JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Screening and genotyping of group B streptococcus in pregnant and non-pregnant women in Turkey

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Abstract

Introduction: The purpose of this study was to investigate group B streptococcus (GBS) colonization, to compare the methods, to determine the relationship between GBS carriage and risk factors, and to genotype the GBS isolates.

Methodology: Recto-vaginal swab specimens were obtained from 500 women, and a questionnaire was administered to each to assess their risk factors for GBS carriage. A culture, GBS antigen test, and polymerase chain reaction (PCR) were performed on all samples. Antibiotic susceptibility testing was performed, and the clonal relationship was determined by pulsed-field gel electrophoresis (PFGE) on all viable isolates.

Results: Of the 500 women, sixty-eight (13.6%) women were GBS carriers, of whom 9.8% were pregnant and 16.5% not. There was a significant difference between GBS carriage and history of premature rupture of membrane (PROM). GBS was isolated from 65 (13%) samples. GBS was positive in 70 (14%) samples by antigen test and in 62 (12.4%) by PCR. Sixty-eight of the 70 positive antigen tests were confirmed by PCR or culture. Fifty-five isolates were resistant to tetracycline, 16 to erythromycin and clindamycin, and 13 to levofloxacin. Thirteen different pulsotypes and 17 sporadic strains were determined by PFGE.

Conclusions: GBS carriage rate in non-pregnant women was higher than in pregnant women. The GBS antigen test was more sensitive than culture and PCR. GBS isolates did not originate from a single clone and contained sporadic strains. There was a significant difference between GBS carriage and history of PROM. Epidemiologic data obtained in this study will help future studies.

Key words: Group B streptococcus; carriage; culture; PCR; GBS antigen test; PFGE.

J Infect Dev Ctries 2016; 10(3):222-226. doi:10.3855/jidc.6190

(Received 03 November 2014 – Accepted 05 November 2015)

Introduction

Streptococcus agalactiae or group B streptococcus (GBS) are members of normal flora in human genitourinary and gastrointestinal systems. GBS is the main agent for serious infections such as meningitis in newborns, asymptomatic bacteriuria, urinary system infections, cystitis, pyelonephritis, chorioamnionitis, postpartum endometritis, pre and postpartum bacteremia, and post-cesarean wound infections in pregnant women [1].

The colonization of GBS in the urogenital or gastrointestinal system of the mother is the most important risk factor for the development of invasive newborn disease [2]. When no precaution is taken, early-onset GBS infections develop in 1–2% of the infants of women with GBS colonization, usually in the first days of life, which often progresses to fulminant disease. The majority of GBS infections are early-onset infections and can be prevented with intra-partum antibiotic prophylaxis [3,4]. The Centers for Disease Control (CDC) recommends screening all pregnant women at 35–37 weeks of gestation and providing intra-partum antibiotic prophylaxis to pregnant women with recto-vaginal colonization, women who previously delivered infants with invasive GBS infection, those identified as having GBS bacteriuria in any trimester of pregnancy, and those with unknown GBS status at the onset of labor but who have risk factors, for the prevention of early-onset neonatal GBS disease [5].

The purpose of this study was to investigate GBS colonization using culture, the GBS antigen test, and polymerase chain reaction (PCR); to compare the methods; to determine the relationship between GBS carriage and the risk factors in childbearing-age women followed up in our hospital and to genotype the GBS isolates.

Methodology

Study design and population

This prospective study was approved by ethical committee of Faculty of Medicine, Selcuk University (number 12, November 2011). A total of 500 women between the ages of 15 and 45 admitted to Selcuk University, Faculty of Medicine, Obstetrics and Gynecology Clinic in 2013 were included in the study. Women with a history of antibiotic use in the last month were excluded. The clinical data collected included age, presence of a intrauterine device, numbers of pregnancies and births, pregnancy week, menstruation period, and history of premature rupture of membrane (PROM).

Specimens

Vaginal samples were obtained from the lower one-third of the vagina with a sterile swab at lithotomy position without using specula. Rectal samples were taken from the rectum, forwarding the swab 2 cm from the anal sphincter to the rectum and rotating 360 degrees. The swabs were placed in Amies transport medium (Cultiplast, Milan, Italy).

