Evaluation of chromogenic medium and direct latex agglutination test for detection of group B streptococcus in vaginal specimens from pregnant women in Lebanon and Kuwait

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This study was undertaken to evaluate chromogenic medium and a direct latex agglutination test (DLA) for detection of Group B Streptococcus (GBS) in the vaginal specimens of pregnant women, and to ascertain the prevalence of GBS in this population in Kuwait and Lebanon. Vaginal swabs, collected from women at 35–37 weeks of gestation, were cultured on 5 % sheep blood agar (SBA), colistin nalidixic acid agar (CNA), Strept B Select chromogenic agar (SBS) as well as Lim enrichment broth in 168 cases in Lebanon while only SBA was used for 1391 samples in Kuwait. In addition, vaginal samples from 102 GBS-positive and 20 GBS-negative women near the time of delivery were collected in Kuwait for evaluation of the DLA test. During the study period, the prevalence of GBS colonization was determined to be 20.7 % (288/1391) in Kuwait while 18.4 % (31) of 168 pregnant women in Lebanon had vaginal cultures positive for GBS. By direct plating of vaginal swabs on the three media used, the isolation rates of GBS were 51.6, 64.5 and 77.4 % on SBA, CNA and SBS, respectively, which increased to 90.35, 93.1 and 96.8 %, respectively, following subculture in Lim broth after 18 h of incubation. The sensitivity of the DLA test was found to be dependent on the density of GBS colonization, resulting in 100 % sensitivity and 100 % specificity for heavy (>10^5 c.f.u. per swab) and moderately heavy (50–100 c.f.u. per swab) growth of GBS. However, for vaginal specimens yielding <50 c.f.u. per swab, the sensitivity, specificity, positive and negative predictive values of the DLA test were 100, 55.5, 63.6 and 100 %, respectively. In conclusion, a chromogenic agar, such as SBS, and a DLA test can be used for rapid detection of GBS in pregnant women. The DLA test, in particular, could prove to be a useful tool for immediate detection of GBS in women near delivery so that intrapartum antibiotic prophylaxis can be initiated.

INTRODUCTION

Early-onset sepsis due to Group B Streptococcus (GBS) remains one of the significant causes of neonatal morbidity and mortality. According to the Centers for Disease Control and Prevention (CDC), GBS is the leading cause of sepsis and septic shock in the newborn, accounting for 15% of all neonatal deaths in the United States. GBS is a Gram-positive bacteria that is commonly found in the gastrointestinal and genitourinary tracts of women. During pregnancy, GBS can colonize the lower genital tract and ascend to the amniotic cavity, leading to ascending infection and birth complications. The CDC recommends routine screening for GBS in asymptomatic women at 35–37 weeks of gestation, and intrapartum prophylaxis for those who test positive. However, currently used screening methods, such as culture on blood agar, can take up to 24 hours to obtain results, which can delay the initiation of antibiotic prophylaxis.

The primary purpose of this study was to evaluate the efficacy of chromogenic medium and a direct latex agglutination test (DLA) for the detection of GBS in vaginal specimens from pregnant women in Lebanon and Kuwait. The study aimed to determine the prevalence of GBS colonization and compare the performance of the DLA test with culture methods.
and mortality in developed and developing countries (Rallu et al., 2006; Louie et al., 2010; Riedlinger et al., 2010). Rectovaginal colonization with GBS has been found to occur in 10–30% pregnant women with 1.8 neonatal infections per 1000 live births reported per year (El Aila et al., 2010). Two earlier studies, one from Kuwait and the other from Lebanon, revealed the carriage rate of GBS to be 16.4 and 17.7% among pregnant women, respectively (Al-Sweih et al., 2004; Seoud et al., 2010). Since the newborn acquires the infection at birth from a GBS-colonized mother, Centers for Disease Control and Prevention (CDC) guidelines recommend use of intrapartum antibiotics to interrupt this vertical transmission. It is, therefore, advisable to perform antenatal screening for GBS colonization in pregnant women, especially at 35–37 weeks of gestation (Yancey et al., 1996).

