Culture systems for isolation of group B Streptococci in parturient women in Jakarta, Indonesia

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ABSTRACT

A total of 465 parturient women were screened for group B Streptococcus (GBS) colonization by using 4 culture systems, direct plating of vaginal and rectal swab samples and enrichment of the samples in Todd-Hewitt broth. Group B Streptococcus was isolated in 8.2%. Direct plating and enrichment culture of vaginal samples (DVS) yielded 5.6% and 1.9% isolates of GBS, respectively. Direct rectal swab (DRS) was positive in 3.2% of the women and enrichment culture of rectal swab (ERS) was positive in 0.9%. Combination of direct plating and enrichment of vaginal swab detected 5.8% isolates of GBS, whereas combination of direct plating and enrichment of rectal swab was positive in 4.1% samples, and combination of DVS + DRS yielded 7.1% isolates of GBS. A total of 427 samples were negative for GBS. Test results of the four culture systems demonstrated that direct plating of vaginal swab was the most sensitive. However, for maximum isolation of GBS, use of selective broth media in addition to direct plating is important.

Key words : Culture systems, group B Streptococcus, parturient women

INTRODUCTION

Colonization with group B Streptococcus (GBS) in genital and gastrointestinal tract of pregnant women is implicated with early-onset neonatal disease. Pregnancy-associated GBS infection is most often manifest during labor or within the first few days of an infant’s life. It can affect the woman or her baby or both. Ascending spread leads to amniotic infection, which can result in maternal sepsis. GBS is also a leading cause of chorioamnionitis and is one of several bacteria now thought to enhance the risk of preterm premature rupture of membranes. Newborn babies can acquire GBS by aspiration of infected amniotic fluid or during passage through the birth canal. In many
industrialized countries GBS has been considered to be the principal cause of sepsis and meningitis during the first week of infant’s life.

Colonization with GBS in the birth canal of pregnant mother was reported to be associated with a 1.4 times increased risk of preterm low birthweight and was present in 16% of the cohort. Published data on GBS colonization among women in developing countries suggests geographical variation in GBS carriage but the range (12% in India and Pakistan to 22% in North Africa and the Middle East) is consistent with the range found in industrialized population. Regan et al. studied cervical colonization with GBS in 6,706 parturients in New York and found that among 123 who delivered at ≤ 32 weeks gestation, 38% were GBS carriers.

A recent report described that the risk of high density/heavy colonization GBS vaginal colonization was associated with a stepwise increase in the risk of intra-amniotic infection and that GBS identified in mid-pregnancy was not associated with intra-amniotic infection at delivery.

The cornerstone of prevention efforts of GBS transmission from mothers to neonates is to identify the bacteria from pregnant women. One recommended approach is to screen all pregnant women for GBS carriage between 35-37 weeks of pregnancy. This requires that vaginal and anorectal swabs be obtained and cultured. Numerous investigators have compared methods of isolating GBS from the genital tract and rectum and these studies are consistent in showing that combined swabs of the vagina and rectum give up to 30% better yield than vaginal swabs alone.

Since bacteriological procedures are still not regularly made as routine in many developing countries including Indonesia, reports on risks of GBS colonization in pregnancy and the efficacy of a particular culture system are not available. The purpose of this study was to detect the present and rate of GBS in pregnant women at admission for delivery, by using specimens from vagina and rectum. Information of these results will be useful in identifying the risk of GBS colonization in association with early onset of neonate infection due to GBS.

METHODS

Subjects
A total of 465 women age between 25-35 years old in the process of delivery who were admitted to Obstetrics and Gynaecology clinic in one State Hospital in Jakarta from May 1999 through June 2000, aged between 25-35 years old, were enrolled. For medical records, a standard clinical form was used. Voluntary, written, informed consent was obtained from the subject prior to enrolment in the study. Participants were excluded from the study if they had multiple gestations or scheduled caesarean delivery.

Sample collection
Specimens were obtained from women who were admitted to the delivery unit with contractions. Vaginal specimen was collected by using Dacron-tipped swab placed into the lower one-third of the vagina and rotating the swabs 360°. A swab sample was also collected from rectum. Each of the swab samples collected from the rectal and vaginal site, was placed in a silica transport medium. Specimens were transported to the Microbiology Laboratory, Medical Faculty Trisakti University, within 4 to 12 hours after acquisition.

Microbiology analysis
Swab sample was removed from silica transport medium, moistened with Todd Hewitt broth and directly inoculated onto a defibrinated sheep blood agar. The swab was then placed in Todd Hewitt broth for enrichment, incubated aerobically overnight at 35°C and then inoculated onto sheep blood agar plate. Blood agar plates were streak-inoculated for 4 zones of isolation and were incubated in a candle extinction jar for CO₂ atmosphere at 35°C for 18-20 h. Thus, there were four culture systems performed, (i) direct plating of vaginal swab (DVS) and (ii) rectal swab (DRS) from silica, (iii) culture of vaginal swab (EVS) and (iv) rectal swab (ERS) after enrichment.

According to Krohn, et al. parturient women with vaginal specimens that were positive for GBS on direct culture on the blood agar were categorized having (i) light colonization (growth in the first or second streak zone), and (ii) heavy colonization...
growth in the third or forth zone) and compared with those of women who were negative for GBS.

Small colonies, grayish to whitish and glisten with beta-hemolytic zone resembling those of Streptococci were tested by using standard culture methods and latex agglutination (Strep Grouping Kit; DIFCO Laboratories, Detroit, MI) for group B Streptococcus.

Data analysis

Significance differences in isolation rates for individual and combined culture procedures were determined by chi-square test. The software package Epi Info version 6.03 (Centers for Disease Control, Atlanta, GA) was used for statistical calculations.

