Iranian Journal of Microbiology
Volume 15 Number 2 (April 2023) 219-224

ORIGINAL ARTICLE

Determination of pilus-islands profile and antibiotic susceptibility of *Streptococcus agalactiae* isolated from urine of pregnant women

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Received: September 2021**, Accepted:** February 2023

ABSTRACT

Background and Objectives: Group B Streptococcus (GBS) is one of the most important causes of neonatal diseases and postpartum fever. GBS infection can be transmitted from the infected mother to her baby during delivery. This bacterium is also involved in causing urinary tract infections and asymptomatic bacteriuria, pyelonephritis, cystitis and urethritis. In addition to capsule, Pilus is known as a virulence factor of GBS. The aim of this study was to evaluate the frequency of pilus islands and antibiotic resistance in GBS isolated from urine of pregnant women in Yazd, Iran.

Materials and Methods: In this cross-sectional study, 33 GBS samples isolated from the urine of pregnant women were studied by the multiplex polymerase chain reaction (PCR) method for the presence of pilus islands PI-1, PI-2a and PI-2b. Antibiotic resistance phenotype of tetracycline, penicillin, gentamicin, erythromycin, levofloxacin and clindamycin was determined by disk diffusion method. Data were analyzed using SPSS, version 16.

Results: PI-1+PI-2a was the most frequent pilus island in the GBS isolates 28 (84.8%) and the frequency of PI-2b was 5 (15.2%). The frequency of PI-1+PI-2a was 50% in serotype III and 25%, 14.3%, 7.1% and 3.6% in serotypes Ia, II, Ib and V respectively (P=0.492). The sensitivity of all GBS isolates to penicillin was 93.9% and highest resistance to tetracycline (97%), clindamycin (24.2%) and erythromycin (21.2%).

Conclusion: Most of the GBS urine isolates examined carried the PI-1+PI-2a gene, which increases bacterial potency in colonization and resistance to the immune system. Penicillin was best choice for prevention.

Keywords: Group B Streptococcus; Pregnant women; Bacterial pili; Antibiotic resistance; Urine; *Streptococcus agalactiae*

INTRODUCTION

Streptococcus agalactia or group B streptococcus (GBS) is a Gram-positive bacterium and is known as an important causes of sepsis, meningitis and pneumonia in infants and urinary tract infections (UTI) in both pregnant and non-pregnant women (1, 2). Also, this bacterium is a critical reason for mortality or morbidity in non-pregnant adults, mostly in the elderly and those with underlying diseases (3).

GBS colonization occurs in the vagina and rectum of 12-42% of women. It is also reported to cause 2

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to 3% of all UTI. This colonization can be transient, intermittent or chronic, and its rate varies in different geographical areas (4, 5).

The power of GBS in producing infection in urinary tract is highly dependent upon the presence of adhesive factors (5). Investigations showed that the capsule is the most important as adherence and virulence factors. Due to antigenic properties of capsular polysaccharide, the GBS has been classified into 10 serotypes as: Ia, Ib, II-IX (6).

In addition to capsule, the pili structure has a mission in GBS colonization, persistence, biofilm formation and invasion to the host's cell tissue (7). Research revealed that GBS represent two pilus island shown as PI-1 and PI-2. PI2 harbors two variants represent as PI-2a and PI-2b, Studies represent that the GBS strain harbor PI-2a and or PI-2b, but, however many contain PI-1 (7, 8).

Although GBS is currently sensitive to penicillin, unfortunately, a significant increase in resistance to macrolides and lincosamides has been observed in this bacterium (9). Therefore, it seems necessary to evaluate the antibiotic resistance of GBS isolated from clinical specimens.

The aim of this study was to determine the frequency of pilus islands and antibiotic resistance in *Streptococcus agalactiae* isolated from urine of pregnant women in Yazd, Iran.

MATERIALS AND METHODS

Study population. Thirty-three previously isolated GBS species from 346 urine samples of pregnant women (2015-2016), identified and serotyped, were further subjected to pilus-island determination using molecular technique. Furthermore, Antibiotic sensitivity test (AST) was performed using the Kirby-Bauer test for all isolates with the selected antibiotic discs. It is worth mentioning that in the previously published study (8), 57 GBS were isolated from vaginal swab samples of the same aforementioned 346 pregnant women who were tested for pilus-island and antibiotic resistance determination. This study was reviewed and approved by the Ethics Committees of Shahid Sadoughi University of Medical Sciences, Yazd, Iran [IR.SSU.MEDICINE.REC.1396.150].