In order to reduce the turnaround time for early detection of GBS during late pregnancy or at the time of labour, several new techniques such as pigmented agars and broths, nucleic acid amplification tests and chromogenic agars have been successfully used, each with their advantages and disadvantages (Rallu et al., 2006; Louie et al., 2010; Riedlinger et al., 2010). The CDC recommends isolation of the organism from vaginal swabs by growth in an enrichment medium such as Lim broth (Todd–Hewitt broth with colistin and nalidixic acid), followed by subculture on sheep blood agar. CDC also recommended the development of media with a colour indicator to signal the presence of GBS, with the aim to improve accuracy of prenatal culture processing at clinical laboratories with limited technical capacity (Centers for Disease Control and Prevention, 1996; Yancey et al., 1996; Schrag et al., 2002; Al-Sweih et al., 2004; El Aila et al., 2010; Seoud et al., 2010). However, a culture method can prove to be costly, labour-intensive and time-consuming with a slow turn-around time; therefore, a rapid antigen detection test may be used to circumvent these issues (Rallu et al., 2006).

Since the culture method remains the gold standard we compared the recovery of GBS on enriched, selective and a chromogenic medium, Strept B Select (SBS), which is also a selective medium used for isolation and presumptive identification of GBS (turquoise–blue colonies), before and after enrichment. In addition, we investigated the use of a direct latex agglutination test on vaginal samples for rapid identification of GBS and compared it to culture results. The study was conducted to compare the results obtained from three hospitals (Saint George, Makased, and Clemenceau Medical Center) in Lebanon to those obtained at one hospital (Farwania) in Kuwait to ascertain the GBS vaginal colonization among pregnant women in the two countries by using different methods in this study and from those used previously (Al-Sweih et al., 2004; Seoud et al., 2010).

METHODS

Study design. Lebanon: Obstetricians at Saint George Hospital, Makased Hospital and Clemenceau Medical Center monitored women at 35–37 weeks of gestation for vaginal colonization with GBS. From September 2011 to October 2012, 168 patients were recruited following written consent. Duplicate vaginal swabs (inserted together at the time of collection) were collected from each patient and processed at Makassed Hospital Laboratory. One of the swabs was inoculated on to 5% sheep blood agar (SBA), colistin-nalidixic acid agar (CNA) (Louie et al., 2010) and a chromogenic medium, SBS (Select; Bio-Rad). The second swab was immersed in the Lim enrichment broth (Todd–Hewitt broth containing 10 mg colistin l⁻¹ and 15 mg nalidixic acid l⁻¹). All media were incubated overnight at 35–37 °C, following which the enrichment broth was subcultured on to all three solid culture media irrespective of GBS growth on primary media. The identification of suspected colonies (beta-haemolytic or non-haemolytic grey colonies) of GBS on SBA and CNA was done by using a latex agglutination test (Streptococcal grouping kit; Oxoid) whereas turquoise colonies on SBS were identified as GBS.

Kuwait: From September 2011 through October 2012 the study was also conducted in Farwania Hospital, which is a tertiary care facility where approximately 7000 deliveries are conducted per year. Data from a single vaginal swab were collected for analysis from 1391 pregnant women at 35–37 weeks of gestation and processed in Farwania Hospital Laboratory. All swabs were cultured only on SBA and incubated at 5% CO₂ for 18 h before identifying beta- or non-haemolytic colonies resembling GBS, which were confirmed by a latex agglutination test (Streptococcal grouping kit; Oxoid). In addition, during a period of 6 months (September 2011 to February 2012), 102 vaginal swabs collected from women in the labour room, which were positive for GBS by culture, and were categorized based on growth as heavy (>100 c.f.u. per swab), moderately heavy (50–100 c.f.u. per swab) and light (<50 c.f.u. per swab), were used for the DLA test. The samples were stored at 4–8 °C until the culture results were available. Twenty GBS culture-negative swabs were also included for DLA test from the same patient group.

