Current Trends in
Microbiology
Vol. 9, 2014

Original Communication

Colonization by *Streptococcus agalactiae* in pregnant women during labor: serotype distribution of the involved strains and performance of PCR assay as a screening test

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ABSTRACT

Group B *Streptococcus* (GBS) is the leading cause of neonatal sepsis in the developed world. Little is known about the epidemiology and microbiological characteristics of the involved strains in the developing countries, where the majority of deaths from neonatal infections occur. The goals of this study were to assess the prevalence of recto-vaginal colonization with Group B *Streptococcus* (GBS) in pregnant women going into labor; to determine the capsular serotype of the recovered strains; and evaluate the performance of a commercial qualitative polymerase chain reaction (PCR) assay as a screening test. Between April and November 2012 we studied 87 pregnant women who delivered at the 'Dr. Manuel Quintela University Hospital'. Recto-vaginal samples were processed by standard microbiological procedures as well as commercial PCR assay for GBS direct detection. Antimicrobial susceptibility tests on the recovered GBS strains were performed by disk-diffusion to penicillin, erythromycin and clindamycin. GBS isolates were serotyped by a previously described PCR procedure which identifies the following serotypes: Ia, Ib, II, III, IV, V, VI, VII and VIII. We recovered GBS in 9 out of 87 women (10.3%; 95% CI: 3.9-16.7). All the recovered GBS were susceptible to the antibiotics tested. The serotype distribution was as follows: serotype III, 3 isolates; serotypes Ia, Ib, IV and V, one isolate of each. The performance values of the commercial PCR assay applied directly to samples were: sensitivity 100%, specificity 97%, positive predictive value 82%, and negative predictive value 100%. If we take all PCR results as true positives, the performance of this PCR assay would be even better than standard culture method. In this case, the prevalence of GBS carriage in this population rises up to 13.2%. Although results are ready in 45 minutes, PCR assay requires the use of sophisticated laboratory equipment and does not allow the determination of the antimicrobial susceptibility pattern or the determination of the capsular serotype of the involved strains.

KEYWORDS: pregnancy, Group B *Streptococcus* colonization, screening assays, PCR, serotypes

INTRODUCTION

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is one of the most common causes of early-onset neonatal infection. The incidence of these illnesses ranges from 0.80 to 3.06 per 1,000 live births in developing

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countries [1]. As recommended by the Centers for Disease Control and Prevention (CDC), all pregnant women between 35-37 weeks of gestation should undergo routine microbiological screening for GBS carriage, and those who are colonized with GBS should receive intrapartum prophylaxis with suitable antibiotics (penicillin or ampicillin) to decrease the risk of vertical transmission [2].

However many pregnant women, especially those from low income households with limited access to health services, reach birth-time without performing routine microbiological screening [3]. In these cases, analysis of risk factors for neonatal infection or the use of rapid tests for screening for GBS carriage are useful tools to make therapeutic decisions. The sensitivity of different commercially available rapid tests based on antigen-antibody reaction to directly detect GBS antigens in recto-vaginal samples ranges between 35 to 50% [3, 4]. These figures preclude their use as first-line screening tests. Hence in 1997 FDA issued a safety alert with regard to direct antigenic test kits for GBS producing false-positive and false-negative results.

Furthermore, there are several commercial tests based on DNA amplification that can be used to detect GBS nucleic acid in vaginal and rectal samples. Published studies on the performance of commercially available PCR assays on non-enriched samples have demonstrated varying sensitivities (range: 62.5%-98.5%) and specificities (range: 64.5%-99.6%) compared with the gold standard involving an enrichment step followed by subculture [5, 6, 7, 8].

The aims of this study were to describe the GBS colonization rate in pregnant women going into labor; to determine the capsular serotype distribution of the recovered strains; and to evaluate the performance of a commercial qualitative PCR assay as a rapid screening test.

MATERIALS AND METHODS

Study design

Between April and November 2012 we studied 87 pregnant women who delivered at the ‘Dr. Manuel Quintela University Hospital’, a 350-bed tertiary hospital attending 700 births per year, most of them with high-risk level (e.g., pregnancy at < 18 years of age, short interval between pregnancies, more than five pregnancies, preeclampsia, placental abruption, immaturity or intrauterine growth restriction, drug abuse, etc.). Most people attending the University Hospital live in low or very low income households.

The study was approved by the School of Medicine Ethical Committee and each woman gave written informed consent.

Detection of GBS carriage by culture; characterization of the recovered strains

Vaginal and rectal samples were obtained by a gynecologist during labor. All the specimens were collected using sterile nylon flocked swabs. The vaginal swab was rolled round against the vaginal wall at the midportion of the vault. For rectal specimens, a swab was carefully inserted approximately 1.5-2.5 cm beyond the anal sphincter and then gently rotated.

The swabs were placed in Todd Hewitt broth (TH) and Todd Hewitt broth with 5% sheep blood (TH-B), both supplemented with gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) [2]. The samples were rapidly transported at room temperature to the Bacteriology and Virology department.