RESULTS

Group B Streptococcus was isolated in 38 (8.2%) of the 465 parturient women who participated in this study, and that only 8 (1.7%) of the parturient women showed heavy colonization. Direct plating (DVS) and enrichment culture of vaginal samples (EVS) yielded 26 (5.6%) and 9 (1.9%) isolates of GBS, respectively. Direct rectal swab (DRS) was positive in 15 (3.2%) of the women and enrichment culture of rectal swab (ERS) was positive in 4 (0.9%) (Table 1). DSV was significantly better than DRS, EVS and ERS (p<0.05) in detecting GBS. Combination of direct plating and enrichment of vaginal swab detected 28 (5.8%) isolates of GBS, whereas combination of direct plating and enrichment of rectal swab was positive in 19 (4.1%) samples, and combination of DVS + DRS yielded 33 (7.1%) isolates of GBS. A total of 427 samples were negative for GBS.

Test results of the four culture systems utilizing direct plating and enrichment demonstrated that DVS was the most sensitive. Of the 38 positive samples for GBS, DVS alone detected 12 (31.6%) isolates, DRS alone was positive in 7 (18.4%), EVS alone was positive in 2 (5.3%) and ERS alone was positive in 3 (7.9%) (Table 2). There were only two samples that were positive on 3 culture systems (DVS+DRS+EVS, and DVS+EV+EVS+ERS). GBS was isolated from both rectal and vaginal samples in 7 (18.4%) parturient.

Table 1. Distribution of group B Streptococcus from vaginal and rectal swab samples of 465 parturient women by various culture system and their combination

<table>
<thead>
<tr>
<th>Culture system</th>
<th>Number (%) of positive GBS isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVS</td>
<td>26 (5.6)</td>
</tr>
<tr>
<td>DRS</td>
<td>15 (3.2)</td>
</tr>
<tr>
<td>EVS</td>
<td>9 (1.9)</td>
</tr>
<tr>
<td>ERS</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>DVS + EVS</td>
<td>28 (5.6)</td>
</tr>
<tr>
<td>DRS + ERS</td>
<td>19 (4.1)</td>
</tr>
<tr>
<td>DVS + DRS</td>
<td>33 (7.1)</td>
</tr>
<tr>
<td>DVS + DRS + EVS + ERS</td>
<td>38 (8.2)</td>
</tr>
</tbody>
</table>

DVS, direct plating of vaginal swab sample
EVS, enrichment culture of vaginal swab
DRS, direct plating of rectal swab sample
ERS, enrichment of rectal swab

Table 2. Test results of culture systems in detecting 38 isolates of group B Streptococcus from vaginal and rectal swab samples

<table>
<thead>
<tr>
<th>Culture system/results</th>
<th>Number (%) of parturient women positive for GBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + -</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>+ + - -</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>+ - + +</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>+ - + -</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>+ - - -</td>
<td>12 (31.6)</td>
</tr>
<tr>
<td>- + - -</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>- - + -</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>- - - +</td>
<td>3 (7.9)</td>
</tr>
</tbody>
</table>

DISCUSSION

Many adults are colonized with GBS in the genital and gastrointestinal tracts but remain free of symptoms. However, women colonized with GBS during pregnancy are at risk of premature delivery and pregnancy-associated GBS disease is most often manifest during labor or within the first few days of an infant’s life: Early-onset disease.

It can affect the women or her baby or both.
Isolation of GBS should be attempted by swabbing both vagina and rectum since the inclusion of rectal swab will increase the yield substantially compared sampling the vagina alone.\(^{(14)}\) Rectal swab sampling is important because the gastrointestinal tract serves as the natural reservoir for GBS and is the likely source of vaginal colonization. Vaginal colonization is unusual in childhood but becomes more common in late adolescence.\(^{(15)}\) Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum.\(^{(14)}\) This study found that only 8 (1.7%) of the parturient women showed heavy colonization. This number is much lower than those reported\(^{(13)}\) which may be due to the culture system used in this study. For enrichment we used regular Todd-Hewitt broth without the addition of gentamicin and nalidixic acid or colistin with nalidixic acid. This non-selective broth medium allowed the growth of normal flora and many non-group B Streptococcus, which may have suppressed the group B Streptococci. It appears that the use of selective broth media is an important factor to obtain high yield of pathogens when one attempt to culture bacteria from non-sterile anatomic sites such as vagina and rectum.

As shown in Table 1, combination of rectal and vaginal swab cultures yielded 33 (86.8%) out of 38 positive samples. This isolation rate of GBS was not different significantly compared with the combination of 4 culture systems (DVS+DRS+EVS+ERS). The combination of DVS and DRS was significantly \((p<0.05)\) than DRS, EVS and ERS. Of all systems, ERS was the poorest with isolation rate of 0.9%, followed with EVS with 1.9%. Enrichment of the swab samples in media without antibiotic supplementation does not appear sensitive for isolating GBS. Recently, it was recommended\(^{(8,13)}\) that for maximum isolation rate of GBS, a supplemented broth medium should be used.

Table 2 shows that only 2 samples were positive for GBS in 3 combinations of systems, DVS, DRS and EVS and DVS, EVS and ERS. These two samples were among those collected from women with heavy colonization suggested that in women with heavy colonization, GBS may be detected when 2 or more culture systems are positive.

Our findings were in agreement with the recommendations made by other investigators\(^{(6,8,13)}\) that both rectal and vaginal swab culture, either performed by direct plating alone or with selective enrichment, should be used to obtain high yield of GBS from pregnant women. Culture screening of both the vagina and rectum for GBS colonization should be performed late in gestation during prenatal care because colonization early in the pregnancy is not predictive of neonatal sepsis.

References