All samples were re-cultured on agar medium and after incubation at 37°C for 24 hours; they were used to extract DNA. In this study, different GBS strains dedicated by Fanrong Kong from the Microbiology Laboratory Service Center (New South Wales, Australia) were used as positive control and sterile water was used as negative control.

Genomic DNA extraction. After culturing GBS overnight in broth, extraction was performed using DNA extraction kit (Gen All, South Korea) and according to the manufacturer's instructions.

Multiplex PCR test for detection of pilus island genes. The presence of GBS pilus islands PI-1, PI-2a and PI2b was evaluated with the multiplex PCR test described by Martins et al. (10) (Table 1). PI-1-All primers were used to confirm PI-1 negative isolates that did not carry the pathogenic island of pilus or specific parts of it. Negative isolates of PI-1 gene were subjected to PCR with PI-1-All primers.

Antibacterial susceptibility testing. To evaluate antibacterial susceptibility, Kirby-Bauer test was performed according to CLSI (2018) using tetracycline (30 µg), penicillin (10 µg), gentamicin (120 µg), erythromycin (15 µg), levofloxacin (5 µg) and clindamycin (2 µg) (MAST, England) antibiotic discs (2018) (11). According to the protocol, 0.5 McFarland suspension was cultured on Müller Hinton agar (Merck, Germany) containing 5% of sheep blood and then antibiotic discs were placed on the medium. Furthermore, inducible clindamycin resistance was identified according to CLSI 2018 guidelines by using the D-Zone test (11). The inhibition zone of each disk was determined after overnight incubation, and the isolate was described as susceptible, intermediate or resistant, also for the control, *Streptococcus pneumoniae* (ATCC 49619) was used.

Statistical analysis. Data analysis was performed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA). To compare the frequency of PI-1, PI-2a and PI-2b genes in different odds ratio groups, Chi-square test was used and a P-value of less than 0.05 were considered statistically significant.

RESULTS

The results of multiplex PCR to determine pilus island genes of GBS isolates showed that the highest frequency was 28 (84.8%) for PI-1+PI-2a, and the fre-

Table 1. The sequencing of primer of pilus islands PI-1, PI-2a and PI2b

Gene	PCR primer	Sequence 5´→3´	Product size (base pairs)
PI1	Forward	GGTCGTCGATGCTCTGGATTC	881
	Reverse	GTTGCCCAGTAACAGCTTCTCC	
PI2a	Forward	CTATGACACTAATGGTAGAAC	575
	Reverse	CACCTGCAATAGACATCATAG	
PI2b	Forward	ACACGACTATGCCTCCTCATG	721
	Reverse	TCTCCTACTGGAATAATGACAG	
PI1-all	Forward	ACCTATGTTGCTGATTCGGCTGAAAATG	684
	Reverse	TACGGACACTTTCTAGTGCCTTTGGATC	

quency of I-2b was 5 (15.2%). Note that, the pilus island gene of PI-2a was not observed (Fig. 1, Table 2).

Frequency distribution of genes encoding pilus islands across serotypes showed that the frequency of serotype III was 17 (51.5%) in GBS isolated, among which, 14 (82.4%) contained PI1+PI2a and 3 (17.6%) were PI2b (Table 2).

Fig. 2. exhibits the sensitivity and resistance of GBS samples. The results demonstrated that 93.9% of isolates were sensitive to penicillin, but 97% isolates were resistance to tetracycline. However, 21.2% and 24.2% of isolates were resistance to erythromycin and clindamycin respectively too. Inducible resistance to clindamycin or D- zone (iMLSB) was not found. Also 7 (21.2%) of isolates harbored cMLSB phenotype (resistant to both clindamycin and erythromycin) and associated with serotypes Ia (2 isolates), II (1 isolates), III (4 isolates). One (3%) of serotype Ia was sensitive to erythromycin but resistance to clindamycin (L phenotype) and M phenotype not found.