After 24 hours incubation at 37 °C all the broths were sub-cultured onto 5% sheep blood agar plates. Suspicious colonies were identified by conventional phenotypic procedures (e.g. colony morphology, β-haemolysis, Gram stain, catalase and CAMP (Christie Atkins Munch-Petersen) tests). Detection of carbohydrate specific group was carried out using a commercial latex agglutination assay kit (Slidex® Strepto Plus B, bioMérieux, Marcy l´Etoile, France).

Antimicrobial susceptibility testing was performed by disk-diffusion for penicillin, erythromycin and clindamycin (Oxoid Ltd., Basingstoke, Hampshire, UK) [9].

Capsular serotype was determined by a previously described PCR assay which identifies the following serotypes: Ia, Ib, II, III, IV, V, VI, VII and VIII [10]. Briefly, 4 or 5 individual GBS colonies were suspended in 1 ml of digestion buffer (10 mM Tris-HCl [pH 8.0], 0.45% Triton

X-100, 0.45% Tween-20) in 1.5 ml tubes. The tubes containing GBS suspensions were heated at 100 °C for 10 min in a water bath and then quenched on ice for 10 min, and centrifuged at 16,000 X *g* in a micro-centrifuge for 2 min to pellet the cell debris. A 4 μL aliquot of the supernatant was added to 21 μL of the PCR mixture reaction (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$, 2 mM of each deoxynucleoside triphosphate), 1.25 U of *Taq* DNA polymerase (HybriPol™, Bioline, UK), and 0.4 μM of each primer (SBS Genetech Co., Ltd) shown in Table 1. The reactions were run in a thermal cycler (Gene Amp® PCR System 2700 Applied Biosystem) with the following cycling conditions: 94 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing for 1 min (see Table 1 for annealing temperature) and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. PCR products were visualized after electrophoresis in 2% agarose gels in 0.5X TBE buffer and ethidium bromide staining.

Direct detection of GBS carriage by PCR

PCR samples were obtained strictly following the manufacturer's instructions and rapidly transported to the Bacteriology and Virology department.

DNA extraction was performed directly from swabs (recto-vaginal non-enriched samples) soaked in 0.5 ml of Vircell® kit sample solution for 10 seconds, heated at 100 °C for 10 min, and then centrifuged to pellet the cell debris, according to manufacturer's instructions. The obtained DNA was stored at -20 °C. We performed a commercial PCR assay (Oligo-EGB Speed, Vircell®), following manufacturer's instructions. The reactions were

Table 1. Oligonucleotide primers used in this study for molecular serotype identification of GBS strains.

Target serotype	Primer	Oligonucleotide sequence (5´-3´)	Product length (bp)	*Annealing* temperature
Ia	IacpsHS	ATACAGTTGTCGTAAAGAAGAAAAC	361	55 ºC
	IacpsHA	TGTTTAGCTTTCCTACCAATATTAG		
Ib	IbcpsHS	TTTAGAAGTCCAGAATTTCATAGAGTC	280	57 ºC
	IbcpsHA	CAAAGAAAGCCATTGCTCTCTG		
II	IIcpsKS	CTCCAGATGGTCTTTGTGAC	432	55 ºC
	IIcpsKA	AAAATTGGTATATTTCCTCTTGAC		
III	IIIcpsHS	CCACATATGAGAATAAGACTTGC	366	56 ºC
	IIIcpsHA	CCTAGTGATAGTACTTTGGTTTCTG		
IV	IVcpsHS	ATAGCCTTTTGACAGGTAGGTT	325	55 ºC
	IVcpsHA	TGTAAATCATCTACACCCCC		
V	VcpsHS	GATGTTCTTTTAACAGGTAGATTACAC	330	55 ºC
	VcpsHA	CTTTTTATAGGTTCGATACCATC		
VI	VIcpsHS	TGTTTTTCTTACAAAGTGGAGTC	279	55 ºC
	VIcpsHA	CCTGTTTTGTTTGATAGCTTCTC		
VII	VIIcpsMS	GTGCAATTAGAGGACAAAAATTTA	293	56 ºC
	VIIcpsMA	CATCGAATCAGGAAAAATAGAT		
VIII	VIIIcpsJS	ATCTCATGGCATGTCTGG	412	55 ºC
	VIIIcpsJA	CATTCGAATAAACAATCTTATTGC		

run in a thermal cycler (Gene Amp® PCR System 2700 Applied Biosystem) with the following cycling conditions: 92 °C for 90 sec; 40 cycles of denaturation at 92 °C for 20 sec, annealing at 55 ºC for 20 sec and extension at 72 °C for 20 sec; followed by a final extension at 72 °C for 2 min. PCR assay results were revealed using the strips provided by the kit. Manufacturer does not indicate which is the GBS target gene amplified by this PCR assay.

RESULTS

The average age of this subpopulation was 23 years with a range of 16 to 41. 40% of these women had ≤ 20 years of age and 10% were ≥ 35 years old.

We recovered GBS strains in 9 out of 87 women (10.3%, 95% CI: 3.9-16.7). All recovered GBS were susceptible to the antibiotics tested.

