SEROTYPE DISTRIBUTION AND ANTIMICROBIAL RESISTANCE PATTERN OF GROUP B STREPTOCOCCUS ISOLATED FROM PREGNANT WOMEN

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ABSTRACT:
Background: Group B Streptococcus (GBS) is an important cause of infection in newborns and pregnant women. Penicillin is usually the choice drug for treatment of GBS infections. The capsular polysaccharide is a major virulence factor in GBS. Nine capsular types have been described; (Ia, Ib, II-VIII). The formulation of GBS vaccines relies on information about the GBS serotypes distribution.

Methods: Vaginal swabs were collected from 382 pregnant women. GBS isolates were identified by standard laboratory methods and serotyping was performed by the Multiplex PCR method. Antibiotic susceptibility of GBS isolates determined using the disk-diffusion method.

Results: Of the 382 vaginal samples, 36 GBS positive cultures were recognized (9.4%). The most common serotypes were III (32.1%), V (21.4%) and IV (14.3%). All isolated GBS were sensitive to penicillin and Cefazolin, resistance to Erythromycin and Clindamycin was found in 5.6% and 8.3% of isolates respectively; that more accurate among serotypes III and V.

Conclusion: Our study confirms the high rate of beta-lactam sensitivity of GBS. The prevalent serotypes in this study (III, V and IV) can be considered in future studies in order to produce multivalent GBS vaccine.

Keywords: Group B Streptococcus; Serotyping; Antibiotic; Pregnant Women; Multiplex PCR.

INTRODUCTION

Group B streptococcus (GBS) has been introduced as a significant pathogen in newborns and pregnant women in the last 40 years, and was identified as the most frequent cause of neonatal infection in the 1970s (1,2). According to the reports, 20-30% of pregnant women are colonized with GBS in western countries (3-5). Women colonization with GBS for the period of pregnancy is notably related to infections in newborns (1,6). GBS can be passed to the baby in the womb or through the birth canal during delivery and cause infections such as sepsis and meningitis (3,5). Penicillin is the drug of choice for prevention and treatment of GBS infections, erythromycin and clindamycin are recommended for patients allergic to betalactams (6-8). Studies performed in many countries, reports that are increasing bacterial resistance to antibiotics and erythromycin and clindamycin resistance levels are higher than 10% (7-10). Maternal immunization against GBS, going to possibly reducing maternal colonization and increasing transfer of antibody to the fetus, is being discovered to prevent GBS related sepsis during early infancy (11,12). The capsular polysaccharide is a major GBS virulence factor and the
main goal for killing antibodies (9, 13). In the last years conjugated multivalent vaccines have been expanded based on the capsular polysaccharide antigen and showed to be very immunogenic, increasing the possibility of the prevention of GBS infection through maternal vaccination (14, 15). Nine capsular serotypes are identified: Ia, Ib, II-VIII and type IX that proposed recently (2, 5, 7, 15, 16, 17). The serotype distribution varies in different geographic regions and over time, so the vaccine is not possible to universally optimal, therefore it is important to be aware of the serotype distribution to determine the composition of multivalent vaccines against the common serotypes (5, 10, 17). There are imperfect information on GBS serotype epidemiology related to recto-vaginal maternal colonization or invasive disease in newborns from developing countries (2). The purpose of this study is detection of antibiotic resistance pattern and serotype distribution of GBS using multiplex PCR in pregnant women.

METHODS AND MATERIALS:
Study population and Bacterial culture:
Vaginal samples were collected from 382 pregnant women at 28-37 weeks of gestation attending to two teaching hospitals in Kashan during December 2011 to November 2012. The vaginal swabs were placed in LIM broth (Todd-Hewitt broth containing 10 µg/ml gentamicin and 15µg/ml nalidixic acid to prevent the growth of normal bacterial flora) then transported to the laboratory and incubated at 37° C for 24 h. A 5µl loop of this broth was a subculture on to 5% sheep blood agar plates, the plates were incubated at 37° C in 5% CO2 and beta-hemolytic colonies were isolated. The identification of GBS was performed using colony morphology, gram staining, catalase test, bacitracin sensitivity, sodium hippurate hydrolysis and CAMP test. The strains were stored in Todd-Hewitt broth containing 15% glycerol at -70° C

Serotyping: DNA was extracted with Fermentas kit according to instructions manufacturer factory. Capsular serotyping was performed with the Multiplex PCR method for serotypes Ia, Ib, II-VIII uses primer sequences reported by Poyart et al (18). The gene encoding dltS was used as a positive control for GBS identification. Tow master mixes were used in separate PCRs for individual capsular types: types Ia, Ib, III and V in tube A, types II, IV, VI, VII and VIII in tube B. A second PCR targeting the GBS-specific dltS gene was also included as a positive control (tube C). The reaction mixtures (25µl) were prepared in separate tubes, consisted of 1µl of each primer pair, 0.25µl of DNA Taq Polymerase, 2.5µl of 10X PCR buffer, 0.75µl of Mgcl₂ (25 mM), 0.5µl of deoxynucleotide triphosphate mixture (2 mM), 5µl of DNA and 8µl, 6µl and 15µl of DNase-free distilled water for tube A, B and C respectively. PCR condition for amplification was performed according to table1. The PCR products were analyzed by electrophoresis in a 2% agarose gel (Figs. 2, 3)

