Rapid diagnosis of vaginal carriage of group B beta haemolytic streptococcus by an enrichment cum antigen detection test

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Background & objectives: Group B beta haemolytic streptococcus (GBS) is a frequent colonizer of the maternal genital tract causing peripartum fever, puerperal sepsis, neonatal sepsis and neonatal meningitis. The conventional methods for detection of maternal colonization take 24-48 h. We made an attempt to standardize a rapid enrichment cum antigen detection test to screen pregnant women for GBS colonization in less than 8 h, so as to enable early institution of measures to prevent neonatal sepsis.

Methods: Vaginal swabs of 100 women >36 wk of gestation were inoculated onto enrichment broth (Todd Hewitt broth with lysed horse blood and antibiotics). After incubation for 1, 2, 4, 6, and 18 h, the broth was cultured on sheep blood agar. In culture positive cases, the enrichment broth was subjected to antigen detection by latex agglutination test (LAT). For further evaluation of the rapid test, another group of 100 pregnant women were screened for GBS carriage by 6 h enrichment broth culture followed by antigen detection test.

Results: Five of the first group yielded GBS on culture and all were positive for GBS antigen after 6 h enrichment. Thirteen of the second group were positive for the antigen, but GBS could be isolated in ten only. This enrichment cum antigen detection test showed sensitivity, specificity, and positive and negative predictive values of 100, 98.4, 83.3 and 100 per cent respectively and could detect as few as $10^3$ cfu/ml organisms. Maternal vaginal carriage of GBS was 7.5 per cent (15/200).

Interpretation & conclusion: Six hours of enrichment followed by antigen detection proved to be a rapid and reliable method for detection of GBS colonization. This test is easy to perform making it an ideal test for screening GBS vaginal colonization at labour and starting chemoprophylaxis, where indicated on the same day, before the woman is discharged.

Key words Group B streptococcus - pregnancy - *Streptococcus agalactiae* - vaginal colonization

The majority of infections caused by group B beta haemolytic streptococcus (GBS) or *Streptococcus agalactiae* are found in post partum women and neonates. GBS is a frequent cause of peripartum fever, puerperal sepsis, neonatal sepsis and neonatal meningitis. Neonatal infections show two epidemiological patterns; the early onset type occurring within the first week of life and the late onset type occurring between 1 week and 3 months of life. Early onset infection is acquired vertically during or shortly before birth from organisms colonizing the maternal genital tract. In the late onset
variety, acquisition of infection may occur vertically during birth or horizontally from contact with the mother, other infants, or nursery personnel carrying GBS. Group B streptococci are harboured in the genital tract of 5-40 per cent of pregnant women and 50-70 per cent of their newborns are colonized. Overall only 1 to 2 per cent of the colonized infants develop clinically apparent infection.

Studies from the West showed that penicillin prophylaxis of parturients reduces the total incidence of GBS infection in neonates by 25-80 per cent. It is important to assess the prevalence of vaginal GBS colonization of pregnant women in the Indian population which will help in evolving guidelines for the prevention of GBS infections in the mothers and neonates of our country.

The conventional method for diagnosis of maternal colonization depends on isolation of the organism from a vaginal swab, which takes 24-28 h. Culture following enrichment remains the most sensitive method for diagnosis of individuals colonized with a small number of organisms. Screening of pregnant women at labour and early institution of antimicrobial prophylaxis demands a rapid test which can detect the presence of GBS in the least possible time. A rapid test that can detect GBS colonization in mother within few hours will enable institution of measures to prevent neonatal sepsis during or just after delivery before discharge from hospital. Various tests such as enzyme immunoassay, latex agglutination test, rapid ELISA, optical immunoassay, Gen-probe, Accuprobe, and Group B streptococcus tests have been evaluated. Till date there is no rapid and sensitive method to replace the conventional method of isolation of GBS and offer a reliable report in less 48 h, especially in patients colonized with a small number of GBS.

In this study, we attempted to screen pregnant women for GBS colonization by culture isolation and standardize a rapid enrichment cum antigen detection test to detect such cases so that the GBS colonization status at labour can be assessed within 8 h and adequate measures to protect the newborn taken timely before mother and baby are discharged.

