DETECTION OF MATERNAL COLONIZATION OF GROUP B STREPTOCOCCUS BY PCR TARGETING Cfb AND ScpB GENES

Marwa Fouad, Sakhar Zakaria, Lobna Metwally, Hasan Aboul-Atta, Mahmoud Kamel

Address(es):
Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez Canal University, Egypt.

*Corresponding author: saharmicrobiology@yahoo.com

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INTRODUCTION
Vertical transmission of group B streptococcus (GBS) from a vagina colonized mother to her infant during labor can cause life-threatening infections in newborn. Maternal GBS colonization is associated with increased risk of transfer to the neonatal intensive care unit in term infants (Brittsen et al., 2015). Neonatal sepsis and pneumonia are the most important GBS-related neonatal infections, followed by meningitis, cellulitis, osteomyelitis and septic arthritis (Schrag et al., 2002). GBS is also associated with invasive and noninvasive infections in pregnant women and non-pregnant adults, especially the elderly or those with underlying medical conditions (Dutra et al., 2014).

The centers of disease control and prevention (CDC) recommended antenatal screening for all pregnant women at 35-37 weeks of gestation for the prevention of early onset GBS disease with vaginal/rectal cultures and selective IAP administration to GBS-positive women (CDC, 2010). Prenatal screening by culture is currently the gold standard method for detection of anogenital GBS colonization. However, cultures require several days (24–72 h) to yield results, thus precluding their use for intrapartum screening and these are only performed at 35–37 weeks gestation (Emonet et al., 2013). For this reason, there is a requirement for a rapid diagnostic test to detect GBS colonization status of women in labour, those in preterm labour or women who have not had prenatal care (Gavino and Wang, 2007).

An ideal screening test for GBS colonization is which could accurately identify pregnant women who carry the bacteria (even low-count bacteria carriers) and presenting a short turnaround time (de-Paris et al., 2011). New rapid molecular-based tests, such as polymerase chain reaction (PCR), can detect GBS within hours. They have the potential to be used intrapartum and to allow for selective IAP in women carrying GBS (Emonet et al., 2013). PCR assays have been developed to detect a variety of genetic targets, including genes encoding C protein, the 16S rRNA, and the 16S-23S spacer region (Bergeron and Ke, 2004).

The objective of our study was to evaluate PCR targeting cfb and scpB genes as a screening method for detection of maternal colonization of GBS compared to culture.

MATERIAL AND METHODS
Detection of GBS by culture
The study included 120 consenting pregnant women, between 35 and 37 weeks of gestation, attending the antenatal clinic of Suez Canal University Hospital in Ismailia, Egypt. Vaginal/rectal swabs were collected from each patient and inoculated into Lim broth; a selective medium consisting of Todd-Hewitt broth supplemented with 10 μg/ml colistin and 15 μg/ml nalidixic acid. Cultures are incubated at 37°C for 24 hours and then subcultured onto CNA medium (Colombia blood agar supplemented with 10 μg/ml colistin and 15 μg/ml nalidixic acid) and incubated at 37°C for 24 hours. Colonies with a narrow zone of beta hemolysis were suggestive of GBS and were further identified by being gram positive cocci, catalase negative, CAMP positive and hippurate hydrolysis positive. Confirmation was done by a streptococcus latex agglutination test (BIOTEC Laboratories).

Detection of GBS by PCR targeting cfb and scpB genes
All the 120 specimens were tested by PCR for detection of GBS using two sets of primers; one targeting the cfb gene which encodes the C5a peptidase. After DNA extraction, PCR reaction for each assay was performed in a 25 μl volume containing 2.4 μl DNA template, 12.5 μl of 2X power Taq PCR master mix (IQAGEN, Germany), 0.7 μM of each primer. The volume for each PCR reaction was completed to 25 μl with nuclease free water. For the cfb PCR, primers published by Ke et al. (2000) were used. For the scpB PCR, the primers described by Dmitriev et al. (2004) were used. The primer sequence and amplicon size for each target gene are shown in Table (1). A negative control consisting of the reaction mixture and nuclease-free water was added in each run.

