (2). several virulence determinants factors are involved in the adhesion to and invasion of host cells and the immune system evasion. It has been demonstrated that

polysaccharide capsule and proteins, such as Cα, Cβ, Rib and the laminin binding protein (LMB), and a number of enzymes (like the C5a peptidase) andoxins /cytolysins, are produced and associated with GBS virulence (3).

To identify GBS, general screening of pregnant womenin35to 39weeksis recommended. The resistance of GBS to antibiotics is variable (2,3).The intensity of congenital infections caused by GBS depends highly on virulence factors (Pathogenic) coded by bacterial genome, including GBS which codes capsule. Genome *SCPB* is used for coding SCPB superficial enzyme which causes damage to neutrophiles and fibrino gene plants which exist in epithelial cells (4).

BCA Gene codes Alpha proteina protein which helps bacteria to enter the host cells (5). Genome IMB codes IMB protein (Laminine connecting protein), which invades and damages the epithelium (6).Genome cycle codes Beta hemo lysinepoison, a poison which damages thetissue, and systemic spread of bacteria and has a role in the formation of meningitides (7).Genome rib super facial protein, a protein which is frequently seen in the invasive says of GBS, other pathogenic factors which interfere in pathogenic process of GBS is beta G protein which is coded by bca genome and it's action is reaction with FC part of immunoglobulin which prevents phagocytosis(8, 9).

Among other GBS pathogenic fibrinogens connecting proteins, such as *FbsB* and *FbsA* which is a superficial protein which is coded by *FbsA* genome.The protein prevents against opsonophagocytosis phenomenon and causes more connection of bacteria to epithelial cells and microvascular cells of human brain (HB MEC)and causes passing of bacteria from blood brain barrier and soared of meningitis(10). FBSB is coded by FbsB genome which is a superficial protein and helpseria to epithelial cells (11, 12).

With respect to the fact that streptococcus Agalactia is an agent of pathogen in human especially in pregnant women and could be transmitted to newborns and therefore jeopardizes their live, the objective of this research is to study the prevalence of GBS bacterium in pregnant women of Yasuj city and to evaluate the resistance of bacterium against antibiotics and frequency of each of pathogen genomes under study in each isolated bacteria.

Methodology

Firstly, with the purpose of vaginal sampling of pregnant women, the Todd Hewitt broth media was prepared, which is a transmitting and specific media containing antibiotics such as gentamycin and nalidixicacid. These media was used to transfer the collected sample from hospital to laboratory and also elimination of unnecessary bacteria(13).

Sampling process

Sterilized swap inserted in the vaginal muscle of pregnant woman and kept inside the vagine for a few moments, then moved to be mixed to vaginal liquid well. the soap taken out and put in the T.H.W media and media culture transfer to laboratory, and kept to 37 centigrade temperature for 24 hours and checked for bacterial growth if the bacteria growth in this media. All clinical isolates were confirmed as *S.agalactiae* by gram staining, catalase tests and CAMP test. CAMP is a specific test to diagnose GBS. After doing these tests the antibiogram test was performed using Muller Hinton Agar media by disk diffusion method to determine the susceptibility and resistant this bacterium against antibiotics(14).The PCR

test was performed on positive clinical bacteria, PCR kit and other things prepared from the Sinagen Company.In this research, five pairs of exclusive primers of ScpB, bca, rib, lmb and*cylE* genes were used to study the frequency of each genome:

Table.1 Primers used for recognition and isolation of genomes:

1 – Specific primer pair pairs*cpb* genome
2 - Specific primer pair pair*ribs* genome
3 - Specific primer pair pair*bca* genome
4 - Specific primer pair pair*lmb* genome
5 - Specific primer pair pair*cylE* genome
DNA Extraction:

DNA extraction by boiling method was used .The sample were taken out of -70 centigrade degrees and after being melted, were cultured on MRS media. After bacterial growth, 2 -3 colonies of bacteria were dissolved in 500 micro liter of distill water and kept in boiling water bath for 10 minutes then put on refrigerator -4 centigrade degree for 10 minutes and then centrifuged in 1200 rpm for 10 minutes and supernatant kept in sterilized microtubes for PCR test. In this method, the destroyedbacteria, Released DNA, the vials containing DNA were kept in a refrigerator.

Proliferation of extracted DNA using PCR reaction

At first, the PCR kite and primers were taken out of the freezer were taken,28 cases were contaminatedwith GBS bacterium and were kept in room temperature for 15 minutes then pilot tests were done using different volumes, temperatures, and plans to obtain the best results.For preparing the main mixture (master mix) and doing PCR test, its total volume was taken in to consideration, with regard to the number of number of sample. Positive and negative control, in addition to one more reaction and was transferred to thermocytermachine, to which proper programgiven in anticipation.To obtain proper quantities of compound used in sequential reactions of PCR, a gradient of different compounds were placed after obtaining proper conditions.PCR reaction was performed.