Material & Methods

Patient profile: A total of 200 pregnant women, admitted during August 1999 to July 2000, for delivery in the clean labour room (CLR) of the Nehru Hospital, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, were investigated. Women with history of antimicrobial therapy or pelvic examination during pregnancy, and those with ruptured membranes were excluded. Name, age, parity, gestation period and presence of complications during previous and present pregnancies were recorded.

Processing of specimens: In the first phase of the study vaginal swabs were collected from 100 pregnant women with a sterile cotton swab and transported to the laboratory in Amie’s transport medium (HiMedia, Mumbai). The swabs were inoculated onto a plate of sheep blood agar (SBA) and put into 5ml of enrichment broth to be incubated at 35°C aerobically with 10 per cent CO\(_2\). The SBA plates were incubated for 18-48 h. From the enrichment broth, 0.1 ml was removed in aliquots after 1, 2, 4, 6 and 18 h of incubation. The aliquots were preserved at 4°C with the addition of 0.01 per cent sodium merthiolate, for antigen detection. From the enrichment broth after 1, 2, 4, 6, and 18 h incubation, a loopful (4 mm diameter) was subcultured on SBA plates and incubated overnight at 37°C in 10 per cent CO\(_2\). The direct plate and the enrichment broth subcultures were examined for the presence of GBS colonies.

Identification of GBS: Beta haemolytic colonies on SBA plates suggestive of GBS were identified by smear microscopy, catalase test, bacitracin susceptibility and CAMP test. They were confirmed by sero-grouping using a latex-agglutination antigen-detection kit (Murex Diagnostics, UK).

Antigen detection: In culture positive cases, the enrichment broth aliquots, preserved earlier after various periods (1, 2, 4, 6, and 18 h) of incubation, were centrifuged (3000g × 10 min) and the supernatant tested for the presence of group B specific antigen using latex agglutination antigen detection kit (Murex®, UK). In culture negative cases
latex agglutination antigen detection test was done on overnight incubated enrichment broth supernatant only.

**Standardization of antigen detection with reference strain:** Graded densities \(10^2-10^8\) cfu/ml of reference strain of *Streptococcus agalactiae* (NCTC 8183) (National Collection of Type Cultures, Colindale, London) in 5 ml of enrichment broth (Todd Hewitt broth, Difco®, USA, containing 10% lysed horse blood, 8 mg/l gentamicin, and 15 mg/l nalidixic acid) were incubated at 37°C aerobically with 10 per cent CO₂. At intervals of 1, 2, 4, 6 and 18 h, 200 µl of broth were taken out and subjected to antigen detection test by latex agglutination test (LAT) and quantitative culture by the method of Miles and colleagues¹⁰.

**Evaluation of culture cum antigen detection method:** In the second phase of the study, the antigen detection test was evaluated in another group of 100 pregnant women admitted during the later half of the study period. The enrichment broth cultures were subjected to antigen detection test and subcultured on SBA plates after 6 h and 18 h of incubation (based on the results of the first phase of the study).

**Statistical analysis:** The association of age and parity of the pregnant women with their colonization status was analyzed by Chi-square test. The sensitivity, specificity, positive and negative predictive values and efficiency of the rapid enrichment cum antigen detection test were evaluated against the gold standard of culture isolation.

**Results & Discussion**

**Standardization of antigen (Ag) detection with reference strain:** In the present study, different concentrations of *S. agalactiae* (NCTC 8183) grown in enrichment broth were used to study the suitability of the broth for Ag detection and the detection limit of LAT kit (Murex® Diagnostics, UK). With a starting inoculum of \(10^3\) cfu/ml, Ag detection was positive after 6 h incubation whereas an inoculum of \(10^4-10^5\) cfu/ml could detect Ag after 4 h incubation. At higher starting concentrations, Ag detection was positive after 2 h \((10^6\) cfu/ml) and 1 h \((10^7-10^8\) cfu/ml). GBS Ag could not be detected with a starting organism load of \(10^2\) cfu/ml even after 18 h incubation (Table I). GBS could be isolated in enrichment broth with an initial organism load of \(10^3\) cfu/ml after 6 h of incubation. Sensitivity of culture isolation increased with higher initial inocula and shorter incubation.