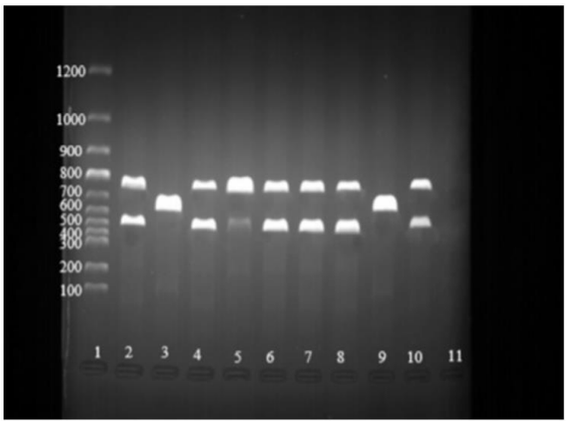

Fig. 1. Gel electrophoresis of multiplex PCR amplification products for determining pilus island genes of GBS isolates. Lane 1 is DNA ladder (100 bp, Fermentas). Lanes 3 and 9 are PI-2b, and lanes 2, 4, 5, 6, 7, 8 and 10 are PI-1+PI-2a and Lane 11 is negative control.

DISCUSSION

In the present study, 33 isolated and serotyped GBS from urine samples of pregnant women were further tested for pilus- island determination. Also, the frequency of antibiotic resistance of the isolates was determined. As Table 2 indicate among 33 GBS, PI-1+PI2a was found to be 28 (84.8%) followed by PI-2b with 5 genotype (15.2%). In a similar study, Desai et al. (12) revealed that among 292 GBS 110 (37.7%) were PI-1+ PI-2a. This controversy in prevalence of pilus- island is probably due to the limited number of GBS tested in our study. In this survey, there was not

Table 2. Distribution of genes encoding pilus islands across serotypes

Pilus island	Capsular serotypes N (%)					
	Ia	Ib	II	III	V	Total
PI-1+PI-2a	7 (100)	2 (100)	4 (66.7)	14 (82.4)	1 (100)	28 (84.8)
PI-2b	0 (0)	0 (0.0)	2 (33.33)	3 (17.6)	0 (0.0)	5 (15.2)
Total	7 (100)	2 (100)	6 (100)	17 (100)	1 (100)	33 (100)

P value= 0.492

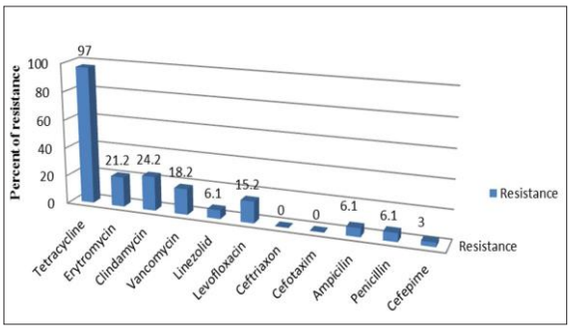

Fig. 2. Antibiotic resistance of GBS isolates

any PI- 1 or PI-2a detected separately but they could isolate PI-1 (205/292) followed by PI-2a with 117/292 strain of GBS. It is important to note that PI-1 + PI-2a are directly correlated with maternal colonization whereas, PI-1 +PI-2b have a critical task with the disease of neonates (13). In addition, GBS, which carries PI-1 along with PI-2a, may cause invasive infections in adults (14). In our previous published work (8), the most frequent of pilus- island with 71.9% were PI-1+ PI- 2a. In our recent study, among 33 GBS strains isolated from urine 84.7% were PI-1 + PI-2a. As mentioned before, the urine GBS strains used for pilus- island determinations were all isolated from the same women whom vaginal swabs were tested for pilus-island type identification. When compared the GBS pilus- island from urine with those detected from the vaginal GBS, it was found that 28 pilus-island were the same as vaginal GBS pilus- island. The remaining 5 pilus- island as PI-2b (1 strain) and PI- 2a (4 strain) found in vaginal GBS, whereas PI-1+ PI2a were recognized from urine GBS isolates. This result represents that there may exist two types of GBS strain in the same niche. Since the publications regarding pilus- island of urine GBS is limited, therefore it seems justified to compare the results with those work reported from study of pilus- island of vaginal GBS. Madzivhandila et al. showed that the pilus type PI1+PI2b (45.1%) was the most prevalent genotype from GBS isolated of vaginal samples, followed by PI2a (29.8%), PI1+PI2a (24.8%) and PI2b (0.2%). The authors reported that the combination of PI-1 + PI2a is higher in colonized GBS compared to invasive isolates (15). Nasr Esfahani et al. reported that the most frequent pilus types were PI1 (38.7%), PI1+PI2a (33.8%), PI1+PI2b (24.1%), and PI2a (3.2%) among non-pregnant adults (16). Also, other studies revealed that PI-1+ PI-2a was the most common pilus island in colonizing isolates (8, 17). The results of this study showed that PI-1+PI-2a and PI-2b were the most frequent pilus islands among urine samples of pregnant women. It seems that combination of PI-1 and one of the PI-2 variants enhances invasiveness of GBS strains (18). Also, PI-1+PI-2a could improve survival of GBS strains by increasing resistance to host immune system, disinfectants used in the hospital environment and starvation (19) while pilus 2b is associated with bacteremia and infiltration of the blood-brain barrier (20). The diversity within PI-2a is likely to increase the colonizing ability of multiple hosts and niches. Support for this hypothesis of high frequencies reported in asymptomatic women (13).