GBS antigen test and bacteriological methods

The samples were inoculated in LIM broth containing 10 µg/mL colistin and 15 µg/mL nalidixic acid, in Todd-Hewitt broth (Becton Dickinson, Sparks, USA), and aerobically incubated at 35°C for 16–24 hours. After incubation, 100 µL of LIM broth was used for the GBS latex agglutination test (bioMérieux, Marcy-l'Etoile, France). *S. agalactia* ATCC 13813 was used as a positive control. Next, 50 µL of broth was subcultured onto chromID Strepto B agar (bioMérieux) and aerobically incubated at 35°C for 48 hours. Pink-red-colored colonies on chromID Strepto B agar were phenotypically identified with CAMP and GBS latex agglutination tests (bioMérieux). Antibiotic susceptibility testing was done using the Kirby-Bauer disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [6]. *S. pneumoniae* ATCC 49619 was used as the control strain.

Molecular methods

DNA isolation from LIM broth was made using a commercial kit (Nanohelix, Daejeon, South Korea) according to the recommendations of the manufacturer. PCR was performed on SensoQuest LabCycler (SensoQuest, Goettingen, Germany) device using *atr*-F 5'-CAA CGA TTC TCT CAG CTT TGT TAA-3' and *atr* -R 5'-TAA GAA ATC TCT TGT GCG GAT TTC-3' primers specific to the *atr* gene (780 bp). PCR amplification steps included an initial denaturation at 95°C for five minutes followed by 30 cycles at 94°C for one minute, annealing at 55°C for 45 seconds, elongation at 72°C for one minute, and a final elongation at 72°C for 10 minutes [7].

GBS strains were genotyped by the pulsed-field gel electrophoresis (PFGE) method using *SmaI* enzyme (10 U/µL) (Fermentas, Vilnius, Lithuania) in CHEF DR-II system (BioRad Laboratories, Nazareth, Belgium) at 14°C, with 6 V/cm² for 20 hours. The initial switch time was 3 seconds, and final switch time was 55 seconds. The gel was stained with ethidium bromide, and DNA bands were imaged and photographed using the Gel Logic imaging system (Kodak Company, Rochester, USA) under UV light. DNA band profiles were analyzed with Gel Compar II software, version 5.0 using the unweighted pair group method with mathematical average (UPGMA) method and the Dice similarity coefficient. A 1% tolerance was used to compare the DNA profiles. If the similarity coefficient was ≥ 70% (contained a minimum of four bands), it was considered similar [8]. *S. agalactia* ATCC 13813 was used as the control strain.

Statistical analysis

A sample was considered as GBS positive if it was positive in culture and/or PCR [9]. The sensitivity, specificity, and positive and negative predictive values of methods were calculated. The Kolmogorov-Smirnov Z test was used to analyze whether the data was parametric. The relationship between GBS carriage and risk factors was evaluated using the Chi-square, McNemar, and Mann-Whitney U tests. $P < 0.05$ was considered statistically significant.

Results

Of the 500 women, 215 (43%) were pregnant, and 285 (57%) were not pregnant. A total 68 (13.6%) women were GBS carriers; 21 (9.8%) of the 68 GBS-positive women were pregnant and 47 (16.5%) were not pregnant. GBS positivity was statistically higher in non-pregnant women than in pregnant women ($p = 0.03$).

There was no statistically significant relationship between GBS carriage and age, numbers of pregnancies and births, pregnancy week, menstruation period, and presence of intrauterine device ($p > 0.05$). GBS colonization was determined in 5 of 13 (38.5%) women who had a history of PROM in previous births and in 55 (11.3%) of 487 women who had no history of PROM. There was a statistically significant relationship

Table 1. Relationship between GBS carriage and risk factors

Risk factors		Number	GBS carriage number (%)
Age	≤ 35	368	42 (11.5)
	> 35	132	26 (19.7)
Numbers of pregnancies	0	59	9 (15.3)
	1	110	13 (11.8)
	2–3	238	32 (13.4)
	> 4	93	14 (15.1)
Numbers of births	0	148	18 (12.2)
	1	128	15 (11.7)
	2–3	194	32 (16.5)
	> 4	30	3 (10.0)
Pregnancy week	≤ 12	26	2 (7.7)
	13–34	74	6 (8.1)
	35–37	52	7 (13.5)
	≥ 38	63	6 (9.5)
Menstruation period	Follicular phase	100	13 (13.0)
	Luteal phase	185	34 (18.4)
Presence of intrauterine device	Yes	26	2 (7.7)
	No	259	45 (17.4)
History of PROM	Yes	13	5 (38.5)
	No	487	55 (11.3)

between history of PROM and GBS positivity (p = 0.022) (Table 1).

