Comparison of a PCR assay to culture standard method for the screening of Group B Streptococcus in pregnant women


ABSTRACT

Group B Streptococcus (GBS) is the most common cause of life-threatening infection in neonates. Guidelines from CDC recommends universal screening of pregnant women for rectovaginal GBS colonization. The objective of this study was to compare the performance of polymerase chain reaction (PCR) targeting the ATR gene in relation to culture using enrichment with selective broth medium (standard method) to identify the presence of GBS in pregnant women. Rectovaginal GBS samples from women at 36 weeks or more of pregnancy were obtained with a swab and analyzed by the two methods. A total of 89 samples were evaluated. Sensitivity of the PCR assay was 100%. ATR primers showed high analytic specificity for GBS identification in our assays. This PCR based test presented a higher prevalence of positive results (35.9% versus 22.5%) in comparison to standard method, providing a diagnostic tool for GBS detection, allowing more accurate and effective intrapartum antibiotic prophylaxis.

Keywords: Streptococcus agalactiae, group B Streptococcus, ATR gene, screening test, accuracy.

Introduction

Streptococcus agalactiae, or Group B Streptococcus (GBS), is a gram-positive coccus, associated to severe invasive disease in newborns. In fact, GBS is considered as one of the major causes of neonatal meningitis and sepsis.1

It is estimated that 5 to 40% of all pregnant women may present rectovaginal colonization with GBS, which is asymptomatic in almost all cases.2 However, GBS may be associated with acute chorioamnionitis, endometritis, and urinary tract infection.3 Moreover, pregnant women may transmit GBS to their newborns during labor and this may constitute the first step for invasive disease in the first week of life.4

The incidence of GBS neonatal infection is 1.8 per 1000 live births.3-5 Clinical syndromes of GBS disease in newborns include sepsis, meningitis, pneumonia, cellulitis, osteomyelitis, and septic arthritis. Other syndromes which have been reported among older children include endocarditis6,7 and epiglottitis.8 Bloodstream infections, with or without pneumonia, are the main manifestation of
neonatal GBS disease and are observed in approximately 90% of cases, while meningitis occurs in around 10%.

Maternal risk factors that predispose a neonate to early-onset GBS infection include preterm delivery, prolonged rupture of membranes (>18 hours), intrapartum temperature of at least 38°C, or prior infant with GBS infection.

Currently, the most effective strategy of reducing early-onset GBS infection is prenatal maternal screening for rectovaginal GBS colonization between the 35th to 37th weeks of gestation. The screening can be performed by sampling the vaginal and anorectal regions with the aid of swab which is submitted to bacteriological culture usually into a selective broth medium (enrichment) followed by subculture onto a sheep blood agar plate, as recommended by The Centers for Disease Control and Prevention (CDC) and The American College of Obstetricians and Gynecologists (ACOG).

Guidelines from CDC also recommends intrapartum chemoprophylaxis for those pregnant women with: (1) positive maternal GBS screening, (2) positive GBS urine culture during the current pregnancy, and (3) a previous infant who had GBS infection. An oral chemoprophylaxis approach with antibiotics is not recommended because it is unlikely to eradicate maternal genital GBS colonization. From 30% to 70% of pregnant patients who received prenatal ampicillin or aqueous penicillin for genital colonization of GBS remained colonized at delivery.

Although the laboratory methods for the identification of GBS have evolved, there remains a clinical need for greater accuracy. Therefore, a screening test with high sensitivity and specificity for GBS to identify pregnant women colonized at delivery would lead to a more precise treatment and reduce unnecessary antibiotic use and emergence of bacterial resistance.

The aim of this study was to establish the sensitivity and the specificity of the polymerase chain reaction (PCR) targeting the ATR gene, in relation to the culture standard method, to identify the presence of GBS in pregnant women.

### Materials and methods

#### Study design

The study was performed in 89 women at 36 or more weeks of pregnancy who attended the primary health care unit in Uruguaiana, Brazil, following the recommendation of the CDC and the STARD.

### Collection of specimens

Specimens of combined vaginal and anal secretions were collected from April 2006 to May 2007 using the technique recommended by the CDC. Briefly, the vagina and the rectum were sampled and the swabs were soaked into the Stuart’s transport medium immediately after the sample was obtained. All the specimens were transported at room temperature. The same specimen was used for the microbiological identification and the PCR assays.