DLA test. The refrigerated swabs (102 culture-positive and 20 culture-negative) were used for direct detection of GBS. A latex agglutination test (Streptococcal grouping kit; Oxoid) was performed after GBS antigen was extracted from the swabs by using 0.4 ml of extraction enzyme on a vortex mixer and incubating swabs in enzyme at 37 °C in a water bath or incubator for 10 min. Before discarding, fluid was expressed from the swabs by pressing against the wall of the tube. A positive control swab immersed in a broth containing GBS strains ATCC 12403 at 0.5 McFarland turbidity and a negative control swab immersed in sterile broth were included with each run of the test.

RESULTS

The prevalence of GBS in Kuwait during the 13-month study period was found to be 20.7% (288/1391) among pregnant women at 35–37 weeks of gestation while the frequency of GBS isolation from 168 similar subjects of the group in Lebanon was 18.4% (31/168). The reason behind the imbalance between the sample size of the Kuwait and Lebanon samples is that Lebanon follows high-risk patient screening, whereas Kuwait follows screening of all pregnant women.

Direct plating of vaginal swabs on the three solid media resulted in GBS isolation rates of 51.6, 64.5 and 77.4% on SBA, CNA and SBS, respectively. Subculture of swabs in Lim broth resulted in a higher isolation rate of GBS. The recovery rates were 90.3, 93.1 and 96.8% on SBA, CNA and SBS, respectively (Table 1).
A DLA test performed on 102 culture-positive samples showed 86 (84.3%) to be reactive with the reagent. However, when the comparison was based on the density of GBS growth on SBA, the sensitivity and specificity of the DLA were 100% with heavy or moderately heavy growth of GBS, whereas the sensitivity, specificity, and positive and negative predictive values when compared for light growth were 100, 55.5, 63.6 and 100 %, respectively (Table 2).

Correlations between the proportions of GBS colonization among 44 specimens growing $\leq 50$ c.f.u. per swab and the DLA test results are shown in Table 3. The DLA test was positive in 52.2% (23/44) and 11.4% (5/44) vaginal swabs growing $\geq 10$ colonies and $\leq 10$ colonies, respectively, whereas the test was negative in 11.4% (5/44) and 25.0% (11/44) vaginal swabs growing $>10$ colonies and $\leq 10$ colonies respectively.

**DISCUSSION**

This research was intended to study the carriage rate of GBS in pregnant women at 35–37 weeks of gestation in Kuwait and Lebanon, and also to investigate culture and immunological methods with a shorter turnaround time for detection of GBS in the vaginal specimens.

A limitation of this study may be the sample size – 168 in Lebanon, where a chromogenic medium (SBS) was evaluated for isolation and identification of GBS, and 122 vaginal swabs from women near labour in Farwania Hospital in Kuwait, which were tested by a DLA for screening of GBS colonization. The urgency to receive information about GBS vaginal colonization is highest in women who are in labour; therefore, DLA testing was limited to that category of women.

Although the CDC recommends rectovaginal sampling for studying carriage rate of GBS in pregnant women, we used only vaginal swabs. Some studies did not find significant differences in detection rates between vaginal and rectal samples (Gupta & Briski, 2004; Nomura et al., 2006). In contrast, there are reports which claim that GBS colonization of rectal samples is 18% to 24% higher than that of vaginal samples and those that find rectovaginal sampling more appropriate than vaginal sampling only. However, several obstetrics departments still rely only on vaginal specimens to assess GBS colonization (El Aila et al., 2010).

There are different selective media formulations to improve isolation of GBS from clinical samples, especially those that are mixed with other microbial flora. It has been shown that addition of horse serum and starch to agar-based media increased the orange/red pigment formation that is already present to some degree on Columbia agar, which is typical for GBS and is not produced by other serotypes (Islam AK, 1977). In order to exploit this property of GBS and improve the quality of the medium in terms of sensitivity, specificity, cost-effectiveness and turnaround time, research in the past couple of decades has resulted in several commercially available chromogenic media, such as those developed by Bio-Rad, bioMérieux, Thermo Fisher and Oxoid/Remel.