GBS was isolated from 65 (13.0%) samples. Seventy (14%) samples were positive by the GBS antigen test and 62 (12.4%) by PCR. Sixty-eight of 70 samples positive by the GBS antigen test were confirmed by culture and/or PCR. Although there was a statistically significant difference between PCR and the GBS antigen test (p = 0.008), there was no statistically significant difference between culture and the GBS antigen test (p > 0.05) or between culture and PCR (p > 0.05). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and comparison of methods are shown in tables 2, 3, and 4.

None of the isolates were resistant to penicillin, ceftriaxone, vancomycin, or linezolid. Fifty-five isolates were resistant to tetracycline, 16 to erythromycin and clindamycin (10 inducible resistant, 6 constitutive resistant), and 13 to levofloxacin.

Table 2. Results of the three assays used for GBS detection

	Culture %	GBS antigen test %	PCR %
GBS positive (n = 68)	13.0	14.0	12.4
GBS negative (n = 432)	87.0	86.0	87.6
Sensitivity	95.6	100	91.2
Specificity	100	99.5	98.6
PPD	100	97.1	100
NPD	99.3	100	98.9

GBS: group B streptococcus; PPV: positive predictive value; NPV: negative predictive value

Of 65 GBS isolates, two did not grow in passages; therefore, 63 strains were genotyped by PFGE. In dendrogram analysis, 13 different pulsotypes (PT) from 46 isolates were found. There were 17 were sporadic strains. Ten isolates in PT4 and six isolates in PT6 had similar genotypes (Figure 1).

Discussion

GBS infections are important health problems for newborns, and screening pregnant women for GBS carriage is important and necessary, as these infections are preventable and treatable. GBS colonization rates in women were 16%–30% in several studies [10-12]. In our study, the GBS colonization rate (16.5%) in non-pregnant women was similar to those found in previous studies. GSB colonization rates in pregnant women have been reported at rates ranging between 2% and 36% worldwide [13-20]. GBS colonization in pregnant women was reported to be between 3% and 15% in recent studies carried out in Turkey [21,22], but was 9.8% in our study. Unlike the other studies reporting GBS increases in pregnancy, in our study, GBS colonization in non-pregnant women (16.5%) was significantly higher than in pregnant women (9.8%) (p = 0.03).

The number of sexual partners, geographic region, race, cultural situation, age, parity, pregnancy week, presence of an intrauterine device, inadequate antenatal

Table 3. Comparison of GBS antigen test, culture, and PCR assays

Total	GBS antigen test		Culture		PCR	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive (n = 68)	68	0	65	3	62	6
Negative (n = 432)	2	430	0	432	0	432
Total (n = 500)	**70**	**430**	**65**	**435**	**62**	**438**

GBS: group B streptococcus; PCR: polymerase chain reaction

Table 4. Comparison of culture and PCR assays

Culture	PCR		Total
	Positive	Negative	
Positive	59	6	65
Negative	3	442	445
Total	**62**	**448**	**500**

PCR: polymerase chain reaction

care, and smoking have been reported as important factors for GBS colonization [23]. In our study, we found no statistically significant relationship between GBS carriage and age, numbers of pregnancies and births, pregnancy week, menstruation period, and presence of intrauterine devices. We identified GBS colonization in 5 of 13 women who described PROM in their previous deliveries, demonstrating a statistically significant relationship between GBS positivity and history of PROM. In a previous study carried out in Turkey on pregnant women, no significant relationship was found between GBS colonization and PROM [22].

The GBS antigen test done directly from LIM broth has been reported to be more sensitive than culture [9,24]. In our study, 68 of 70 samples positive by the GBS antigen test were confirmed by culture and/or PCR. Our results showed that there was no statistically significant difference between GBS antigen test and culture, but there was a significant difference between the GBS antigen test and PCR. The GBS antigen test had the highest sensitivity of the methods used in our study but had two false-positive results.