### Microbiological method for the identification of GBS

For the identification of GBS, the swabs were incubated into 2 ml BHI broth (Difco, Detroit) supplemented with peptone 3 (2 g/L), gentamicin (8 μg/mL) and nalidixic acid (15 μg/mL) and incubated at 36 °C in 5% CO₂ for 18-24 hours. The swabs where then cultured onto sheep blood agar plates (BioMérieux, Marcy L’Étoile, France), incubated at 36 °C in 5% CO₂ for 24 hours and inspected for characteristic GBS colonies. If these colonies were not identified, the plates were reincubated and inspected for another 24 hours. If suspected colonies were present, they were subcultured in thioglycollate broth for 12 hours and then submitted to Gram stain. Whether the Gram stain indicates gram-positive cocci arranged in pairs and chains, they were submitted to CAMP test. The specimens positive for the CAMP test were considered presumptive GBS.

### Identification of GBS Using PCR Assay

For the PCR assays, the swabs were first cultured into BHI broth (Difco, Detroit) supplemented - as described above. The cultures were centrifuged and the pellets were washed in PBS buffer and then incubated with 500 μL of LiCl 5M for 30 minutes at room temperature. DNA-extraction was performed with the kit Wizard Genomic DNA Purification (Promega corp, Madison, WI, USA), according to the manufacturer’s instructions.

The PCR reaction was carried out using specific primers for the glutamine transporter protein (atr), selected from the Streptococcus agalactiae Multi Locus Sequence Typing (MLST) website (http://pubmlst.org/sagalactiae/). A mix of 25 μL was prepared as follows: 0.2 mM of dNTPs (ABgene®, Epson, UK); 0.4 μM of primer ATR forward (5'-GCATTCTCTCAGCTTTGTTA-3'); 0.4 μM of primer ATR reverse (5'-AAGAAATCCTCTTTGCGGAT-3') (Invitrogen Life Technologies, Grand Island, NY); 1x PCR buffer with 1.5 mM of MgCl₂ (JMR Holdings, London, UK);
1.0 U of Super-Therm DNA polymerase (JMR Holdings, London, UK); 2 µl of DNA. For the amplification, the reaction mixtures underwent denaturation at 94 °C for 1 minute, followed by 30 cycles of 1 minute at 94 °C, 45 seconds at 55°C for primer annealing, 1 minute at 72 °C for elongation, with a final period of extension at 72 °C for 10 minutes (model PTC-100 thermocycler, MJ Research TM, Waltham, USA). Subsequently, 10 µl of the amplified reaction mixture was visualized with ethidium bromide after gel electrophoresis in 2% agarose gel. The samples presenting a 779 bp amplicon were considered positive for GBS.

Purified GBS genomic DNA obtained from a clinical isolate (characterized by sequencing - below) was used as a positive control.

The investigator who performed the PCR assay was blinded to the results of the culture.

Molecular characterization of GBS genomic DNA

The amplified PCR product of a purified GBS genomic DNA was sequenced with the chain terminators method (Amersham Biosciences ET Terminator Kit) by automatic sequencement using MegaBACE 1000 following the manufacturer’s protocols. The ATR sequence obtained was compared to all known sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information, Bethesda, MD (http://www.ncbi.nlm.nih.gov/BLAST/), ensuring the specificity of the ATR primers for the identification of S. agalactiae. This DNA was used as a positive control in all experiments.

Specificity Assay for the ATR Primers

A mix of purified genomic DNA was obtained from cultures of clinical isolates of Streptococcus pyogenes, Streptococcus uberis, Enterococcus faecalis and Escherichia coli with and without S. agalactiae. The mixes were subsequently submitted to the PCR reaction with the ATR primers.

In an additional test, the purified genomic DNA was obtained from cultures of clinical isolates of Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter sp., Serratia sp., Salmonella sp., Proteus mirabilis, Citrobacter sp. and Morganella morganii and were submitted to the PCR reaction with the ATR primers.

Statistical analysis

We estimated the sensitivity and the specificity, using enrichment culture with selective broth medium as the standard method as recommended by CDC guidelines.\(^4\) Kappa coefficient evaluated the agreement between the methods.\(^18\) The statistical analysis was performed in SPSS\(^\text{®} \) v.12.