<table>
<thead>
<tr>
<th>Cycle number</th>
<th>Time</th>
<th>Temperature</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 Minutes</td>
<td>94° C</td>
<td>Hot start</td>
</tr>
<tr>
<td>40</td>
<td>45 Second</td>
<td>94° C</td>
<td>Denaturation</td>
</tr>
<tr>
<td>40</td>
<td>54° C</td>
<td>Annealing</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>72° C</td>
<td>Extension</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 Minutes</td>
<td>72° C</td>
<td>Final extension</td>
</tr>
</tbody>
</table>

Antimicrobial susceptibility: Antimicrobial susceptibility testing was performed by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Susceptibility to penicillin, ampicillin, erythromycin, clindamycin and cefazolin was recorded.
RESULTS:
Of the 382 vaginal swabs from pregnant women 36 (9.4%) isolates were identified as the group B *streptococcus* (GBS). Serotypes of group B streptococcus strains isolated in this study were III (32.14%), V (21.43%), IV (14.3%) and followed by Ia (10.7%), VI (10.7%), Ib (7.13%) and VII (3.6%). Types II and VIII were not found in this study (Fig. 1).
All of GBS isolates were susceptible to penicillin and cefazolin. There was a semi-sensitive strain to ampicillin with capsular serotype V. Resistance to erythromycin was observed in 2 isolates (5.6%; serotypes III, V). Resistance to clindamycin was found in 3 isolates (8.3%, serotypes III, V, V); Table 2.

Table 2: Antibiotic resistance pattern of Group B *Streptococcus*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Semi sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>36 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>35 (97.2%)</td>
<td>1 (2.8%; V)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>36 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>29 (80.5%)</td>
<td>5 (13.9%; III, III, IV, V)</td>
<td>2 (5.6%; III, V)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>30 (83.4%)</td>
<td>3 (8.3%; Ib, III, V)</td>
<td>3 (8.3%; III, V, V)</td>
</tr>
</tbody>
</table>

Fig. 1: Distribution of serotypes of group B streptococcus isolated from pregnant women.

Fig. 2: PCR specific group B streptococcus (*dlt*: 952bp)
DISCUSSION:
The epidemiology of GBS serotypes not only varies in different geographical areas but also changes over time and hence conjugate vaccines developed for major disease causing GBS serotypes may not be universally optimal(19-21). Although there are extensive data on maternal colonizing serotype distribution from industrialized countries (22,23) there are limited data comparing the serotype distribution from colonized mothers from industrializing countries (24,25). The most common serotypes isolated in this study were III(32.1%), V(21.4%) and IV(14.3%) respectively. The predominance of these serotypes among the pregnant women is largely consistent with reports in the literature (26,27). Studies performed in European, North American and Latin American countries have demonstrated that serotypes III, V and Ia are more frequently found (6,28-30). A recently published meta-analysis of geographical GBS serotype distribution of carriage isolates, showed that in Europe, the middle East , Africa, Australia and Asia serotype III predominated (31) The rate of occurrence of serotype IV in different part of the world is low (6,26,32) however, we isolated GBS expressing this capsular polysaccharide at a relatively high frequency(14.3%). In a study conducted in Madigan , Ia(28.5%), III(27%), V(17%) were the most common serotypes respectively, types VII and VIII not found in this study(16). In a similar research that Performed in Zimbabwe , types III(38.8%), V(24%) and Ia(15.7%) reported as the most common serotypes respectively(33). Also in Korea, III(29.8%), V(27.7%) and Ia(17%) were the most prevalent (10).
The check of GBS serotypes Prevalence was performed in southeast of Iran (Kerman), the rate of colonization was 9.1% and III(41.8%), Ib(25.4%) and II(14.5%) was the most common serotypes respectively . Type IV was not found in this study (34). In our study also type III was the most common serotype that confirms it. In kerman study serotyping was performed by antiserum kit for serotypes Ia-V, therefore not comparable to present study directly. In the other region of Iran (Tabriz), the rate of GBS colonization was 5.2% and types V(19.5%), Ia(17.6%) and II(14.2%) was the most common serotypes respectively(35). In contrast to our study serotypes III and IV had a low prevalence too, in this study was used antiserum kit for the GBS serotyping. In order to identify of serotypes Ia-V. In the other study performed in Ardabil, the rate of colonization was 13.3% . Serotype distribution was identified using the dedicated antiserums for serotypes Ia-VIII (36). GBS serotypes strains isolated in this study were V(19.6%),II ,IV(12.5%), III,VI(10.7%), Ib(8.9%), Ia (7.1%) and VII,VIII(5.3%) .Type III was found less in comparison to our study. Type II had a relatively high prevalence while not found in our study .This different finding confirms the high degree of variability of serotypes in distinct regions, even within the same country. In this study, we used the Multiplex PCR method for the detection of GBS serotype distribution that is more sensitive and infrastructure than three recent studies.
Our study confirms the high level of beta-lactam susceptibility of GBS (26, 37). Erythromycin and clindamycin resistance was detected in3.6% and7.15 % of the GBS isolates respectively. A relation between capsular serotype and antibacterial resistance in GBS isolates is reported; antibiotic resistance occurred usually among serotypes III and V; this is consistent with reports in another place (4,10,38). In this study, although a higher rate of
resistance to erythromycin and clindamycin was found in the serotype III and V the difference were not significant.

CONCLUSION:
The differences in serotype distribution among various populations may reflect differences in pathogenesis among the serotypes. Therefore, monitoring the serotype distribution is important for complete surveillance of GBS infections and for vaccine formulation.

REFERENCES