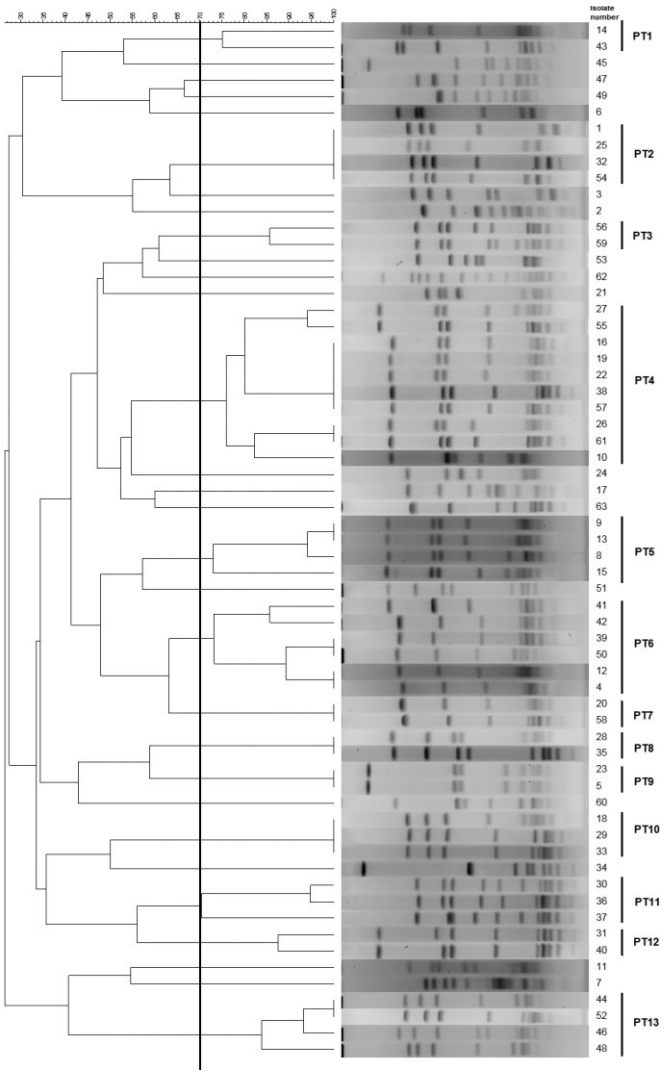
Previous studies have reported that PCR done on aliquots from LIM broth was more sensitive than culture [9,24-27]. In our study, 62 (12.4%) samples were positive by PCR from LIM broth. There was a significant difference between PCR and GBS antigen test, but not between PCR and culture. There was no statistically significant difference between culture, the GBS antigen test, and PCR. Culture was more sensitive than PCR.

PFGE is considered to be the golden standard of molecular typing methods and can determine clonal relationships between strains and indicate genetic origins or clonal diffusion [28,29]. In our study, PFGE showed that 63 GBS isolates were in 13 different pulsotypes; 10 isolates in PT4 and 6 isolates in PT6 had similar genotypes. We determined that our GBS isolates did not originate from a single clone but were distributed in different clones within 17 sporadic strains.

Conclusions

The GBS carriage rate in non-pregnant women was higher than that in pregnant women in our study. The GBS antigen test was faster and more sensitive than were culture and PCR. Analysis of demographic characteristics showed that there was a positive relationship between GBS positivity and history of PROM. GBS isolates did not originate from a single clone, were distributed in different clones, and contained sporadic strains. This study is the first in Turkey in which GBS was screened by three different methods and the isolates genotyped by PFGE. We believe that recto-vaginal screening for GBS in pregnant women at 35–37 weeks of pregnancy will decrease GSB-related morbidity and mortality rates in newborns in Turkey. Epidemiologic data obtained in this study are believed to help future studies.

Figure 1. Dendogram and PFGE imaging of group B Streptococci



Acknowledgements

This study was supported by Selcuk University Scientific Research Projects Coordinator ship. A part of this study was

presented as a poster in the 16th International Congress on Infectious Diseases (April 2014) in Cape Town, South Africa.

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Conflict of interests: No conflict of interests is declared.