The efficacy of commercially available rapid assays like immunoassays and LAT to detect pure suspensions of Group B streptococcus in vitro has already been investigated.¹¹⁻¹³. In the present study, GBS Ag could be detected with an initial organism load of \(10^3\) cfu/ml after 6 h incubation. Sensitivity was higher with higher organism densities and

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**Table I Viable counts and antigen positivity of *Streptococcus agalactiae* (NCTC 8183)**

<table>
<thead>
<tr>
<th>Inoculum (cfu/ml)</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>18 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^8)</td>
<td>2×10⁷(+)</td>
<td>6×10⁷(+)</td>
<td>1.8×10⁸(+)</td>
<td>2×10⁸(+)</td>
<td>1.4×10⁹(+)</td>
</tr>
<tr>
<td>(10^7)</td>
<td>3×10⁹(+)</td>
<td>6×10⁹(+)</td>
<td>9×10⁹(+)</td>
<td>1.4×10⁹(+)</td>
<td>1×10⁹(+)</td>
</tr>
<tr>
<td>(10^6)</td>
<td>4×10⁵(−)</td>
<td>8×10⁵(+)</td>
<td>8×10⁶(+)</td>
<td>3×10⁷(+)</td>
<td>2×10⁷(+)</td>
</tr>
<tr>
<td>(10^5)</td>
<td>3×10⁴(−)</td>
<td>7×10⁴(−)</td>
<td>6×10⁵(+)</td>
<td>4×10⁶(+)</td>
<td>4×10⁶(+)</td>
</tr>
<tr>
<td>(10^4)</td>
<td>4×10³(−)</td>
<td>6×10³(−)</td>
<td>5×10⁴(+)</td>
<td>3×10⁵(+)</td>
<td>3×10⁵(+)</td>
</tr>
<tr>
<td>(10^3)</td>
<td>(−)</td>
<td>5×10²(−)</td>
<td>1×10³(−)</td>
<td>4×10⁴(+)</td>
<td>3×10⁴(+)</td>
</tr>
<tr>
<td>(10^2)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>4×10²(−)</td>
<td>3×10²(−)</td>
</tr>
</tbody>
</table>

Results of antigen detection test are in parentheses.
shorter incubation periods. Compared to culture isolation the rapid assays reported very low sensitivity ranging from 10 to 90 per cent with higher values of 30-100 per cent in heavily colonized cases (>10⁴ cfu/ml)³,⁵,¹¹,¹⁴,¹⁵. Such wide variations in sensitivity reflect the lack of efficacy of different commercially available assays. All studies have however, reported high specificities for these tests (81 -100%). The reported low sensitivity for the LAT may be due to detection of Ag directly from the vaginal swabs³,¹⁶ or after enzyme (pronase B) extraction¹⁷. Multiplication of the GBS in enrichment broth along with release of antigen in the medium may have contributed to higher sensitivity of antigen detection test in this study. Several other studies have reported high sensitivity for LAT performed after enrichment in LIM broth (82%)³, pre-incubation in selective broth medium (91.8%)¹⁸,¹⁹ and one hour incubation for enzyme extraction (88%)²⁰. The sensitivity of available rapid assays in vitro ranges from 10⁶ to 10⁸ cfu/ml, immunoassays being more sensitive than LAT¹¹. The limit of detection of Ag by LAT in the present study was 10³ cfu/ml in pure culture (reflecting higher sensitivity of culture isolation at bacterial counts below 10⁵ cfu/ml). Approximately, 15 per cent of neonates with invasive disease are born to women with vaginal colonization with small number of organisms, stressing the need for test systems for intrapartum detection of low levels of colonization³,⁹.

Patient profile: The study population comprised 200 pregnant women at ≥ 36 wk of gestation with 89 primi- and 111 multigravidae over a period of 12 months (August 1999 to July 2000). In the first phase, 100 pregnant women (44 primi- and 56 multigravidae), age ranging from 21 to 32 yr (mean 25.26 ± 2.2 yr), were investigated. The clinical profile included bad obstetric history (BOH) (17), cervicitis (6), vaginitis (11) and diabetes mellitus (3 women, including one with gestational diabetes and one with concurrent vaginitis). Sixty three women did not have any associated medical complaints. The second phase included 45 primi- and 55 multigravidae with an age range of 20-32 yr (mean 24.85±3.1 yr). The clinical profile in this group included BOH (16), cervicitis (4), vaginitis (11) and diabetes (2). The remaining subjects (67) did not have any co-existing illness.