Results and Discussion

Among 250 pregnant women inYasuj, 28 cases were contaminated with GBS bacterium (11.2%).
The results of antibiogram test of 28 cases are shown in table 2:
The PCR results obtained from 28 positive samples showed the existence of SCPB genome in 23 samples (82.1%), *bca* genome in 16 (57.1%), *rib* genome in 14 (50%) *cylE*genome in 9 (32.7%) of samples, which are shown in table 3.
Considering the obtained data among 28 positive samples available in seven(7) samples 3 genomes SCPB,*bca* and *rib* were simultaneously present and 16 samples simultaneous presence of two(2) genomes (*SCPB*)and (*bca*) were observed.

Conclusion

For assessment and coping with GBS, different antibiotics have been tested that the resistance and susceptibility of these bacteria are variableas shown previously. In the present study the frequency of pathogenic genomes were obtained to be SCPB(82.1%),*bca*(57.1%) *rib*(50%) lmb(32.1%) *cylE*(21.4%),and in both studies SCPb had the frequency and highest effects in the pathogenic property of GBS.on the other hand the majority of subjects showed more susceptibility to Vancomycin, Cefalotin, Cephasolin, and Gentamycin respectively.

Table.2 Number and percentage of resistance, Intermediate and susceptibility of GBS to antibiotics

Antibiotic	Susceptibility	Intermediate	Resistance
Gentamycin	25(89%)	3(11%)	_
Colexacillin	12(42%)	16(58%)	_
Erythromycin	_	4(14%)	24(86%)
Vancomycin	28(100%)	_	_
Oxacyline	9(32%)	19(68%)	_
Penicillin	3(11%)	16(58%)	9(32%)
Cefalotin	28(100%)	_	_
Clindamycin	3(11%)	22(78%)	3(11%)
Chloramphenicol	13(46%)	1(4%)	_
Cephasolin	27(96%)	1(4%)	_
Rifampicin	_	25(89%)	3(14%)
Amikacin	_	6(22%)	22(78%)

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Table.3 Number (percent) of pathogenic genomes recognized in total samples of GBS

Genome	No. Presence in 28 samples	percent
SCPB	23	82.1%
Bca	16	57.1%
rib	14	50%
cylE	6	21.4
lmb	9	32.1%

It has been postulated that a total of 309 genital GBS strains cultured from vaginal/rectal all isolates were susceptible to penicillin, but high rates of resistance were found to both erythromycin (25%) and clindamycin (22%),15. In another study the results of the minimum inhibitory concentrations (MIC) tests showed all isolates (56 GBS subjects) were susceptible to ampicillin, vancomycin and penicillin. According to the disk diffusion test, 47 (83.9%), 8 (14.2%) and 7 (12.5%) isolates were resistant to Co-trimoxazole, ciprofloxacin and ceftriaxone respectively (16).

These results are in agreement with studyKimura K*et al* were collected 141 GBS isolates from vaginal specimens of 122 pregnant women in a hospital in Kobe, Japan. Of the 141 GBS isolates, 139 were subjected to antimicrobial susceptibility testing based on the results of screening for PRGBS by the disk diffusion method. All 139 isolates were susceptible to penicillin G, ampicillin, cefotaxime, cefepime, and meropenem (17).

According to Sherman K[1] study all 158 isolates were penicillin G sensitive. Inducible macrolide-lincosamide-streptogramin B (MLSB) resistance was observed in 13.9% of isolates. Constitutive MLSB resistance was observed in 12.7% of isolates (18). A total of 62 GBS strains were randomly selected for in vitro susceptibility testing to penicillin G, ampicillin, tetracycline, levofloxacin, gatifloxacin, ciprofloxacin, quinupristin-dalfopristin, linezolid, vancomycin, rifampicin,

trimethoprim- sulfametoxazol, nitrofurantoin, gentamicin, clindamycin and erythromycin, and determination of resistance phenotypes. No resistance to penicillin, ampicillin, quinupristin-dalfopristin, linezolid, and vancomycin was found.

Of the isolates examined 96.8%, 98.3%, 46.8%, and 29.0% were susceptible to rifampicin, nitrofurantoin, trimethoprim-sulfametoxazol and tetracycline, respectively (19). Generally speaking with respect to recent studies and the results that we obtained during our research in the city of Yasuj, one of the most important ways to better recognition of GBS is more research on virulence genomes of this bacterium, through which we can recognize the pathogenic genomes of the bacteria and study the way of coping with them.

Moreover the effects of geographical areas and different races and also in methodical use of different antibiotics on the rate of prevalence and resistance antibiotics (Especially important antibiotics such as penicillin) and GBS virulence must be studied.

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