Clinical diagnosis of group B streptococci by *scpB* gene based PCR

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**Background & objectives:** The goal of the present study was to improve and simplify the diagnosis of *Streptococcus agalactiae* (group B *Streptococcus*, GBS) infection for routine clinical practice.

**Methods:** A total of 71 clinical samples were tested by microbiologic culture, counter immunoelectrophoresis (CIE) and PCR described in the literature. Southern hybridization was accomplished with the Enzo™ “DNA Labeling and Detection Kit”, Roche (Germany). The computer techniques were used for selection of the specific primers and for analysis of the sizes of PCR products.

**Results:** The primers for the regions around the 51 bp deletion in C5a peptidase gene (*scpB*) of GBS were selected. PCR analysis revealed the 255 bp amplification fragment in GBS, 306 bp fragment in groups A and G streptococci (GAS, GGS) and did not reveal any fragments in other bacterial species. Among 71 urine and serum clinical samples tested, none were found to be GBS positive by microbiologic culture, 16 samples by CIE, 36 by PCR. The specificity of amplification was confirmed by Southern hybridization.

**Interpretation & conclusion:** The 51 bp deletion in *scpB* gene in comparison with *scpA* and *scpG* genes can be used as a diagnostic tool for identification of GBS. The 51 bp deletion based PCR proved to be faster and more reliable test than microbiologic culture or CIE.

**Key words** Diagnosis - group B streptococci - *scpB* gene

*Streptococcus agalactiae* (group B *Streptococcus*, GBS) is a well known causative agent of neonatal invasive diseases. A significant increase of GBS infections and GBS carriage by the healthy people has been reported. The accurate identification of GBS can be useful for the prevention and control of the disease. Genetic approaches for identification of GBS being more sensitive in comparison with culture method, have certain disadvantages. The goal of the present study was to develop PCR based approach employing primers to *scpB* gene in order to improve and simplify the diagnosis of GBS infection for routine clinical practice.

**Material & Methods**

A total of 71 (23 urine and 48 serum) samples were collected from pregnant women in Beijing Children’s Hospital in 1997-1998.

The presence of streptococcal antigens in the clinical samples was detected by counter immunoelectrophoresis (CIE). The GBS was grown either in Todd-Hewitt broth or on 1.5 per cent blood agar at 37°C overnight.

Most of the molecular genetic procedures were carried out according to standard protocols. The
bacterial DNA from the clinical samples was prepared by the phenol/chloroform extraction. Southern hybridization was accomplished with the Enzo™ “DNA Labeling and Detection Kit”, Roche (Germany). PCR was performed using the primers: 5’-ACAACGGAAGGCCGCTACTGTTCC - 3’ (forward) and 5’-ACCTGGTTTTTGACCTGAACTA - 3’ (reverse). The PCR was performed as following: denaturation at 94°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for 1 min.

After electrophoresis the sizes of DNA fragments were calculated using 100 bp ladder (Bio-Rad Laboratories) as DNA molecular size standards.

**Results**

The primers for the regions around 51 bp deletion in scpB gene in comparison with group A streptococcal scpA gene were designed. After PCR analysis the size of GBS amplification fragment was equal to 255 bp while the GAS and GGS amplification fragments were equal to 306 bp (Fig.). Other streptococcal and staphylococcal species and *Escherichia coli* tested by scpB gene based PCR did not reveal any amplification bands.

![PCR analysis employing primers for the regions around the deletion in scpB gene. Lane 1: negative control; Lane 2: 100 bp ladder; Lane 3: GAS 306 bp amplification fragment; Lane 4: GGS 306 bp amplification fragment; Lane 5: GBS 255 bp amplification fragment.](image)

**Discussion**

It is well known that classical microbiological culture method used for bacterial identification is associated with several disadvantages. Usually the culture method requires several days while for successful treating of the patient the rapid diagnosis is required. Sometimes even before diagnosis the patients are treated with strong antibiotics. In this case microbiological method of pathogen identification is completely uninformative.

PCR analysis is often used for the rapid identification of different bacteria and takes significantly shorter time in comparison with routine culture method. Sensitivity of PCR detection is very high and even few cells can be detected by PCR.

Accurate PCR diagnosis requires selection of species-specific pairs of primers. The species-specific regions of 16S rRNA gene and 16S-23S intergenic spacer are often used for identification of bacteria and PCR diagnosis of infections. Nevertheless, high homology of ribosomal operons in different species can end up with false positive results. For example, this

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**Table.** Analysis of the clinical samples by microbiological culture, CIE and PCR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Culture negative</th>
<th>CIE positive</th>
<th>CIE negative</th>
<th>PCR positive</th>
<th>PCR negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (48)</td>
<td>16</td>
<td>14</td>
<td>18</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Urine (23)</td>
<td>16</td>
<td>20</td>
<td>35</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>
method caused the misidentification of *Streptococcus agalactiae* and *Streptococcus difficile* as the same specie.

In our opinion some other species-specific regions, e.g., virulence genes, could be the candidate PCR targets. In the present study the virulence gene *scpB* encoding C5α peptidase was chosen as such a target. Previously, C5α peptidase genes were found only in group B streptococci (*scpB* gene), group A streptococci (*scpA* gene) and group G streptococci (*scpG* gene). *ScpB* gene being extremely homologous to *scpA* and *scpG* genes has a 51 bp deletion at the 3'-end of the gene. In our study this deletion was considered as GBS specific diagnostic marker.

The primers for the regions around the deletion were designed. As expected, the size of GBS amplification fragment was equal to 255 bp while GAS and GGS amplification fragments were equal to 306 bp (Fig.). DNA templates from other bacterial species used as negative controls in our experiments (i.e., other groups of streptococci, staphylococci and *Escherichia coli*) did not produce any amplification band. These results indicate specificity and possible value of the *scpB* gene based approach for GBS identification.

The samples were repeatedly tested by all three methods to confirm the results. The low level of GBS detection determined by culture method and CIE can be explained by the various reasons, e.g., small amount of bacterial cells in the samples, antibiotic therapy etc. PCR diagnosis revealed the higher level of detection. The specificity of PCR was confirmed by Southern hybridization.

The data presented in this paper indicate that 51 bp deletion in *scpB* gene can be used as a diagnostic tool for identification of GBS. The *scpB* gene based PCR proved to be faster and more reliable test than microbiologic culture or CIE and can be considered as a very useful approach for the accurate diagnosis of infection.

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**References**


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