GBS colonization by culture isolation: In the first phase, of the 100 pregnant women, whose vaginal swabs were subjected to enrichment and subculture, GBS could be isolated in one woman after 24 h incubation and in 4 after 48 h. The sensitivity of Ag detection improved with longer incubation, the cumulative positivities after 1, 2, 4, 6 and 18 h being 1, 2, 4, 5 and 5 cases respectively.

GBS colonization detected by rapid antigen detection and culture isolation: In the first group, all culture positive cases were positive for Ag detection after 6 h enrichment. Accordingly, in the second group, Ag detection was carried out after 6 and 18 h enrichment only. Thirteen women were detected to have vaginal GBS colonization by LAT after 6 h and 18 h of incubation. GBS could subsequently be isolated in 10 of these cases, in 7 on first subculture and in 3 by repeated subcultures and enrichment. In three Ag positive women, no GBS could be isolated in spite of prolonged and repeated enrichment and subcultures. Eighteen of 200 women were positive by LAT whereas 15 were positive by isolation. There were 3 likely false positives and no false negative cases, showing that rapid Ag detection had a sensitivity of 100 per cent and a specificity of 98.37 per cent (Table II). LAT being independent of the viability of the organism, the probability of higher sensitivity of LAT than the gold standard of culture cannot be ruled out. Direct culture of vaginal swabs without enrichment yielded

<table>
<thead>
<tr>
<th>Test results</th>
<th>Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag positive</td>
<td>15 (True positive)</td>
<td>3 (False positive)</td>
<td>18</td>
</tr>
<tr>
<td>Ag negative</td>
<td>0 (False negative)</td>
<td>182 (True negative)</td>
<td>182</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>185</td>
<td>200</td>
</tr>
</tbody>
</table>

Sensitivity = 100%, Specificity = 98.4%, Positive predictive value = 83.3%, Negative predictive value =100%
GBS in 3 cases only, again emphasizing the importance of enrichment.

**Analysis of carriage rate with parity, age and clinical status:** In the study population, there were 89 primigravidae and 111 multigravidae. Five of 89 primigravidae (3 in first phase and 2 in second phase) had vaginal GBS carriage confirmed by both culture isolation and Ag detection. In the multigravida group (111) of the 13 LAT positive cases (2 in first phase and 11 in second phase) 10 were confirmed by culture isolation.

The overall rate of colonization was 7.5 per cent (15/200) and was higher in multi- (10/111; 9.0%) compared to primigravidae (5/89; 5.6%), but the difference was not statistically significant. GBS colonization was present in 8 of 130 (6.2%) women with no associated complaints, 2 of 33 with BOH, 2 of 5 with diabetes (gestational and pre-gestational), 3 of 22 with vaginitis and none with cervicitis. Greater association of GBS colonization (13%) was seen in women with symptoms of vaginitis e.g., purulent vaginal discharge, pruritus and erythema. The mean age of the study population was 25 yr (range 20 to 32). The number of pregnant women was maximum (n=95) in the age group 23-25 yr but many (7 of 15) women with vaginal carriage belonged to the age group 29-31 yr. GBS colonization was present in 13.5 per cent (10/64) of women above 25 yr against 4.1 per cent (5/121) younger women and the difference was statistically significant (P<0.05).

The prevalence of GBS colonization in pregnant women has been estimated in many studies and shown to be 13.4 per cent in Netherlands11, 13.5 per cent in Norway21, 12 per cent in Greece22 and 24 per cent in Saudi Arabia18. The low rate of colonization (7.5%) in the present study may be a reflection of the stringent selection criteria which excluded women with ruptured membranes and history of prior pelvic examination. Higher prevalence rates have been reported in studies that included women with ruptured membranes4,23. The social and cultural background may also contribute to the low prevalence since frequent intercourse and multiple sexual partners have been associated with increased risk of colonization24. Some studies estimated colonization by both vaginal and rectal cultures3,25. Single vaginal culture and lack of rectal culture can partly explain the low prevalence in our study. Positive rectal cultures are of doubtful clinical relevance since GBS is normally present in the gut flora. Few available reports of maternal vaginal colonization from India also show low figures of 9.66 per cent26 and 12 per cent27 whereas the incidence of early onset neonatal disease has been reported to be 0.17/1000 live births28.

The rapid antigen detection test is easy to perform and may be an ideal test for screening GBS vaginal colonization at labour for starting chemophylaxis, where indicated, on the same day. The association of various factors with the carriage rate of GBS needs to be investigated in larger studies.

**References**


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