The serotype distribution was as follows: serotype III, 3 isolates; serotypes Ia, Ib, IV and V, one isolate of each. We could not establish the serotype of 2 strains.

Commercial qualitative PCR assay detected 2 additional GBS-colonized women.

The performance values of commercial qualitative PCR assay compared with enriched culture for detecting GBS found in this study were: sensitivity 100%, specificity 97%, positive predictive value 82% and negative predictive value 100% (see Table 2).

None of the infants born from GBS-colonized women developed infectious complications. Also, none of the colonized women developed complications after birth.

DISCUSSION

The intrapartum use of antibiotics in pregnant women colonized with GBS has been shown to be very successful at reducing the incidence of GBS early-onset neonatal disease. However, detection of the appropriate candidates for prophylaxis has been a major challenge. Although a clinical risk-based approach appeared to be easier for gynecologists/obstetricians to implement, demonstration of recto-vaginal GBS carriage at the time of delivery is by far the most powerful indicator of risk for early-onset neonatal disease.

The prevalence of GBS carriage determined by culture method in this subpopulation of pregnant women (10.3%, 95% CI: 3.9-16.7) was similar to that reported previously in our country and neighboring Argentina [11, 12].

There is very little published data on the susceptibility pattern of the recovered GBS strains in Uruguay; and no information was available about the serotype distribution [11].

All the recovered GBS strains were susceptible to penicillin, and consequently this antibiotic should be the first-line drug for intrapartum therapy in this subpopulation of pregnant women.

We found a variety of serotypes. Our data suggest a predominance of GBS serotype III in this population, as reported in Korea by Lee *et al.* [13]; however, more local studies including a larger number of GBS isolates are required to confirm this trend.

Taking conventional culture as the gold standard, the PCR assay showed an identical sensitivity for detecting GBS (see Table 1). These figures support that this PCR assay can be used as a direct

Table 2. Performance of a commercial qualitative PCR assay compared with conventional microbiological methods to detect GBS in recto-vaginal samples.

		Culture results		
		Positive	Negative	Total
PCR results	Positive	9	2	11
	Negative	0	76	76
	Total	9	78	87

screening test in populations with GBS carriage prevalence close to 10%.

Considering the above mentioned criteria, PCR assay results were interpreted as false positive in 2 cases (see Table 2). However, these results could be truly positive due to the presence of GBS in very low amounts (below the sensitivity of the conventional culture methods); colonization with non-hemolytic GBS strains; suppression of the growth of GBS by the enteroccoci present in the vaginal and rectal flora; or revealing remains of DNA of dead bacteria in the samples. In that sense, one of the women who showed positive PCR assay and negative culture results had received ampicillin for urinary tract infection a few days before delivery.

Considering all these facts, the performance of PCR assay would be even better than standard culture methods used in this study. If we take all PCR assay results as true positives, prevalence of GBS carriage would be slightly higher. In this population the prevalence of GBS carriage was found to be 10.3% by culture method and rises up to 13.2% by PCR assay. In that sense, some researchers claim that the culture method showed lower sensitivity, given that studies of confirmed early neonatal GBS infection have demonstrated disease in neonates born from mothers yielding negative tests for GBS by culture method [14, 15].

Hypothetically, the use of a procedure with increased sensitivity, like quantitative or qualitative PCR assays, would result in an increase in the number of pregnant women receiving antibiotic prophylaxis and, above all, could further reduce the incidence of early-onset neonatal GBS disease beyond the reduction seen by use of the standard culture [16].

PCR assay results are ready in 45 min, allowing health personnel to make therapeutic decisions quickly. However, the assay does not allow the determination of the antimicrobial susceptibility or the determination of the capsular serotype of the involved strains. Also it requires the use of expensive and sophisticated laboratory equipments. This last fact precludes its universal application for direct screening of GBS carriage in pregnant women treated in public health hospitals of developing countries, because for them the economic resources for purchasing reagents and equipment are scarce. As suggested by Rallu *et al*., an alternative to this situation is to apply rapid tests based on antigen-antibody reactions to detect GBS on incubated broths. These procedures are more sensitive than the standard culture-subculture method, easy to perform, inexpensive, results are ready in 15-20 minutes, and they do not require the use of sophisticated laboratory equipment [17]. However, as it is the case with PCR assays, these procedures do not allow the determination of the antimicrobial susceptibility pattern or the capsular serotypes of the involved strains.

CONCLUSIONS

The prevalence of GBS colonization in this subpopulation was similar to that previously reported in the region. All the recovered GBS strains were susceptible to penicillin, erythromycin and clindamycin. The capsular serotype III was the most frequently found. Our results support the use of qualitative PCR assays as a rapid tool for direct screening of GBS carriage in pregnant women.

ACKNOWLEDGMENTS

This work was supported by a CSIC-PAIE program grant to DC, GR and GV (University Research Committee, Uruguay/2011), Universidad de la República (UdelaR).

CONFLICT OF INTEREST STATEMENT

All the authors agree with this publication and declare that they don't have conflicts of interest.

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