The prevalence of maternal GBS colonization in our study was similar to that reported in the literature previously as 11.4–26.5% in both developed and developing countries (Yancey et al., 1996). Interestingly, our result for Kuwait was higher than what was reported earlier as 14.2 and 16.4% in 2002 and 2004, respectively (Al-Sweih et al., 2004). In addition, an earlier study from Lebanon had reported the prevalence of vaginal colonization as 17.7% in all pregnant women in 2010. This increase in the rate of GBS detection could be attributed to use of different culture protocols (Al-Sweih et al., 2004; El Aila et al., 2010; Seoud et al., 2010).

**Table 1.** Number of GBS culture-positive samples among Lebanese women detected on different culture media, before and after enrichment in Lim broth

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>GBS positive sample (n)</th>
<th>GBS isolation rate (%)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-En</td>
<td>Post-En</td>
<td>Pre-En</td>
</tr>
<tr>
<td>SBA</td>
<td>16</td>
<td>28</td>
<td>9.5</td>
</tr>
<tr>
<td>CNA</td>
<td>20</td>
<td>29</td>
<td>11.9</td>
</tr>
<tr>
<td>SBS</td>
<td>24</td>
<td>30</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Pre-En, pre-enrichment; Post-En, post-enrichment.

**Table 2.** Correlation between light GBS growth ($\leq 10$–$<50$ c.f.u. per swab) on SBA with DLA test in vaginal samples of Kuwaiti women

<table>
<thead>
<tr>
<th>GBS culture-positive samples (%; n=44)</th>
<th>GBS (c.f.u. per swab)</th>
<th>DLA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 (52.2)</td>
<td>$&gt;10$</td>
<td>Positive</td>
</tr>
<tr>
<td>5 (11.4)</td>
<td>$&gt;10$</td>
<td>Negative</td>
</tr>
<tr>
<td>11 (25.0)</td>
<td>$\leq 10$</td>
<td>Negative</td>
</tr>
<tr>
<td>5 (11.4)</td>
<td>$\leq 10$</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Table 3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of DLA test as compared with different densities of vaginal colonization of GBS in 102 Kuwaiti women in labour

<table>
<thead>
<tr>
<th>Level of GBS density (c.f.u. per swab) (n)</th>
<th>Sensitivity (%)</th>
<th>DLA test specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (&gt;100) 28</td>
<td>28/28 (100)</td>
<td>20/20 (100)</td>
<td>28/28 (100)</td>
<td>20/20 (100)</td>
</tr>
<tr>
<td>Moderate (50–100) 30</td>
<td>30/30 (100)</td>
<td>20/20 (100)</td>
<td>30/30 (100)</td>
<td>20/20 (100)</td>
</tr>
<tr>
<td>Low (&lt;50) 44</td>
<td>28/28 (100)</td>
<td>20/36 (55.5)</td>
<td>28/44 (63.6)</td>
<td>20/20 (100)</td>
</tr>
</tbody>
</table>

There are only a few studies which examined the use of chromogenic media for rapid detection of GBS as a screening measure in pregnant women (Gupta & Briski., 2004; Smith et al., 2008; Tazi et al., 2009; Louie et al., 2010). Previous evaluation of SBS medium has demonstrated excellent sensitivity for the detection of GBS from vaginal or vaginal-rectal swabs (Tazi et al., 2009; Louie et al., 2010). Comparison of GBS yield on SBS medium with PCR for the cfb gene directly from the enrichment broth (Streptococcus selective broth) produced similar results, indicating high sensitivity of this chromogenic medium (Louie et al., 2010).