As Table 2 shows, the most isolates (82.4%) contain PI1 + PI2a and belong to serotype III. Khodaei et al. demonstrated that 67% of GBS isolates were PI1+PI2a and were generally seen in serotype III (21). In another study, 57.9% of the isolates belonged to serotype III, of which 90.9% contained PI1 + PI2a. The authors mentioned, serotype III of vaginal GBS isolates contained all pilus types except PI-2b, while our findings showed that 17.6% of serotypes III contained PI-2b (8). Different studies have stated an association between serotype III and neonatal meningitis (6, 22).

The results of sensitivity and resistance of GBS samples to the selected antibiotics demonstrated that the majority of isolates were resistant to tetracycline, erythromycin and clindamycin, and the lowest resistance was to ceftriaxone, cefotaxime and cefepime. Penicillin resistance was found in 6.1% of isolates (Fig. 2). On the contrary Gizachew et al. reported that among 1974 GBS detected from pregnant women, 33.6% isolates showed resistance to penicillin (23). But in Iran, Khodaei et al. showed that all of GBS isolates except one were found to be sensitive to penicillin (21). In another study, Nabavinia et al. reported that 10.5% of GBS strains were penicillin-resistance, and 96.5% were resistant to tetracycline which concurred with the present study. The frequency of D-zone (iMLSB) was 21.1% while, the D-zone phenotype was not found in the present study (8). In a study conducted by Bolukaoto et al. (24) 100% of the GBS isolates were sensitive to penicillin, vancomycin and ampicillin, and 21.1% and 17.2% of the isolates were resistant to erythromycin and clindamycin, respectively. The cMLSB phenotype was found in 69% and the M and L phenotype in 6.8% of the isolates, which showed an increase in antibiotic resistance compared to the present study.

In a study by Genovese et al. (2), Which was performed on 3494 GBS isolated from pregnant women, most of erythromycin resistance GBS isolates (77.7%) showed cMLSB and L phenotype was found in serotype V (77.8%) and Ia (22.2%), whereas in the present study, all of erythromycin resistance GBS isolates (21.2% of total isolates) showed cMLSB and L phenotype was associated only with serotype Ia, which may be due to the small sample size of the present study. Penicillin and ampicillin are the main drugs for the inhibition and treatment of GBS infection. The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists distributed aligned guidelines in 2019 and 2020 for the prevention of perinatal GBS infection, the main suggested method to prevent perinatal GBS disease was maternal intrapartum antibiotic prophylaxis based on antenatal screening for GBS colonization. Penicillin, ampicillin and cefazolin are suggested for prophylaxis (25). On the other hand, resistance to these antibiotics among GBS isolated from pregnant women has been established in some countries (26-28). The findings of this study support the idea of developing a vaccine globally effective against this opportunistic bacterium based on situational, geographical and racial conditions. The observed resistance to tetracycline, erythromycin and clindamycin and the existence of the genes encoding virulence determinants among GBS isolates indicate the necessity for continued monitoring of GBS to prevent the expansion of infections.

This cross-sectional study was carried out on a small sample size; therefore, the obtained data cannot be generalized to other populations. The various studies over the past five decades, indicate that resistance of GBS against penicillin, erythromycin and clindamycin are noticeably increasing and the possibility of appearance of new resistance strain highlight the need for ongoing monitoring of GBS population to help promote immunization and preventive strategies. Therefore, it is suggested that further clinical trial studies be conducted on the field.

CONCLUSION

The obtained results showed that 28 (84.8%)/33 GBS isolated from urine of pregnant women contained pili PI-1+ PI-2a. Hence, this type of pilus-island has an important role in GBS invasion and pathogenicity; therefore, special attention is required to prevent the prevalence of GBS species among pregnant women.

Base on the results of this study, the screening program for treatment of pregnant women who are infected is strongly recommended during the 35-37 weeks of pregnancy.

ACKNOWLEDGEMENTS

We are grateful to Fanrong Kong for sending GBS control strains.

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