In addition, a reference S. agalactiae (ATCC 12386) strain was used as positive control.
Table 1 Primer sequence and amplicon size for each target gene

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer Sequences</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfb gene</td>
<td>5'-TTTACACGCTGTATTAGAAGTA-3'</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>5'-GTACCTGAAACATTACCTTGAT-3'</td>
<td></td>
</tr>
<tr>
<td>scpB gene</td>
<td>5'-ACCATGGAAGGCTTCTAGTTC-3'</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>5'-ACCTGGTTTTGACCTGAAGTA-3'</td>
<td></td>
</tr>
</tbody>
</table>

Amplifications were carried out in a Thermocycler (Eppendorf, USA) and consisted of initial denaturation step at 94˚C for 3 minutes followed by 33 cycles of 45 seconds at 94˚C, 45 seconds at 57˚C, and one minute at 72˚C. These are followed by an extension step at 72˚C for 7 minutes. The amplicons obtained were run on 2% agarose gel with 0.5 µg/ml ethidium bromide in a Tris-borate-EDTA buffer. The gels were run in an electrophoresis gel tank at 100V for 30 minutes. After electrophoresis the sizes of DNA fragments were calculated using 100 bp ladder as DNA molecular size standards. Each gel run contained a negative control and a positive control. Finally, the DNA was visualized and photographed using a Gel Documentation System (BioSpectrum 310 Imaging System, USA).

Evaluation of PCR assay

The specificity and sensitivity of PCR assay using the previously mentioned primers were evaluated compared to culture. The analytical sensitivity (i.e. the detection limit or the minimal number of genome copies that can be detected) of each assay was determined by testing serial 10-fold dilutions of purified genomic DNA from a reference GBS strain (ATCC 12386), containing from 10^7 to 10^3 CFU/ml and starting with a concentration of 38.1 ng/µl. The stock DNA concentration was measured using the nanodrop technique (NanoDrop ND 1000 spectrophotometer). Each 10-fold dilution was added directly to the PCR reaction mixture before PCR amplification for each of the cfb and scpB genes. Amplifications were carried out using the same conditions used in the two PCR assays carried out before. A negative control was included in the reaction for both genes. After gel electrophoresis, the DNA was visualized and photographed. The analytical sensitivity of each of the two PCR assays was determined as the least concentration of genomic DNA at which the PCR gave a detectable band.

RESULTS AND DISCUSSION

Eighteen samples out of 120 were identified as GBS by culture. The rate of maternal colonization of GBS by the culture method was 15%. Twenty eight specimens (23.3%) were positive for GBS using the cfb PCR assay. Positive specimens showed specific bands of approximately 153bp in size (Figure 1). Twenty six specimens (21.7%) were positive for GBS using the scpB PCR assay. Positive specimens showed specific bands of approximately 255bp in size (Figure 2).

Although culture methods are the current standard for prenatal GBS screening, the implementation of more sensitive molecular diagnostic tests may be able to further reduce the risk of early-onset GBS infection (Buchan et al., 2015). Using the culture method, this study reported that the rate of GBS colonization in pregnant women was 15%. This rate varies greatly among countries. It was reported to be 7.98% in Italy (Puccio et al., 2014), 7.2% in Ethiopia (Woldu et al., 2014), 14% in Denmark (Peterson et al., 2014), 20% in USA (Page-Ramsey et al., 2013), 21.8% in Taiwan (Lee and Lai, 2014), 20.7% in Kuwait and 18.4% in Lebanon (Ghuddar et al., 2014). These variations in colonization rates relate to intrinsic differences in populations and to lack of standardization in culture methods employed for ascertainment. Also, a change in the prevalence over time, or real population differences account for some of the disparity in these reported prevalence rates. In spite of the great variation of prevalence rates, Le and Heath (2013) reported that the serotype distribution of GBS isolates is similar in Africa, Western Pacific, Europe, the Americas and the Eastern Mediterranean regions and has not changed over the past 30 years. This study showed that PCR using cfb and scpB genes was more sensitive for detection of GBS than the culture method as the rate of detection was 15% by the culture method compared to 23.3% by cfb PCR assay and 21.7% by scpB PCR.
ASSOCIATED WITH AN INCREASED RATE OF INFANTS TRANSFERRED TO THE NEONATAL CARE UNIT

CONCLUSION

Although more expensive than the standard culture method, the PCR technique targeting cfb and scpB genes is rapid, specific and has a higher sensitivity in detecting GBS carriers during pregnancy with the scpB PCR assay being more sensitive than the cfb PCR assay. PCR allows for accurate diagnosis of GBS which will be translated into more rational use of antibiotics and more effective treatment of carrier females leading to reduction of newborn morbidity and mortality. Yet, the cost-effectiveness of such PCR tests need to be more elucidated by further studies to see if it can be used as a routine screening method in centers with maternity wards.

REFERENCES


Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B Streptococcus carriage in pregnant women. J Clin Microbiol, 44(3), 725–728. http://dx.doi.org/10.1128/jcm.44.3.725-728.2006