Guidelines published by the CDC endorse the use of a selective broth medium, such as Lim broth, to maximize recovery of GBS from vaginal and rectal specimens (Jones et al., 1983, Dunne & Holland-Staley, 1998; Schrag et al., 2002). However, variable results have been reported using different media preparations while studying direct plating versus subculture from Lim broth. Some studies found comparable sensitivity of direct plating on a differential (Granada) medium compared with Lim broth enrichment (Gil et al., 1999; Votava et al., 2001), whereas other reports (De la Rosa et al., 1992; Rosa-Fraile et al., 1999) suggested that direct plating on chromogenic and or selective media was significantly less sensitive (Overman et al., 2002; Nomura et al., 2006). Comparing direct plating on SBS or CNA (bioMérieux) to that of plating on the same media after Lim broth enrichment was found to be equally sensitive with no significant advantage for enrichment (Tazi et al., 2009; El Aila et al., 2010; Louie et al., 2010). In contrast, our study showed that enrichment of vaginal specimens resulted in better detection of GBS on all three media albeit with higher yield on SBS as compared with SBA or CNA. In an earlier study (Dunne & Holland-Staley, 1998) it was shown that while broth enrichment and direct plating on a selective medium (CNA) were found to be equally sensitive, neither method was sufficiently reliable as the sole method for recovery of GBS from vaginal, cervical and rectal specimens. This observation, as well as ours, provides further evidence to support the current recommendations proposed by the CDC.

Limitations of this method are the delay incurred in finalizing the report and competitive overgrowth of Enterooccus spp. in the enrichment broth reducing the number of GBS by approximately 2 log_{10} (Dunne & Holland-Staley, 1998). False-positive results have been obtained on chromogenic media, which would require suspected GBS colonies to be confirmed by additional biochemical tests, latex agglutination or molecular techniques (Tazi et al., 2009; El Aila et al., 2010).

It is important clinically to identify GBS colonizing women near delivery to eliminate the risk of intrapartum exposure of the neonate to GBS. We used a rapid direct method to detect GBS antigen in the vaginal swabs of women in labour because only 40% of colonized women carry GBS continually throughout the course of their pregnancy while others may be colonized intermittently or transiently (Overman et al., 2002). It has been suggested that an LPA-enzyme extraction (Streptex) method for direct antigen detection for GBS is positive in only heavily colonized (>10^4 c.f.u per swab) women, presenting the sensitivity and specificity of 63 and 99.7%, respectively (Konnick & Edberg, 2002). In two other analogous studies, sensitivity and specificity were reported as 85.7 and 99.3%, respectively, by Wald et al. (1987) and 95.2 and 99.3 %, respectively, by Lotz-Nolan et al. (1989). In both these studies, a different method of antigen extraction, viz. LPA-micronitrous acid extraction (Bactigen; Wampole Laboratories), was used, which may explain improved sensitivity of the test. In our study, we examined the association of semiquantitative culture of the vaginal swabs on SBA and DLA for GBS antigen extraction. Although we observed 100 % sensitivity even when the GBS colony count was <50 c.f.u per swab, the specificity and positive predictive value dropped to 55.5 and 63.6 %, respectively. This may prove to be a limitation of the DLA test since about 40 % of the women who are lightly colonized may escape detection. However, it has been shown that only women who are heavily colonized often deliver heavily GBS-colonized infants subjecting them to a higher risk of developing early-onset GBS disease (Ancona et al., 1980; Bobitt et al., 1980; Boyer et al., 1983; Park et al., 2001). The advantage of antigen detection methods over culture is that non-viable GBS, which may result because of the use of antibiotics or feminine hygiene products, can be detected while the culture remains negative. Since a DLA test is simple to perform and requires minimal technician training, this method is easily adaptable to small satellite laboratories located in peripheral community clinics.

CONCLUSION

Although optimum recovery of GBS could be achieved by using both chromogenic agar with a selective enrichment broth and a direct antigen detection method, it may prove
to be labour-intensive and not cost-effective. However, judicious use of these techniques, for example, using culture for women at 35–37 weeks of gestation and a DLA test at the time of delivery may help in reducing the workload, cost and reduction in turnaround time for the results.

REFERENCES