Results

Analytical specificity of the ATR Primers for GBS identification

All assays performed with the mix of DNA with S. agalactiae proved to be positive by the PCR with the ATR primers. Conversely, all assays performed with the mix of DNA without S. agalactiae rendered negative results in the PCR. Moreover, all clinical isolates of other species also presented PCR negative (Figure 1).

Performance of PCR for GBS detection

A total of 89 pregnant women were screened for GBS colonization using enrichment culture and the molecular method (PCR). Among them, 32 (35.9%) were identified as carriers of GBS on the basis of the results of PCR, as compared with 20 (22.5%) on the basis of enrichment culture.

All samples positive according to the culture were also positive by the PCR technique, thus the sensitivity of the PCR assay was 100%. Among the 69 samples culture-negative for GBS, 12 were positive by PCR and 57 were negative in both methods which indicates a specificity of 82.6% of the molecular method (Figure 2). The index of agreement (Kappa) between the techniques was 0.68.

Discussion

This study compared two screening methods (microbiological – enrichment culture and molecular - PCR) for the identification of GBS-colonized women.

At the present, the standard method for the diagnosis of GBS colonization consists of culturing combined vaginal and anal secretions in a selective enrichment broth.\(^4,19\) Although rapid tests have been developed for GBS detection, such as antigen-based tests, they however are neither sensitive nor specific enough to substitute bacterial culture.\(^4,9,11,12\) Latex tests, for example, presents 70%-90% sensitivity for heavily colonized women, but less than 50% sensitivity for those lightly colonized. This is a cause of concern as many
infected neonates were born to lightly colonized women, as showed in a clinical study conducted by Baker (1996). These studies have led to the 2002 CDC recommendation, which advises that the screening of pregnant women should use optimal microbiologic methods in the antepartum period with the effort to identify all pregnant GBS carriers. In our study, we used specific primers (ATR primers) for GBS detection in a PCR assay, a strategy that has not

![Image of PCR assay results](image_url)

**Figure 1** - Results for PCR assay with the ATR primers to the mix of DNA with and without *S. agalactiae* (A) and for the cultures of clinical isolates (B). Lane M- 100-bp molecular-size standard. Line W/O- mix of DNA without *S. agalactiae*. Line W- mix of DNA with *S. agalactiae*. Line 1 - *Proteus mirabilis*. Line 2 - *S. agalactiae*. Line 3 - *Staphylococcus aureus*. Line 4 - *Staphylococcus epidermidis*. Line 5 - *Acinetobacter* sp. Line 6 - *Serratia* sp. Line 7 - *Salmonella* sp. Line 8 - *Morganella morganii*. Line 9 - *Citrobacter* sp.

![Image of diagnostic accuracy study](image_url)

**Figure 2** - Flow diagram of the diagnostic accuracy study of a PCR assay to identify the presence of GBS in pregnant women.
been described in the literature. In fact, these ATR primers presented high analytical specificity for GBS detection in our assays. The amplification is performed after culturing the specimens into selective enrichment broth, consisting of an improvement in our methodology leading to an extremely specific methodology for GBS detection through the use of these primers. In addition to its accuracy, this test is performed in a moderate time (24-36 hours) when compared to culture-based methods (48-72 hours considering the standard method).

Our results indicated that the PCR technique proved to be as sensitive as the culture method. We also found a specificity of 82.6% for the PCR in comparison to the culture and this indicates that the PCR is, therefore, very useful as a screening method. It is important to consider, however, that the culture may provide false negative results which may lead to a more serious consequence for the patient than false positive results. Other studies have demonstrated truly early neonatal GBS infection in neonates born to mothers negative for GBS by culture.\textsuperscript{21,22}

Therefore, it is possible to speculate that the PCR for GBS detection in pregnant women is preferable to the culture method as the molecular method presented a considerable higher prevalence of positive results (35.9% versus 22.5%). Clinical studies on the impact of positive PCR results are warranted in order to assess the real importance of the positive results of this molecular method.

We demonstrated that the use of selective enrichment broth followed by PCR targeting the ATR gene is an excellent test for GBS screening in pregnant women. This PCR methodology may provide a diagnostic tool for GBS detection, potentially allowing more accurate and effective intrapartum antibiotic prophylaxis and lower infant mortality and morbidity.

References